Acta Horticulturae
Number 1371

Proceedings of the VII International Date Palm Conference

ISHS

KHALIFA INTERNATIONAL AWARD FOR DATE PALM AND AGRICULTURAL INNOVATION

Editors A. Zaid, G.A. Alhadrami and S. Mitra

Acta Horticulturae 1371
June 2023

PROCEEDINGS OF THE

VII International Date Palm Conference

Abu Dhabi, United Arab Emirates

March 14-16, 2022

Conveners
A. Zaid
G.A. Alhadrami

ISHS Division Tropical and Subtropical Fruit and Nuts

ISSN 0567-7572 (print) 2406-6168 (electronic)
ISBN 978 94 6261 370 6, Acta Horticulturae nº. 1371
Price for non-members of ISHS: € 116,–
Published by ISHS, June 2023

Executive Director of ISHS: P. Vanderborght
Technical Processing: S. Franssens

ISHS Secretariat, PO Box 500, 3001 Leuven 1, Belgium – https://www.ishs.org

Printed by Drukkerij Graphius, Eekhoutdriesstraat 67, B-9041 Gent, Belgium

Photograph on the front cover*:*
Second place wining photo in the Date Palm International Photography Competition, 2010. Courtesy of Mr. Khaled bin Ali Al-Maktom. ©Khalifa International Award for Date Palm and Agricultural Innovation.

Editors and Editorial Board

VII International Date Palm Conference

Conveners

A. Zaid, *Khalifa International Award for Date Palm and Agricultural Innovation (KIADPAI), United Arab Emirates*
G.A. Alhadrami, *United Arab Emirates University, United Arab Emirates*

High Committee

H.E. Sheikh Nahayan Mabarak Al Nahayan, *Chairman of Khalifa International Award for Date Palm and Agricultural Innovation Board of Trustees, United Arab Emirates*
A. Zaid, *Khalifa International Award for Date Palm and Agricultural Innovation (KIADPAI), United Arab Emirates*
H.H. Al Kaabi, *Abu Dhabi Quality and Conformity Council, United Arab Emirates*

Scientific Committee

G.A. Alhadrami, *United Arab Emirates University, United Arab Emirates*
B. Chowdhary, *United Arab Emirates University, United Arab Emirates*
F. Hoffmann, Chair of the Scientific Committee, *Khalifa International Award for Date Palm and Agricultural Innovation (KIADPAI), USA*
H. Hughes, *Colorado State University*, *USA*
Y. Martel, *Khalifa International Award for Date Palm and Agricultural Innovation (KIADPAI), Canada*
J.I. Cubero, *Universidad of Córdoba, Spain*
S. Al Shakir, *Date Palm International Expert*, *Iraq*
A. Ben Abdallah, *International Center for Agricultural Research in the Dry Areas (ICARDA)*, *Tunisia*
A. Ouda Ibrahim, *Date Palm International Horticulture Expert*, *Iraq*
F. Al Ansari, *United Arab Emirates University, United Arab Emirates*
T. Yaseen, *FAO-Regional Office for Near East and North Africa, Italy*
M. Meihiar, *Ministry of Climate Change and Environment (MOCCAE), United Arab Emirates*
S. Al Yammahi, *Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), United Arab Emirates*
Z. Hammami, *International Center for Biosaline Agriculture (ICBA), United Arab Emirates*
S.F. Sharabassi, *International Date Palm Expert, Egypt*
A.K. Barakat, *Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), Syria*
A. Haddad, *Jordan Dates Association (JODA), Jordan*
N.S.A. Al Khalfan, *Association of Agricultural Research Institutions in the Near East and North Africa (AARINENA), Jordan*
M.A. Salem, *United Arab Emirates University, United Arab Emirates*
S. Maqsood Ahmed, *United Arab Emirates University, United Arab Emirates*
S. Kurup, *United Arab Emirates University, United Arab Emirates*
A.J. Cheruth, *United Arab Emirates University, United Arab Emirates*
Z. Ahmed, *United Arab Emirates University, United Arab Emirates*
A. Basarir, *United Arab Emirates University, United Arab Emirates*

Organizing Committee

A. Zaid, Chair, *Khalifa International Award for Date Palm and Agricultural Innovation (KIADPAI), United Arab Emirates*
M. Alaidaroos, *FAO-Regional Office for Near East and North Africa, Italy*
W. Abouelhassan, *FAO-Subregional Office for the Gulf Cooperation Council states and Yemen, Italy*
A. Khabbab, *International Fund for Agricultural Development (IFAD), Italy*
A. Oihabi, *Date Palm International Expert, Spain*
M.A. Al Dhanani, *Ministry of Climate Change and Environment (MOCCAE), United Arab Emirates*
A. AlSaad, *Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), United Arab Emirates*
M.M. Al Kaabi, *United Arab Emirates University, United Arab Emirates*
A. Rasheed Ezhikkotayil, *United Arab Emirates University, United Arab Emirates*
G. Jawad Aljabri, *International Center for Biosaline Agriculture (ICBA), United Arab Emirates*
K. Taiba, *Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), Syria*
N. Ammar, *ACSAD, Syria*
A. Kamal Elfar, *Arab Federation for Dates, Egypt*
I. Al Juboori, *Arab Society for Plant Protection, Lebanon*
E. Idriss, *Sudanese Date Palm Society, Sudan*
T. Elsir Musa, *KIADPAI, United Arab Emirates*
E. Saad, *KIADPAI, United Arab Emirates*
A. Karkouti, *KIADPAI, United Arab Emirates*
A. Al Kaabi, *KIADPAI, United Arab Emirates*
E. Shatnawi, *KIADPAI, United Arab Emirates*
Y. Alantari, *KIADPAI, United Arab Emirates*
S. Nasr, *DPFS, United Arab Emirates*
R. Burgos, *KIADPAI, United Arab Emirates*
S. Lakshan, *KIADPAI, United Arab Emirates*
B. Alnsour, *KIADPAI, United Arab Emirates*
A. Mohamed, *KIADPAI, United Arab Emirates*
R. Bhaskaracharya, *United Arab Emirates University, United Arab Emirates*
M.A. Elhoumaizi, *Mohammed Premier University, Morocco*
W. Wakil, *International Organisation for Biological and Integrated Control – West Palaearctic Regional Section (IOBC-WPRS), Pakistan*

Partners

UNITED ARAB EMIRATES
PRESIDENTIAL COURT
SUPPORT SERVICES DIVISION

الإمارات العربية المتحدة
ديوان الرئاسة
قطاع الخدمات المساندة

Food and Agriculture Organization of the United Nations

UAEU
جامعة الإمارات العربية المتحدة
United Arab Emirates University

IFAD
INTERNATIONAL FUND FOR AGRICULTURAL DEVELPMENT

جائزة خليفة الدولية لنخيل التمر والابتكار الزراعي
KHALIFA INTERNATIONAL AWARD FOR DATE PALM AND AGRICULTURAL INNOVATION

جمعية أصدقاء النخلة
DATE PALM FRIENDS SOCIETY

ICARDA

هيئة أبوظبي للزراعة والسلامة الغذائية
ABU DHABI AGRICULTURE AND FOOD SAFETY AUTHORITY

ARAB AUTHORITY FOR AGRICULTURAL INVESTMENT & DEVELOPMENT
الهيئة العربية للاستثمار والإنماء الزراعي

DPGN

AARINENA
Association of Agricultural Research Institutions in the Near East and North Africa

وزارة التغير المناخي والبيئة
MINISTRY OF CLIMATE CHANGE & ENVIRONMENT

ISHS

جامعة الدول العربية
المنظمة العربية للتنمية الزراعية

CFS
COMMITTEE ON WORLD FOOD SECURITY

IsDB
البنك الإسلامي للتنمية
Islamic Development Bank

THE LEAGUE OF ARAB STATES
THE ARAB CENTER FOR THE STUDIES OF ARID ZONES AND DRY LANDS
ACSAD 1968 أكساد

نزرع للغد
ICBA
AGRICULTURE FOR TOMORROW

المصرف العربي للتنمية الاقتصادية في إفريقيا
BADEA

FOREWORD

It is our pleasure to introduce this conference proceedings which enlists organizers and sponsors, as well as organizing and scientific committees and detailed program, and presented scientific articles of the VII International Date Palm Conference which is held under the patronage of His Highness President of the United Arab Emirates (May God protect him), and organized by Khalifa International Award for Date Palm and Agricultural Innovation, in cooperation with the Presidential Court, UAE University, Abu Dhabi Agriculture and Food Safety Authority, International Center for Biosaline Agriculture, Ministry of Climate Change & Environment, Date Palm Friends Society, Food and Agriculture Organization of the United Nations (FAO), FAO Regional Office for Near East and North Africa (FAO-RNE), Arab Organization for Agricultural Development (AOAD), Association of Agricultural Research Institutions in the Near East and North Africa (AARINENA), International Center for Agricultural Research in the Dry Areas (ICARDA), Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD), International Fund for Agricultural Development (IFAD), Date Palm Global Network, Committee on World Food Security (CWFS), in the period from 14 to 16 March 2022 at Emirates Palace Hotel in Abu Dhabi.

We seize this opportunity to express our personal appreciation of the continuous support rendered by H.E. Sheikh Nahayan Mabarak Al Nahayan, Minister of Tolerance and President of the Award's Board of Trustees, as well as organizers and sponsors of this conference. Their contribution is indeed a remarkable example of corporate responsibility.

We sincerely hope that the conference did achieve its objectives and be up to the expectations of His Highness the UAE President, and all interested institutions and individuals.

A. Zaid and G.A. Alhadrami
Conveners

PREFACE

The papers contained in this volume of *Acta Horticulturae* report the scientifically reviewed Proceedings of the VII International Date Palm Conference. Keynote speakers and authors of selected contributed oral and poster presentations were given the opportunity to submit a manuscript for publication.

The manuscripts were reviewed by the Editors and members of the Editorial Board. Only those papers judged suitable for publication following the authors' consideration of reviewer suggestions appear in this volume of *Acta Horticulturae*.

The ISHS acknowledges and appreciates the contribution of all editors and reviewers. They have made a significant contribution to improving the quality of this publication.

The ISHS Board of Directors

LIST OF CONTENTS

Technical practices of date palm

General topics on date palm

LIST OF AUTHORS

The origin and geographical distribution of the 'Mejhoul' date cultivar

A. Zaid[1,a] and A. Oihabi[2,b]

[1]Secretary General Khalifa International Award for Date Palm and Agricultural Innovation, UAE; [2]International Expert, Morocco.

Abstract

The 'Mejhoul' date palm originated from the Tafilalet Valley in Morocco, which is administratively known as the Errachidia Province. Origination was confirmed by DNA analysis of several samples of 'Mejhoul' palms collected from diverse areas including Morocco, Egypt and the USA. This study shows that the 'Mejhoul' cultivar is a landrace variety of Morocco. During the 17th century, 'Mejhoul' was known as a high-quality date and its fruit was sold at a higher price than other cultivars in the markets of England and Spain (Wright, 2016). At that time, most of the dates brought to Europe came from Tafilalet (Hodel and Johnson, 2007). Unfortunately, the occurrence of the Bayoud disease, caused by a soil-born fungi (*Fusarium oxysporum* f. sp. *albedinis*) which was described scientifically for the first time in 1919 by Foex and Vayssière intensively destroyed the Moroccan date plantations. 'Mejhoul' was among the most sensitive cultivars to the disease and therefore, fresh 'Mejhoul' dates disappeared from the European market after its production decreased significantly.

Keywords: 'Mejhoul', Morocco, date palm, *Phoenix dactylifera*

INTRODUCTION

'Mejhoul', the "Jewel of Dates", is known for its attractive appearance, large size, brownish colour, succulent and juicy flesh, and excellent taste due to its maple syrup-like flavour. It is currently the most important and desired date on the international market, as well as the most expensive compared to other date cultivars. 'Mejhoul' dates have natural sugar crystals that give its skin a slight shimmer, and when you bite into one, you'll immediately notice the rush of flavours hinting at wild honey, cinnamon and caramel. They are truly one of nature's most delectable treats, tasting as if they have come right out of the oven.

The 'Mejhoul' date palm originated from the Tafilalet Valley in Morocco, which is administratively known as the Errachidia Province (Figure 1). Origination was confirmed by DNA analysis of several samples of 'Mejhoul' palms collected from diverse areas including Morocco, Egypt and the USA (Elhoumaizi et al., 2006). This study shows that 'Mejhoul' is a landrace cultivar of Morocco.

During the 17th century, 'Mejhoul' was known as a high-quality date and its fruit was sold at a higher price than other cultivars in the markets of England and Spain (Wright, 2016). At that time, most of the dates brought to Europe came from Tafilalet (Hodel and Johnson, 2007). Unfortunately, the occurrence of Bayoud disease, caused by a soil-born fungi (*Fusarium oxysporum* f. sp. *albedinis*) which was described scientifically for the first time in 1919 by Foex and Vayssière (Malençon, 1950) intensively destroyed the Moroccan date plantations. 'Mejhoul' was among the most sensitive cultivars to the disease and therefore, fresh 'Mejhoul' dates disappeared from the European market after its production decreased significantly.

[a]E-mail: zaidabdelouahhab@gmail.com

[b]E-mail: oihabi@gmail.com

Figure 1. Map of Morocco showing the location of the Tafilalet Oasis (around Errachidia).

THE INTERNATIONAL JOURNEY OF THE 'MEJHOUL' DATE CULTIVAR

The first journey to the US

In 1927, the French colonial authorities appointed a scientific commission to investigate Bayoud disease further. The commission included Walter Swingle from the US, as well as Vayssière, Maire, Régnier, Killian, De Lepiney, and Emberger from France (Chabrolin, 1930). The commission conducted its investigations in Colombachar, Erfoud, Errachidia (Ksar Souk), and Boudnib. During their stay in this late date oasis, which accounted for some 9,000 date palms made mainly of the 'Mejhoul' cultivar, Swingle was impressed by the cultivar which he considered as one of the best dates grown anywhere. He purchased six standard size offshoots and was offered five small ones (Swingle, 1945) which he imported to the US. Arriving in Washington DC five weeks later, the 11 offshoots were fumigated and quarantined in Nevada, where no date palms were growing, but which had a suitable climate for date palm cultivation. This important quarantine period lasted from 1927 until 1936. Nine of the 11 offshoots survived to the end of the quarantine period, and they were then transferred (along with the 64 new additional offshoots they produced) to a US Department of Agriculture (USDA) research station at Indio in California. In 1944, the USDA station at Indio started distributing offshoots to growers in California and Arizona. The Bard Company of California took 24 offshoots from USDA and even now, 99% of date palm growing in Yuma and the Bard Valley are of the 'Mejhoul' cultivar (Wright, 2016).

It is worth noting that the US 'Mejhoul' industry, as well as the 'Mejhoul' industry of several other countries (including Namibia, South Africa, Israel, Australia and Jordan) originated from the 1927 'Mejhoul' importation from Morocco.

Locating the mother date palm collected by Walter Swingle

Dr. Mohammed Aziz Elhoumaizi conducted a sound survey and site visit to the Tafilalet region, aiming to locate the original mother 'Mejhoul' tree, from which the US 'Mejhoul' industry originated. The region where the offshoots were collected is called Rahat Almaa. The geographical position of the mother plant is 31°56'15.1 N, 3°36'10.1 W.

The 'Mejhoul' cultivar's second journey to various countries

The introduction of the 'Mejhoul' date palm to Mexico was achieved through offshoots imported from the US to the San Luis Rio Colorado, Mexicali valley in 1968. Currently, 'Mejhoul' represents 94% of the total date production in Mexico (Salomon, 2021). Figure 2 represents the major Mejhoul growing areas in Mexico and the US.

Between 1978 and 1981, Israel imported 9,000 Mejhoul offshoots from California.

In the early 1990s, the Republic of South Africa (RSA) imported hundreds of 'Mejhoul' offshoots from California. Then, due to the development of the production of date palm plantlets derived from tissue culture, RSA and Namibia imported thousands of 'Mejhoul' plantlets. Namibia also imported 'Mejhoul' from different tissue culture laboratories from France and England.

Figure 2. Major 'Mejhoul' date growing areas in US and Mexico.

In 1995, Jordan imported the country's first 'Mejhoul' offshoot from California. Currently, Jordan has about 500,000 'Mejhoul' productive trees.

In 2006, Palestine imported 'Mejhoul' plantlets derived from tissue culture from different sources (Al Banna, pers. commun.). 'Mejhoul' cultivars were also introduced in other countries such as Australia, Peru, Chile and the Sudan.

Egypt has recently planted thousands of 'Mejhoul' plantlets derived from tissue culture and aims to planting five million date palms of different cultivars, including 'Mejhoul'. In 2008, Morocco initiated the 'Green Morocco Plan' programme, and has already planted three million date palms, of which 'Mejhoul' enjoys a 70% share. Morocco is also planning for a second extension of its date palm plantations (of about five million date palms) with a special focus on 'Mejhoul' (Generation Green Plan 2020-2030).

The 'Mejhoul' journey from Boudnib Valley in Morocco to the different date-producing countries around the world is represented in Figure 3.

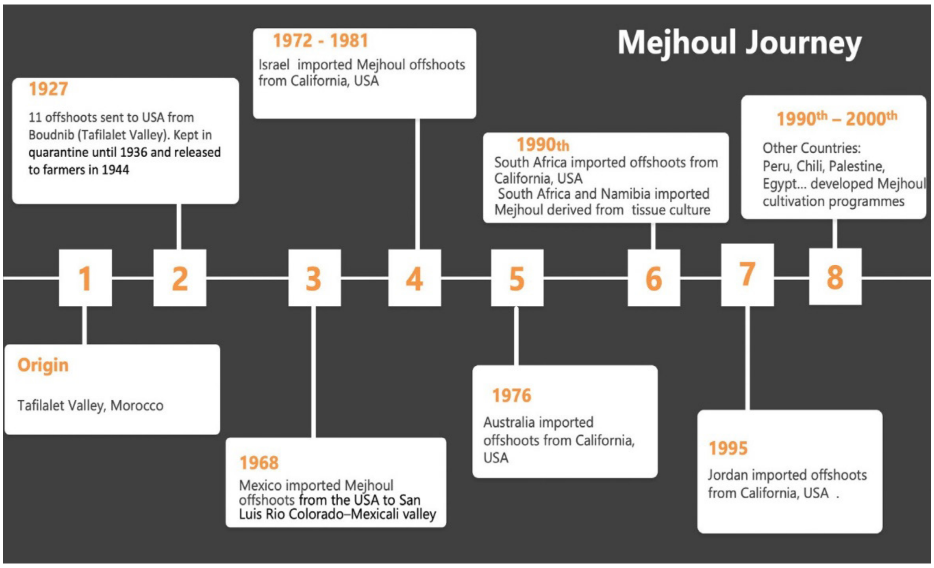


Figure 3. 'Mejhoul' journey from Morocco to different date-producing countries. Source: Wright (2006).

GLOBAL 'MEJHOUL' PRODUCTION

'Mejhoul' dates represent 94% of the total dates produced in Mexico, and 85 and 70% of Israeli and Jordanian date production, respectively. Table 1 summarises global 'Mejhoul' date production in 2020 which is estimated at 108,498 t (B. Glasner, R. Salomon, A. Haddad, and M. Al Banna, 2021, pers. commun.).

Table 1. 'Mejhoul' date production by country (in t).

Country	Quantity (t)	Share (%)
Israel	45,000	41.48
USA	16,000	14.75
Mexico	14,898	13.73
Palestine	12,000	11.06
Jordan	10,000	9.22
Morocco	3,500	3.23
SA & Namibia	3,000	2.77
Egypt	3,000	2.77
Peru	500	0.46
Australia	100	0.09
Others	500	0.46
Total	**108,498**	**100.00**

'Mejhoul' date production is expected to increase enormously during the forthcoming years, mainly through the introduction of several million date palm trees in new large areas of Moroccan and Egyptian date plantations.

Literature cited

Chabrolin, Ch. (1930). Les maladies du palmier dattier. J. Agric. Tradit. Bot. Appl. *107*, 557–566.

Elhoumaizi, M.A., Devanand, P.S., Fang, J., and Chao, C.-C.T. (2006). Confirmation of Medjool date as landrace variety through genetic analysis of Medjool accession in Morocco. J. Am. Soc. Hortic. Sci. *131* (*3*), 403–407 https://doi.org/10.21273/JASHS.131.3.403.

Hodel, D.R., and Johnson, D.V. (2007). Imported and American Varieties of Dates in the United States. Pub 3498 (Oakland: University of California).

Malençon, G. (1950). Le Bayoud maladie fusarienne du palmier dattier en Afrique du Nord. Fruits d'Outre-Mer. *5* (*8*), 279–289.

Salomon, R. (2021). Research for the improvement of Medjool date in Mexico. Paper presented at: 1st International Medjool Date Colloquium (Mexico) (Webinar).

Swingle, W.T. (1945). Introduction of the Medjool Date from Africa into the United States, Vol. 22 (Date Grower's Institute), p.15–16.

Wright, G.C. (2016). The commercial date industry in the United States and Mexico. HortScience *51* (*11*), 1333–1338 https://doi.org/10.21273/HORTSCI11043-16.

‘Mejhoul’ cultivation in the USA

G.C. Wright[a]

University of Arizona - Yuma Agriculture Center, 6425 W. 8th Street, Yuma, AZ 85364, USA.

Abstract

Dates have been grown in the United States for over 200 years. Large plantings began about 125 years ago and the ‘Mejhoul’ was imported successfully in 1927. The US ‘Mejhoul’ industry began with a 23-ha planting in 1945, near Bard, CA, and has grown to several thousand ha today. Trees are planted in several types of soils, at spacings ranging from 7.5 to 9 m². Irrigation is by flood or pressurized systems, and most fertilization is applied via the irrigation water. ‘Mejhoul’ dates are pollinated and thinned, then bagged before harvest. Harvest begins in August and continues through October. Few pests are problematic on ‘Mejhoul’. Harvested dates are sorted for color, dried to consistent moisture, sorted again for quality, and then shipped fresh or frozen if needed. US ‘Mejhoul’ dates are exported to Canada, Australia, Mexico, and other countries, while the US imports ‘Mejhoul’ dates from Mexico, Israel, and other countries. Date consumption in the US is low but has increased by 50% since 2012 due to active marketing programs.

Keywords: history, horticultural practices, production, marketing, exports

INTRODUCTION

Spanish priests were the first to introduce the date palm to the United States (US) in the late 1700s (Toumey, 1898; Trent and Seymour, 2010). Palms were planted across both California and Arizona wherever the climate was favorable. These early plantings came from seed. However, US date consumption in the late 1800s and early 1900s exceeded the domestic supply, so most dates were imported (Hopper, 2013). As early as the 1820s, American ships carried US goods to ports on the Arabian Peninsula, and in exchange they picked up dates and other commodities. By 1925, US date imports exceeded 8,000 metric t.

In response to the demand and the need to find suitable crops for the desert, the United States Department of Agriculture (USDA) organized importations of thousands of date palm offshoots, chiefly from Algeria, Tunisia, Egypt and Iraq to California and Arizona (Toumey, 1898; Nixon, 1950; Hilgeman, 1972). Private individuals also imported thousand more. Many cultivars were imported, including ‘Barhi’, ‘Deglet Noor’, ‘Fard’, ‘Hayany’, ‘Khadrawy’, ‘Sayer’ and ‘Zahidi’ (Wright, 2012).

The first arrival of the ‘Mejhoul’ cultivar into the US was in 1912, but it is unknown what happened to them (Wright, 2016). ‘Mejhoul’ dates were imported successfully into the US in 1927 by Dr. Walter Swingle (Swingle, 1945). Swingle was visiting Boudenib, Morocco, where the Sharif of the local oasis sold him 11 apparently disease-free offshoots which were packed in wooden crates and shipped to the US, arriving five weeks later. Because of the threat of Bayoud disease, the offshoots were fumigated upon arrival and placed in quarantine in Nevada for eight years, where nine of the 11 survived. After quarantine, the original nine and many additional offshoots were moved to a USDA facility in California. The entire US ‘Mejhoul’ industry, and the ‘Mejhoul’ industries of several other countries, originate from the importation of 1927.

MASS PRODUCTION IN THE US

The US date production industry began with a 23-ha planting in 1945, near Bard, California, across the river from Yuma, Arizona. Stanley Dillman and Al Coleman were the first to plant the ‘Mejhoul’ cultivar from 22 offshoots received from the USDA (Berryman, 1972).

[a]E-mail: gwright@arizona.edu

They developed several horticultural practices which are still practiced. Today, the US date industry encompasses 6,700 ha, all in California and Arizona (USDA, 2021). The 2020 value of the industry was estimated at $ 189,000,000. Because the USDA does not distinguish between cultivars, it is not possible to make a full comparison between the different cultivars, but the author estimates the 'Mejhoul' total is about 55%.

Dates are planted on both alluvial river-bottom soil and sandy upland soil (Figure 1). Since the 'Mejhoul' cultivar makes many offshoots, tissue culture-derived palms are not common. Traditional planting of the 'Mejhoul' palm is about 9×9 m square, but newer plantings are being established at a density of 8×8, and 7.5×7.5 m. One male palm is planted for every 49 females. There are no specific male palms identified as superior pollinators.

Figure 1. Imperial date gardens, Yuma Arizona, USA.

Growers employ either flood or pressurized drippers or micro sprinklers (Figure 2) to irrigate the palms. Irrigation rates vary by tree size and season and can be as much as 500 to 600 L of water tree^{-1} day^{-1} in the summer. Conventional fertilisers are applied through the irrigation water, and growers regularly apply nitrogen, potassium, and boron. Other macro- and micronutrients are not as commonly applied. Organic dates receive composted chicken or steer manure, or liquid forms of organic fertilizers, containing humic acids, seaweed, and other compounds.

DATE CULTIVATION BY SEASON

Workers clean the palms in January, removing thorns and old leaves. Male palms bloom in early March, and once the spathe cracks, the male flowers and pollen are extracted and dried with fans and heat. Growers dilute pollen with flour, talc, or cornstarch. Female palms bloom in mid-to late March, and a mature palm will produce 20 to 25 bunches. Each emerging bunch is forced to curve downward by tying them to leaves below. Bunches are pollinated as many as four times a season using squeeze bottles, blow pipes, or modified air blowers.

Thinning the fruit begins in April. In areas with higher humidity, such as Arizona, workers remove about 70% of the fruit, leaving fruit spaced at about 2-3 cm apart on the strands. Where humidity is less of a problem, the strands are cut so that six to ten fruit remain. After the center strands are removed, 35 to 40 strands remain on the bunch. Workers support the increasingly heavy bunches by tying them to nearby leaf petioles for support.

Figure 2. 'Medjool' date irrigated with pressurized drippers near Bard, California, USA.

Workers cover the bunches with cotton or nylon bags in late July. These bags retain fruit that might drop early, protect the fruit from birds, insects, and animals, protect the ripening fruit from rain and provide ventilation to the bunch. Some growers also insert metal rings into the bunches to spread out the strands, improve ventilation and reduce the chance of fermentation.

Harvest begins in late August; each tree is harvested three to four times, every 10-14 days as the fruit do not all mature uniformly. Harvest is finished by October. Each mature palm can produce as much as 100 to 125 kg of fruit. At the packing house, unmarketable fruit are eliminated, then the rest are gently washed then sorted by maturation. This sort allows the fruit to be segregated according to the amount of time needed to dry them to 16-21% moisture content. Fruits are dried from one to seven days at about 65°C. Most dates are dried using forced hot air, but a few are dried traditionally in the sun. Following drying, the dates are washed again then sorted according to size and external appearance. Finally, they are packaged and kept refrigerated or frozen until sold. Because of the use of the bags, the heat applied during drying, and the frozen storage, no additional fumigation is necessary.

'Mejhoul' dates grown in Arizona and California have no significant pest problems. Carob moth (*Ectomy eloisceratoniae*) infestations can be eliminated by the steps noted above, or by using insect pheromones and insecticides (Mafra-Neto et al., 2013). The palm weevil (*Rhyncho phorusvulneratus*) has been eliminated from the US (Hoddle et al., 2016) although the South American palm weevil (*Rhynchophorus palmarum*) has been advancing northward in the coastal areas of Mexico and Southern California since 2010 (Hoddle et al., 2021). Date growers and Agriculture Department officials are monitoring for the arrival of this pest. Black scorch (*Thielaviopsis punctulata*) is an occasional problem and the Bayoud disease has been kept out because of phyto-sanitary regulations. No date palm offshoot has been imported to the US since 1929.

US date production is low compared to other date-producing countries. This author estimates that the US is now producing about 25,000 metric t of 'Mejhoul' dates annually. Date Pac is a large grower cooperative in Yuma Arizona that produces pesticide-free and organic 'Mejhoul' dates. Date Pac claims its dates are sold in 90% of all the supermarkets in the US. There are other smaller date packinghouses that distribute their fruit to local, regional, and international markets. USDA statistics show that US dates are exported to Canada, Australia, Mexico, and the UK. Dates are imported into the US from Tunisia, Algeria, Israel, and Mexico (Agricultural Marketing Resource Center, 2018). US table date fruit consumption (not processed) was about 75 g person^{-1} year^{-1} in 2019 (Statista, 2021). However, increasingly aggressive marketing programs are leading to more buying (Grebitus and Hughner, 2021). This level of consumption represents a 50% increase over 2012.

Literature cited

Agricultural Marketing Resource Center. (2018). https://www.agmrc.org/commodities-products/fruits/dates.

Berryman, E. (1972). Medjool date production in Bard, California. Date Grower's Inst. *49*, 10.

Grebitus, C., and Hughner, R.S. (2021). Consumer demand and preferences for Medjool dates grown in Arizona. Arizona Food Industry Journal *June 2021*, 7.

Hilgeman, R.H. (1972). History of date culture and research in Arizona. Date Grower's Inst. *49*, 11–14.

Hoddle, M. S., Kabashima, J. N., Millar, J. G. and Dimson, M.(2016). The palm weevil *Rhynchophorus vulneratus* is eradicated from Laguna Beach. California Agric. *71* (*1*), 23–29.

Hoddle, M.S., Hoddle, C.D., and Milosavljević, I. (2021). Quantification of the life time flight capabilities of the South American palm weevil, *Rhynchophorus palmarum* (L.) (*Coleoptera*: *curculionidae*). Insects *12* (*2*), 123 https://doi.org/10.3390/insects12020126. PubMed

Hopper, M.S. (2013). The globalization of dried fruit - transformations in the eastern Arabian economy, 1860-1920s. In Global Muslims in the Age of Steam and Print, J.L. Gelvin, and N. Green, eds. (Berkeley, CA: University of California Press), p.158–181.

Mafra-Neto, A., et al (2013). Manipulation of insect behaviour with specialized pheromone and lure application technology (SPLAT). In Pest Management with Natural Products, J.J. Beck, J.R. Coates, et al, eds. (Washington, DC: American Chemical Society), p.31–58.

Nixon, R.W. (1950). Imported varieties of dates in the United States. USDA Circular *834*, 144.

Statista. (2021). Annual consumption of table dates in the United States from 2012 to 2019. https://www.statista.com/statistics/936362/date- consumption-us/.

Swingle, W.T. (1945). Introduction of the Medjool date from Africa into the United States. Date Grower's Inst. *22*, 15–16.

Toumey, J.S. (1898). The Date Palm. Arizona Agric. Exp. Sta. Bull. *29*, 102–150.

Trent, H., and Seymour, J. (2010). Examining California's first palm tree: the Serra palm. J. San Diego Hist. *56* (*3*), 105–120.

USDA. (2021). Noncitrus Fruits and Nuts - 2020 Summary. https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/sf269213r/6t054c23t/ncit0521.pdf.

Wright, G.C. (2012). Date cultivation in Arizona and the Bard Valley. J. Am. Pomol. Soc. *66* (*3*), 110–117.

Wright, G.C. (2016). The commercial date industry in the United States and Mexico. HortScience *51* (*11*), 1333–1338 https://doi.org/10.21273/HORTSCI11043-16.

The 'Mejhoul' cultivar in the Kingdom of Morocco: origin, geographical distribution, and international market

M. Bachri[a]

National Agency for the Development of Oasis and Argan Zones (ANDZOA), Morocco.

Abstract

The origin of the 'Mejhoul' cultivar belongs to the Tafilalet area, and it is this date cultivar that attracted the attention of many regional and international investors. This in turn led to the introduction and spread of the 'Mejhoul' cultivar in various regions in the Kingdom of Morocco, as well as other countries across the world, such as the USA, Republic of Namibia, South America, and Australia.

Keywords: 'Mejhoul', Morocco, date palm, *Phoenix dactylifera*, trading

INTRODUCTION

Date palms are one of the oldest fruit trees in the world, having emerged since the end of the second era, spreading across the Persian Gulf to North Africa. Trade between the Maghreb countries and the Middle East played an important role in the value gained by date palm trees, which led to the spread of date palm cultivation in these areas using date seed. Dates became the main source of food supply that travelers carried during their journeys across the countries. Date palm cultivation then reached the Islamic Republic of Mauritania, during the first century, and to Sudan in the fifth century. Date palm cultivation also spread into semi-desert areas, with dates the main source of food to travelers crossing the desert areas.

Date palm cultivation was introduced to the USA during the 18th century, by biology travelers, however, date palm cultivation did not spread there until the1900s, in California (Toutain, 1965).

Dr. Abdul-Jabbar Al-Bakr, in his book 'The Date Palm: a Review of Its Past, Present Status and the Recent Advances in Its Culture Industry and Trade', noted that the original source of the 'Mejhoul' date cultivar was Tafilalet, Wadi Ziz. The 'Mejhoul' cultivar was first introduced to the USA by Mr. Swingle in 1927, from the Moroccan town of Boudnaib, 90 km from Errachidia city. It was then planted separately in the State of Nevada, until found free from pests and diseases, before being transferred to Indio, California in 1932. In 1957, The Date Palm Experiment Station in Indio started efforts to increase 'Mejhoul' cultivation, after the significant results, and its excellent commercial qualities.

The 'Tafilalet book', is a contribution to the Moroccan history where during the 17th and 18th centuries, Al-Arabi Mizin spoke about the presence of the 'Mejhoul' cultivar in the Aoufous region. Of the harvest traditions at the time, Al-Arabi Mizin wrote: "As for the harvest in autumn, they started it on the first day of October, except for the 'Mejhoul', which was harvested till the 17th of September".

All these sources prove the 'Mejhoul' date cultivar originated from the Tafilalet area. 'Mejhoul' fruits originating from this region are the result of the selections made by date growers throughout the years. The Tafilalet region is also known as the home of the International Forum of Moroccan dates, and replaced the annual 'Date Season', which was organized by the late King Mohammed V "May God bless his soul". Today, some of the people who lived during the time, still remember the first session held in the 1940s, after World War II, in the Erfoud/Errachidia region.

All this information proves the origin of the 'Mejhoul' cultivar belongs to the Tafilalet

[a]E-mail: bachri.andzoa@gmail.com

area, and it is this date cultivar that attracted the attention of many regional and international investors. This in turn led to the introduction and spread of the ‘Mejhoul’ cultivar in various regions in the Kingdom of Morocco, as well as other countries across the world, such as the USA, Republic of Namibia, South America, and Australia.

GEOGRAPHICAL DISTRIBUTION

The geographical distribution of ‘Mejhoul’ production in Morocco includes two main plantation areas located on the banks of Wadi Ziz, Griss, Todgha and Draa, as well as expansions of ‘Mejhoul’ plantations in the Errachidia area (Figure 1). These plantations have developed significantly under the Green Morocco scheme, which allocated important support to date growers in both traditional and commercial sectors. These efforts will also continue as part of the Moroccan new Green Generation Strategy.

The geographical boundaries of the date production areas of the ‘Tafilalet Mejhoul’ are:

- Northern parts of Midelt and Figuig areas;
- Eastern parts of Figuig and the Algerian border;
- Western parts of both Tinghir and Zagora regions;
- The southern Algerian border.

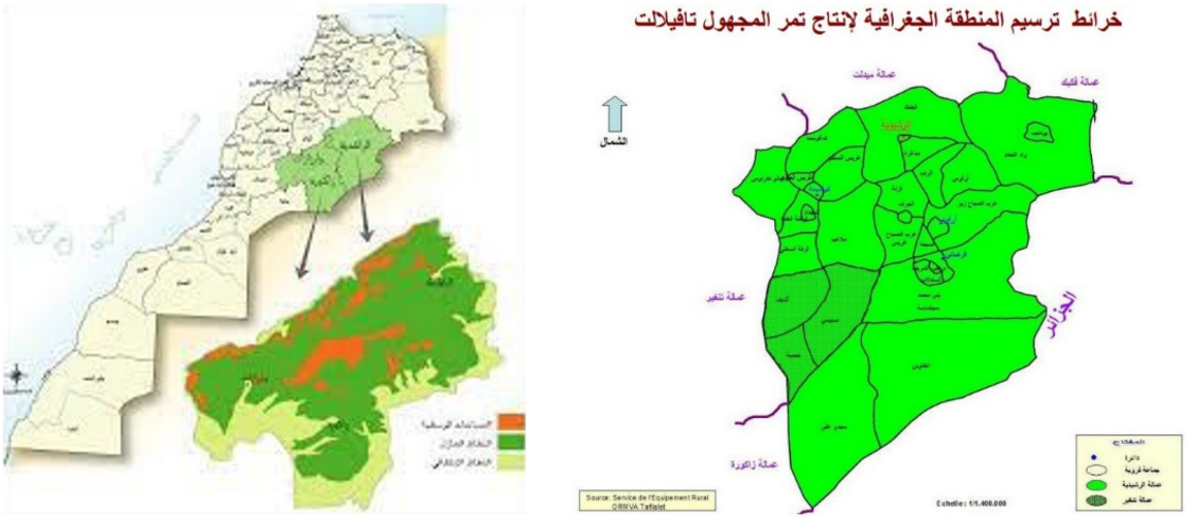


Figure 1. Maps of geographical distribution of ‘Mejhoul’ in Tafilalet area.

EXPECTED DATE PRODUCTION ACCORDING TO TYPE, DIVERSE EXPECTATIONS, AND FUTURE MARKETING PROSPECTS

Taking into account the new modern ‘Mejhoul’ plantations, the number of available date cultivars in Morocco is set to change significantly in the coming years. At present, ‘other’ date cultivars account for 90% of the total production, but this is expected to significantly reduce, being replaced by higher quality date cultivars, such as the ‘Mejhoul’, ‘Najda’, and ‘BouFeggous’ cultivars, which are estimated to eventually take over more than 50% of the total Moroccan dates production.

INTERNATIONAL MARKETS

The Kingdom of Morocco exported 3,288 t of dates, worth 55.9 million Moroccan Dirhams, in 2020, compared to 17.6 t exported in 2011, worth 0.4 million Moroccan Dirhams. However, of this quantity, the production of ‘Mejhoul’ date was very low. Morocco is expecting a production of 70,000 t of ‘Mejhoul’ dates, to be presented in the national and international markets, by 2028.

Edaphoclimatic requirements of the 'Mejhoul' date cultivar

A. Meddich[1,2,a] and A. Oihabi[3,b]

[1]Center of Agrobiotechnology and Bioengineering, Research Unit Labelled CNRST (Centre AgroBiotech-URL-CNRST-05), "Physiology of Abiotic Stresses Team", Cadi Ayyad University, Marrakech, 40000, Morocco; [2]Laboratory of Agro-Food, Biotechnologies and Valorization of Plant Bioresources (Agrobioval), Faculty of Sciences Semlalia, Cadi Ayyad University, PO Box 2390, Marrakech, 40000, Morocco.

Abstract

'Mejhoul' date palm is considered the pillar of the oasis ecosystem and one of the most economically important perennial plants in arid and semi-arid areas. It is able to tolerate severe environmental conditions (drought and salinity) through its aptitude to being cultivated in diverse soil and climatic conditions. In this work, we present the main planting areas of 'Mejhoul' in the northern and southern hemispheres, for which, thermal coefficients and relative humidity were described. 'Mejhoul' prefers doses of cold in winter and heat is important from fruit set to harvest, for around five to six months per year. In 'Mejhoul' plantation areas, the lowest minimum temperature varies from 3 to 9°C, while the highest maximum temperature varies between 38 and 42°C. The flowering process starts at 18 to 20°C which coincides with the period of January/February in the northern hemisphere and June/July in the southern hemisphere. 'Mejhoul' dates require low air relative humidity during the fruit development and maturation periods. Furthermore, the combined effect of different climatic conditions (heat, annual temperatures and relative humidity averages) impact the characteristics of 'Mejhoul' fruit.

Keywords: edaphoclimatic, temperature, relative humidity, 'Mejhoul', date palm

INTRODUCTION

'Mejhoul' dates represent an important source of income as well as a staple food for the local population in arid and semi-arid regions (El Bakouri et al., 2021). 'Mejhoul' is the most known and demanded in the world (Ibourki et al., 2021) and its fruits are a good source of essential nutrients. Moreover, the commercial value of the fruits of 'Mejhoul' is higher than that of other date palm cultivars traded in the world. While all date palm cultivars tolerate various soil types, the 'Mejhoul' cultivar particularly grows better on free drainage sands or sandy loams with good moisture-holding capacity. It also accepts different pH levels but prefers neutral value. Several scientific studies have shown that the average pH in most date-growing areas varies between 7 and 8.5. Alkaline and saline soil negatively affect the productivity and the quality of 'Mejhoul' fruits (Hasanaoui et al., 2010; Zhen et al., 2020). The 'Mejhoul' date palm tolerates drought and can continue to grow under severe water stress. For its optimal production, its water requirements are important and vary from 10,000 to 20,000 m^3 ha^{-1} depending on different factors such as climatic conditions, irrigation management, age, soil texture and type of fertilizers used. 'Mejhoul' date palms are known as drought-resistant (Elfeky and Elfaki, 2019). However, for optimal productivity, appropriate and quality irrigation is needed, as irrigation management plays an important role in the fruit's development and quality (Mohammed et al., 2020). The implementation of adequate good agricultural practices (GAP) plays an important role in the morphological and nutritional characteristics of 'Mejhoul' dates (Ranasinghe et al., 2022). Water salinity reduces yields and growth of 'Mejhoul' trees (Alsmairat et al., 2019; Sperling et al., 2014), which remain tolerant to salt concentrations between 3 and 10 g L^{-1}, depending on the age of the date palm and the

[a]E-mail: meddich@uca.ma
[b]E-mail: oihabi@gmail.com

prevalent soil characteristics (Ait-El-Mokhtar et al., 2020; Alsmairat et al., 2019). Currently, and due to their ability to adapt to different climatic and environmental conditions, 'Mejhoul' is present in many parts of the world. It is known worldwide for its large size, pleasant taste and excellent quality (Pérez-Pérez et al., 2021). The objective of this study was to investigate the impact of certain environmental conditions of different localities in the world (temperature, relative humidity, heat) on the adaptive capacity of 'Mejhoul' and the maturity of its fruit.

METHODOLOGY

In this study, we have located on a map the main localities of the Mejhoul plantations. Eleven locations were studied including El karamah (Jordan), Mexical (Mexico), Al Madinah Al Munawara (Saudi Arabia), Boudinb (Morocco), Keetmanshoop (Namibia), Wahat Baharia (Egypt), Jericho (Palestine), En Yahav (Israel), Alice Springs (Australia), Coachella Valley and Yuma (United States of America) (Figure 1).

Figure 1. Mejhoul planting areas.

The 30-year average minimum and maximum temperatures in the different localities of 'Mejhoul' were illustrated (Figure 2). Also, the average relative humidity for the period 2016-2020 was presented for the different sites studied (Figure 3).

In addition, the combined effect of different climatic conditions (heat, annual temperatures and relative humidity averages) was evaluated (Table 1). The cumulative heat units are calculated for the seven-month period of March to September.

RESULTS AND DISCUSSION

Climatic factors (temperature and relative humidity)

The 'Mejhoul' date palm prefers doses of cold in winter and heat is important from fruit set to harvesting, for around five to six months per year. This is confirmed by Figure 2, which represent, the average maximum and minimum temperatures of some of the main 'Mejhoul' growing areas around the world. Thus, in these areas the lowest minimum temperature varies from 3 to 9°C, while the highest maximum temperature varies between 38 and 42°C. The flowering process starts at around 18 to 20°C (called 'zero vegetative value') which coincides with the period of January/February in the northern hemisphere and June/July in the southern hemisphere.

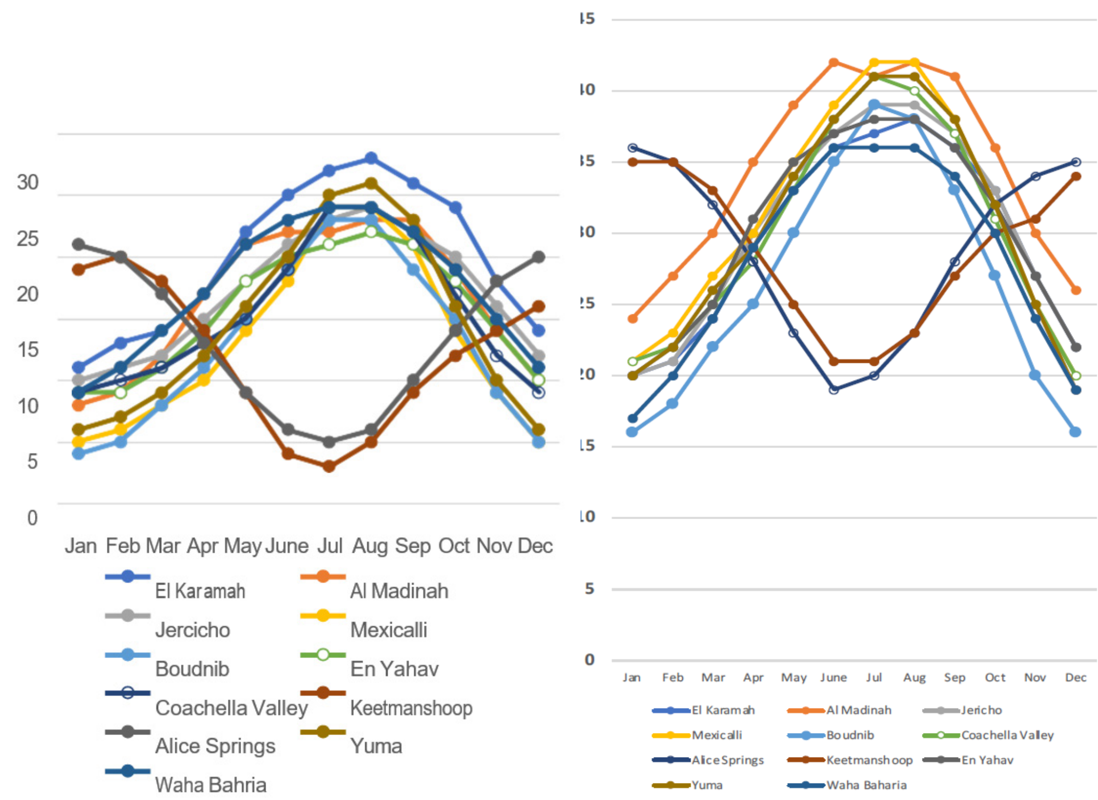


Figure 2. Average 30-year minimum (left) and maximum (right) temperature (°C). The main date-growing areas are characterized by low temperature during the period preceding the flowering season. This allows the induction of the flowering process. In the represented areas, the minimum observed average temperature varies from 4°C in the northern hemisphere (Boudnib, Morocco), 3°C in the southern hemisphere (Keetmanshoop, Namibia) and 10°C (Jericho, Palestine). The flowering of the 'Mejhoul' date palms starts in general when the air temperature goes above 18°C. In most date-producing areas this occurs around end of January/February in the northern hemisphere and June/July in the southern hemisphere, respectively. In instances where the temperature drops below 17°C after the fruit set period, a second date palm flowering can appear. During the fruit maturation period, the climate in 'Mejhoul' producing areas is hot, particularly July to August, in the northern hemisphere, varying between 36°C at Al Waha Al Baharia in Egypt, and 42°C at Al Madinah Al Munawara in Saudi Arabia and Mexicalli in Mexico, while temperatures reach around 36°C in the represented areas in the southern hemisphere, during January/February.

Combination effect of different climatic conditions

Table 1 shows how the combination of different climatic conditions of a given area significantly impacts date fruit maturation and quality, depending on the location of the date plant.

The Al Madinah area in the Kingdom of Saudi Arabia (KSA) is characterized by hot temperatures (above 40°C) for four months (June-September), a heat unit of 4,370°C and a very dry atmosphere with an annual relative humidity average of 23%. Consequently, the 'Mejhoul' fruits produced in this area are dry (Figure 4), mainly when the irrigation is not adapted to suit these climatic conditions.

The Boudnib area in Morocco has an annual average temperature of 19.8°C, with a heat unit of 3,057°C. The combination of this temperature factor with a relative humidity of 31%

makes the 'Mejhoul' fruits of this area soft (Figure 5).

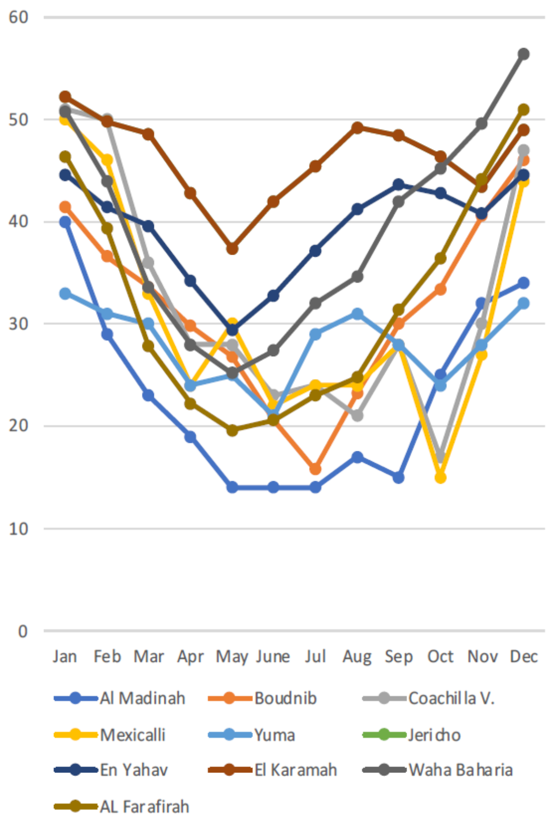


Figure 3. Average relative humidity % for the period 2016-2020. 'Mejhoul' dates require low air relative humidity (RH) during the fruit development and maturation periods. High RH levels may have a negative impact on fruit quality, mainly by causing serious problems of skin separation. Therefore, the date-growing areas are mainly characterized by a dry climate during the June-August period, with RH of 14% at Al Madinah Al Mounawara in the Kingdom of Saudi Arabia to 50% RH at El Karamah in the Hashemite Kingdom of Jordan.

Table 1. Heat units, annual temperatures and relative humidity averages.

	Cumulative heat units (°C)[a]	Average annual temperature (°C)	Average RH (%)
Boudinb	3,057	19.79	31
Waha Baharia	3,117	22.83	39
El karamah	3,300	24.79	46
Jericho	3,300	23.65	46
EN Yahav	3,514	21.75	39
Keetmanshoop	3,614	20.29	25
Coachella Valley	3,637	22.83	32
Yuma	3,720	22.54	28
Alice Springs	3,767	22.08	32
Mexicall	3,974	22.17	31
Al Madinah	4,370	25.54	23

[a]The cumulative heat units are calculated for seven-month period of March to September.

Figure 4. Dry 'Mejhoul' dates produced at Al Madinah Al Mounawara, KSA.

Figure 5. Soft 'Mejhoul' dates produced in Boudnib, Morocco.

In some areas of the Middle East where the air relative humidity is high, fruits are of dark color (Figure 6).

Figure 6. 'Mejhoul' dates of dark color from Jordan Valley, Hashemite Kingdom of Jordan.

‘Mejhoul’ fruits produced in the studied areas have different characteristics due to combinations of edaphoclimatic factors such as soil properties, including physico-chemical characteristics, cumulative heat units and relative humidity during the fruit development and maturation. In consequence, the ‘Mejhoul’ fruits produced in these areas may show different characteristics. This includes fruit color (high rate of relative humidity combined with high temperature provides dark fruits), fruit sugar content, total phenols, vitamins and antioxidants (Hasanaoui et al., 2010; Mahawar et al., 2017; Salomón-Torres et al., 2019). The ‘Mejhoul’ fruit characteristics are also impacted by prevalent agricultural practices, such as fertilization, irrigation management, fruit thinning, and bunch management. Harvesting practices, as well as postharvest handling, also play a crucial role in the quality of the final product.

CONCLUSIONS AND FUTURE WORK

The ‘Mejhoul’ producing areas around the globe present a diversity of the combinations of the cumulative heat units during the period from fruit set to fruit maturation, and the average air relative humidity mainly during the fruit development and maturation. Therefore, the ‘Mejhoul’ fruits produced in the studied regions present different fruit characteristics as a result of the above combinations. Therefore, it is essential to build the capacities of ‘Mejhoul’ producers on good agricultural practices (GAP) applied to ‘Mejhoul’ cultivation to ensure the production of high-quality fruits that match with the targeted market standards. These agricultural practices should be adapted to the particular characteristics of each ‘Mejhoul’ production area, mainly knowing that ‘Mejhoul’ has good elasticity and adaptability to various edapho-climate conditions.

Literature cited

Ait-El-Mokhtar, M., Fakhech, A., Anli, M., Ben-Laouane, R., Boutasknit, A., Wahbi, S., and Meddich, A. (2020). Infectivity of the palm groves arbuscular mycorrhizal fungi under arid and semi-arid climate and its edaphic determinants towards efficient ecological restoration. Rhizosphere *15*, 100220 https://doi.org/10.1016/j.rhisph.2020.100220.

Alsmairat, N., Qudah, T., Asi, N., and Mehyar, G.F. (2019). Effect of drying process on physical and chemical properties of "Medjool" date palm fruits. Fresenius Environ. Bull. *28*, 1552–1559.

El Bakouri, Z., Meziani, R., Mazri, M.A., Chitt, M.A., Bouamri, R., and Jaiti, F. (2021). Estimation of the production cost of date fruits of cultivar Majhoul (*Phoenix dactylifera* L.) and evaluation of the Moroccan competitiveness towards the major exporting regions in the world. Agric. Sci. *12* (*11*), 1342–1351 https://doi.org/10.4236/as.2021.1211086.

Elfeky, A., and Elfaki, J. (2019). A review: date palm irrigation methods and water resources in the kingdom of Saudi Arabia. J. Eng Res. Reports *9*, 1–11 https://doi.org/10.9734/jerr/2019/v9i217012.

Hasanaoui, A., Elhoumaizi, M.A., Hakkou, A., Wathelet, B., and Sindic, M. (2010). Physico-chemical characterization, classification and quality evaluation of date palm fruits of some Moroccan cultivars. J. Sci. Res. *3* (*1*), 139 https://doi.org/10.3329/jsr.v3i1.6062.

Ibourki, M., Azouguigh, F., Jadouali, S.M., Sakar, E.H., Bijla, L., Majourhat, K., Gharby, S., and Laknifli, A. (2021). Physical fruit traits, nutritional composition, and seed oil fatty acids profiling in the main date palm (*Phoenix dactylifera* L.) varieties grown in Morocco. J. Food Qual. *2021*, 5138043 https://doi.org/10.1155/2021/5138043.

Mahawar, M., Jalgaonkar, K., Kumar, M., Singh Meena, V., and Bhushan, B. (2017). Determination of some physical properties of date palm fruits (cv. Khadrawy and Medjool). Acta Geophys. *24*, 217–223.

Mohammed, M.E.A., Alhajhoj, M.R., Ali-Dinar, H.M., and Munir, M. (2020). Impact of a novel water-saving subsurface irrigation system on water productivity, photosynthetic characteristics, yield, and fruit quality of date palm under arid conditions. Agronomy (Basel) *10* (*9*), 1265 https://doi.org/10.3390/agronomy10091265.

Pérez-Pérez, B.D., García Vázquez, J.P., and Salomón-Torres, R. (2021). Evaluation of convolutional neural networks’ hyperparameters with transfer learning to determine sorting of ripe Medjool dates. Agriculture (Switzerland) *11* (*2*), 115 https://doi.org/10.3390/agriculture11020115.

Ranasinghe, M., Manikas, I., Maqsood, S., and Stathopoulos, C. (2022). Date components as promising plant-based materials to be incorporated into baked goods: a review. Sustainability (Switzerland) *14* (*2*), 605 https://doi.org/10.3390/su14020605.

Salomón-Torres, R., Ortiz-Uribe, N., Valdez-Salas, B., Rosas-González, N., García-González, C., Chávez, D., Córdova-

Guerrero, I., Díaz-Rubio, L., Haro-Vázquez, M.D.P., Mijangos-Montiel, J.L., et al. (2019). Nutritional assessment, phytochemical composition and antioxidant analysis of the pulp and seed of medjool date grown in Mexico. PeerJ *7*, e6821 https://doi.org/10.7717/peerj.6821. PubMed

Sperling, O., Lazarovitch, N., Schwartz, A., and Shapira, O. (2014). Effects of high salinity irrigation on growth, gas-exchange, and photoprotection in date palms (*Phoenix dactylifera* L., cv. Medjool). Environ. Exp. Bot. *99*, 100–109 https://doi.org/10.1016/j.envexpbot.2013.10.014.

Zhen, J., Lazarovitch, N., and Tripler, E. (2020). Effects of fruit load intensity and irrigation level on fruit quality, water productivity and net profits of date palms. Agric. Water Manage. *241*, 106385 https://doi.org/10.1016/j.agwat.2020.106385.

The actual and future of the production and marketing of Jordanian dates

A. Haddad[a]

Jordan Dates Association (JODA), Jordan.

Abstract

This paper reviews the reality of production and marketing of Jordanian dates and their cultivars, with a focus on the cultivar, where it shows the areas of cultivation, the evolution of its production, their areas, tenure rates, and categories of producer farmers. This paper also shows the local consumption rates of local and imported dates across time periods. A time line showing the annual increases as well as the most important countries and markets to which these dates are exported, as well as the most important competitors in these markets from the 'Mejhool' dates producers around the world, the most important of which are America, Israel, Palestine and Morocco. The global market for 'Mejhool' dates, after which the study is moved to review the possibilities and prospects of expansion in the cultivation and production of 'Mejhool' in Jordan, the factors affecting this expansion and the challenges faced by this sector, such as water scarcity, competition in marketing, high production costs, especially labor, in addition to pests and epidemic diseases, the most important of which is the red palm weevil. The study, based on specialized studies, showed the strategic goals of this sector, the vision and the strategic plan that can achieve these goals and recommendations, which included in the most important what included addressing challenges and obstacles such as water, employment, combating the palm weevil, conducting studies and research necessary for this, adopting promotional programs and strengthening the role of the Dates Association in helping to improve this sector and enable it to do so.

Keywords: dates, palm, farmers

INTRODUCTION

This presentation sheds light on the reality and future of the cultivation and production of 'Mejhool' dates in Jordan and the constraints and challenges it faces and how to overcome them. The presentation was based on the reality of production and marketing of dates in Jordan, including competitiveness in global markets, the presentation includes also recommendations for achieving the strategic objectives for date sector in Jordan

MATERIALS AND METHODS

The author of the paper relied on secondary data available in local and international statistical information centers in addition to relying on his personal experience during the past two decades through his work as a researcher and as president of the Jordanian Dates Association and through direct supervision of palm plantations spread in Jordan and keeping pace with the development of the date palm sector in Jordan during the 20 past years (Table 1; Figures 1-17).

Market segmentation and parameters

Demographics; geographics; behavioral; psychographic.

Factors arranged in order of importance that affects the decision to buy dates in the European markets: trademark; the quality; source of origin; prices; appearance; seasonality; cultivar; freshness; taste; texture; food safety; packaging; contents; attractiveness.

[a]E-mail: haddadanwar3013@gmail.com

Table 1. Jordanian dates competitive standards.

Parameter	Result
Jordan's ranking among the date exporting countries	Jordan ranks thirteenth among the countries exporting dates in the world
Competitive prices of Jordanian dates exported in some international markets	Jordan is not considered competitive in terms of price in the global market for dates, as it was ranked 12th among countries exporting this commodity
Apparent competitive advantage (RCA)	Jordan dates have a clear competitive advantage, but they are in the tenth position
Comparative advantage of date production in Jordan	A study of the value chain of dates in Jordan showed that Jordan has a comparative advantage in production
Market penetration coefficient	The Netherlands is the most penetrable market (i.e., it can be exported to) followed by France, Denmark, and Belgium

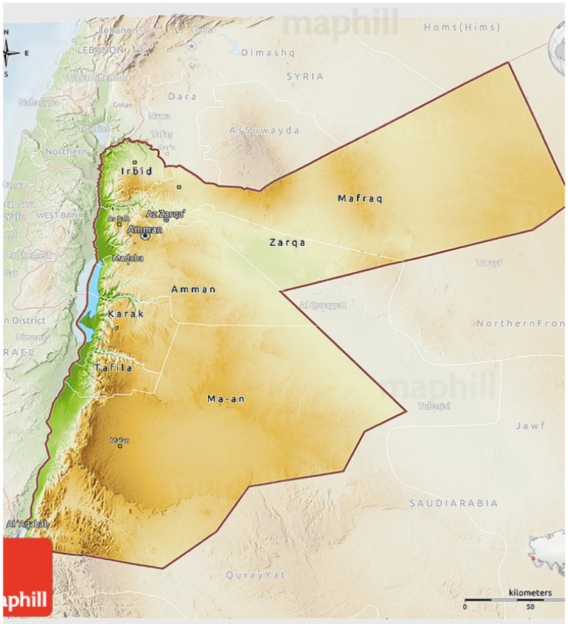


Figure 1. Where do we grow date palm in Jordan?

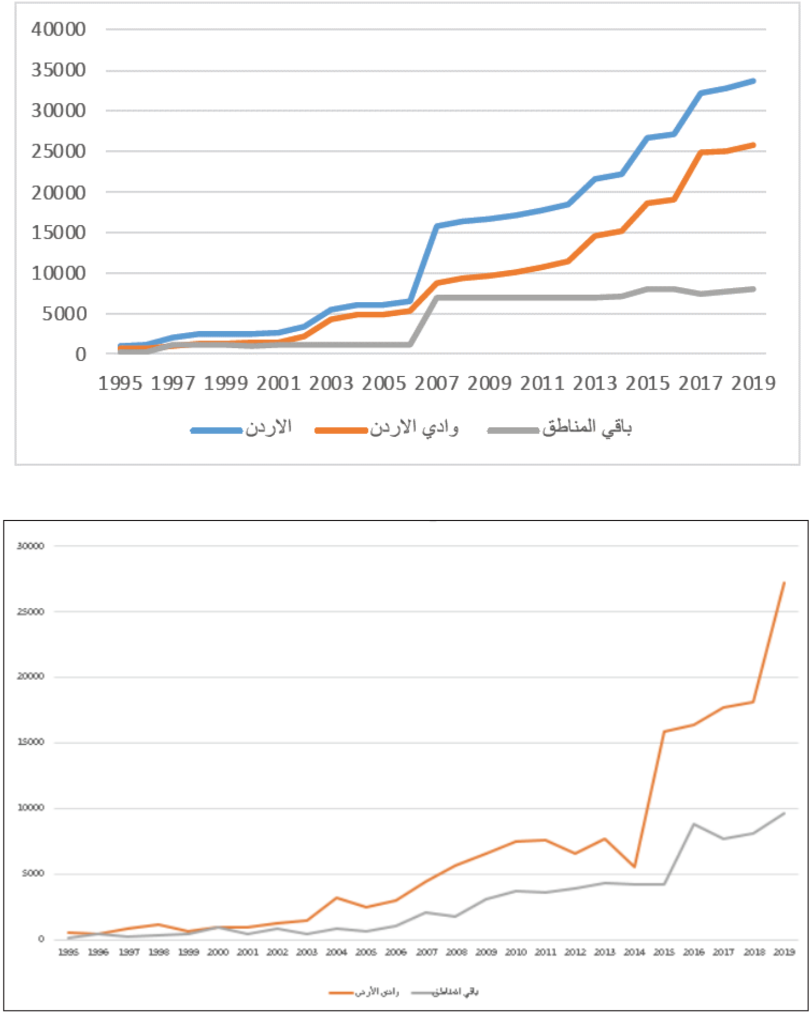


Figure 2. Development of date palm cultivated area (top) and production (bottom) 1995-2019.

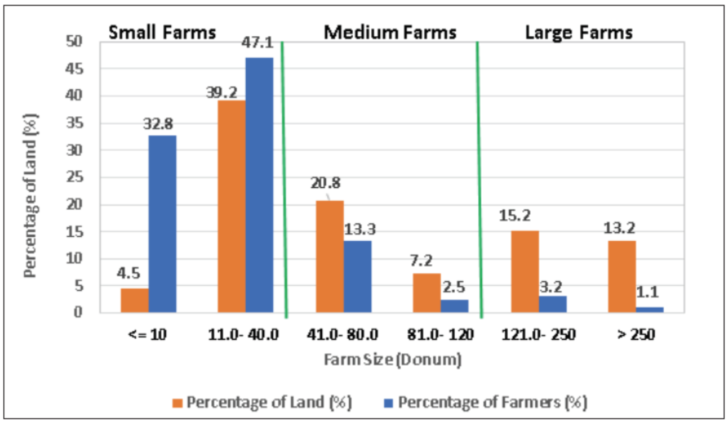


Figure 3. Distribution of date producers in Jordan according to farm size in Jordan Valley, 2016.

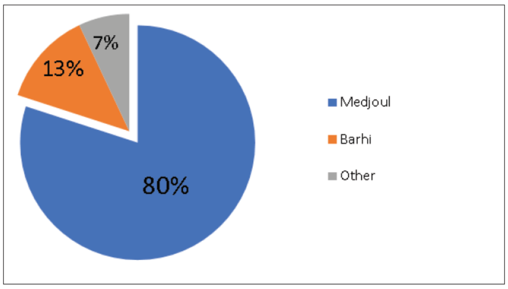


Figure 4. % Distribution of date palm area of cultivars grown in Jordan.

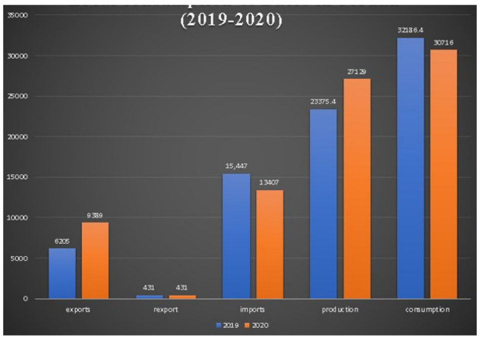


Figure 5.Local consumption of dates in Jordan (2019-2020).

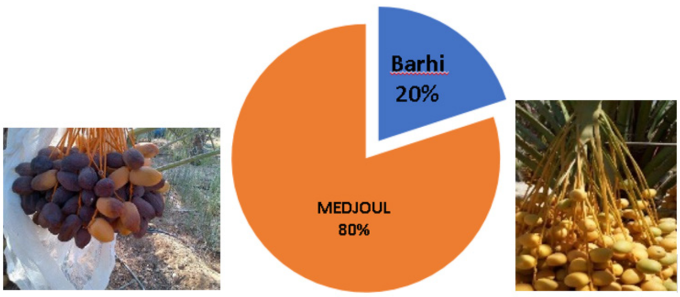


Figure 6. Jordanian exported date cultivars.

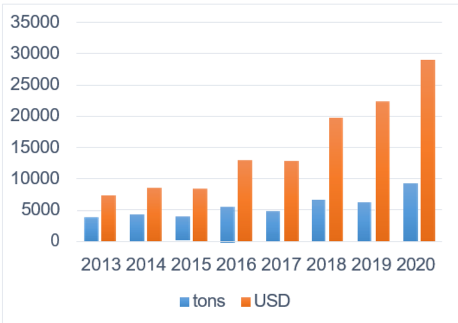


Figure 7. Exported quantities and values of Jordan dates to the global market (2013-2020).

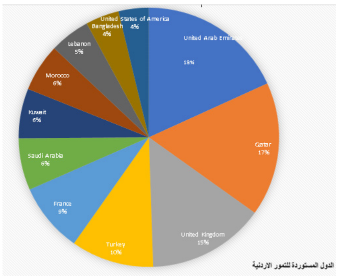


Figure 8. Countries importing Jordanian dates and % of imported quantities 2020.

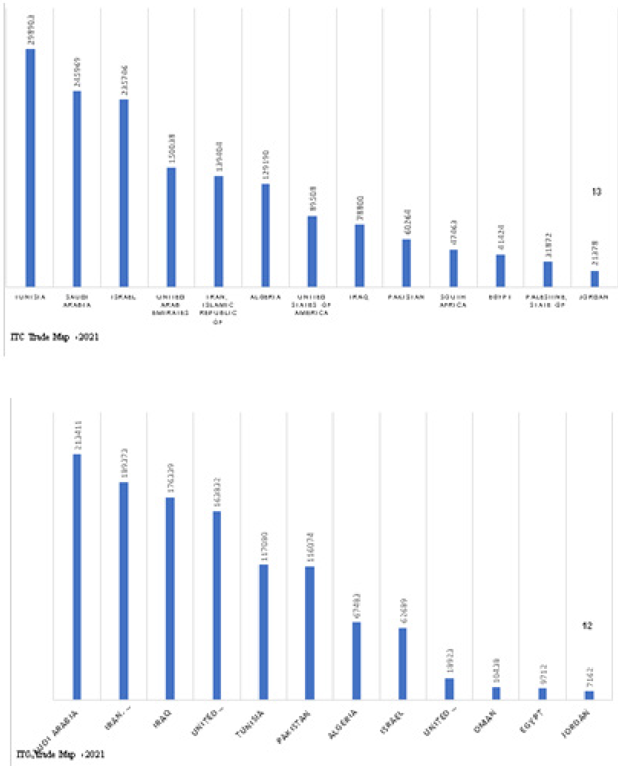


Figure 9. Ranking of Jordan as an exporter of dates to the global market in 2020 in MT (top) and $ (bottom).

Competitiveness \ Market Importance			
%	LOW	Medium	High
LOW	• Don't Enter • S9 - Indonesia/Jakarta • Adults (16 – 65)	• Don't Enter • S6 - Russia • Adults (16 – 65)	• Enter later • S11 – EU non Islamic • Kids (0 – 15)
Medium	• Don't Enter • S7 – India • Adults (16 – 65)	• Enter later • S10 – EU – Islamic • Adults (16 – 65)	• Enter first • S5 – EU – Islamic • Kids (0 – 15)
High	• Enter Latter • S-12 – EU non - Islamic • Adults (16 – 65)	• Enter first • S8 - MENA &Turkey • Adults (16 – 65)	• Enter first • S3 - MENA &Turkey • Kids (0 – 15)

Figure 10. % markets importance and Jordanian date competitiveness.

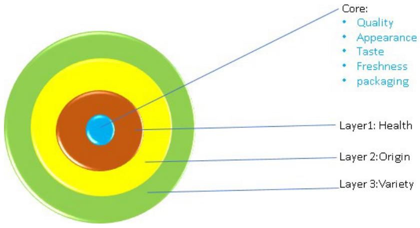


Figure 11. Factors affecting positioning of Jordan dates in the global market.

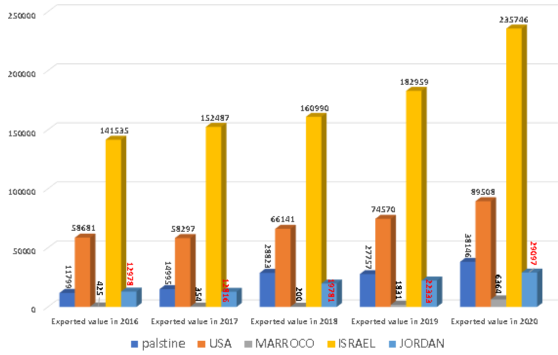


Figure 12. Jordan exports compared to the 5 top countries in exporting 'Medjoul' to the world $(000).

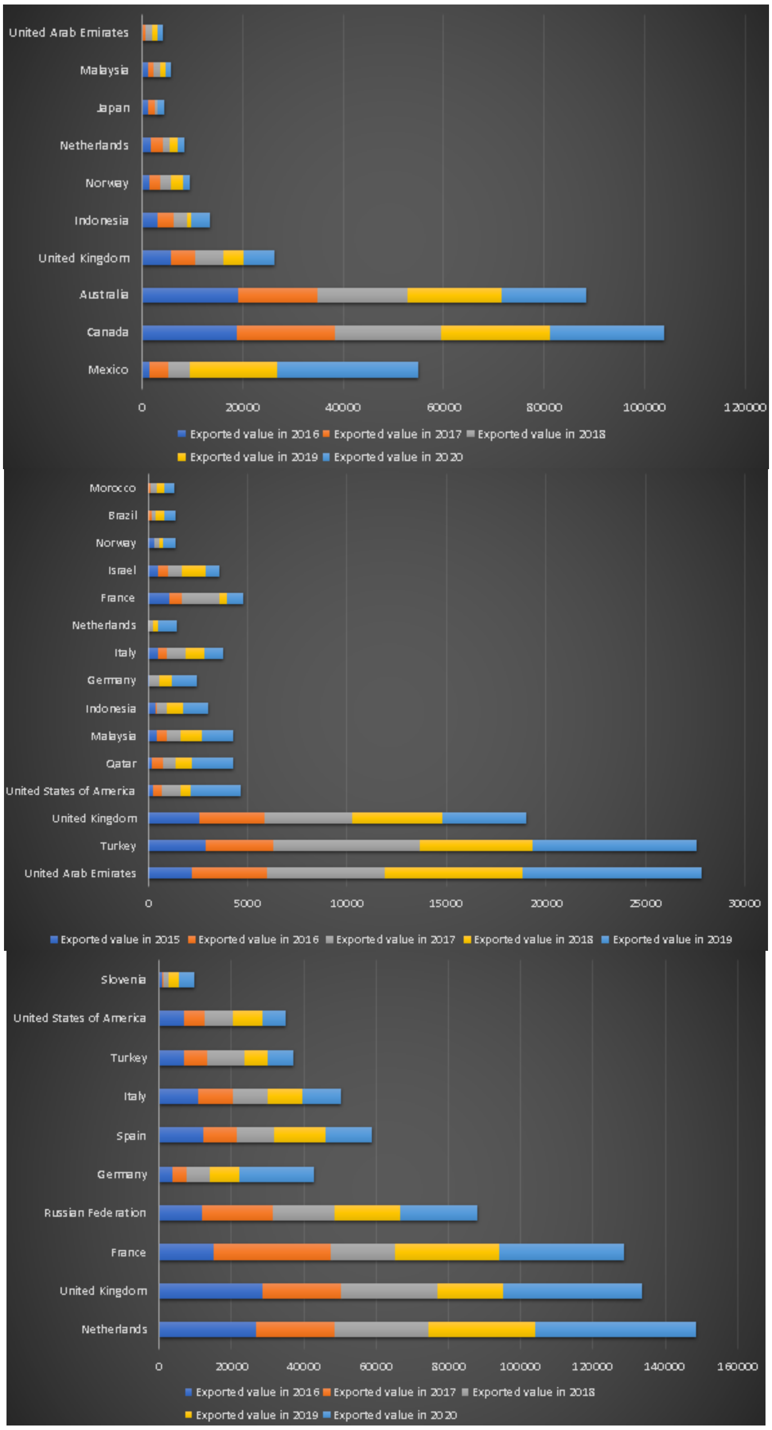


Figure 13. List of markets importing US dates (top), Palestinian dates (center) and Israeli dates (bottom) ($).

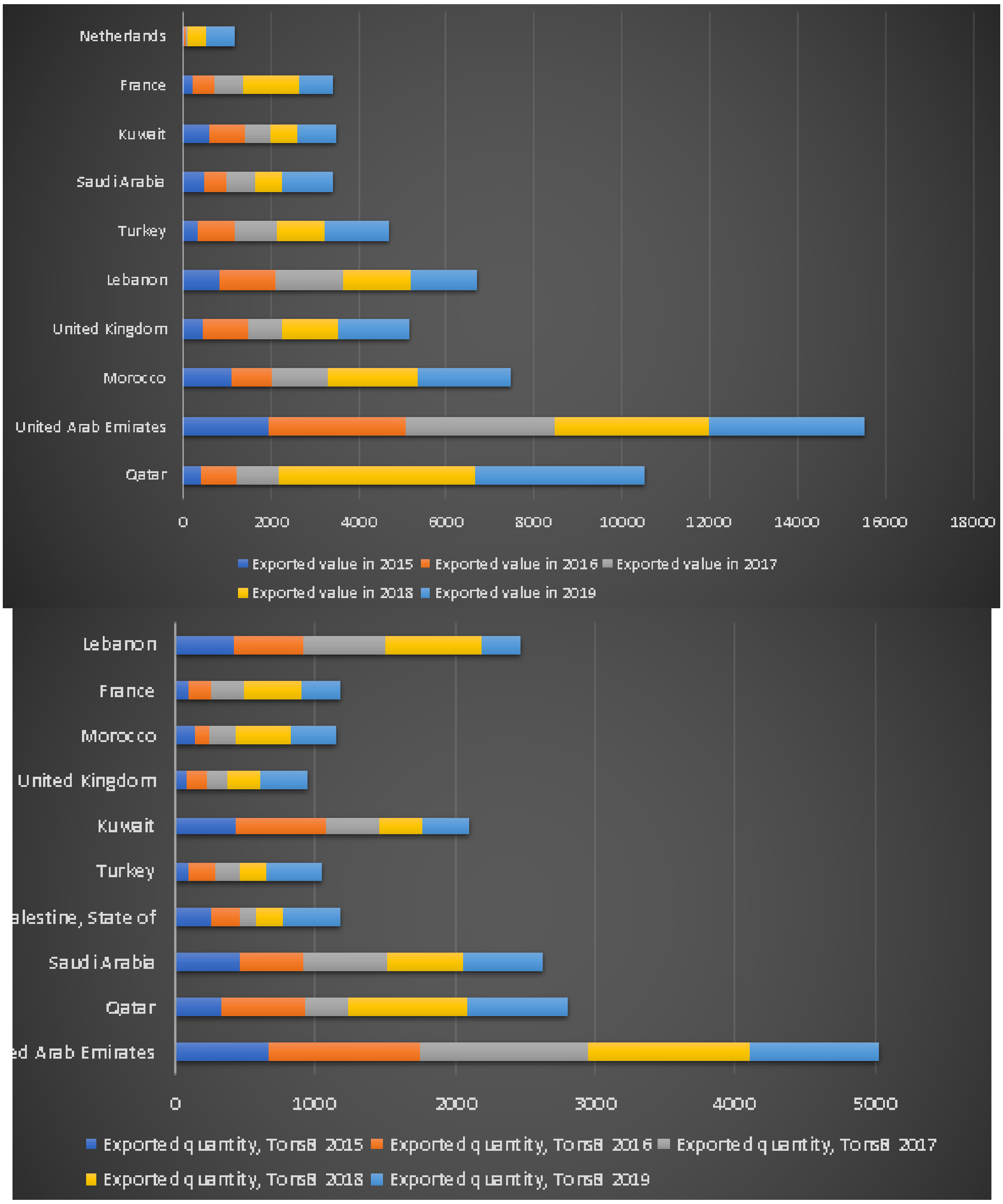


Figure 14. List of markets importing Jordanian dates $ (top) and MT (bottom).

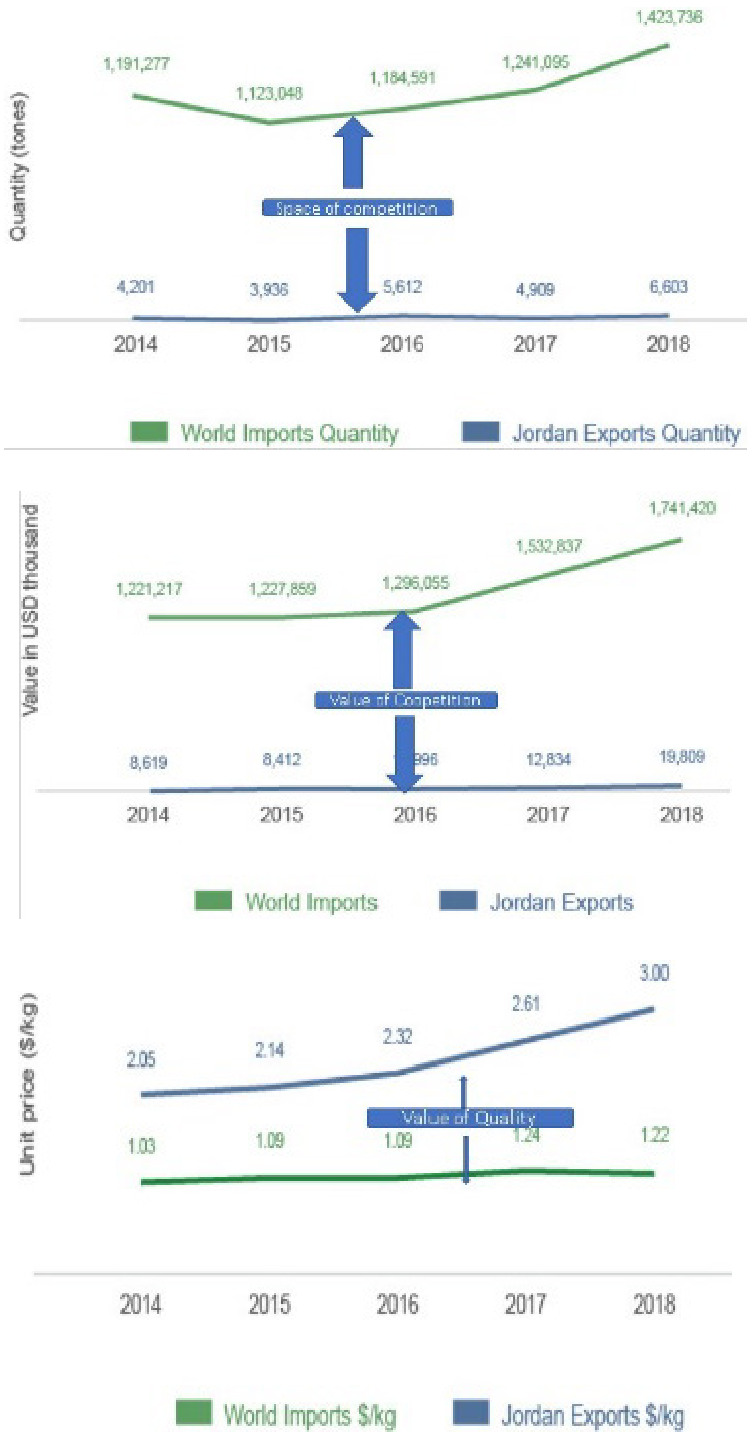


Figure 15. World's import vs. Jordan's export quantities (top), export values (center) and export unit price of dates (bottom).

Figure 16. Factors affecting future of the date palm cultivation and production in Jordan: Water availability and quality; climate and climate change; competitiveness; quality and safety; cost of production and transportation.

Figure 17. Expansion in date palm planting area and water limitations.

DATE SECTOR STRATEGY

Vision

A growing, sustainable, comprehensive, highly efficient, and competitive production of Jordanian dates.

Mission

Expand the production and export of dates in a sustainable manner by increasing production horizontally and vertically, developing the value chain, and providing an enabling and regulatory environment to raise the competitiveness of Jordanian dates, in order to increase the contribution of the agricultural sector to the GDP, and enhance food security and nutrition.

The following strategic objectives have been suggested:

1. Strengthening the Jordanian precedence and leadership in the production and export of the 'Mejhoul' cultivar, and dates in general, and raising its competitiveness aiming to make dates an important source of national income;
2. In order to achieve sustainability, it is suggested to regulate the production, processing, marketing of Jordanian dates, maintaining the current markets, expand horizontally by opening new global markets, including those in the Far East, in order to achieve sustainability;
3. Enhancing research and development through an integrated research and mentoring program aimed at conducting applied research to address the productivity problems experienced by palm growers and transferring the results of research, innovations and modern technologies to farmers;
4. Optimizing the use of natural resources and raising the efficiency of their use, especially irrigation water, to increase the productivity and production of dates in a sustainable manner;
5. Elimination of the red weevil and controlling palm pests by applying an integrated package for crop service and expansion operations using digital palm cultivation applications;
6. Providing the appropriate environment for investment in the date palm sector, and supporting and encouraging investment, especially for small farmers, to raise the efficiency of the dates value chain and maintain its sustainability;
7. Building human capacities and research and technical personnel specialized in the fields of production, processing, marketing and export of dates.

Action plan and programs

1. Create enabling environment for expansion In dates production, processing and export;
2. Institutionalization of dates palm sector in Jordan;
3. Establishing national program for building the national capacity in dates production, postharvest processing, and marketing;
4. Integrated pest management program for red palm weevil;
5. Implementation program of applied research to determine the best productive and preventive practices and the needs of palm from environmental resources and improve post-harvest operations to raise productivity and production of the palm tree and reduce the negative impact of climate change on dates;
6. Establish a national program for the expansion in dates palm plantation;
7. Establish a national fund on date palm development in Jordan;
8. Strategy within a strategic marketing plan;
9. The program to increase the capacity of the current workshops and establish new workshops for the packaging and packaging of dates;
10. Exploitation and utilization program of surplus dates and by-products of palms and dates;
11. Program for the development and improvement of local packaging materials;

12. Jordanian Organic Dates Production Program;
13. Creation of tissue culture laboratory;
14. Expansion program in areas eligible for date palm cultivation (Al-Azraq, Ghor Al-Safi and Wadi Araba);
15. The Jordanian Dates Association Capacity Building Program as a representative of date palm producers in the Hashemite Kingdom of Jordan.

DISCUSSION AND CONCLUSIONS

1. The available information and data indicated that the date palm sector and date production have witnessed a great development during the past 20 years, as a significant increase is observed in the areas planted with date palms, which recorded their highest jump in the period after 2006, and this was clearly reflected on the production in 2014, where the planted areas in 2019 exceeded 30 thousand dunams, mostly in the Jordan Valley, and production reached about 30,000 t, 80% of which are of the high-value 'Mejhool' cultivar, in addition to 13% of the 'Barhi' cultivar and the rest of the other cultivars. The growth rates in areas and production are estimated at 10-12% annually;
2. Farmers in Jordan face a number of challenges, the most important of which are water scarcity, lack of trained workers, high wages, random expansion of planted areas, pests and epidemic diseases, the most important of which is the red palm weevil infestation, and the lack of research and studies to meet these challenges, as well as strong competition in the global market;
3. Jordan exports about 50% of its production of dates to more than 15 countries around the world, most of which are of the 'Mejhool' cultivar. It ranks 12th in terms of quantity and 13th in terms of the value of dates exported among the countries producing/exporting dates, while Jordan imports about 14,000 t annually and estimates a consumption rate per capita at about 3.3 kg annually, which is one of the low rates in the region, and Jordan competes with four countries producing 'Mejhool' in the world and occupies fourth place in terms of the value of its exports of dates;
4. The Khalifa International Award contributed to the preparation of a strategy for this sector. The Jordan Dates Association is working in cooperation with the Ministry of Agriculture, partners, and palm farmers to implement it, the strategy recommended organizing expansion in the sector, diversifying cultivars, and improving product quality;
5. The markets of the Middle East, North Africa, and Europe were among the most important markets for Jordanian dates, according to the results of the marketing study conducted by the association with the support of the FAO.

RECOMMENDATIONS

1. Adopting and implementing the proposed programs to implement the palm sector strategy;
2. Giving absolute priority to the project to combat the red palm weevil, with the aim of declaring Jordan a clean country from this scourge by 2030;
3. Agreement with the Ministry of Water and Irrigation, and the Jordan Valley Authority, to provide water for irrigation in the necessary quantity and quality at the right time;
4. Conducting promotional programs for Jordanian dates (especially 'Mejhoul') to enhance competitiveness and increase the volume of date exports;
5. The necessity of organizing and supporting the palm sector due to its economic and developmental importance through institutionalizing this sector;
6. Strengthening the role of the Jordan Dates Association and enhancing its capabilities to be able to perform the roles required of it in the various items of the strategy, and its distinguished role in the development of the palm sector in general;
7. Reviewing the framing of a system for contracting between major producers, owners of workshops and small farmers, according to a tight system;

8. Improving the infrastructure necessary for the date palm sector, especially roads, modernizing irrigation networks and providing energy sources;
9. Giving importance to small farmers in terms of developing their production and marketing capabilities, allocating extension programs, transferring technologies, weevil control and crop service operations;
10. Establishing a knowledge platform for the Nakheel sector aimed at developing and applying modern technologies;
11. Conducting a field survey to inventory the areas of fruitful date palms and their cultivars in all production areas;
12. Reconsidering the size of the palm production unit on the basis of profitability, efficiency and investment requirements.

ACKNOWLEDGEMENTS

I would like to extend my sincere thanks to the study team that prepared the strategy for the date palm sector in Jordan, which is considered a guiding guide for the future of this sector, as well as to the Khalifa Award for date palm and agricultural innovation that supported and funded this work, also thanks to the FAO, which funded the study of the positioning of Jordanian dates in the global market and identified the way towards better marketing for Jordanian dates, as well as to the work team in the Jordan Dates Association, which contributed to collecting and coordinating information.

‘Mejhoul’ dates cultivation in Morocco

M.A. Elhoumaizi[a]

Mohammed I University, Faculty of Sciences, Department of Biology, PO Box: 717, Oujda 60000, Morocco.

Abstract

Native to Morocco the ‘Mejhoul’ cultivar is one of the most important date cultivars around the world. It is commonly known as the queen of dates, which offer the best value in the market. In this paper we presented the origin, importance and the future of the ‘Mejhoul’ cultivar in Morocco. The ‘Mejhoul’ cultivar is extensively planted in various Moroccan oases (old and new plantation), due to its great ability to adapt. Morocco is expecting the production of 70,000 t of ‘Mejhoul’ dates, to be presented in the national and international markets, by 2030.

Keywords: date palm, ‘Mejhoul’, Morocco, cultivation

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), is one of the oldest and ancient crops in southwestern Asia and northern Africa. Although date palm is mostly cultivated for fruit, it is also grown in many countries as an ornamental plant or as a landscape tree (Chao et Krueger, 2007). The date palm tree offers a wide range of benefits. It contributes significantly to environmental balance. It thrives in severe climatic conditions.

In Morocco, the date palm is grown in several zones located on the southern side of the Atlas Mountains along rivers and around water points. Date palm is grown mainly in regions situated in the southern and northeastern parts of the country. The date palm is the pivot of the oasis ecosystem of the Moroccan Saharan and pre-Saharan regions and the providential tree for more than 4 million inhabitants.

Date palm production area in Morocco is estimated to be close to 60,000 ha in 2019, concentrated mainly in the oases of Draâ-Tafilalet (77%), Sous Massa (15%), Oriental (5%) and Guelmim Oued Noun (4%) (Figure 1) (MAPMDREF, 2021a). Consumption of dates fruit is mainly concentrated in oases in eastern and southeastern Morocco, where dates represent an essential component of the food diet for the population. In the cities, dates consumption become important during the Ramadan fasting month and Achoura, another religious feast (Toutain, 1973; Chetto et al., 2005).

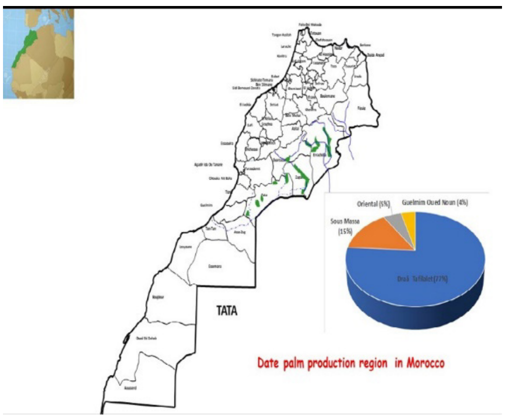


Figure 1. Date palm region in Morocco.

[a]E-mail: Elhoumaizi@yahoo.fr; m.elhoumaizi@ump.ac.ma

Total date production from Morocco was reported to be 143,000 t in 2021 (MAPMDREF, 2021a), which comprised of over 400 cultivars including a high proportion of 'Khalts' (mainly originated from seed) and other high-value such as 'Boufeggous', 'Mejhoul', 'Bouskri', and 'Aziza Bouzid' (Figure 2) (Hasnaoui et al., 2012). Morocco continues to import 60,000 t of dates annually, mainly from Tunisia, especially 'Deglet Noor', which is the most marketed cultivar (about 90%) in Europe (APIA, 2008). Annual consumption of dates is estimated at 2.82 kg person^{-1}. However, in more than 68% of cases, this consumption is done on an occasional basis, particularly in the month of Ramadan.

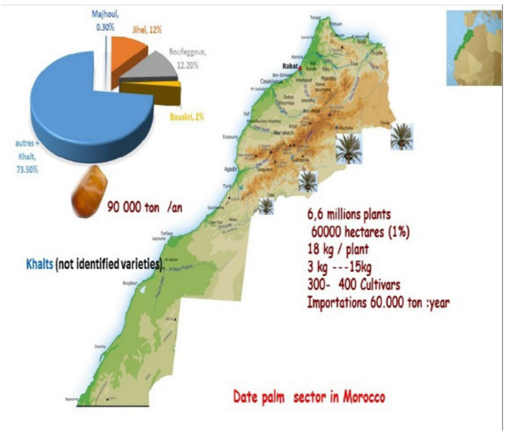


Figure 2. Date palm production area in Morocco.

The area of land dedicated to the date palm had a net increase of 25%, from 48,000 ha in 2008 to 60,000 ha in 2019. Production increased by 50%, from 68,000 t between 2003 and 2007 to 102,000 t between 2010 and 2019 (MAPMDREF, 2021a). These figures are likely to change in the next years due to the fact that the new intensive specialized and mostly mono-varietal date plantations, rapidly developing at the oasis outskirts, are mainly of the 'Mejhoul' cultivar and only in some cases, also few other valuable cultivars are planted. The recently developed 'Nadja' cultivar, which resists Bayoud disease, could also take place in some heavily infested areas (Sedra, 2003).

HISTORY OF MOROCCAN CULTIVAR 'MEJHOUL'

There are an estimated 1000 cultivars of date grown worldwide. Date palm cultivars were continuously subjected to the natural selection imposed by their immediate environment, geographical locality, and agricultural practices. 'Mejhoul', 'Medjoul' or the 'Medhjoul' cultivar (meaning unknown) dates are one of the most popular date cultivars in Morocco, as well as in many parts of the world. 'Mejhoul' date is originally from Boudenib in Morocco but largely spread around the world (Figure 3). It is of high commercial value and is considered to be one of the best exported dates with regard to its fruit quality and size in comparison with other cultivars. It was enjoyed exclusively by royalty and reserved for lavish celebrations, thus inspiring the titles "The King of Fruits" and "The Diamond of Fruits". 'Mejhoul' currently represents 0.3% of the total date production in Morocco and 70% of the new plantations developed within the Green Morocco Plan (GMP).

Up until 1927, Morocco was the exclusive grower of 'Mejhoul' date palm. The introduction of the 'Mejhoul' date to the western world came when a disease nearly wiped out the date palm trees in the oases of southern Morocco, and to save the delicious fruit from extinction, 11 date palms were sent to the United States in the 1927 by Dr. Walter Swingle. These offshoots were planted, and nine survived. In 1934, these date palms were moved to Coachella Valley in southern California. Those offshoots are now responsible for the millions of 'Mejhoul' dates found all over California and in many parts of Arizona and the world. The propagation of the cultivation of the 'Mejhoul' cultivar was carried out in two periods, firstly by offshoots (before 1995) and later by vitro-plants (Figure 4).

Figure 3. Date palm production area in Morocco.

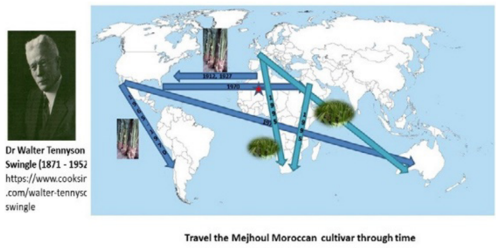


Figure 4. ‘Mejhoul’ Moroccan date travel through time.

DISTRIBUTION OF THE MEJHOUL IN MOROCCO

Traditional oasis

In the old plantations the ‘Mejhoul’ cultivar is mainly cultivated in the region of Errachidia almost 90% (ORMVAT, 2011). The Erfoud region remains the main supplier of ‘Mejhoul’ in Morocco. The ‘Mejhoul’ date production is concentrated especially at traditional palm groves (Figure 5). Concerning the agronomic aspect, the productivity of ‘Mejhoul’ cultivar varies according to the conditions of irrigation, the state of the plantations, etc.; however, the average productivity is estimated at 42 kg plant^{-1}. ‘Mejhoul’ cultivar is subjected to various phyto-sanitary problems that hinder its development and extension principally Bayoud disease. In 2011 the total number of ‘Mejhoul’ tree is estimated at 151,261 of which 72,584 are productive with an average annual production of 2900 t (Table 1) (ORMVAT, 2011).

Table 1. ‘Mejhoul’ cultivar distribution in Moroccan traditional oasis (2011).

Regions	Tree number	Productive trees	%	Average yield tree^{-1}	Production (t)
Erfoud	67250	32850	49	40	1314
Kheng	13900	7425	53	40	297
M’daghra	11300	3740	51	40	150
Ferkla-Tinejdad	10250	5263	51	40	211
Oued Naâm	8906	4500	59	40	180
Aoufous	8800	5628	52	40	225
Goulmima-Ghéris	8000	1398	17	40	56
Mellab-Touroug	7000	4150	59	40	166
Rteb	6710	4300	64	40	172
Figuig	4050	300	7	27	8
Alnif	3545	1950	9	40	78
Mezguita Agdez	800	600	75	52	31
Aïn Chouater	750	480	40	64	12
Total	**151261**	**72584**	**42**	**45**	**2900**

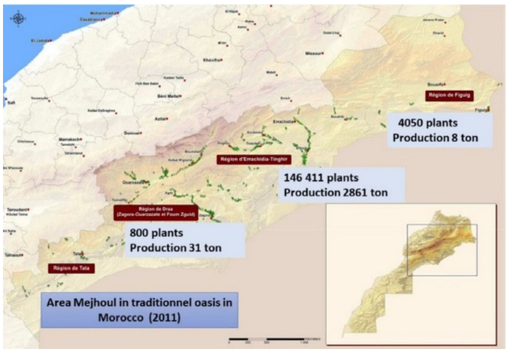


Figure 5. Area 'Mejhoul' cultivar distribution in traditional oasis area (Chapron et al., 2014).

New plantations

The Moroccan Government developed the Green-Morocco Plan (2008-2020) (GMP), a national agriculture strategy that has the aim of improving food security and promoting inclusive economic growth through strengthening the resilience, sustainability and competitiveness of the agriculture sector and tackling structural inequalities (MAPMDREF, 2021a). The GMP had for objectives concerning the date palm sector.

- Planting 3 million palms in 10 years;
- Make Morocco a date exporting country, producing 185,000 t in 2030.

Date production in Morocco has multiplied by 2.3 times, with a record date yield of 149,000 t in 2020. The number of date palm trees in Morocco is estimated at 6.9 million, of these 2.7 million were planted within the framework of the GMP (118% of the objective set). This includes 1.5 million for the densification of traditional oases and 1.1 million in extensions, especially in the Meski-Boudnib region.

Date production has multiplied by 2.3 with a record campaign of 149,000 t in 2020. Actually, the number of palm trees is estimated at 6.9 million, among 2.7 million were planted within the framework of the Green Morocco Plan (118% of the objectives set), including 1.5 million for the densification of traditional oases and 1.1 million in extensions, especially in the region: Meski-Boudnib axis (ORMVAT, 2020).

The most important 'Mejhoul' new plantation is based in the axis level of the Meski-Boudnib (Figure 6) (Errachidia region), the profile composed by 'Mejhoul' (79.3%), 'Bouffegouss' (16.1%), males (1.8%), 'Najda' (1,2%). The rest is composed of the cultivars 'Bouskri', 'Abou- Ijjou', 'Boufeguous Gharass', 'Aziza Bouzid' and 'OumNhal' which represented (1.5%). However, the presence of foreign cultivars from other countries (Tunisia, Saudi Arabia, etc.) namely the 'Deglet noor', 'Barhi' and 'Khalass' should be noted. In 2011 in the region Errachidia in old oasis and new plantations more than 862019 'Mejhoul' trees were planted (Table 2; Figure 7) (ORMVAT, 2020).

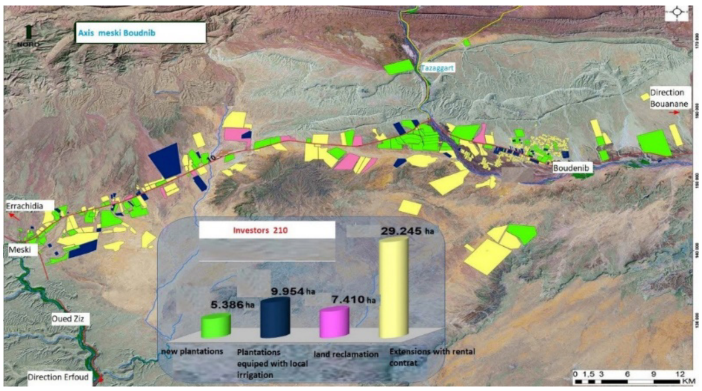


Figure 6. Distribution of 'Mejhoul' in new plantation in axis Boudnib-Maski.

Table 2. 'Mejhoul' cultivar distribution in Moroccan traditional oasis and new plantations in Errachidia region oasis (ORMVAT, 2020).

Region	Tree number
Oued Naâm	439552
M'Daghra	145231
Erfoud	119304
Kheng	28197
Ferkla Tinejdad	25189
Bouânane	25165
Goulmima Gheriss	21609
Mellab	12500
Aoufous	12400
Ain Chair	10086
RTeb	8200
Aghbalou	7893
Alnif	4250
Tadighoust	2443
Total	**862019**

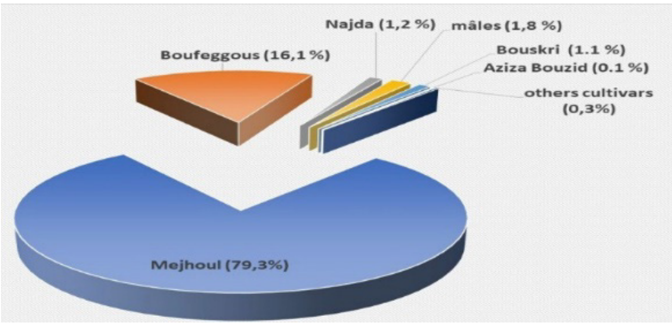


Figure 7. Cultivars distribution in new plantation (Meski-Boudenib).

Traditional propagation via offshoots is simply not enough to meet the present-day date palm tree demands for GMP. Conversely, micro propagation has shown promising signs as a better large-scale propagation method for cultivating date palm trees. Morocco has the largest number of date palm tissue culture laboratories internationally, which provides the necessary number of vitro plants. In Morocco there are eight tissues culture laboratories producing in average 400,000 plants year^{-1} (80% 'Mejhoul') (Figure 8).

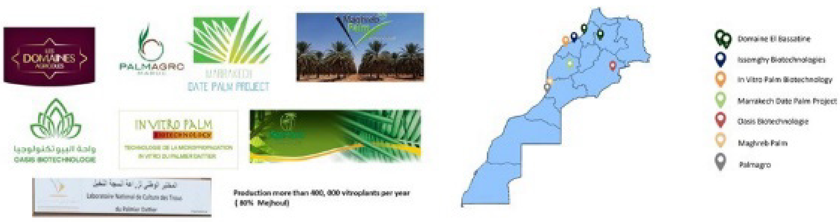


Figure 8. Tissue culture laboratories in Morocco.

In February 2020, the Government of Morocco launched its second strategic plan for agriculture. The new plans represent a continuity of the Green Morocco Plan (GMP), launched in 2008. The new plan, named "Generation Green", sets out an agricultural development strategy through 2030 (MAPMDREF, 2021b). It has two major pillars:

- Develop a new agricultural middle class representing between 350,000 and 400,000 households by supporting young entrepreneurs through the mobilization of one million ha of collective lands;
- Promote human and social development.

The national date palm planting program provides for the planting of 5 million palm trees as part of the Generation Green strategy (2020-2030), including 4 million in the Draa-Tafilalet region, with 2.4 million trees for the densification of oases traditional and 1.6 million trees for extensions (Figure 9).

Figure 9. Green Morocco plan and Generation Green in Morocco (2010-2030).

Geographical indication 'Mejhoul' Tafillaet dates

Given its high commercial value and traditional position in the domestic market, the 'Mejhoul' dates of Tafilalet are positioned within the core of the development strategy of the GMP. This cultivar of date was the first one to receive a labeling of a distinctive sign of origin and quality as (PGI) geographical indication in 2010 (Figure 10). PGI name used to identify a product as originating from a territory, region or locality, where a quality, reputation or other characteristic of that product can be attributed essentially to that product, and that production and/or processing and/or processing takes place within the defined geographical area (MAPMDREF, 2019).

Figure 10. Geographical indication 'Mejhoul' Tafillaet dates (MAPMDREF, 2019).

Geographical area of the distribution of 'Mejhoul' date palm is estimated at 32,500 km^2, concentrated mainly in Errachidia province. The geographical area which is granted the right to produce 'Mejhoul' dates of Tafilalet is composed of 30 rural communities spread across the provinces of Errachidia and Tinghiri in the oasis of Tafilalet. This delimitation is sufficiently wide to generate a variability of 'Mejhoul' dates including the effect of the micro environment associated with knowledge of farmers (MAPMDREF, 2019).

“Mejhoul Road”

The “Mejhoul Road” is the nucleus for the development of eco-tourism in the oases. It has evolved from a tourism circuit to a territorial brand. The idea of the Sustainable Territorial Development Program of Tafilalet Oasis is to protect the oasis heritage and nature by introducing tourism products enhancing this goal. The main product is “Mejhoul Road”, which is initiated to create in the area a specific image and to enhance the eco-tourism efforts (Figure 11) (Programme des Oasis du Tafilalet, 2016).

Figure 11. “Mejhoul Road” (Programme des Oasis du Tafilalet, 2016).

CONCLUSIONS

The ‘Mejhoul’ cultivar is extensively planted in various Moroccan oases (old and new plantation), due to its great ability to adapt. Morocco is expecting the production of 70,000 t of ‘Mejhoul’ dates, to be presented in the national and international markets, by 2030. In terms of new agricultural practices, Morocco is also approving good agricultural practices and mechanisms, as well as following the newest international methods for the development of modern irrigation systems, digital processes and pest forecasting and diseases control. Morocco is committed to develop the date industry, as well as its agricultural methods, to ensure higher productivity of date crops, and to increase the quality and reputation of Morocco’s dates within international markets. Morocco’s has proximity to Europe, the most important global market for exporting ‘Moroccan’ Mejhoul dates.

Literature cited

APIA. (2008). Étude de Positionnement Stratégique de la ‘Deglet Nour’ Tunisienne et de la Promotion de Ses Exportations à Long et Moyen Terme (Agence de Promotion des Investissements Agricoles de la Tunisie), pp.194.

Chao, C.T., and Krueger, R.R. (2007). The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. HortScience *42* (*5*), 1077–1082 https://doi.org/10.21273/HORTSCI.42.5.1077.

Chapron, H.A., Rahmani, M., Benmokhtar, A., and Bouabid, R. (2014). Etude du Positionnement Commercial des Variétés de Dattes Marocaines (Morocco: Ministère de l’Agriculture et de la Pêche Maritime, Office Régional de Mise En Valeur Agricole de Ouarzazate).

Chetto, A., Harrak, H., and Elhachmi, N. (2005). Le Marketing des Dattes au Maroc Défaillances, Préférences et Attentes (Morocco: Edit. INRA, Al Watania-Marrakech), pp.157.

Hasnaoui, A., Elhoumaizi, M.A., Borchani, C., Attia, H., and Besbes, S. (2012). Physico-chemical characterization and associated antioxidant capacity of fiber concentrates from Moroccan date flesh. Indian J. Sci. Technol. *5* (*7*), 1–7 https://doi.org/10.17485/ijst/2012/v5i7.4.

MAPMDREF. (2019). Produits Agricoles Labellisés au Maroc, Edition 2019.

MAPMDREF. (2021a). Filiere Palmier Dattier (Ministère de l’Agriculture, de la Pêche Maritime, du Développement Rurale et des Eaux et Forêts), https://www.agriculture.gov.ma/fr/filiere/palmier-dattier.

MAPMDREF. (2021b). Generation-Green – 2020-2030 (Ministère de l’Agriculture, de la Pêche Maritime, du Développement Rurale et des Eaux et Forêts), https://www.agriculture.gov.ma/fr/ministere/generation-green-2020-2030.

ORMVAT. (2011). Bilan Phoenicicole au Titre de la Campagne Agricole 2009-2010.

ORMVAT. (2020). Bilan Phoenicicole au Titre de la Campagne Agricole 2019-2020.

Programme des Oasis du Tafilalet. (2016). Synthèse des Réalisations 2006-2016 (PNUD), pp.216.

Sedra, M.H. (2003.) Le Palmier Dattier Base de la Mise en Valeur des Oasis au Maroc Techniques Phoénicicoles et Création d'Oasis (Morocco: INRA, Imprimerie Al Watania-Marrakech).

Toutain, G. (1973). Productions du Palmier Dattier 3: Destination de la Datte Marocaine (Al Awami), p.147–159.

‘Mejhoul’ cultivation in the Arab Republic of Egypt

A.A. El-Kady[1] and S.F. El-Sharabasy[2,a]

[1]Food and Agro-Industries Technology Center (FAITC), Ministry of Trade and Industry, Arab Republic of Egypt; [2]Central Lab. of Date Palm - International Expert, Ministry of Agriculture and Land Reclamation, Arab Republic of Egypt.

Abstract

Egypt is considered the largest producer of palm dates in the world. Different cultivars of dates are produced all over Egypt’s areas. In the last 15 years, the ‘Mejhoul’ cultivar had a significant expansion in different governorates in Egypt because of the advantages of this high marketing value semi-dry cultivar. More than 700,000 ‘Mejhoul’ date palm trees have been planted through a mega-national project in Egypt, where the Egyptian Government and the political leadership pay special attention to the date palm production sector in Egypt.

Keywords: ‘Mejhoul’ cultivar, semi-dry cultivar, national projects in Egypt, date palm, Giza governorate, date palm industry

INTRODUCTION

Egypt occupies the first position as the largest producer of palm dates in the world, with an annual productivity of about 1.65 million t (Table 1) from about 16 million palm trees, equivalent to nearly 18% of the global production estimated as 9 million t, and about 25% of the Arab production of dates estimated at 6.5 million t (Abdullah, 2018). Due to the large area of Egypt (1 million km^2) and the diversity of the climate, many cultivars of dates are produced. The semi-dry date cultivars – which are mostly desired in the global markets – represent about 17% of Egypt’s date palm production, and is considered the main pillar in the strategy of developing the dates’ sector and export. The popularity of semi-dry date cultivars has contributed to the great expansion in the cultivation of the ‘Mejhoul’ cultivar, and some other cultivars of high market value in the last 15 years, especially in the regions of Giza, El-Wadi El-Gedid, Aswan and Minya Governorates (Hosny, 2016; El-Sharabasy and Rizk, 2019).

Table 1. Cultivar groups, their productivity and their approximate percentage of date palm in Egypt.

Cultivar group	Total production (t)	Percentage of total production (%)
Soft	900,000	53
Semi-Dry	290,000	17
Dry	34,000	2
Unidentified cultivars[a]	476,000	28
Total	**1,700,000**	**100**

[a]Unidentified cultivars resulting from date palm seeds.

THE PREFERENCE FOR ‘MEJHOUL’

The ‘Mejhoul’ cultivar (Figure 1) has many advantages, including the late ripening time that has earned it the top spot in competition with other early-ripening cultivars (Zaid and Oihabi, 2022). In terms of specifications, it has a beautiful shape, large size and an appropriate sweetness ratio, which makes it desirable for export, especially to Europe and America. In terms of agriculture, ‘Mejhoul’ trees are not exposed to a large amount of environmental stresses that affect the pollination process at the beginning of the season, which reduces production costs and leads to good fruiting ratio (Panoff, 2019). The central Egypt region is

[a]E-mail: sharabasydates@yahoo.com

considered one of the best areas for growing the 'Mejhoul' palm, and it starts from Giza Governorate in the north to Assiut Governorate in the south, and from South Sinai Governorate in the east to the western oases of Giza and El-Wadi El-Gedid and Matrouh governorates in the west. 'Mejhoul' can be grown in many other areas in Egypt (Hosny, 2016), but some additional techniques need to be applied to reach high quality. It is necessary to provide suitable environmental conditions for 'Mejhoul' cultivation, especially the appropriate weather conditions, where it thrives in areas with moderate temperatures and humidity, as it grows best between 13 to 37°C. It is not suitable for cultivation in areas with a lot of rain in the summer, especially during the stage of fruit ripening, and also in areas with long winters. 'Mejhoul' trees are grown in many types of soil, but it is preferable to be planted in sandy or yellow lands with good drainage to obtain a good quality crop, while heavy black soil causes a decrease in the quality of the crop.

Figure 1. A field of an adult date palm, 'Mejhoul' cultivar.

'MEJHOUL' CULTIVATION

'Mejhoul' cultivation goes through several stages of growth (Figures 2 and 3), which ends when the fruit reaches the semi-dry ripening stage (moisture content 22-28%), in which the fruit develops a wrinkled shape, distinctive color, and becomes suitable for consumption and marketing. Thanks to the appropriate climatic conditions, Egypt is one of the best countries for cultivating 'Mejhoul'. But Egypt also has strong advantages due to the availability of production inputs, human resources and trained labor at an appropriate cost, and the necessary land areas. Egypt's geographical location, shipping lines, and trade agreements with many countries of the world also allow for increased competitiveness in exporting the products, beside the availability of a large and growing local market due to the annual increase in the population and the growing awareness of the consumption of palm dates in general because of its high nutritional and health value.

THE 'MEJHOUL' INDUSTRY IN EGYPT

Egypt includes tens of investment farms specialized in the cultivation of date palms and the production of dates, containing hundreds of thousands of 'Mejhoul' palms cultivated in the last 15 years. These farms rely mainly on the import of seedlings that are subjected to strict control by the Central Administration of Agricultural Quarantine at the Egyptian Ministry of Agriculture and Land Reclamation, which is responsible for ensuring the quality of imported seedlings, and to prevent the transmission of pests and diseases. Most of these modern farms apply organic or clean farming systems to ensure the production of chemical free dates, and most of these farms are certified to international quality certificates such as Global Gap - GRASP.

Figure 2. Fruit punch of 'Mejhoul' cultivar in camry stage (left) and in the rutab stage (right).

Figure 4. Fruit punch of 'Mejhoul' cultivar in the (harvest) stage.

INNOVATIONS IN DATE CULTIVATION

Cold stores, sorting and packing stations for these farms are also available according to the latest technologies, most of them are certified to international quality certificates such as BRC - ISO22000 - FSSC22000. One of these refrigerated warehouses with a storage capacity of 4,000 t was established in El-Bahariya Oasis - Giza Governorate as a part of the continuous fruitful cooperation with Khalifa International Award for Date Palm and Agricultural Innovation (KIAAI) with a grant from the UAE. The number of 'Mejhoul' date palm trees in Egypt is currently about 1.4 million, of which about 600,000 are fruitful, with an average productivity ranging between 60 and 70 kg palm^{-1}. The total productivity at the present time reaches more than 3,000 t annually, increasing at an annual rate due to the increase the number of fruitful palms cultivated in the last few years, and due to the new Egyptian and Arab investments in this sector (FAO, 2019).

NATIONAL PROJECT OF DATE PALM CULTIVATION IN EGYPT

The Egyptian Government encourages investment in Egypt in general, and the political leadership pays special attention to the date palm cultivation and date production sector in Egypt. This is in recognition of the cultivars of dates with high market value, as the date palm sector is considered one of the most promising sectors for achieving the sustainable development and for increasing exports. Therefore, the Egyptian Government supports its exports by several mechanisms, including providing financial support for exports. In this regard, since 2018, the establishment of the largest date palm farm in the region has started on an area of 40,000 acres that accommodates 2.5 million date palm trees in El-Wadi El-Gedid and Aswan Governorates for the cultivation of different types of date palms, where in the last two years, more than 700,000 'Mejhoul' date palm trees have been planted. This mega-project which is gaining the attention of the political leadership in Egypt also establishing refrigerated and frozen warehouses, sorting and packaging stations, and various production lines to maximize the added value that can be derived from the date palm.

Literature cited

Abdullah, B.A. (2018). The Strategy for Developing the Palm and Dates Sector in Egypt (Food and Agriculture Organization of the United Nations).

El-Sharabasy, S., and Rizk, R. (2019). Atlas of Date Palm in Egypt (Food and Agriculture Organization of the United Nations).

FAO. (2019). Dates Value Chain Development in Egypt. https://www.fao.org/egypt/programmes-and-projects/dates-palm/fr/.

Hosny, S.S. (2016). Comparative study of some semi-dry Arabian date palm cultivars grown in Mounofia governorate. Egyptian Journal of Agricultural Research *94* (*4*), 859–873 https://doi.org/10.21608/ejar.2016.153131.

Panoff, L. (2019). Everything You Need to Know About Mejhoul Dates, J. Kubala, ed. (Healthline), https://www.healthline.com/nutrition/Mejhoul-dates.

Zaid, A., and Oihabi, A. (2022) Mejhoul, the Jewel of Dates Origin, Distribution and International markets. A Scientific Book of E-library of Khalifa International Award for Date Palm and Agricultural Innovation. https://www.kiaai.ae/en.

‘Mejhoul’ cultivation in the Republic of South Africa

M. McCubbin[1,a] and C. Edmonds[2,b]

[1]Pasture Valley Pty Ltd., PO Box 1129, Nhlangano, Eswatini, South Africa; [2]Boschendal Estate, Franschhoek, 7690, Cape Province, South Africa.

Abstract

Date fruit is highly valued in many areas of the world. The date palm can withstand harse environments and is grown in deserts where not many other plants can be grown. In the Republic of South Africa, new plantations have started up near the Orange River and the date palm industry is growing as more plantations are established. Of all the cultivars planted, ‘Mejhoul’ is by far the most favoured for its large size, sweet taste and soft texture. Plants are sourced through tissue culture or off shoots and planted in a 8×8 m spacing. In South Africa the flowering time is around July to September and pollination is mostly by hand. Pests such as the red palm mite and more recently the greater date moth has been identified. A production of 90-100 kg of fruit per mature palm is expected. The larger companies use exporters to export their fruit while some fruit is also sold on the local markets especially during Ramadan.

Keywords: date palm, ‘Mejhoul’, cultivation

INTRODUCTION

The ‘Mejhoul’ cultivar (or ‘Medjool’ as referred to locally) of date palms is by far the most favourite and most cultivated variety in South Africa. The large size, sweet caramel taste, soft chewy texture and good appearance make it ideal for the South African market and export markets. The dates are a source of fibre, iron, calcium, magnesium and potassium and are a good energy source.

Date palm cultivation in South Africa first originated in Pella, as missionaries planted them around their station around 1882. Various cultivars, such as ‘Khadrawy’, ‘Deglet Noor’, ‘Barhi’ and ‘Mejhoul’ were planted at Klein Pella near Kakamas. Today, 87 ha of date palms remain, with 90% of the plantation being of the ‘Mejhoul’ cultivar at Karsten Farms.

Newer plantations have started up on the Orange River, with Southern Farms planting date palms of ‘Mejhoul’ and ‘Zamli’. Southern Farms has cultivated 200 ha of Mejhoul, making up 24,600 trees planted to date. Smaller clusters of date palms have been grown elsewhere, such as Tankwa Karoo with around 50 plants and others on the Limpopo River near Mussina.

CONDITIONS FOR PLANTING

The soils used for date palm planting can be placed into two main classifications, deep alluvial soils along the river banks of the Orange River, and coarse sandy soils in all the other areas away from the river. The micro climate along the river – with slightly higher humidity and better water retention in the soils – produces top quality areas for the date palms to grow. Although older plantations started with a 10×10 m spacing, most of the new plantings have been established with 9×9 m spacing, while even newer orchards have moved to a higher density spacing of 8×8 m. The trend is to plant with the higher density the further one gets away from the river, to create better micro climates in the orchards.

New planting material is either tissue culture palms that are imported or from offshoots taken from existing orchards (Figure 1). Tissue culture trees are planted in 50- to 100-L bags and kept in the nursery for one to two years, planted out when they are 1 m tall. Offshoots are planted in 100-L bags and planted out after one year, depending on their growth and root development.

[a]E-mail: pastval@mweb.co.za
[b]E-mail: charles25e@icloud.com

The Orange River is the main source of water supply for date palm cultivation in South Africa, and the abundance of water assists in optimal production. The irrigation systems used in South Africa for date palms are mainly bubblers, micro and drip irrigation.

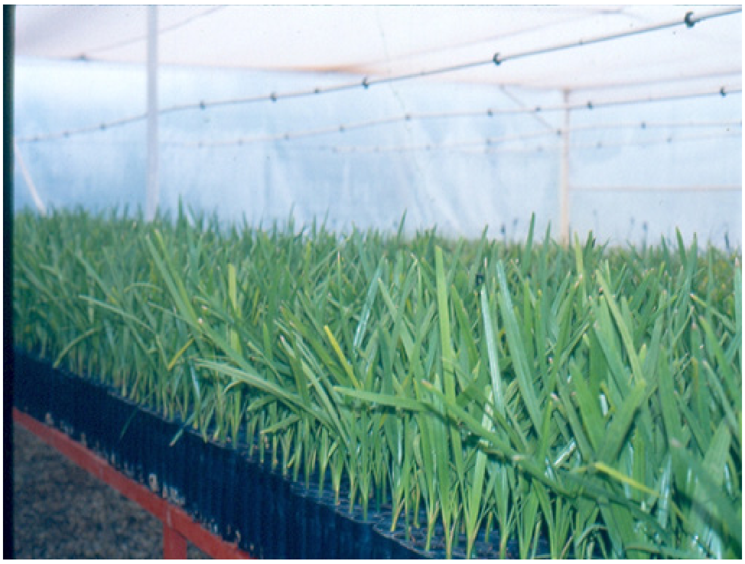


Figure 1. Tissue culture date palms produced in South Africa grown in torpedo pots ready for hardening.

DATE PALM CULTIVATION

The date palm's flowering time in South Africa takes place from the end of July to September. All pollination is done by hand. The pollen is mainly imported from the UAE. Most of the pollen imported is of the male 'Ghunami' cultivar. Fruit thinning is determined by the size specification of the target market the fruit is being grown for, although the best prices and higher consumer demand are for Large and Jumbo sizes. At pollination, the heart of the bunch is removed and the bottom 10 cm of all the spikelets are cut off to reduce the crop load. The heart of the bunch is removed to help with ventilation and even the ripening of the fruit. The fruit is spaced on the spikelets to reduce compaction of the bunches. Once fruit set has been determined, the bunches are thinned to between 30 and 45 spikelets per bunch with 10-12 fruit per spikelet. At optimum production 90-100 kg of fruit tree^{-1} can be expected.

The cultivation of dates is not without problems in South Africa. Pests such as white scale and fruit fly are present causing losses in production. The greater date moth (*Arenipses sabella*) is a new pest that is becoming a problem. The moth causes damage to the bunch bases or ends, which affects fruit yield and quality. The red palm mite (*Raoiella indica*) has also recently been reported to be seen on date palms.

Fungal diseases, such as black scorch (*Thielaviopsis punctulata*), are also prevalent. Black scorch fungi penetrate the leaves, inflorescence, heart, trunk and bud, causing black and hard lesions. The leaves will have a charcoal-like appearance which will bend, die back and rot.

THE GROWING SEASON

The dates grow under hot and dry climatic conditions and the heat provides an ideal growing climate for the date palms. However the low humidity and dry climate in turn poses a major problem, with stage skipping of the fruits in the ripening process before harvest time. This affects the quality of the fruit. The dates are normally harvested between mid-February to March. The harvested fruit are then brought into a pack house and cooled immediately after picking.

With a moisture content range of 19-24%, the 'Mejhoul' date fruits are sorted into various categories such as Premium (loose skin 0-10%), Choice (loose skin 10-15%), Supreme, Large and Jumbo to give some examples. The fruit are packed for national fresh produce markets and supermarkets locally as well as for export. Dates are available in 200-g punnets, 400-g pouches and punnets, 1-kg and 5-kg boxes. The 'Mejhoul' fruits for export are frozen and shipped at -18°C. When frozen, the shelf life of dates can be up to one year.

MARKETING AND EXPORT

Some of the date-producing organisations use exporters to market their fruit while other organisations do their own marketing, for example through Southern Cross Marketing where the target markets are predominately the UK and EU countries such as The Netherlands, Spain, Germany and Portugal. A small percentage of fruit also reaches countries such as the UAE, Singapore and Cambodia. Around 20% of the fruit of lower quality is sold on the South African market before or during Ramadan.

In summary, the cultivation of date palms in South Africa is expanding and growing. Having fresh dates 'out of season' to the northern hemisphere date-growing areas has great advantages for South African date palm growers. Although other cultivars such as 'Zamli' are also grown, 'Mejhoul' still remains the cultivar of choice for the growers.

'Mejhoul' cultivation in Australia

D. Reilly[a] and A. Reilly

Gurra Downs Date Company Pty. Ltd., PO Box 1029, Loxton 5333 South Australia, Australia.

Abstract

Australia is a large continent with highly variable climatic zones, from tropical and desert country to snow alps. Dates are a relatively new introduction to Australia so production is small. Historically, industry development has been limited by lack of suitable elite date palm genetics, leadership and a skilled workforce. In the last 20 years Gurra Downs has introduced many cultivars into Australia. 'Mejhoul' has been an outstanding performer and is now present in many locations around the continent. After several years of proven fruit production and with an exponential growth of offshoots being traded by farmers, significant increases in production volumes are expected in coming years.

Keywords: Australia, date palm, Gurra Downs, 'Mejhoul'

INTRODUCTION

The first documented importation of the 'Mejhoul' date cultivar into Australia was in 1976, with offshoots introduced from the USA. A very small number of 'Mejhoul' palms were planted at Pukatja (Ernabella), an aboriginal reserve in the far north of South Australia, along with 'Deglet Noor' and many seedlings. In 1991, a Northern Territory government-led date palm initiative was undertaken in Central Australia at a research farm known as the Arid Zone Research Institute, located at Alice Springs (Figure 1). The date palm was selected as a crop that should be suited to this hot environment. Tissue culture plants were imported from England and France, along with 'Mejhoul' offshoots from California (McEllister, 1991).

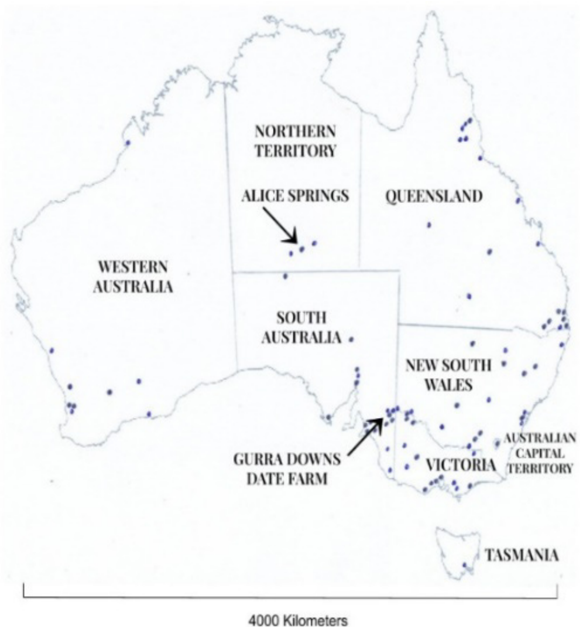


Figure 1. The locations where the 'Mejhoul' cultivar is currently grown in Australia.

[a]E-mail: reilly@gurradowns.com.au; www.gurradowns.com.au

In 1991, Jim and Trudi Luedi established the Tamara Date Garden, located to the south east of Alice Springs on the edge of the Simpson Desert. They ran their Desert Fruit Company until retiring in 2006. Another nearby farm, Arid Gold, was also producing 'Medjoul' for many years.

CLIMATE AND CULTIVATION CHALLENGES

It was thought the central Australian date industry would expand and be the main location for date production. However, there are challenges from high humidity and summer rainfall influenced by the monsoon season 1500 km to the tropical north. High input costs of labour and freight also limit growth in this remote location. Today, the Desert Fruit Company is cooperatively owned and produces very good quality dates in drier years, with their 'Mejhoul' considered as being as good as anywhere in the world.

In 1996, Dave and Anita Reilly decided to plant date palms on their farm in the Riverland of South Australia. However, at that time there was no available nursery stock in Australia. They established the Gurra Downs Date Company and in 2001, commenced importing tissue culture plants from Date Palm Developments in the UK. A date palm nursery was subsequently established, as well as an evaluation site to assess cultivar performance.

The Riverland is a semi-arid environment. Date palms had never been grown commercially in this region. There was no guarantee that any cultivar grown in this location was going to replicate yield and quality performance achieved in other date-growing countries. For Gurra Downs, the strategy was to introduce and evaluate as many world-leading cultivars as possible, with the aim of selecting the most suited cultivars for Riverland conditions (Reilly and Reilly, 2014). Twenty years on, it is most fortunate that the 'Mejhoul' has proven to be one of two leading selections.

The Riverland climate is characterised by cool springs, hot dry summers, mild autumns and cold winters. One of the biggest challenges in selection is finding a cultivar that will successfully set fruit in low spring temperatures. The 'Mejhoul' has consistently proven it has the capacity to set fruit at a lower temperature point than most other cultivars.

Summer/autumn rainfall is the enemy of the date grower. Some cultivars suffer fruit spoilage easily during high humidity events. Rain-induced fruit damage has disqualified many cultivars from commercialisation. However, 'Mejhoul' has proven to be reasonably tolerant of this occasional rainfall and high humidity. The percentage of fruit spoilage from rain events has been observed to be directly linked to temperature. The hot arid interior of Australia ripens the 'Mejhoul' dates during the summer months of February to March when temperatures are highest. Fruit spoilage is more prevalent in these conditions than in southern Australia, where the fruit ripens later in the autumn season of April to June, when temperatures are lower.

CULTIVATION PRACTICES

Gurra Downs has learned when growing 'Mejhoul', to thin bunches to no more than ten per tree. Although the tree can produce 250 kg of fruit in a season, this has the tendency to convert the tree to a bi-annual bearing habit, and makes it unlikely to flower the following year. By limiting the yield to 80-90 kg palm^{-1}, to ensure repeat flowering the following year, a large fruit size is still being achieved that is welcomed in the marketplace.

Since 2004, Gurra Downs has distributed date palms across the Australian continent to more than 400 farmers. Date palms are now being grown in districts where they have never been grown before. Many cultivars have been trialled, with some successful, and others not. One common thread among all farmers who are having success in growing dates is that it is consistently the 'Mejhoul' cultivar proving to be widely suited.

THE FUTURE OF THE 'MEJHOUL' IN AUSTRALIA

Expansion of the Australian date industry is gaining momentum. Many areas selected to grow dates are in existing large horticultural districts like the Riverland. These districts are supported by good infrastructure with transportation, water security, availability of electricity for irrigation pumps, access to a workforce, and proximity to airports and shipping ports

(Reilly and Reilly, 2014).

A characteristic of the ‘Mejhoul’ is that it comes into production in a shorter number of years than most other cultivars. It also produces many offshoots, which is important for an expanding industry. The tree delivers heavy crops of large-size fruit, making it well known by the Australian public in the domestic marketplace. ‘Mejhoul’ dates are displayed prominently in supermarket fresh fruit aisles, usually imported from the USA, Mexico and more recently, Jordan and Israel (Reilly et al., 2010).

In a global comparison, the Australian ‘Mejhoul’ industry is very small but expanding. After 20 years of evaluating many cultivars, the ‘Mejhoul’ genetics are providing Australian farmers with the confidence to increase production. The next stage of industry development is to invest capital in postharvest facilities; to mechanise fruit handling and packing, as well as processing equipment to manufacture products like date paste and syrup.

Gurra Downs is very proud to be Australia’s largest producer of the ‘Mejhoul’ date cultivar. This magnificent tree has provided a great source of interest and excitement, not only for the family business but also for many other Australian farmers.

Literature cited

McEllister, F.V. (1991). Date Production in Central Australia - an Agronomic and Economic Evaluation. Technical Bulletin 162 (Northern Territory Dept. Primary Industries).

Reilly, D., and Reilly, A. (2014). Developing a date industry in Australia. Emir. J. Food Agric. *26* (*11*), 1000–1013 https://doi.org/10.9755/ejfa.v26i11.18987.

Reilly, D., Reilly, A., and Lewis, I. (2010). Towards an Australian Date Industry: an Overview of the Australian Domestic and International Date Industries. Publication No.10/174 (Rural Industries Research and Development Corporation).

‘Mejhoul’ cultivation in Mexico and other South-American countries

R. Salomón-Torres[a]

Unidad Académica San Luis Rio Colorado, Universidad Estatal de Sonora, Km.6.5 carretera a Sonoyta, SLRC, Sonora, 83500, Mexico.

Abstract

In Mexico, the crop of ‘Mejhoul’ date palm found the best climatic conditions for its development in the San Luis Rio Colorado, Sonora and Mexicali, Baja California valleys. By 2020, both areas achieved 14,898 t of ‘Mejhoul’ dates. The ‘Mejhoul’ cultivar represents 94% of the national date production, making Mexico the second-largest producer in the American continent, and the fourth largest producer in the world. In South America, its cultivation is very little developed. However, it has good development prospects in the medium term.

Keywords: ‘Mejhoul’, training, harvester, family gardens

INTRODUCTION

The modern date industry was established in Mexico in 1968, in the San Luis Rio Colorado Valley (32°18’19”N, 114°56’43”W), with the first ‘Mejhoul’ date plantation using offshoots imported from Yuma, Arizona (Johnson et al., 2015). Over time, this crop spread to the Mexicali valley (32°22’27”N, 115°07’13”W), with offshoots imported from southern California. Finally, it expanded to the arid regions of north western Mexico. Date palm cultivation in Mexico is 97% concentrated in these two valleys, with the production of the ‘Mejhoul’ cultivar standing out with 94% of the national production (Ortiz-Uribe et al., 2019). Likewise, the Mexican companies that produce dates are well organized. Each year, they improve their cultivation techniques, gain more experience to obtain better yields, and continue the expansion of the planted areas.

‘MEJHOUL’ CULTIVATION

The Mexican date industry is very small compared to the larger producing countries of this fruit, largely due to the fact that it is not a high priority crop. Production has increased by almost 50% during the last two years. In 2020, 15,849 t of dates were harvested in 2,504 ha, of which 14,898 t are attributed to the ‘Mejhoul’ cultivar (SIAP, 2021). With this, Mexico becomes the fourth-largest ‘Mejhoul’ date producer in the world after Israel, the US and Palestine (Krueger, 2015). However, almost a thousand ha have not yet entered into production, which would represent around 7,000 additional t of ‘Mejhoul’ dates in the short term, with which Mexico could become the second largest producer (Table 1). The average yields of ‘Mejhoul’ date palm are between 6.78 and 7.90 t ha^{-1}, however, some harvesters that carry out the best crop practices have achieved up to 12 t ha^{-1}.

The establishment of 1 ha of ‘Mejhoul’ date palm is estimated at $ 28,000 for the first year. For its second year, $ 9,500 and from the third year, it remains at approximately $ 4,500. This means that between seven and eight years, the crop becomes highly profitable (SCSA, 2021).

The cost of labor in handling the palms and packing the ‘Mejhoul’ date is very low in relation to the costs in the US, which gives Mexico a greater competitiveness and higher profits. This advantage has been seized by some US companies, which send several thousand tons of ‘Mejhoul’ dates for packaging to Mexico, which are then returned to the US for commercialisation.

[a]E-mail: ricardo.salomon@ues.mx

Table 1. Date production in Mexico in 2020.

State	Surface planted (ha)	Surface harvested (ha)	Production (t)	Yield (t)	Production value ($ millions)
Baja California	1,740.00	755.00	5,972.93	7.90	38.20
Baja California Sur	373.13	369.13	602.01	1.63	1.17
Coahuila	16.81	16.81	25.75	1.53	0.06
Sonora	1,363.94	1,363.94	9,248.35	6.78	21.27
Total	**3,493.88**	**2,504.88**	**15,849.04**	**7.34/1.58**	**60.70**

The 'Mejhoul' date is exported mainly to the US and Australia, and to a lesser extent to countries such as The Netherlands, Spain, UK, Canada, Italy and Argentina, among others. In 2019, 5,550 t were exported with a value of $ 19.5 million.

DATE PALM CULTIVATION AND PRACTICES

In Mexico, there are no significant problems with pests or diseases with date palm. The agricultural-sanitary authorities of Mexico offer free training for the good management of safety and management of pests and diseases.

The small and medium-sized date harvesters have been integrated into associations called the Date Product System to improve the competitiveness of the crop with better cultural and commercial practices, as well as negotiating with the government programmes aimed at supporting date production. Likewise, constant training is offered to harvesters with the intervention of researchers, field technicians and government analysts, to stimulate the improvement of the quality of the 'Mejhoul' date. Finally, internal consumption has recently been stimulated through fairs, tourist routes and exclusive agricultural exhibitions for dates, where fresh fruit, gourmet dishes, beverages and a great diversity of by-products derived from 'Mejhoul' dates are offered (Ortiz-Uribe et al., 2019).

Water scarcity has not yet been a climate change problem in this date-producing region of Mexico. However, some preventive measures have been taken to conserve water, such as cementing irrigation canals and some parts of the Colorado River (which comes from the US and feeds both valleys), in order to avoid water seepage. According to data from the Ministry of Agriculture, a higher yield has been reported in plantations where drip irrigation systems are used compared to those that use flood irrigation systems (SADER, 2021). With this, the government hopes to encourage the use of drip irrigation systems to save irrigation water.

Given the climatic conditions of this region of Mexico, the transplantation of offshoots commonly has a mortality rate between 20 and 30% (SCSA, 2021). To avoid this risk, very few harvesters have opted for the acquisition of in vitro seedlings. However, there is much distrust on the part of the harvesters, since the yields of the palms produced by this method are not known and those that are planted in Mexico are still very young.

Within bunch thinning activities, 50 to 70 strands per bunch, 10 to 14 dates per strand, and 16 to 20 bunches (Figure 1A) per adult palm are commonly left behind (Salomon-Torres et al., 2017). Most of the plantations are irrigated, the common method of pollination is by means of dry pollen mixed with flour (Salomón-Torres et al., 2018) and very few companies have production of organic Mejhoul date (Figure 1B, C).

Research efforts are focusing on improving fruit quality through the use of growth bio-stimulators, efficient pollen management, reduction of mortality in the transplantation of offshoots, and the method of pollination in liquid suspension, it is in its first stage of experimentation. Likewise, the quality of the 'Mejhoul' date produced in Mexico has recently been evaluated, concluding that it has the same or better nutrients and antioxidant properties than that produced in other parts of the world (Salomón-Torres et al., 2019).

Figure 1. Some aspects of the agricultural management of organic date 'Mejhoul' in Mexico. a) Preparation of the inflorescence for pollination; b) Harvest of the date 'Mejhoul'; c) Organic plantation of 'Mejhoul' date in the Valley of Mexicali, Mexico, with the bunch in bags.

OTHER SOUTH-AMERICAN COUNTRIES

Date palm cultivation can be found in limited specific areas in Colombia, Venezuela, Argentina, Brazil, Peru and Chile. These last two countries have regions with agro climatic conditions that have allowed the successful cultivation of the 'Mejhoul' date, which is currently produced in family gardens, not in commercial quantities (Escobar and Valdivia, 2015).

In Chile, date palms are mostly found in the extreme north of the country and do not constitute an important industry. The interest in this crop began in 1965-1970, when the Chilean government introduced offshoots from the US to the Tarapacá Region (Escobar and Valdivia, 2015). Likewise, in 2020, 1,100 in vitro 'Mejhoul' palms from California were introduced to Chile, with which it is intended to develop a new agribusiness associated with the production of 'Mejhoul' dates (Generación M, 2020).

In 2018, the visit of harvesters and technicians from Honduras and Panama was received in Mexico, in order for them to know the agronomic characteristics of the 'Mejhoul' date crop, to implement it as a production alternative in their respective arid regions. Harvesters in Argentina are currently analyzing alternatives to import 'Mejhoul' offshoots from Mexico to that country.

CONCLUSIONS

Being a recently introduced crop, and one of less importance, there is still much to do to increase the production of 'Mejhoul' dates in Mexico. It is necessary to develop further research to improve yields, quality and decrease mortality after transplantation. Farmers should focus more on quality production than quantity. A more active participation of the government of Mexico is required, in terms of economic support for its cultivation, as well as a wide advertising campaign to stimulate the internal consumption of 'Mejhoul' date.

Literature cited

Escobar, H.A., and Valdivia, R.G.J. (2015). Date palm status and perspective in South American Countries: Chile and Peru. In Date Palm Genetic Resources and Utilization, Vol. 1: Africa and the Americas, 1st edn, J.M. Al-Khayri, S.M. Jain, and D.V. Johnson, eds. (London, UK: Springer), p.487–506.

Generación M. (2020). Ingresan al país más de mil palmeras para producir dátiles made in Chile. https://www.elmostrador.cl/generacion-m/2020/10/12/ingresan-al-pais-mas-de-mil-palmeras-para-producir-datiles-made-in-chile/ (accessed September 4, 2021).

Johnson, D.V., Al-Khayri, J.M., and Jain, S.M. (2015). Introduction: date production status and prospects in Africa and the Americas. In Date Palm Genetic Resources and Utilization, Vol. 1: Africa and the Americas, 1st edn, J.M. Al-Khayri, S.M. Jain, and D.V. Johnson, eds. (London, UK: Springer), p.3–18.

Krueger, R.R. (2015). Date palm status and perspective in the United States. In Date Palm Genetic Resources and Utilization, Vol. 1: Africa and the Americas, 1st edn, J.M. Al-Khayri, S.M. Jain, and D.V. Johnson, eds. (London, UK: Springer), p.447–485.

Ortiz-Uribe, N., Salomón-Torres, R., and Krueger, R. (2019). Date palm status and perspective in Mexico. Agriculture *9* (*3*), 46 https://doi.org/10.3390/agriculture9030046.

SADER. (2021). Secretaria de Agricultura y Desarrollo Rural - Baja California. https://www.gob.mx/agricultura/bajacalifornia (accessed September 1, 2021).

Salomon-Torres, R., Ortiz-Uribe, N., Villa-Angulo, R., Villa-Angulo, C., Norzagaray-Plasencia, S., and García-Verdugo, C.D. (2017). Effect of pollenizers on production and fruit characteristics of date palm (*Phoenix dactylifera* L.) cultivar Medjool in Mexico. Turk. J. Agric. For. *41* (*5*), 338–347 https://doi.org/10.3906/tar-1704-14.

Salomón-Torres, R., Ortiz-Uribe, N., Sol-Uribe, J.A., Villa-Angulo, C., Villa-Angulo, R., Valdez-Salas, B., García-González, C., Monroy, C.G.I., and Norzagaray-Plasencia, S. (2018). Influence of different sources of pollen on the chemical composition of date (*Phoenix dactylifera* L.) cultivar Medjool in Mexico. Aust. J. Crop Sci. *12* (*6*), 1008–1015 https://doi.org/10.21475/ajcs.18.12.06.PNE1213.

Salomón-Torres, R., Ortiz-Uribe, N., Valdez-Salas, B., Rosas-González, N., García-González, C., Chávez, D., Córdova-Guerrero, I., Díaz-Rubio, L., Haro-Vázquez, M.D.P., Mijangos-Montiel, J.L., et al. (2019). Nutritional assessment, phytochemical composition and antioxidant analysis of the pulp and seed of medjool date grown in Mexico. PeerJ *7*, e6821 https://doi.org/10.7717/peerj.6821. PubMed

SCSA. (2021). Secretaria del Campo y Seguridad Alimentaria del Estado de Baja California. http://www.scsa.gob.mx/ (accessed September 3, 2021).

SIAP. (2021). Servicio de Información Agroalimentaria y Pesquera. https://nube.siap.gob.mx/cierreagricola/ (accessed September 3, 2021).

Date palm cultivation in the Republic of India

B.N. Mohanan[1,a] and A.S. Batra[2,b]

[1]Utilities and Services, Atul Ltd., India; [2]Business Development, Atul Ltd., India.

Abstract

With average imports of more than 310,000 metric t each year, India is the world's largest dates importer and constitutes nearly 30% of total global imports. Iraq, UAE, Islamic Republic of Iran and Pakistan are the major date suppliers to India. With a population of 1.4 billion, India has tremendous market potential for dates, which is evident from the increasing imports trend. For its contribution towards establishing a modern and scientific date industry in India, Atul has been awarded the Khalifa International Date Palm Award 2009, Siemens Ecovatives Award 2010, Peacock Eco Innovative Award 2014, Make in India Award 2015 and Rashtra Vibhushan Award 2018. Atul has contributed in providing high quality tissue culture date palm planting material over more than 8,000 acres in India. With the support of the Indian government, and continued good performance of high-quality tissue culture date palms, Indian farmers of hot and arid climatic zones, especially in Jaisalmer, Barmer and other districts of western Rajasthan are being encouraged to cultivate the 'Mejhoul' cultivar.

Keywords: 'Mejhoul', date palm, India, *Phoenix dactylifera*

INTRODUCTION

With average imports of more than 310,000 metric t each year, India is the world's largest dates importer and constitutes nearly 30% of total global imports (Figure 1). Iraq, UAE, Islamic Republic of Iran and Pakistan are the major date suppliers to India. With a population of 1.4 billion, India has tremendous market potential for dates, which is evident from the increasing imports trend.

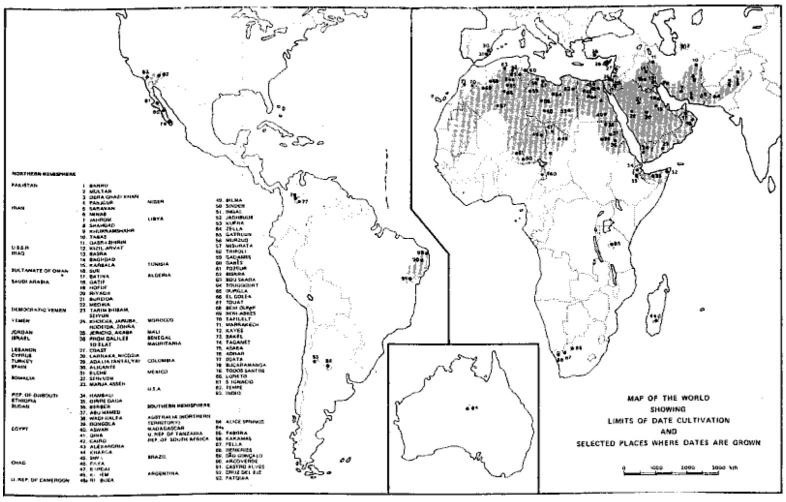


Figure 1. Map of the world showing limits of date cultivation and selected places where dates are grown.

[a]E-mail: bn_mohanan@atul.co.in
[b]E-mail: Ajitsingh_Batra@atul.co.in

Acta Hortic. 1371. ISHS 2023. DOI 10.17660/ActaHortic.2023.1371.11
Proc. VII International Date Palm Conference
Eds.: A. Zaid, G.A. Alhadrami and S. Mitra

Several cultivars of dates make a significant contribution towards the volume of dates in the Indian market, including: 'Mazafatti', 'Safawi', 'Deglet noor', 'Fard', 'Khunezi' and dried dates of other cultivars. These dates are being imported at a price of $ 0.4 to 1.5 kg^{-1} (FAOSTAT).

However, due to increased awareness and structured marketing efforts by several importing companies, 'Mejhoul' – known as the emperor of dates – has been established as one of the premium date cultivars in India. Jordan and Israel are the top two 'Mejhoul' exporting countries to India. Dates from Israel and Jordan are being imported to India at a markedly higher cost than other cultivars, of between $ 5 and 7 kg^{-1} (FAOSTAT).

Before partition in 1947, India was one of the top date-growing countries, with dates grown primarily from the great desert of Thar. However, partition left the entire date growing region in today's Pakistan (Figure 2, highlighted with arrow). After partition, India lost this bio-diversity and date planting materials. This meant India's date industry could not grow until 2007-2008, due to unavailability of high quality planting material.

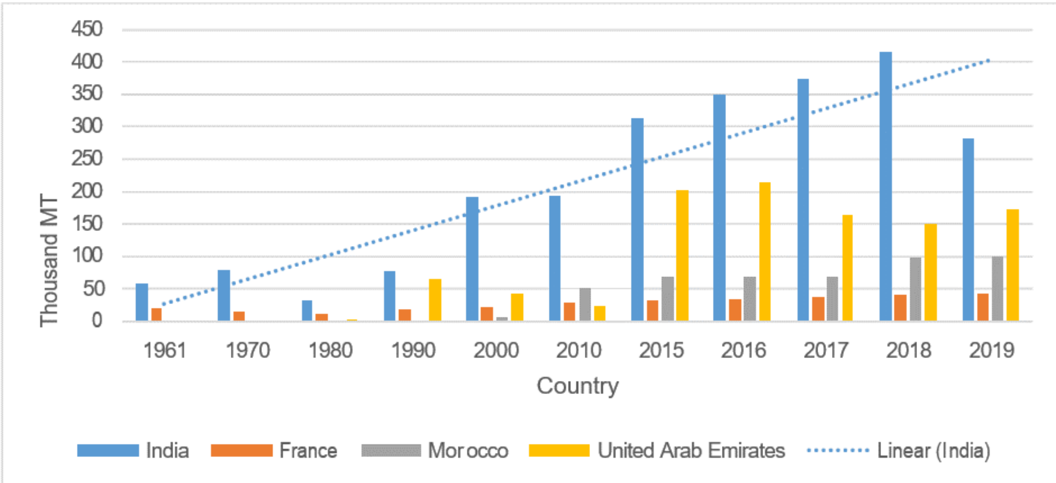


Figure 2. Date growing regions. Source: FAOSTAT.

'GREENING THE DESERTS'

Atul Ltd., along with the Indian government, introduced the 'Greening the deserts' initiative, which introduced tissue culture-raised date palms to India. Atul demonstrated successful cultivation of various cultivars of date palms in arid and semi-arid agro-climatic zones of India by establishing the nation's largest tissue culture-raised date palm demonstration farms in Jaisalmer and Bikaner districts, Rajasthan, spread over more than 250 and 125 acres, respectively.

'Mejhoul', was one of the ripened cultivars (rhutab and tamar stage), which performed well in the demonstration trials in the Thar desert of western Rajasthan, while fresh fruit cultivars like 'Barhee', 'Khunezi' also showed excellent results as fresh fruit cultivars (khalal stage) in other regions, including the Rann of Kutch of North Gujarat.

To ensure a continuous supply of high quality true-to-type tissue culture-raised planting material of date palms, Atul also set up Atul Rajasthan Date Palms, a state-of-the-art tissue culture production unit, as a joint venture with the Government of Rajasthan under a public-private partnership model with overseas technology transfer from Date Palm Research and Development Center, UAE University, Al-Ain, UAE. To expand globally, Atul acquired a majority stake in Date Palm Developments UK (DPD), one of the world's oldest and largest date palm tissue culture commercial units. Further, it has set up a third production unit of tissue culture date palms at Panoli, Ankleshwar, Gujarat, India.

For its contribution towards establishing a modern and scientific date industry in India, Atul has been awarded the Khalifa International Date Palm Award 2009, Siemens Ecovatives

Award 2010, Peacock Eco Innovative Award 2014, Make in India Award 2015 and Rashtra Vibhushan Award 2018. Atul has contributed in providing high quality tissue culture date palm planting material over more than 8,000 acres in India.

The 'Mejhoul' cultivar has shown good commercial feasibility in arid regions of India, where rain is scarce. Indian farmers have adopted improvised postharvest technologies to deliver high quality standards of 'Mejhoul'. According to the Indian climate, the 'Mejhoul' tree starts flowering during February and March. Upon pollination, the fruit develops and enters into the khalal stage between May and June. Further, it enters the rhutab stage between July and August, and is finally harvested in rhutab/partial tamar stage (Figure 3, left). Conversion of rhutab to tamar is done after harvesting by simply drying under the sun or using solar dryers (Figure 3, middle). The appearance of the Indian 'Mejhoul' is light brown to dark brown, depending on which stage it is harvested (Figure 3, right).

Figure 3. Fruit bunch management (left); drying of the harvested 'Mejhoul' (middle); appearance of the Indian 'Mejhoul' (right).

The size and weight of Indian 'Mejhoul' dates varies from 3 to 5 cm and 8 to 20 g, respectively. There is scope of achieving a larger 'Mejhoul', if the thinning of bunches is implemented timely, during berry development in the kimri stage. Indian farmers are adapting fruit bunch management and fruit sorting and grading techniques to improve on quality. After drying, dates are then sorted, washed, graded, packed according to size and quality and packed in retail packs (Figure 4). The Indian-grown 'Mejhoul' fetches prices in home markets of INR 300 (approximately $ 4) to INR 1500 (approximately $ 20) kg^{-1}.

Figure 4. Grading and packing of 'Mejhoul'.

With the support of the Indian government, and continued good performance of high quality tissue culture date palms, Indian farmers of hot and arid climatic zones, especially in Jaisalmer, Barmer and other districts of western Rajasthan are being encouraged to cultivate the 'Mejhoul' cultivar. Hence the area under cultivation of 'Mejhoul' is gradually increasing in India.

Regional and international marketing of ‘Mejhoul’ dates

A. Oihabi[1,a] and A. Zaid[2,b]

[1]Date Palm Production and Value Chain Expert, Alicante, Spain; [2]Secretary General of Khalifa Int’l Award for Date Palm and Agricultural Innovation, Abu Dhabi, United Arab Emirates.

Abstract

The data about date production provided by the specialized regional and international organization does not distinguish between date cultivars. Therefore, the information provided in this paper was gathered with the most appreciated cooperation of members of local agencies in the main ‘Mejhoul’ producing countries. Based on the collected data, ‘Mejhoul’ dates production in 2020 was estimated to 105,489 t which represented 1.16% of the global date production. Six countries led by Israel and the USA produced 99.95% of the total ‘Mejhoul’ production and exported to the international market 63.27% of their total production. This situation is expected to change tremendously within the forthcoming years due to the important extension of ‘Mejhoul’ cultivation in some date producing countries mainly Morocco and Egypt. This paper describes the geographical distribution of the main destinations of the ‘Mejhoul’ dates exported by the different producing countries as well as the marketing standards used by these late. It also discuss the challenges faced by the ‘Mejhoul’ sectors with regards to its increasing production.

Keywords: dates marketing, ‘Mejhoul’, *Phoenix dactylifera*, date palm

INTRODUCTION

Dates are produced in 40 countries around the world, with an annual production of 9,075,466 t. Of this total, 1,836,827 t (20.24%) are distributed to the international market, generating a total income of USD2 billion (FAOSTAT, 2020). The remaining dates quantities are used for local consumption and waste, which represents up to 40% of the date production in some date-producing countries(AOAD, 2018).

The available data provided by international organisations about dates production and international marketing does not specify the share of specific date cultivars. Therefore, it is impossible to distinguish the relative weighting of different date cultivars, unless the information is communicated by the concerned agencies in the targeted date-producing country.

The information about ‘Mejhoul’ production and marketing reported in this chapter has been gathered with the most appreciated cooperation of members of specialized agencies and research centers in the main ‘Mejhoul’ date-producing countries such as Morocco, Israel, the US, Jordan, Mexico, Palestine, South Africa, Namibia, Egypt, Peru, and Australia.

THE PRODUCTION AND INTERNATIONAL MARKETING OF ‘MEJHOUL’

The estimated ‘Mejhoul’ production of the top ten date-producing counties (at the global level) for the year 2020 is summarized in Table 1. It estimates total ‘Mejhoul’ date production at 105,498 t which represents 1,16% of the total world dates’ production. Israel is the leader of this cultivar’s production at the international level with 42.65% of the world production followed by USA and Mexico with respectively 15.17 and 14.12%.

[a]E-mail: oihabi@gmail.com

[b]E-mail: zaidabdelouahhab@gmail.com

Table 1. Estimated 'Mejhoul' date production 2020 (Glasner (Israel), Salomon (Mexico), M. Al-Banna (Palestine), 2021, pers. commun.).

Country	Quantity (t)	Share (%)
Israel	45,000	42.65
US	16,000	15.17
Mexico	14,898	14.12
Jordan	10,000	9.48
Palestine	9,000	8.53
Morocco	3,500	3.32
South Africa and Namibia	3,000	2.84
Egypt	3,000	2.84
Peru	500	0.47
Australia	100	0.09
Others	500	0.47
Total	**105,498**	**100**

STANDARDS AND MARKETING

'Mejhoul' dates are the most appreciated of date cultivars, and the most expensive at the international level. It is also one of the rare cultivars with specific marketing standards, including three grades: medium (15-18 g date^{-1}), large (19-22 g date^{-1}) and jumbo (23-27 g date^{-1}). Some exporters also include a fourth super jumbo category (+27 g date^{-1}).

The price of 'Mejhoul' dates varies at the international level between $ 7 and 10 kg^{-1} for the medium to jumbo grades, while retail prices vary from one country to the other, with packaging quality having a significant impact. Figures 1 to 4 represent a sample of 'Mejhoul' selling prices in different markets around the world. In various European countries, the cost the consumer pays for 1 kg of 'Mejhoul' is around € 20.

Figure 1. 'Mejhoul' dates in Malaga (left) and in Alicante (right), Spain.

Figure 2. 'Mejhoul' dates in Morocco (left) and in Marseille, France (right).

Figure 3. Price of ‘Mejhoul’ compared to ‘Barhi’ and ‘Deglet Nour’ in a market in Alicante, Spain.

Figure 4. ‘Mejhoul’ dates in Al-Madinah Al-Munawara, Saudi Arabia, main ‘Mejhoul’ exporting countries.

‘MEJHOUL’ INTERNATIONAL MARKETING

As indicated in the introduction, the data exposed in this article were gathered thanks to the collaboration of members of specialized agencies and research institutions in the main ‘Mejhoul’ dates producing countries.

Main ‘Mejhoul’ exporting countries

Table 2 presents the volume of dates exported by the main ‘Mejhoul’ date-producing countries. These countries represent 90% of the volume of ‘Mejhoul’ produced in 2020 at global level and together export an average of 63.27% of their total ‘Mejhoul’ date production.

Table 2. Volume of ‘Mejhoul’ dates exported reported to the produced volume in 2020. Export sources: B. Glasner (Israel), USDA (US), R. Salomon (Mexico), A. Haddad (Jordan) and M. Al-Banna (Palestine) (pers. commun.).

Country	Mejhoul production (t)	Mejhoul export (t)	Mejhoul as % of total date export/production
Israel	45,000	29,000	64.44
US	16,000	13,262	82.88
Mexico	14,898	8,165	54.81
Jordan	10,000	7,511	75.11
Palestine	9,000	4,000	44.44
Total/average	**94,898**	**61,938**	**63.27**

The international 'Mejhoul' date market is led by Israel and the US, which together represent a 67% share of the total volume exported by the main 'Mejhoul' date-producing countries. Palestine is the country exporting the lowest share of its 'Mejhoul' production (44.44%). This is mainly related to the problems facing the sector, including insufficient infrastructure and other challenges of the respective date sector value chain.

The main 'Mejhoul' date-producing countries are low date producers overall. A total production of 142,581 t in 2019 represents 1.57% of the world's date production (FAOSTAT, 2020). Figure 5 shows 'Mejhoul' dates represent the largest share of exported dates from these countries, particularly the US and Jordan, with a 90 and 80% share of 'Mejhoul' dates, respectively. In terms of volumes, Israel is exporting 46.82% of the total volume exported by the five main exporting countries followed by the US (21.41%), Mexico (13.18), Jordan (12.13%) and Palestine (6.46%).

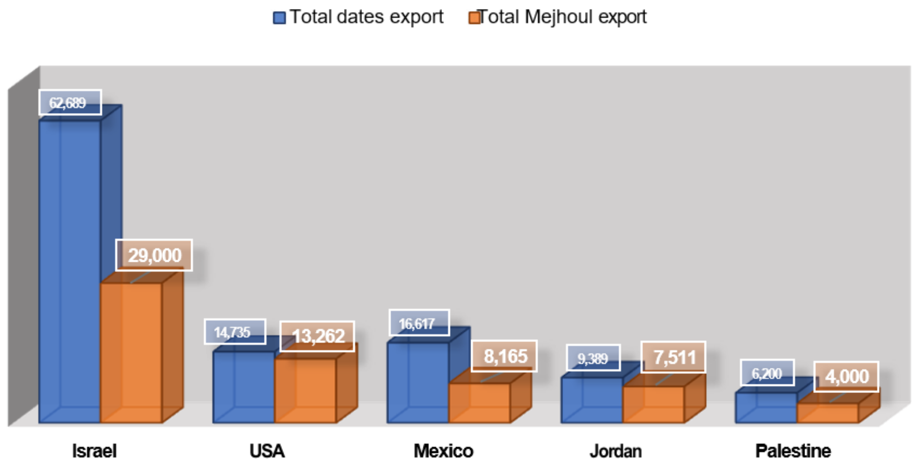


Figure 5. Share of 'Mejhoul' dates among the total dates exported by the main Mejhoul-producing countries.

Unfortunately, no official information is available with regards to the incomes generated by Mejhoul dates handled via the international market.

'MEJHOUL' DATE DESTINATIONS

Figure 6 presents the destination of the dates exported by the main 'Mejhoul' date-producing countries. The dates produced by these countries are directed to different destinations around the world, although the UK is a common partner to the five countries, there is a specific geographical distribution depending on the origin of the 'Mejhoul' dates. This distribution can be summarized as follows:

- The US and Mexico mainly trade with Australia, USA and Mexico.
- Jordan mainly deals with Arab countries.
- Israel focuses on European countries such as the Netherlands, France, the UK and Germany;
- Palestine's main trading partners are the UAE, Turkey and the UK.

CONCLUSIONS

Currently, 'Mejhoul' date production is mainly limited to five countries, led by Israel and the US. The total production of Mejhoul dates represents only 1.16% of the world's total date production, but the 'Mejhoul' is the most sought after and expensive date on the international market. Unfortunately, there is no data related to the economics of the marketing of this important cultivar provided by any international organization. Therefore, it is not possible to measure its financial share of the income of the US$ 2 billion generated by the international date market.

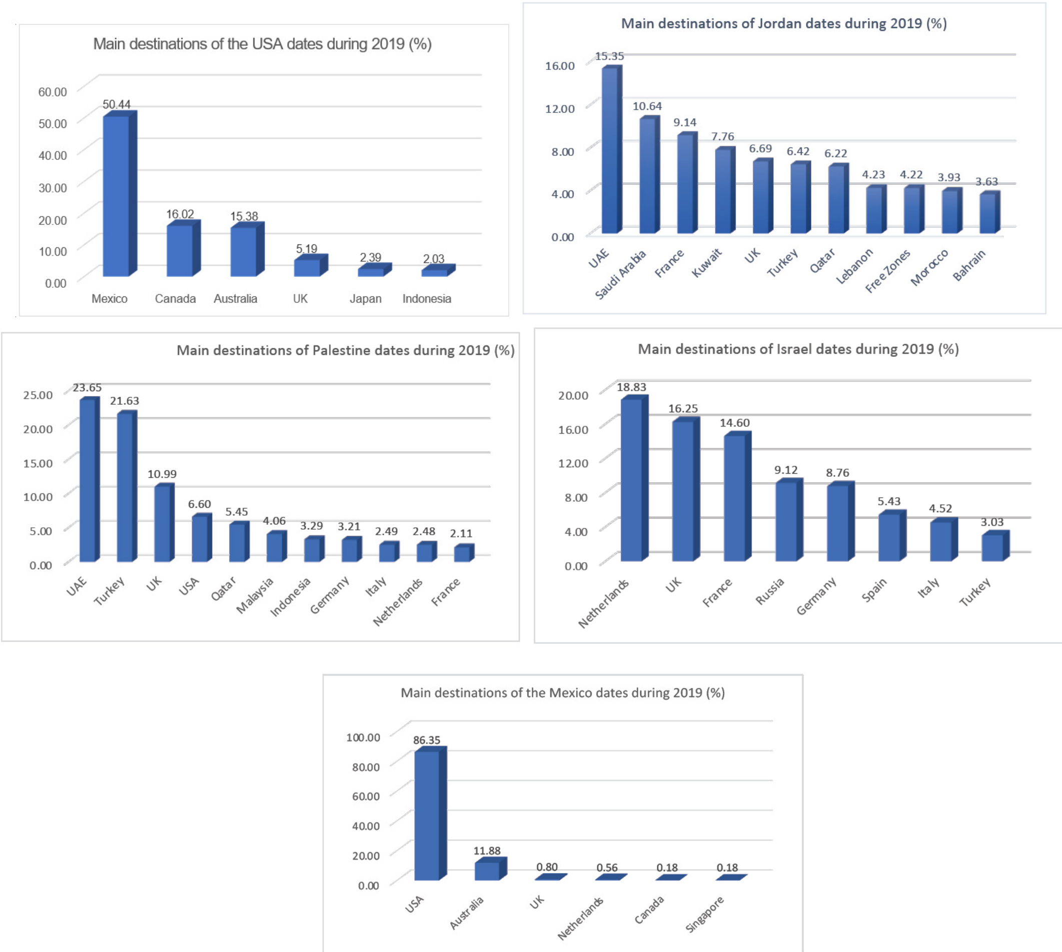


Figure 6. The destination of the dates exported by the main 'Mejhoul' date-producing countries.

Information and statistics provided in this chapter are expected to change completely within the next five years, due to the important increase in the areas planted with 'Mejhoul' in several countries in the MENA region. Indeed, as part of its 'Green Morocco 2008-2020' program, the Kingdom of Morocco has planted more than three million date palms, including at least 70% of the 'Mejhoul' cultivar. The extension of the 'Mejhoul' plantations in Morocco will continue with a new program:'Generation Green 2020-2030', which has a specific focus on increasing the number of date-packing facilities. Egypt has also started a very ambitious program of planting several million date palms, with 'Mejhoul' as the main cultivar. The entrance in production of all these newly planted date palms should make Morocco and Egypt the main 'Mejhoul' world producers, and will put more pressure on Israel and the US as the current international market leaders.

Literature cited

AOAD. (2018). Report on the Date Value Chain in the Arab Region.

FAOSTAT. (2020). FAO.org.

Red palm weevil: governance and regional phytosanitary systems

M. Chouibani[1,a], S. Brunel[2], M. El Akel[1] and L. Abaha[1]

[1]Near East Plant Protection Organization (NEPPO), Bâtiment C INRA, Angle Avenues Hassan II et Ibn Al Ouazzane, Rabat, Morocco; [2]Secretariat International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00153, Rome, Italy.

Abstract

The red palm weevil (RPW) is a major trans boundary pest affecting date palms worldwide. The weevil is causing devastating damage to more than 40 palm species worldwide including date palm, affecting production, the environment and the livelihoods of millions of farmers. The annual losses in date palms in the Mediterranean countries is estimated at 480 million Euros. RPW is a pest of big concern for many countries and is considered as quarantine pest. Despite the efforts done by countries to prevent its introduction or its spread, the lack of specific regulations/guidelines on phytosanitary measures to regulate the palm trade, especially at the entry points has been pointed out by the FAO RPW Eradication Strategy clearly as gaps and challenges in most of national policies and regulatory systems explaining the failure to control RPW. Long-distance dissemination of many pests/pathogens occurs mostly via infected/infested propagative material. The effective control of RPW requires more coordination with the NENA region. NEPPO will develop in this context 1) a regional standard of a harmonized phytosanitary measures (at borders and inspection on the territory; 2) a regional standard protocol for available and cost-effective treatments of offshoots and ornamentals (at border); and 3) a pest free area protocol to help the extension of date palm area and to provide other areas with safe date palm offshoots.

Keywords: RPW, phytosanitary measures, regional standard, PFA

INTRODUCTION

Date palm is one of the most important crops and main staple in North Africa and Near East countries. It covers 1,178,514 ha (Figure 1). The main harvested areas are situated in Iraq, Algeria, Saudi Arabia, Iran, and Pakistan (FAOSTAT, 2022). For many Near East and North African (NENA) countries, the date palm has significant socio-economic importance (Mousavi et al., 2009).

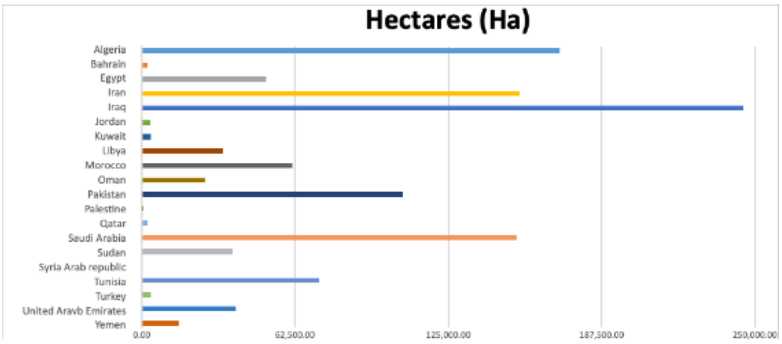


Figure 1. Harvested area (ha) with date palm in North Africa and Near East area (FAOSTAT, 2022).

[a]E-mail: hq.neppo@gmail.com

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera: Curculionidae*) is a key pest affecting date palms across the world. The weevil is causing devastating damage to more than 40 palm species worldwide including date palm, affecting production, the environment, and the livelihoods of millions of farmers. The annual losses in the Mediterranean countries are estimated at 480 million Euros (FAO2017b). In North Africa, while Algeria and Sudan are still free from RPW (Figure 2), the pest occurs on Canary Island palm (*P. canariensis* Chabaud) in Morocco and Tunisia and is still contained in the north of the countries and maintained far from the oases. Mauritania managed to eradicate the pest in the oasis of Tidjikja in Tagant Wilaya in 2017 (FAO, 2017a).

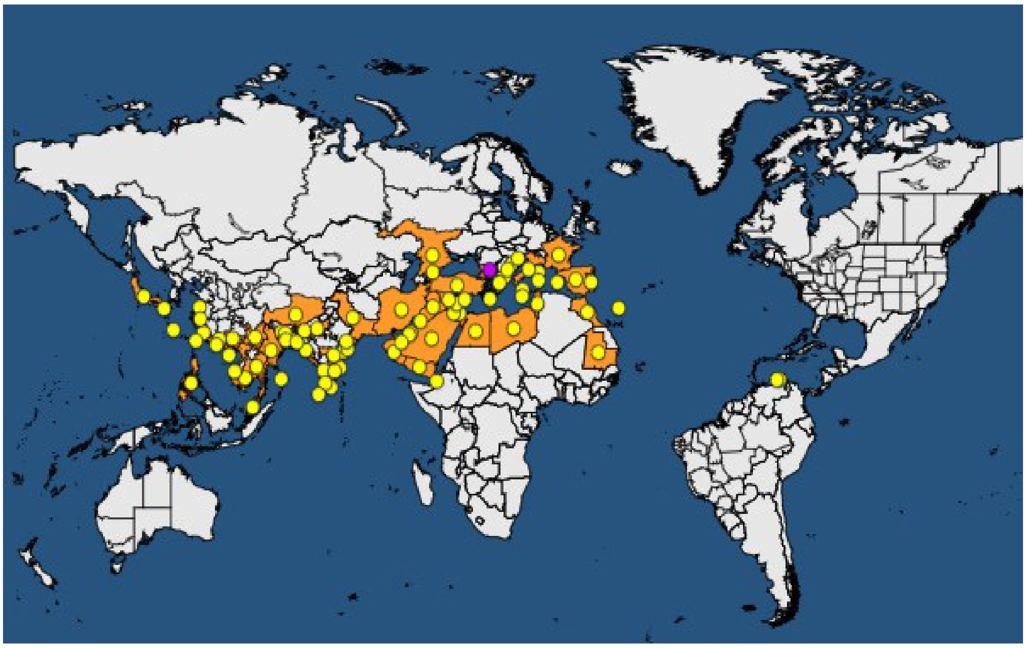


Figure 2. World distribution of red palm weevil *Rhynchophorus ferrugineus* (EPPO, last updated 30/06/2020).

HOW IS THE PEST MITIGATED IN THE NENA REGION?

The biological characteristics of the pest make it complex to detect at an early stage and render its management very difficult. A survey prepared by Near East Plant Protection Organization (NEPPO) in October 2021 was sent to20 NENA countries: Algeria, Bahrain, Egypt, Iraq, Iran, Jordan, Kuwait, Lebanon, Libya, Mauritania, Morocco, Oman, Palestine, Qatar, KSA, Syria, Sudan, UAE, Tunisia, and Yemen. The survey concerned the status of the pest, the availability of a contingency plan with the surveillance plan, the phytosanitary measures implemented within the country and at borders, and the availability of nurseries and certified propagative material. Thirteen countries responded to the questionnaire, providing interesting information about this pest.

The questionnaire highlights that the pest occurs in most countries, but the status of this pest is not reported according to the International Standard for Phytosanitary Measures (ISPM 8) (Determination of pest status in an area) (FAO, 2021b). This could reflect a need for training on how to better implement this ISPM.

Eleven countries considered RPW as a quarantine pest according to the IPPC glossary (ISPM 5) (FAO, 2021c), except for Palestine and the United Arab Emirates where the pest is not considered as such. Only two countries (Lebanon and United Arab Emirates - UAE) have conducted a pest risk analysis (PRA) for this pest according to ISPM 11 (Pest risk analysis for quarantine pests (FAO, 2013)).

To control any emergent pest such as RPW, a contingency plan describing the actions to be implemented in response to any eventual outbreak or detection of a pest would be considered very useful and would help the National Plant Protection Organization (NPPO) to be well prepared and to react promptly and efficiently to stop the spread of the pest. Only 46% of the countries (Iraq, Morocco, Oman, Palestine, Syria, and Tunisia) drafted a contingency plan. However, 10 countries (77) developed a surveillance plan (Iraq, Jordan, Lebanon, Morocco, Oman, Palestine, Syria, UAE, and Tunisia) in the implementation of which stakeholders are involved (55%). Most countries organized a training session for their NPPOs staff on red palm weevil on detection, monitoring, and eradication.

Import of date and ornamental palms

The questionnaire indicates that most countries did not import date and ornamental palms during the last three years. Only three countries (Egypt, Lebanon, and UAE) import such commodities. Eight countries (Oman, Tunisia, Egypt, Jordan, Yemen, Lebanon, Iraq, and Morocco) implement specific phytosanitary requirements for this type of palms: all consignments should be originating from “certified” nurseries in free areas from RPW, in addition, to be accompanied by an import permit and a phytosanitary certificate. In some countries, importation of host plants of RPW and date palm propagative material is banned except for tissue culture with less than 5 cm in diameter (UAE).

Phytosanitary measures within infested areas

In infested countries, the NPPOs reported that they implement phytosanitary measures to contain RPW. An infected area is considered a quarantine area and no plants should move from this infested area. Inside an infected area, all palms are subject to chemical treatment and mass pheromone trapping to survey RPW. Infested palms are destroyed in all countries. However, Illegal/unregulated movement of infested palms was reported within the countries and between countries, mainly in the context of instability in the region (in Iraq, Libya, Syria, and Yemen) where NPPOs are not able to follow up on the movement of palms.

Origin of date plants

Having healthy plant propagation material is one of the main measures supporting the quarantine strategy. It is pivotal to prevent the detrimental effects of RPW or any other pests. According to the survey, offshoots originate from private or owned farms, local nurseries, and tissue culture facilities in some countries. Nine countries have date palm nurseries. Only Libya, Jordan, and Yemen do not have any. However, except for Morocco, there are no certified nurseries ensuring that a certified propagative material truly meets the plant health requirements.

Regional phytosanitary systems

Despite the efforts done by countries to prevent the introduction of RPW or its spread, the lack of specific regulations/guidelines on phytosanitary measures to regulate the palm trade, especially at the entry points has been clearly pointed out by the FAO RPW Eradication Strategy as gaps and challenges in most of the national policies and regulatory systems, explaining the failure to control RPW (FAO, 2017b). The FAO RPW Eradication Strategy also noted the poor implementation of phytosanitary (quarantine) measures for transferring planting materials for new farms or gap-filling in the existing farm, between regions within the country and inadequate protocols and certifications for export / import of ornamental and exotic palms.

A pest free area (PFA) ensuring the absence of RPW, according to ISPM 4 (requirements for the establishment of pest free areas) (FAO, 2021a) should be identified in the exporting country and in the country. This PFA should be submitted to an annual survey by the producers and an annual official inspection by the NPPO to reduce pest challenges. In the exporting countries, an audit of the identified PFA could be undertaken by the importing country.

Long-distance dissemination of many pests/pathogens occurs mostly via infected/infested propagative material. The main element of the quarantine system is a free certified propagative material. Whether offshoots or tissue culture plants, the mother plant is the key in the production chain of palm and should be planted in a pest free area. The selection of the mother plant should be done under the supervision of the official authority, registered, coded, and mapped. A traceability system should be established to facilitate the follow-up and the control of the pathway of the propagative material.

The effective control of RPW requires more coordination within the NENA region. NEPPO is developing in this context:

1. A regional standard for harmonized phytosanitary measures for palms (at borders and inspection on the territory). This standard will also take into consideration other pests of concern such as Bayoud (*Fusarium oxysporum* f. sp. *albedinis*) and date palm phyto plasma disease (Wijam). Regarding the biological characteristics of RPW, we should import only palm propagation material less than 5 cm in diameter from PFA, preferably in test tubes. The importing country should have the required facilities for acclimatization;
2. Regional standard protocol for available and cost-effective treatments of offshoots and ornamentals of date palms (at the border);
3. Pest free area protocol, according to ISPM 4 (FAO, 2021a), to help the extension of date palm area and to provide other areas with safe date palm offshoots. These PFA should be at least far from an infested area by 50 km surrounded by a buffer area. The buffer area should be free from RPW hosts.

Literature cited

FAO. (2013). International Standard for Phytosanitary Measures (ISPM 11): Pest Risk Analysis for Quarantine Pests (adopted in 2001, revised in 2004 and 2013).

FAO. (2017a). Innovative participatory approach to control red palm weevil on date palms in Mauritania. http://www.fao.org/food-chain-crisis/ how-we-work/plant-protection/red-palm-weevil/en/.

FAO. (2017b). Framework Strategy for Eradication of Red Palm Weevil ISPM 5: Glossary of Phytosanitary Terms (updated as needed) in Scientific Consultation and High-Level Meeting on Red Palm Weevil Management (Rome, March 29-31, 2017).

FAO. (2021a). International Standard for Phytosanitary Measures (ISPM) 4: Requirements for the Establishment of Pest Free Areas (International Plant Protection Convention 1995).

FAO. (2021b). International Standard for Phytosanitary Measures (ISPM) 8. Determination of Pest Status in an Area (International Plant Protection Convention) (adopted in 1998, revised in 2021).

FAO. (2021c). International Standard for Phytosanitary Measures (ISPM) 5: Glossary of Phytosanitary Terms (International Plant Protection Convention 2021).

FAOSTAT. (2022). https://www.fao.org/faostat/en/#home (accessed February 8, 2022).

Mousavi, M., Mousavi, A., Habashi, A.A., and Arzani, K. (2009). Optimization of physical and biological parameters for transient expression of *uidA* gene in embryogenic callus of date palm (*Phoenix dactylifera* L.) via particle bombardment. Afr. J. Biotechnol. *8* (*6*), 3721–3730.

Using microwave technology against red palm weevil: an innovative sustainable strategy to contrast a lethal pest of date palms

R. Massa[1,2,a], F. Schettino[1,3], G. Panariello[1,3], M.D. Migliore[1,3], D. Pinchera[1,3], G. Chirico[2], C.J. D'Silva[3], R. Griffo[4] and T. Yaseen[5]

[1]ICEmB-Inter-University research Center on the Study of the Interactions between ElectroMagnetic Fields and Biosystems, Italy; [2]Dep. of Physics "Ettore Pancini", Univ. of Naples Federico II, Via Cintia - CMSA, 80126, Naples, Italy; [3]Dep. of Electrical and Information Engineering "Maurizio Scarano", Univ. of Cassino and Southern Lazio, Cassino, Via G. Di Biasio, 43, 03043 Cassino (FR), Italy; [4]Plant Protection Service of Campania Region, Centro Direzionale, Isola A6, 80143 Naples, Italy; [5]Regional Plant Protection Officer FAO-RNE for the Near East and North Africa Region, Cairo, Egypt.

Abstract

The invasive red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera: Curculionidae*), is currently considered one of the most serious pest insects threatening the sustainability of the date palm sector (*Phoenix dactylifera* L.). Several control methods have been applied; however, concern is raised regarding the treatments that can cause significant environmental pollution. In this context the use of microwaves is particularly attractive, and our team has shown that microwave heating is a promising and eco-compatible solution to fight the spread of weevil in the case of *Phoenix canariensis* palm. The effectiveness of the method strongly relies on a suitable modeling of the process and design of the microwave heating system, which in turn depends on the accurate electromagnetic and thermal characterization of all the materials involved. In our previous studies the feasibility to combat the red palm pest in *Phoenix canariensis* Hort. ex Chabaud palm using microwave heating systems was shown and an electromagnetic-thermal model was developed to better control the temperature profile inside the palm tissues. The aim of this paper is to present our approach and MITHRIL (MIcrowave Technology against Red palm weevil) project: a feasibility study on the use of microwaves to contrast RPW diffusion in a wider scenario by extending the results obtained with *Phoenix canariensis* palm to *Phoenix dactylifera* (date palm). The study will provide information for the development of a microwave heating strategy as an additional tool that, in an IPM approach, could contain or hopefully eliminate RPW that poses a serious threat to date palm cultivation.

Keywords: microwave heating, *Rhynchophorus ferrugineus*, *Phoenix dactylifera*, electromagnetic-thermal model, integrated pest management

INTRODUCTION

Date palm, *Phoenix dactylifera* L. (*Arecaceae*, or *Palmae*) is an important perennial and dioecious species cultivated mostly in arid areas of the world: the major proportion of world's total date palm production is in the Middle East and North Africa, but date palms have also been introduced in Australia, India, Mexico, Southern Africa, South America, Pakistan and the USA during the last decades (Wakil et al., 2015). In particular, the Gulf region of the Middle East accounts for about 30% of the world's date production, with Saudi Arabia and the United Arab Emirates being the region's top producers (FAOSTAT, 2012). The crop has a high socioeconomic importance not only due to its food value, but also its capacity to provide many other products such as shelter, fiber, clothing, aesthetic beauty, and furniture (Al-Shawaf et al., 2013).

The earliest report on the insect pests of date palm dates back to 1920, when Buxton

[a]E-mail: rmassa@na.infn.it

documented the insect pests of date palm in Mesopotamia (nowadays Iraq). Since then, the list has increased (Carpenter and Elmer, 1978; Blumberg, 2008), up to the recent review by El-Shafie and Faleiro (2020), who listed 112 species of mites and insects worldwide associated with date palms, including 22 species attacking stored dates. Among the arthropod pests listed in this report, only ten are classified as major, and *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera: Curculionidae*), or red palm weevil (RPM) is currently considered one of the most serious. The Food and Agriculture Organization of the UN has identified RPW as a category-1 pest of date palm in the Middle East.

As a matter of fact, RPW appears to be a very difficult insect to manage by means of traditional approaches (El-Sufty et al., 2007; Arab and El-Deeb, 2012). It damages more than 29 different palm species in the world, but particularly date palms, coconut palms and oil palms in Africa, South-East Asia and the Middle East (Wakil et al., 2015), causing huge economic losses (Güerri-Agulló et al., 2010). Its infestation was recorded in 50% of date producing countries (Qayyum et al., 2020), causing yield losses up to 0.7-10 t ha^{-1} (Singh and Rethinam, 2005).

Studies have shown that date palm cultivars with high sugar content enhance the RPW growth and development, while cultivars with high calcium content with hard tissue inhibits its growth and development (Al-Dosary et al., 2016). The RPW undergoes complete metamorphosis, progressing through a four-stage life cycle: egg, larva, pupa and adult. The entire life cycle takes about 3-4 months to be completed.

Adult females live for several weeks during which they can lay as many as 300 eggs in cracks and crevices on soft palm tissue. In coconut and date palm, ovi position usually occurs in young palms below 20 years old. The eggs hatch in about 6 days and the neonate larvae start feeding and move toward the interior of the palm, usually through wounds, caused by pruning green growing fronds, feeding of other insects, and other types of physical damage. In fact, larva is the most destructive stage of the RPW, causing serious damage to the palm tree by tunneling the tissue of the trunk, eventually killing it. Moreover, the larvae are inaccessible, hard to detect and difficult to treat with chemical pesticides. They are characterized by dark prominent heads, and pass through three to seven in stars, growing into mature grubs that migrate to the periphery of the trunk where they build cocoons, entering the pupal stage. Finally, adults emerge from the cocoons a few weeks later.

The weevil often completes multiple generations in the same tree host before the tree collapses and the adult weevils abandon it to seek uninfested palm trees. Because of the concealed nature of the larval and pupal stages, infestation of the date palm stem is often hard to detect until the palm tree is dying. Early clues are provided by the drying of the youngest fronds due to larvae feeding, which damages the meristem, resulting in larval excrement accumulation at the base of fronds or at a point of injury.

RPW can only be effectively fought by resorting to an integrated pest management (IPM) strategy, defined as an ecosystem approach to crop production and protection, which combines different management strategies and practices to grow healthy crops and to minimize the use of pesticides (FAOSTAT, 2012). A successful IPM program requires proper identification of the pest and knowledge of its biology, ecology, sampling and monitoring of its population for developing appropriate actions and identifying thresholds (El Bouhssini and Faleiro, 2018). IPM approaches combine elements of plant resistance, chemical, semiochemical, biological and microbial control. In this context, an assessment of the pest complex and associated biological control agents is essential.

IPM of *R. ferrugineus* in date palm comprises several strategies, but resorts mainly on early detection of infested palms, pheromone trapping, preventive and/or curative chemical treatments. The other components of the IPM strategy for *R. ferrugineus* are crop and field sanitation, implementing strict quarantine protocols, identifying and eliminating hidden breeding sites, including neglected and closed gardens, periodic validation of the control strategy based on weevil captures in traps and infestation reports, training of farmers and other stakeholders on the latest best IPM practices (Faleiro et al., 2019). However, there are major concerns regarding the environmental pollution caused by chemical treatments, especially in public areas where ornamental palms are grown. Indiscriminate use of chemical compounds is heavily contributing toward environmental toxicity and posing threat to the ecosystem.

In this respect, microwave heating is proposed as an eco-friendly green protocol (Massa et al., 2017). The basic idea of the microwave approach is very simple: increasing the temperature of the palm tissues and/or insect until it dies, or it is strongly affected being the ability of reproduction of the survived weevils influenced. Advantages of microwave disinfestation include speed, efficiency and the absence of toxic, hazardous or polluting residues. Moreover, insects are not likely to develop a resistance to radiation as they often do to chemical insecticides. Finally, the technique was recognized to be harmless for the environment, the workers, and the plants (Suffert et al., 2018).

Microwave heating has vast applications in the field of food processing such as cooking, drying, pasteurization and preservation of food materials (Chandrasekaran et al., 2013). Microwave disinfestation of grain and pulse is considered a safe and competitive alternative method to fumigation as it avoids environmental pollution (Yadav et al., 2014).

Microwave wood treatment has been considered as an alternative treatment, more environmentally friendly, in the implemented ISPM15 Regulation of Wood Packing Material in International Trade of the FAO. It is the standard describing phyto-sanitary measures that reduce the risk of introduction and spread of quarantine pests associated with the movement in international trade of wood packaging material made from raw wood (International Standards for Phytosanitary Measures, 2019).

The aim of this paper is to show the impact of the correct characterization of the materials involved: an adequate awareness of this impact will allow to extend the results obtained with *Phoenix canariensis* palm to *Phoenix dactylifera* (date palm), which is the aim of MITHRIL (MIcrowave Technology against Red palm weevil) project. A correct estimation of the thermal and electromagnetic parameters is necessary to set up an electromagnetic-thermal model that will allow to identify the power, time and schedule necessary to obtain the RPW lethal temperature at a certain depth, depending on the assumed date palm infestation level (Massa et al., 2019).

BACKGROUND

Parameters relevant to dielectric microwave heating

1. Physical parameters.

The idea of using microwaves against RPW is based on the fact that microwave radiation should be able to induce a lethal thermal dose in the pest, or impair their re-productivity or longevity, without harming the plant tissues.

Microwaves are electromagnetic waves oscillating in the frequency range between 300 MHz (0.3 GHz) and 300 GHz, or equivalently, with wavelengths ranging from as long as 1 m to as short as 1 mm. They are non-ionizing radiation and lie between radio frequency and infrared radiation. In the air they travel with the speed of light and reflection, transmission and absorption can occur when they interact with a medium. For instance, they can be reflected by metals, transmitted through non polar materials (glass, most plastics, ceramics), and absorbed by polar materials like water inducing an increase of temperature.

The microwave thermal effect (microwave dielectric heating) is largely employed for the heating and processing of food, in synthetic chemistry, in production of industrial materials as well as in biomedical applications. As a matter of fact, microwave heating is based on the transformation of alternating electromagnetic field energy into thermal energy by affecting polar molecules of a material. All the matter is made up of atoms and molecules that are electrically neutral, in polar medium the electric charge distribution is not symmetric. In presence of the electromagnetic field, these molecules can move, oscillate and/or rotate and tend to align themselves with the field (polarization). In the Debye interpretation (Debye, 1929), the heat is generated by frictional forces occurring between a polar molecule, whose rotational velocity has been increased by coupling with the microwave radiation, and neighboring molecules. As result, while in conventional heating, depending on the medium thermal conductivity, the thermal increase, induced by an external source, is related to the heat flux into the material from the surface, in the case of microwaves the heating source is inside the material, and an increase of temperature occurs in the area where the radiation is able to propagate (volumetric heating), but the heat flux would successively spread in the rest of the material by means of thermal conduction. The efficiency of conversion of microwave energy into thermal energy depends upon both the dielectric and thermal properties of the material (thermal conductivity, specific heat capacity, and material density).

Dielectric properties are the electrical characteristics of a material. They depend on the frequency of the applied electric field, temperature of the material and the number of polar molecules in the volume of the material. Two parameters are commonly used for describing the electromagnetic properties of the materials of interest: the relative permittivity, and the equivalent electric conductivity (s). The first is related to the ability of materials to 'store' electromagnetic energy and it is reported as a value relative to that of free space (e0 = 8.86×10^{-12} F/m), the latter measures the capacity of a material to dissipate the electromagnetic energy, due to all operating dielectric relaxation mechanisms and ionic conduction, transforming it into heat. Both depend on frequency, moisture content and temperature. Review (Gabriel et al., 1996; Kuang and Nelson, 1998) provide more detail on the basic theory of these processes.

To summarize, an electromagnetic wave impinging on a material will be partially reflected, but part of the radiation will penetrate the material and will transfer a percentage of its energy, that will be dissipated and converted into heat according to the electrical conductivity (s) and electric field intensity. The dissipation process subtracts power from the electromagnetic wave, that will attenuate more rapidly with greater s, and the penetration depth indicate the distance over which the 63.2% of the traveling wave energy (or power) is deposited in the material. The relative rate of increase of temperature (DT/Dt [°C/s]) is strictly related to the electric field strength, and the electric conductivity, specific heat and mass density of the medium.

2. Physical environment and biological parameters.

Other parameters to be considered are the ambient temperature and that of the palm. In the case of *Phoenix canariensis* palm the equivalent conductivity and heat capacity able to simulate the heating and the cooling process (including the effect of several processes such as change of conductivity due to some slow water diffusion in the palm tissue) were estimated in Massa et al. (2017). The data of a low thermal diffusion confirm that the palm tree protects itself from the extremes (Faci and Benziouche, 2021). *Phoenix canariensis* palm is used largely as ornamental in the Mediterranean area, where a mean annual temperature of above 19°C is expected, however a difference of 2 to 6°C between the outer atmospheric temperature and that inside RPW infested palms (in winter and summer) can occur, as the result of the fermentation process occurring when relatively high populations of larvae coincide within one single palm (Dembilio, and Jacas, 2011).

In the case of date palm, this cultivar can adapt to a temperature range from zero to 50.4°C. Temperatures allowing vegetative activity of the date palm, overall, are between 10 and 40°C. They are 18°C for flowering and 25°C for maturity. The internal temperature of the apical bud varies from 4 to 5°C during the day. It can be 11 to 14°C higher than the lowest external temperatures and 17 to 18°C lower than the highest temperatures, with the safeguard of internal tissues from high thermal excursions.

Concerning the insect, time for 100% kill (LT_{100}) of larvae and adults at different temperatures was estimated in (Massa et al., 2011). The adult insects were much more sensitive to heat than the larger larvae. LT_{100} resulted 20 min at 50°C and only 4 min at 80°C, while 30 min at 50°C were necessary for larvae weighting 5-6 g. These data are consistent with that reported in Peng et al. (2016). They observed that, in general, the longevity of adult RPW decreased with increasing temperature: both female and male longevity declined at 36°C, in addition this temperature suppresses mating frequency and sperm transfer, as well as fecundity.

3. Palm dimensions.

P. canariensis is always described as having a thick trunk or stipe (50-120 cm in diameter) of columnar aspect and of uniform thickness, and a height of 12-15 m, although centenary palms can reach more than 30 m (Sosa et al., 2021) (Figure 1).

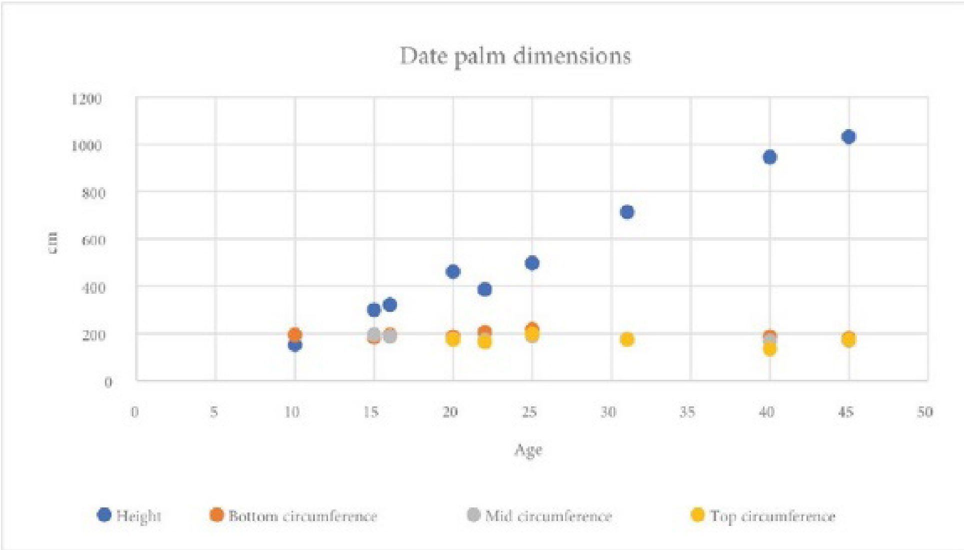


Figure 1. Date palm dimensions for different ages. Data from Jahromi et al. (2007).

P. dactilifera date palm tree commonly grows to a height of about 10 to 15 m and features a slender trunk of more or less constant diameter (about 60 cm) from the base to the crow. These results can be useful for determining the size, reach and general requirements of a special date palm services machine (Jahromi et al., 2007).

MATERIALS AND METHODS

Electromagnetic characterization of the materials involved in the microwave heating process

Measurements of dielectric constant and conductivity of the *P. canariensis* palm tissues and of RPW were carried out with the method of the open-ended coaxial line. This is one of the simplest and the most reliable technique employed for measuring electromagnetic properties of liquid, solid and gel like media as well as biological systems (tissue, cells) within wide radio frequency/microwave frequency band, at different temperatures and in a non-invasive way. It consists in the measurement of the reflection coefficient, strictly related to the permittivity, at the probe-sample surface when the cable is immersed into the liquid or in direct contact with the medium. Measurements were carried out in the 500 MHz-20 GHz band (Massa et al., 2014). The moisture content of the palm tissues was considered being a parameter that can depend on the season of the treatment and/or the phase of the treatment. The moisture content is evaluated as $MC = (M_{water}/M_{sample}) \times 100$, where M_{water} is the mass of water in the sample and M_{sample} is the mass of the oven-dried sample. Being the sample non-homogenous, measurements were repeated with the probe located in different positions either on the healthy/damaged slice of palm or on the insect (larva, adult, puparia chamber) (Figure 2) and the average value was calculated.

Figure 2. Experimental set up. Left: coaxial cable connected to the Vector Network Analyzer; center: measurement of a wood slice; right: measurement of the adult insect.

Numerical simulations

In order to assess the power distribution in the palm, and consequently the temperature distribution, numerical simulations have been performed. Both electromagnetic and thermal simulations have been performed by means of CST Microwave Studio, which implements the finite integration technique (FIT). The computation volume is discretized with a tetrahedral mesh, where Maxwell equations are numerical solved. Mesh dimensions have been chosen equal to one twentieth of the wavelength, which is appropriate for an accurate description of electromagnetic phenomena.

The radiating system was designed as a flanged rectangular waveguide (WR340) placed at 75 mm from the trunk of the palm, modeled as a dielectric cylinder. Two different models have been considered (Figure 3), depending on the health conditions of the palm: healthy palm is supposed to be a homogeneous dielectric cylinder of radius 24 cm with electromagnetic characteristics depending on the moisture content, whereas damaged palm has been modeled as two concentric cylinders, the inner of radius 21.5 cm representing the healthy palm and the outer (2.5 cm thick) the damaged palm. The electromagnetic parameters are summarized in Table 1 (left column). In any case the stimulated power was 500 W, whereas dissipated power was calculated by means of CST features.

Table 1. Overview of analyzed cases and main results.

Case	Power (W) dissipated into the palm	Time (s) to reach the lethal temperature at a depth of 2 cm	Maximum depth (mm) at which lethal temperature is reached after 300 s
Healthy palm only ε healthy_palm = 31.5-11.5j	162.55	284	20.8
Healthy palm only ε healthy_palm = 18.28-7.89	190.61	240	23.6
Damaged-healthy palm ε damaged_palm = 53.13-9.55j ε healthy_palm = 31.5-11.5j	136.13	327	18.2
Damaged-healthy palm ε damaged_palm = 48.57-9.11j ε healthy_palm = 31.5-11.5j	142.37	312	19.1
Damaged-healthy palm ε damaged_palm = 53.13-9.55j ε healthy_palm = 18.28-7.89	132.99	335	17.5
Damaged-healthy palm ε damaged_palm = 48.57-9.11j ε healthy_palm = 18.28-7.89	139.95	318	18.7

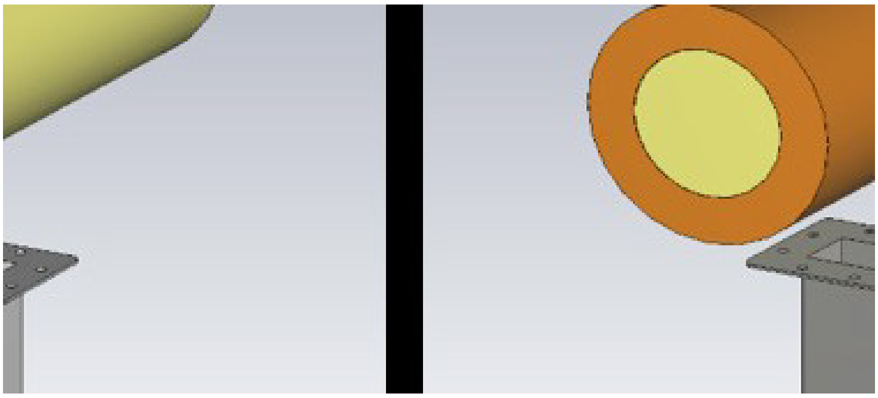


Figure 3. Numerical model. Left: healthy palm; right: damaged palm.

Thermal simulations have been realized by means of the transient temperature solver of CST: the output power loss of electromagnetic simulations represents the heat source of thermal simulation. The thermal parameters of the palm tissues have been taken as in Massa et al. (2017).

RESULTS AND DISCUSSION

Measurements on healthy and rotten *Phoenix canariensis* palm tissues and on the palm weevil in different stages of its life indicate a high water content in the samples and, consequently, a penetration depth of the order of few centimeters. In damaged tissues the volume fraction of free water resulted higher than that of the bulk vegetation-bound water mixture. At 2.45 GHz the penetration depth was about 2.5 cm for the healthy palm and about 1 cm in the damaged palm tissue. This result is due to the higher equivalent conductivity that resulted lower in the healthy tissues compared to that in the damaged tissues. This factor entails the higher penetration depth in infested portion and the possibility to protect healthy area being the layer involved a couple of centimeters. Concerning the insect, the penetration depth was higher in the adult (few centimeters) with respect to the pupa chamber and the larva again due to the different water content in the three different life stage. These results were confirmed in laboratory tests. Larvae at 2.45 GHz absorb more microwave power than adults. Exposures for different times up to 20 min at 0.06 W cm^{-2} impaired larvae and not adults (data not shown), while after few seconds (5 to 30 s) at 2 W cm^{-2} a sort of ablation occurred for larvae and re-productivity ability, of both male and female adults, was reduced (Massa et al., 2019).

In Figure 4 the depth at which the lethal temperature (55°C) is reached is plotted as a function of time for the different cases of Table 1. Almost 6 min are needed to rise the temperature of the palm within a depth of 2 cm. From Figure 4 it is apparent the lethal temperature in the outer region is first reached in healthy palms. This is due to the higher real permittivity of damaged tissues, resulting in a higher reflection of the electromagnetic waves impinging on the trunk. This is consistent with the power dissipated into the palm, which is much higher for healthy tissues (Table 1, second column). Roughly speaking, power transfer into the palms is more efficient for healthy tissues, whereas in damaged palms more power is reflected. However, the layers involved in the heating processes are substantially where RPW (eggs adults puparia chambers) live with the safeguard of internal healthy tissues.

Finally, in Figure 5 a typical example of the temperature distribution inside the palm trunk is shown.

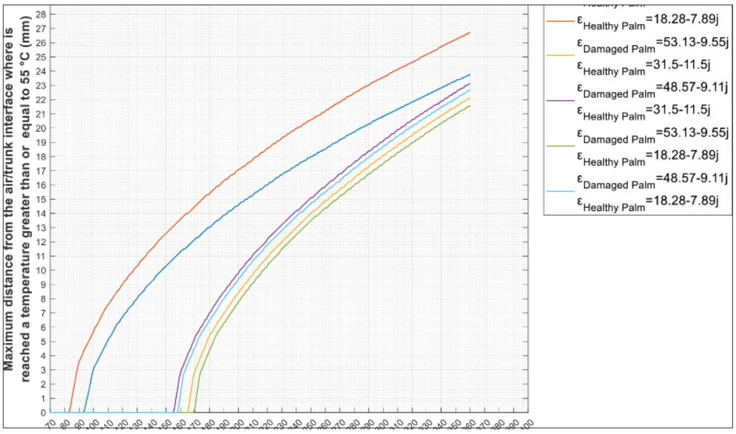


Figure 4. Depth of the lethal temperature as a function of time for the different cases listed in Table 1.

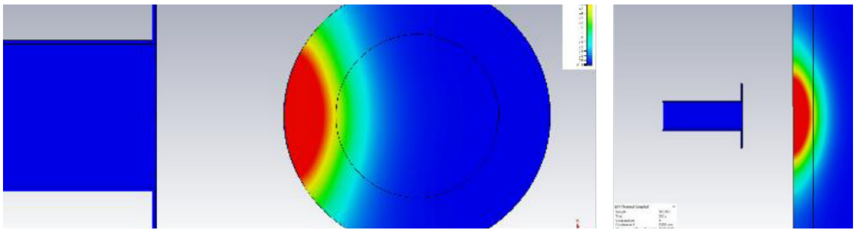


Figure 5. An example of temperature distribution inside the palm. Left: top view; right: lateral view.

CONCLUSIONS

In this paper the dependence of the heating process on the parameters of palm tissues has been shown. As a general result, the lethal temperature can be reached after an adequate time in all the damaged region of the trunk, nevertheless in order to develop a suitable disinfestation protocol especially effective for date palms an accurate estimation of thermal and electromagnetic parameters is needed. This will be achieved during MITHRIL project, since little or no information is present in the literature about thermal and electromagnetic properties of date palms, which have a quite different water content and fibers composition with respect to *P. canariensis* palm.

ACKNOWLEDGEMENTS

We would like to thank the Food and Agriculture Organization of the United Nations who supported this work in the framework of "Evaluation of selected control technologies for sustainable management of Red Palm Weevil" under the FAO's Programme on RPW Eradication.

Literature cited

Al-Dosary, N.M.N., Al-Dobai, S., and Faleiro, J.R. (2016). Review on the management of red palm weevil *Rhynchophorus ferrugineus* Olivier in date palm *Phoenix dactylifera* L. Emir. J. Food Agric. *28* (*1*), 1.

Al-Shawaf, A.M., Al-Shagag, A., Al-Bagshi, M., Al-Saroj, S., Al-Bather, S., Al-Dandan, A.M., Abdallah, A.B., and Faleiro, J.R. (2013). A quarantine protocol against red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleptera*: *Curculiodae*) in date palm. J. Plant Prot. Res. *53* (*4*), 409–415 https://doi.org/10.2478/jppr-2013-0061.

Arab, Y.A., and El-Deeb, H.M. (2012). The use of endophyte *Beauveria bassiana* for bio-protection of date palm seedlings against red palm weevil and Rhizoctonia root-rot disease. Scientific Journal of King Faisal University *13* (*2*), 1433.

Blumberg, D. (2008). Review: date palm arthropod pests and their management in Israel. Phytoparasitica *36* (*5*), 411–448 https://doi.org/10.1007/BF03020290.

Carpenter, J.B., and Elmer, H.S. (1978). Date palm production and pest management challenges. In Pests and Diseases of the Date Palm (United States Department of Agriculture).

Chandrasekaran, S., Ramanathan, S., and Basak, T. (2013). Microwave food processing - a review. Food Res. Int. *52* (*1*), 243–261 https://doi.org/10.1016/j.foodres.2013.02.033.

Debye, P.J.W. (1929). Polar Molecules (Dover Publications).

Dembilio, O., and Jacas, J.A. (2011). Basic bio-ecological parameters of the invasive red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*), in *Phoenix canariensis* under Mediterranean climate. Bull Entomol Res *101* (*2*), 153–163 https://doi.org/10.1017/S0007485310000283. PubMed

El Bouhssini, M., and Faleiro, J.R., eds. (2018). Date Palm Pests and Diseases- Integrated Management Guide (Beirut, Lebanon: International Center for Agriculture Research in the Dry Areas).

El-Shafie, H.A.F., and Faleiro, J.R. (2020). Red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*): global invasion, current management options, challenges and future prospects. In Invasive Species - Introduction Pathways, Economic Impact, and Possible Management Options, H. El-Shafie, ed. (London, United Kingdom: IntechOpen), p.3–31.

El-Sufty, R., Al-Awash, S.A., Al Amiri, A.M., Shahdad, A.S., Al Bathra, A.H., and Musa, S.A. (2007). Biological control of red palm weevil, *Rhynchophorus Ferrugineus* (*Col*.: *Curculionidae*) by the entomopathogenic fungus *Beauveria bassiana* in United Arab Emirates. Acta Hortic. *736*, 399–404 https://doi.org/10.17660/ActaHortic.2007.736.36.

Faci, M., and Benziouche, S.E. (2021). Contribution to monitoring the influence of the air temperature on some phenological stages of the date palm (cultivar 'Deglet Nour') in Biskra. J. Saudi Soc. Agric. Sci. *20* (*4*), 248–256 https://doi.org/10.1016/j.jssas.2021.02.004.

Faleiro, J.R., Ferry, M., Yaseen, T., and Al-Dobai, S. (2019). Overview of the gaps, challenges and prospects of red palm weevil management. Arab J. Pl. Prot. *37* (*2*), 170–177.

FAOSTAT. (2012). Food and Agricultural Commodities Production. https://www.fao.org/faostat/en/#home.

Gabriel, C., Gabriel, S., and Corthout, E. (1996). The dielectric properties of biological tissues: I. Literature survey. Phys Med Biol *41* (*11*), 2231–2249 https://doi.org/10.1088/0031-9155/41/11/001. PubMed

Güerri-Agulló, B., Gómez-Vidal, S., Asensio, L., Barranco, P., and Lopez-Llorca, L.V. (2010). Infection of the red palm weevil (*Rhynchophorus ferrugineus*) by the entomopathogenic fungus *Beauveria bassiana*: a SEM study. Microsc Res Tech *73* (*7*), 714–725. PubMed

International Standards for Phytosanitary Measures. (2019). Regulation of Wood Packaging Material in International Trade ISPM 15 (International Plant Protection Convention, FAO), Adopted 2018; published 2019.

Jahromi, M.K., Jafari, A., Rafiee, S., and Mohtasebi, S.S. (2007). A survey on some physical properties of the date palm tree. Agric. Technol. Thail., p.317–322.

Kuang, W., and Nelson, S.O. (1998). Low-frequency dielectric properties of biological tissues: a review with some new insights. Trans. ASAE *41* (*1*), 173–184 https://doi.org/10.13031/2013.17142.

Massa, R., Caprio, E., De Santis, M., Griffo, R., Migliore, M.D., Panariello, G., Pinchera, D., and Spigno, P. (2011). Microwave treatment for pest control: the case of *Rhynchophorus ferrugineus* in *Phoenix canariensis.* Bull. OEPP *41* (*2*), 128–135 https://doi.org/10.1111/j.1365-2338.2011.02447.x.

Massa, R., Migliore, M.D., Panariello, G., Pinchera, D., Schettino, F., Caprio, E., and Griffo, R. (2014). Wide band permittivity measurements of palm (*Phoenix canariensis*) and *Rhynchophorus ferrugineus* (*Coleoptera Curculionidae*) for RF pest control. J. Microw. Power Electromagn. Energy *48* (*3*), 158–169 https://doi.org/10.1080/08327823.2014.11689880.

Massa, R., Panariello, G., Pinchera, D., Schettino, F., Caprio, E., Griffo, R., and Migliore, M.D. (2017). Experimental and numerical evaluations on palm microwave heating for Red Palm Weevil pest control. Sci Rep *7* (*1*), 45299 https://doi.org/10.1038/srep45299. PubMed

Massa, R., Panariello, G., Migliore, M.D., Pinchera, D., Schettino, F., Griffo, R., Martano, M., Power, K., Maiolino, P., and Caprio, E. (2019). Microwave heating: a promising and eco-compatible solution to fight the spread of red palm weevil. Arab Journal Plant Protection *37* (*2*), 143–148 https://doi.org/10.22268/AJPP-037.2.143148.

Peng, L., Miao, Y., and Hou, Y. (2016). Demographic comparison and population projection of *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) reared on sugarcane at different temperatures. Sci Rep *6* (*1*), 31659 https://doi.org/10.1038/srep31659. PubMed

Qayyum, M.A., Saleem, M.A., Saeed, S., Wakil, W., Ishtiaq, M., Ashraf, W., Ahmed, N., Ali, M., Ikram, R.M., Yasin, M., et al. (2020). Integration of entomopathogenic fungi and eco-friendly insecticides for management of red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Saudi J Biol Sci *27* (*7*), 1811–1817 https://doi.org/10.1016/

j.sjbs.2019.12.018. PubMed

Singh, S.P., and Rethinam, P. (2005). Trapping- a major tactic of BIPM strategy of palm weevils. Coconut Research and Development Journal *21* (*01*), 34 https://doi.org/10.37833/cord.v21i01.401.

Sosa, P.A., Saro, I., Johnson, D., Obon, C., Alcaraz, F., and Rivera, D. (2021). Biodiversity and conservation of *Phoenix canariensis*: a review. Biodivers. Conserv. *30* (*2*), 275–293 https://doi.org/10.1007/s10531-020-02096-1.

Suffert F., Escobar Gutiérrez A., Ollivier L., Rochat D., Silvie P., et al. (2018) Stratégies de Lutte contre le Charançon Rouge du Palmier (Anses: Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail).

Wakil, W., Faleiro, J.R., and Miller, T.A., eds. (2015). Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges (Switzerland: Springer International Publishing).

Yadav, D.N., Anand, T., Sharma, M., and Gupta, R.K. (2014). Microwave technology for disinfestation of cereals and pulses: an overview. J Food Sci Technol *51* (*12*), 3568–3576 https://doi.org/10.1007/s13197-012-0912-8. PubMed

Red palm weevil management strategy, the way forward

J.R. Faleiro[1,a], T. Yaseen[2] and M.A. Bob[2]

[1]Independent Consultant-Red Palm Weevil, Goa, India; [2]Food and Agriculture Organization of the United Nations - Regional office in Near-East and North-Africa, Cairo, Egypt.

Abstract

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier, is a key pest of date palm in the NENA Region. The current RPW-IPM strategy revolves around the core IPM components of manually inspecting palms to detect infestation, use of food baited pheromone traps in monitoring and mass trapping programmes, preventive and curative chemical treatments, removal and disposal of severely infested palms, implementing pre and post entry phytosanitary measures, etc. The ongoing FAO Programme on RPW eradication in the NENA region provides a global platform to plan, execute and evaluate national RPW control programmes by offering research and technical support to on-going RPW control programmes in the region including: i) testing of advanced cost effective and user-friendly early detection systems; ii) encouraging farmer participation in area-wide control of the pest by carrying out detailed socio-economic studies. The FAO RPW eradication program targets the implementation of farmer field schools for enhancing farmers engagement in RPW-IPM program and to lay the basis for technology transfer and adoption of improved technologies; iii) standardize phytosanitary measures at borders and recommend phytosanitary treatment protocols for offshoots and palms besides establishing harmonized certification program for the production, conservation and use of certified (true-to-type and healthy) propagative material of date palm; iv) address gaps in promoting RPW control tactics including the development of fumigation technique for use in curative and quarantine treatment of date palm offshoots, evaluate semiochemical mediated technologies (dry and smart traps, attract and kill, etc.), standardize guidelines for RPW centric GAP, evaluate protocols for chemical control, microwave technology and eradication of severely infested palms; v) field evaluation of remote sensing technology to locate palms and detect RPW infestations; vi) provide overview of the field situation in real time using GIS based Susa Hamra app on automatic information on palm location, trap captures and evolution of palm infestation, for monitoring and evaluation to measure the efficacy of the regional and national control strategy; and vii) build capacities of all stakeholders for the efficient control of RPW in the region. Based on the above, this paper gives an overview on the RPW management strategy and the way forward.

Keywords: *Rhynchophorus ferrugineus*, IPM, early detection, trapping, quarantine, GIS

INTRODUCTION

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (*Coleoptera: Curculionidae*) is a key pest of palms (*Arecaceae*) in diverse agro-ecosystems the world over. In the Near-East and North-Africa (NENA region) RPW is a threat to the livelihood security of date palm farmers in rural communities (FAO-CIHEAM, 2017). The pest has its home in South and Southeast Asia where it is a key pest of coconut, *Cocos nucifera*. During the mid-1980s RPW was reported on date palm (*Phoenix dactylifera* L.) from the United Arab Emirates in the Gulf region of the Middle East. Subsequently, first reports of RPW invasion came from other Gulf countries of the Middle East. During 1993 RPW attack was reported on date palm in Egypt in North Africa

[a]E-mail: jrfaleiro@yahoo.co.in

and later during 1995 it was detected for the first time on *P. canariensis* from Spain in Europe. Currently RPW is being reported from nearly 50 countries with a host range of 40 palm species (EPPO, 2020). Since the mid-1980s RPW has spread rapidly in the Gulf region of the Middle East, some Maghreb countries in North Africa and the Mediterranean basin countries in Europe. Ecological niche modeling (Fiaboe et al., 2012) suggests that the pest is likely to expand its geographical range still further.

Considering the vast host range of this pest, it is essential to ensure strict pre- and post-shipment phytosanitary measures are in place. FAO recently has published detailed guidelines on phytosanitary regulations to be adopted to ensure movement of RPW free planting material within national borders and also from one country to another (Chouibani, 2020). Import and movement of infested plant material within a country are the main pathways to the introduction and spread of *R. ferrugineus*. The concealed nature of the pest and infestation makes detection of infested palms extremely difficult. There are few estimates on the losses incurred due to infestation by RPW. Direct losses due to RPW attack can be attributed to the value of the destroyed palms, the loss in yield and cost of implementing the control program.

Infestation begins when gravid female weevils lay eggs into palm tissue that hatch into damage inflicting larvae which bore and tunnel the palm. Abraham et al. (1998) reported the following damage symptoms in date palm as: i) oozing of brownish fluid together with frass (palm tissue excreted by feeding grubs) which has a typical fermented odour; ii) drying of infested offshoots; iii) tunneling of palm tissue by grubs; iv) presence of adults and pupae at the base of fronds; v) pupae on the ground around an infested palm; vi) drying of outer leaves and fruit bunches; and vii) toppling of the trunk in case of very severe and extensive tissue damage.

The current RPW-IPM strategy revolves around the core IPM components of manually inspecting palms to detect infestation, use of food baited pheromone traps in monitoring and mass trapping programmes, preventive and curative chemical treatments, removal and disposal of severely infested palms, implementing pre and post entry phytosanitary measures.

CURRENT RPW-IPM STRATEGY

The pest is managed by employing an integrated pest management (IPM) strategy comprising of several tactics that mainly revolve around: i) regular inspection of palms to detect infestations; ii) capturing adult weevils using food baited pheromone traps; iii) preventive and curative chemical treatments; and iv) removal/eradication of severely infested palms. These RPW-IPM components are complemented by phytosanitary (quarantine) measures to regulate the movement of planting material, capacity building and extension activities. Additionally, removal of hidden breeding sites particularly in closed gardens, adopting good agronomic practices related to field sanitation, palm density, irrigation practices, frond and offshoot removal, etc., and deploying effective biological control agents (fungi and nematodes) that can reach the pest and also that are sustained in the field have been recommended.

Periodic validation of the strategy based on trap capture data and infestation reports is vital for the judicious use of men and material particularly in an area wide RPW-IPM program.

The strategy supported with adequate resources, with systematic planning, good coordination and involvement of all stakeholders can lead to the eradication of RPW. The failure to manage RPW in most of the countries can be attributed to the lack of awareness and systematic and coordinated control actions or management strategies that involve all stakeholders, which is related to inadequate human and financial resources available to combat the pest (Faleiro, 2020).

FAO PROGRAMME ON RPW ERADICATION IN THE NENA REGION

Despite some success stories in a few countries, RPW control programmes currently being implemented have by and large not been successful in containing the spread or controlling the pest. The failures in the control programmes can be particularly attributed to difficulties in detecting infested palms early in the stage of attack, challenges and constraints facing application of quarantine measures and lack of awareness and commitment of farmers and other stakeholders in the control programmes.

To overcome the current lacunae in the RPW control program a trust fund project was developed by FAO for governments and partners to commit resources for the implementation of global, regional and national initiatives supporting the implementation of the 'FAO Programme on Red Palm Weevil Eradication in the NENA region'. This project facilitates the cooperation and coordination of efforts at regional, inter-regional and global levels for supporting the integrated and sustainable management of programmes to control RPW and reduce its devastating effects on the environment and food security, and socio-economic impact on rural communities. The project will align activities along three major thematic areas:

1. Research: develop ecologically sound integrated management methods such as, the use of innovative technologies for improved detection, surveillance and management of RPW, field-testing of quick, user friendly and cost-effective technologies, and the establishment of good cooperation between research institutes and technology developers;
2. Capacity development: here efforts concentrate on involving farmers and relevant stakeholders in the management of RPW based on a thorough understanding of the various agricultural realities and socio-economic situations in each country;
3. Transfer of knowledge and technology: the experience and expertise that currently exists in affected countries will be called upon to facilitate the work and improve the effectiveness of the program. It will be crucial to ensure the involvement of all relevant stakeholders, especially farmers and young professionals. Positive experiences learnt on the control of RPW in the region need to be transferred and executed in other member countries.

The expected overall result of the FAO's program for Red Palm Weevil Eradication is to contain the outbreak of RPW in the Near-East and in North-Africa, by coordinating the effort to help farmers manage the RPW in order to limit crop damage to a minimum and to avoid a further spread of the pest.

RPW-IPM: THE WAY AHEAD

The ongoing FAO Programme on RPW eradication in the NENA region provides a global platform to plan, execute and evaluate national RPW control programmes by offering research and technical support to on-going RPW control programmes in the region that will strengthen the RPW-IPM components including:

1. Testing of advanced cost effective and user-friendly early detection systems: currently visual detection of infested palms is the only reliable method adopted, which is cumbersome and costly. However, systematic periodic inspection of palms every 45-60 days, can effectively allow to reduce the pest population below the economic threshold and even to eradicate the pest. One of the main objectives of the proposed research program is to scientifically assess, in the field, the efficiency and practical feasibility of the technologies proposed for use at field level besides using geo-tagging or by remote sensing to facilitate the permanent registration and monitoring of the infested palms. Research is underway in several countries to develop cost effective and user-friendly early detection systems. Detection devices based on acoustic, chemical signatures and spectral imaging detection are some of the promising techniques that will be tested;
2. Encourage farmer participation in area-wide control of the pest by carrying out detailed socio-economic studies: in several countries area wide RPW control programmes are state subsidized with little or no participation by the farmers in the control program which in the long run are not sustainable and also do not control the pest to the desired level. The project aims to have a better understanding of the socio-economic consequences of the RPW problem in the farming systems in the affected countries and develop an intervention plan based on comprehensive socio-economic assessment and quantification;
3. Phytosanitary measures and certified offshoot program: the project envisages to

standardize phytosanitary measures at borders to develop a date palm RPW free area protocol and recommend phytosanitary treatment protocols for offshoots and palms besides establishing harmonized certification program for the production, conservation and use of certified (true-to-type and healthy) propagation material of date palm;

4. Address gaps in promoting RPW control tactics: currently there are several deficiencies in the RPW control tactics. The project aims to evaluate fumigation technique for use in curative and quarantine treatment of date palm offshoots, evaluate semiochemical mediated technologies (dry and smart traps, attract and kill, etc.), standardize guidelines for RPW centric GAP, evaluate protocols for chemical control, microwave technology and eradication of severely infested palms;
5. Field evaluation of remote sensing technology to locate palms and detect RPW infestations: the project will evaluate the recent advances in remote sensing technology in identifying tree level information to help reduce on-farm data collection for early detection of suspected and infested trees. Partners will evaluate on scientific basis the efficiency and feasibility for application inside area wide RPW control programmes of the use of remote sensing for the detection of RPW infested palms;
6. Susa Hamra app on automatic information on palm location, trap captures and evolution of palm infestation: GIS based digital monitoring system can facilitate data collection from the field, map and analyze this data in almost real time to support the decision-making process and improve the national RPW management strategies by providing a clear overview on the situation on field. FAO's Susa Hamra app has been developed and will be tested through the project in the region. Furthermore, the Susa Hamra app envisages developing spatial models to map, predict and forecast potential RPW risks based on updated automatic information on palm location and evolution of palm infestation (coming from Susa Hamra data points, and trap data);
7. Build capacities of all stakeholders for the efficient control of RPW in the region: capacity building for stakeholders and farmers and improved access for sustainable management practices for RPW is an important component of the project and will support countries to promote the participatory training approach for municipalities and farmers to enable them to diagnose the pest and facilitate the application of sustainable management practices. Work with countries to promote the engagement of all stakeholders (farmers/farmer cooperatives, NGOs, MOA officials, other law enforcement agencies, etc.) in raising the awareness and implementation of the preventive measures. Also, facilitate farmer participation in the RPW control program to minimize dependence on governmental support through different participatory approaches such as "Farmer Field Schools" and through a network of technical field teams and awareness campaigns.

CONCLUSIONS

The overarching goal of the project is to contribute to the ongoing efforts for the eradication of RPW in the NENA Region. Specifically, to sustainably manage RPW in the targeted countries by refining existing RPW-IPM tactics and developing new technologies, besides equipping national and regional stakeholders to sustainably manage RPW through increased monitoring, better implementation of pre and post entry of quarantine regimes and encouraging a farmer participatory approach for the control of this dreaded pest.

Literature cited

Abraham, V.A., Al-Shuaibi, M.A., Faleiro, J.R., Abozuhairah, R.A., and Vidyasagar, P.S.P.V. (1998). An integrated management approach for red palm weevil, *Rhynchophorus ferrugineus* Olivier, a key pest of date palm in the Middle East. Sultan Qaboos Uni. J. Sci. Res. Agric. Sci. (Melb.) *3*, 77–83.

Chouibani, M. (2020). Guidelines on phytosanitary inspections. In Red Palm Weevil: Guidelines on Management Practices, M. Elkakhy, and J.R. Faleiro, eds. (Rome: FAO), https://doi.org/10.4060/ca7703en.

EPPO. (2020). *Rhynchophorus ferrugineus*. EPPO Datasheets on Pests Recommended for Regulation.

https://gd.eppo.int (accessed May 15, 2020).

Faleiro, J.R. (2020). Guidelines on RPW pheromone trapping with respect to trap design, trap density and servicing. In Red Palm Weevil: Guidelines on Management Practices, M. Elkakhy, and J.R. Faleiro, eds. (Rome: FAO).

FAO-CIHEAM. (2017). Current Situation of Red Palm Weevil in the NENA Region (Current Situation of Management Practices, Challenges/Weaknesses and Available Research and Technologies for Its Improvement). http://www.fao.org/3/a-ms664e.pdf (accessed May 14, 2020).

Fiaboe, K.K.M., Peterson, A.T., Kairo, M.T.K., and Roda, A.L. (2012). Predicting the potential worldwide distribution of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) using ecological niche modeling. Fla. Entomol. *95* (*3*), 659–673 https://doi.org/10.1653/024.095.0317.

The efficacy of some local isolates of the fungus *Beauveria bassiana* (*Ascomycota: Hypocreales*) on the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera: Curculionida*)

E. Mohamed[1,a], M. Hasan[1], T. Yaseen[2], M. Moflih[3], A.H. Hasan[3] and W. Almatni[4]

[1]Arab Centre for the Studies of Arid Zones and Dry Land (ACSAD), Damascus, Syria; [2]Food and Agriculture Organization of the United Nations - Regional office in Near East and North Africa, Cairo, Egypt; [3]General Commission for Scientific Agricultural Research, Damascus, Syria; [4]Debbane AGRI Company, Damascus, Syria.

Abstract

The efficacy of four local isolates of the entomopathogenic fungus *Beauveria bassiana* (*Ascomycota: Hypocreales*) was tested. There were b58 isolated from moor soil (Al Heffah region), K isolated from citrus orchards soil (Al Bassah region), Bb isolated from *Zeuzera pyrina* larva (Oramo region) and Br isolated from red palm weevil adult in Lattakia governorate. Experiments were carried out on adults and fifth L5 larvae stage of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) at a concentration of 10^7 spores mL^{-1} of each isolate. Results showed that Br isolate is the most effective on adults and L5 larvae stage. Mortality rate of insects was 100% on the last day of experiment, while the Bb isolate was the least effective. The LC_{50} values ranged between 2.4 and 6.8 days for L5 larvae and between 4.3 and 13.2 days for adults at the end of the experiment. The efficacy of different concentrations (10^4, 10^6, 10^8 spores mL^{-1}) of Br isolate was tested on adults and L3, L7 larvae stage of red palm weevil then. Morality and LC_{50} were taken for each of them. Results showed that the third larvae stage is the most sensitive to the fungus. Mortality rates were 85, 100 and 100% for the three suspension concentrations of the Br isolate (10^8, 10^6, 10^4 spores mL^{-1}), respectively, whereas they were 58, 87 and 90% for the seventh larvae stage within 7 days of the experiment. The LC_{50} for third instar larvae was 0.15×10^2 and 1×10^3 spores mL^{-1} for seventh instar larvae. While the adult mortality rates during the 14 days of the experiment were 54, 83 and 100% for each concentration (10^8, 10^6, 10^4 spores mL^{-1}), respectively, and the LC_{50} was 7×10^3 spores mL^{-1}. However, mortality rates in the control for L3, L7, and adult weevils were always 0%.

Keywords: biological control, entomopathogenic fungus, Syria

INTRODUCTION

Rhynchophorus ferrugineus (Olivier) is one of the world's invasive pests of palm trees because of its ability to adapt and adapt to different conditions and because of the near-total absence of the insect's natural vital enemies in newly colonized places (Lo Verde et al., 2015).

The insect spread throughout the Mediterranean basin and in Syria, where it was first recorded in 2005 on canary palms in the Bouqa area of Latakia province. The main damage to the insect is mostly due to larvae that feed and develop within the palm trunk and destroy the tree-carrying vessel system, and the top bud in canary palms. Control and prevention of the insect is very difficult because larvae are protected for their presence within the leg. Because many European countries restrict the use of chemical pesticides in urban areas where insect injuries occur mainly, and although natural bio-enemies have registered the insect, little has been studied to assess their effectiveness. As vital control factors for the insect, special attention has been paid to entomopathogens, including entomopathogenic nematodes.

Some entomopathogenic nematodes are *Steinernema carpocapsae* (Weiser) (Llácer et

[a]E-mail: eyadm2009@gmail.com

al., 2009; Dembilio et al., 2010). Other entomopathogenic fungi are mainly like *Beauveria bassiana* (Balsamo) Vuillemin, and, to a less extent fungi like *Metarhizium anisopliae* (Deadman et al., 2001; Ricaño et al., 2013).

Beauveria bassiana (Balsamo) Vuillemin (*Hypocreales: Clavicipitaceae*) is an entomopathogenic fungi, several research has proven its efficacy to causs deaths of RPW in laboratory (Gindin et al., 2006; Güerri-Agulló et al., 2010; Ricaño et al., 2013). Güerri-Agulló et al. (2011) explained that treating palm crown area, petiole with the isolate BP203 of *B. bassiana*, reduced the number of RPW in southeastern Spain.

In another laboratory study conducted in Egypt to test the local isolation of *B. bassiana* obtained from infected insect *Gryllotalpa gryllotalpa* consecutive $6{\times}10^2$ to $6{\times}10^7$ spores mL^{-1} were tested and the results showed that the highest mortality rate occurred in larvae aged L3 treated with the fungus and the final mortality rate reached 100%, while the mortality rates in larvae at the age of L7 were between 45 and 75% of the remaining individuals which formed cocoons that all died in the dormancy phase, and the treatment of adult insects showed that the highest concentrations can kill the adult insects in 7 days shorter than low concentrations which take 11 days (El Husseini, 2019). Field applications using *B. bassiana* mushroom spores have shown that it can be successful in control the insects of the red palm weevil (Sewify and Fouad, 2006; Sewify et al., 2014; El-Akad et al., 2016).

This study was conducted to evaluate the efficacy of four local *B. bassiana* isolates on RPW adult and larvae aged L5, assessing the efficacy of different concentrations of those isolates as well as determining mortality rate and the LD_{50} of each isolate.

MATERIALS AND METHODS

The research was carried out in Latakia province in 2019/2020 at the Economic Insect Laboratory at the Agricultural Scientific Research Center in Latakia and at the ACSAD Al-Sen research station in Latakia province. Four isolations of *Beauveria bassiana* (b58, K, Bb, Br) locally isolated from the Latakia region were used to study to evaluate their ability to cause the highest mortality rate of RPW. The source and geographic location of *Beauveria bassiana* isolates are mentioned in Table 1.

Table 1. Source of *Beauveria bassiana* isolations and geographic location used in experiments to control red palm weevil, *R. ferrugineus*.

Geographic location	Source	Isolate code
Al-Haffe	Soil	b58
Al-Bassa	Soil	K
ERAMO	Apple steep borer	Bb
Madina al Nakhil	RPW adult	Br

RPW insects were collected from infected palm trees during periodic cutting operations in Latakia province, and the samples were placed in sterile plastic boxes (20×15×7 cm) containing holes for ventilation. The insects were transferred to the insect laboratory at the Agricultural Scientific Research Center in Latakia province daily and fed on pieces of sugarcane periodically and monitored daily to get rid of any infected or unhealthy insects for two weeks to get healthy insects for laboratory as described by Saleem et al. (2019). Insects were reared under laboratory conditions 27±2°C, 70±5% relative humidity and 12/12 h light/darkness.

Beauveria bassiana single spore isolates were grown at on petri dishes containing potato dextrose agar, three dishes of each isolate and then incubated at 26°C for 15 days in the dark to reach the full sporulation stage. To collect spore suspension 10 mL of sterile distilled water with 0.05% Tween 80, were added to each dish. Spore suspension was filtered across two layers of sterile filtration paper and then the concentration was determined by counting the spores using a micrometer slide. The screw suspension was adjusted by adding distilled water plus Tween 80 solution at a concentration of 0.05% to reach the target concentrations for the test.

The rate of germination of the collected spores was estimated by inoculation of two petri dishes with a drop of the spore suspension for each isolation. Then the dishes were incubated at a temperature of 26°C for 24 h and then at least 100 spores from each dish were examined. The spore was considered to be swollen if the length of the germination tube exceeded half the length of the spore.

First experiment

RPW insects were placed in small plastic boxes 12×6 cm with perforation for ventilation and fed by parts of the pulp of the palm tree at the rate of four larvae or adults per box (as repetitions), and then sprayed with 5 mL of *Beauveria bassiana* spore suspension at 10^7 spores mL^{-1} using a small hand spray, and the control repetitions were sprayed with 5 mL Tween 80 water per repeater. Each trial for one isolate contains 4 larval and two RPW adults, each composed of 3 repetitions. Each repetition contains 4 larvae in phase V or 4 adults with the control. Observations and notes were taken daily for 10 days in case of larval treatment and for 14 days for RPW adults.

Second experiment

One isolation *B. bassiana* (Br) and three concentrations of the spore suspension were used (10^4, 10^6, 10^8 spores mL^{-1}) and three stages of the red palm weevil (larval stage L3, L7 and adult insects) and each repeater was sprayed with 5 mL of concentration of spore suspensions, while the controls were sprayed with Tween 80 water. Each treatment was composed of four reputations as well as for the control. Each repetition was composed of four insects (larvae or adults) are treatment of the third larval age, treatment of the seventh larval age, treatment of adult insects, control. The treated insects were placed at a temperature of 25±2°C and lighting (16/8 h) (light/darkness). The number of live and dead insects were taken after 2, 4, 6, 8, 10, 12, and 14 days after the start of the experiment on adults and daily until the 7th day on larvae and the number of live and dead insects was recorded in each box and in the case of the dead insect is transferred to a sterile petri dish containing a filter sheet moisturized with distilled water added antibiotic to follow the development of the injury and to ascertain whether the cause of death is an innate infection.

Statistical analysis

The value of both LC_{50} for the concentrations and LT_{50} for the time of killing was calculated using the Ldp Line statistical program. The differences at the lowest significant difference level were compared at the probability level of 0.01 using the Duncan test.

RESULTS AND DISCUSSION

The efficacy of *Beauveria bassiana* isolates

The results showed 100% of RPW larvae died using b58 and Br isolates at the 10th day after treatment, RPW adults recorded 100% mortality rate after 14 days of treatment.

Yasin et al. (2019) showed that the decrease of deadly killing time and the increase in mortality are closely linked to the concentration of conidia, where the value of deadly LT_{50} half-time decreased when the concentration of conidia increased from 10^6 to 10^9 spores mL^{-1}.

All tested *Beauveria bassiana* isolates were efficient in infecting RPW larva and adult with clear differences in their efficacy. The results showed that (Br) isolate is more efficient than (Bb, K, b58), which originally isolated from naturally infected RPW. This isolate caused a 100% death on the fifth larvae stage using 10^7 spores mL^{-1} on the 10th day after infection, and 100% death on adults by the same concentration at the last day of the experiment, while the isolation was Bb was less efficient as compared to the rest of the isolations (Table 2). These findings are in agreement with what was reported earlier that the isolates obtained from the host are more efficient than commercial or isolated or the one obtained from the soil or from different hosts. Ricaño et al. (2013) tested the efficacy of ten isolates of *B. bassiana* from several species of beetles in the Mediterranean environment and observed that the isolations taken from the red palm weevil were more pathogenic to the larvae and adults of the RPW.

Table 2. Mortality rate of red palm weevil (RPW) after treatment with *B. bassiana* after 10 days on larvae and 14 days after adult.

Treatment	Adult RPW (%)	Larva at L5 (%)
b58	75 a	100 a
K	92 a	92 a
Bb	58 ab	83 a
Br	100 a	100 a
Control	0 c	16.7 b

The values of the LD_{50} time of RPW treated with different *B. bassiana* isolations (LT_{50}) ranged between 4.2 and 6.8 days for larvae after 10 days of treatment and between 4.3 and 13.2 days for adults after 14 days of treatment focusing 10^7 spores mL^{-1} *B. bassiana*. The LD_{50} was significantly lower when the insects were treated with Br isolation than both on fifth stage larvae and adults. While Bb isolation required more time to cause the death for the same numbers of insects, whether larvae or adults (Table 3). Figure 1 shows toxicity lines that show the values of the LD_{50} time of *B. bassiana* isolations studied on fifth stage of larvae (Figure 1A) and adults (Figure 1B) of the RPW.

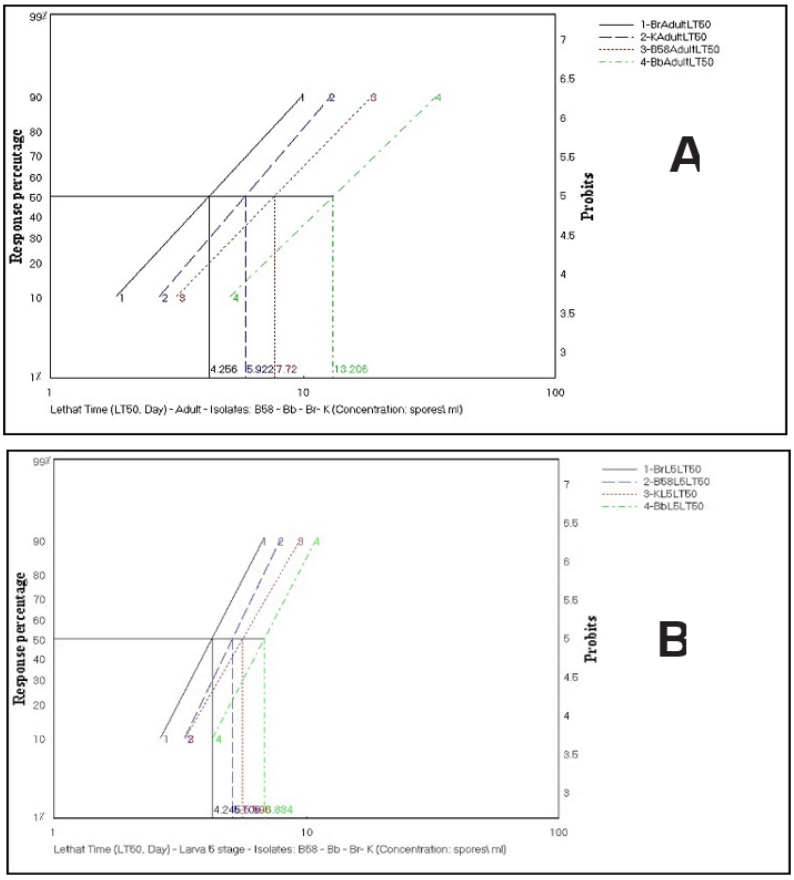


Figure 1. Propite values, toxicity lines and half-deadly time of mushroom isolations *B. bassiana* (Br, b58, K, Bb) tested on 5-year-old larvae L5 (A) and adult insects (B) of red palm weevil. The days followed by the same character in each column have no significant difference between them at the probability level of 1%.

Table 3. The LD_{50} time of RPW insects after treatment with *B. bassiana* after 10 days on larvae and 14 days after adult.

Isolate	Larva of RPW at L5 after 10 days			RPW adults after 14 days		
	LT_{50} time	Min	Max	LT_{50} time	Min	Max
Br	4.2 a	3.9	4.6	4.3 a	3.6	4.9
b58	5.1 ab	4.7	5.5	5.9 ab	5.2	6.6
K	5.5 b	5.1	6.1	7.7 b	6.8	8.6
Bb	6.8 c	6.3	7.4	13.2 c	11.5	16.8

The effect of BR isolate in the third and L7 stage of RPW

The third stage larvae were significantly affected by the three concentrations of *B. bassiana* used after 3 and 7 days after treatment (Figure 2), with mortality percentage after 3 days (28, 44, 60%) of the three concentrations viz., 10^4, 10^6, 10^8 spores mL^{-1}, respectively, while the LC_{50} at the concentration of 5×10^6 spores mL^{-1} reached 85 100 and 100%, respectively, after 7 days of treatment. The LD_{50} concentration was 0.15×102 spores mL^{-1}. As for the larvae of the last age, the results showed that the effect of *B. bassiana* is also significant with a death rate of 32, 38 and 42% of the three concentrations used (10^4, 10^6, 10^8 spores mL^{-1}), respectively, with a LD_{50} concentration equal to 3×10^{10} spores mL^{-1}, and were 58, 87, and 90%, after 7 days of treatment the LD_{50} was 1×10^3 spores mL^{-1}, and all above treatments are significantly different from the control and this is in agreement with El Husseini (2019).

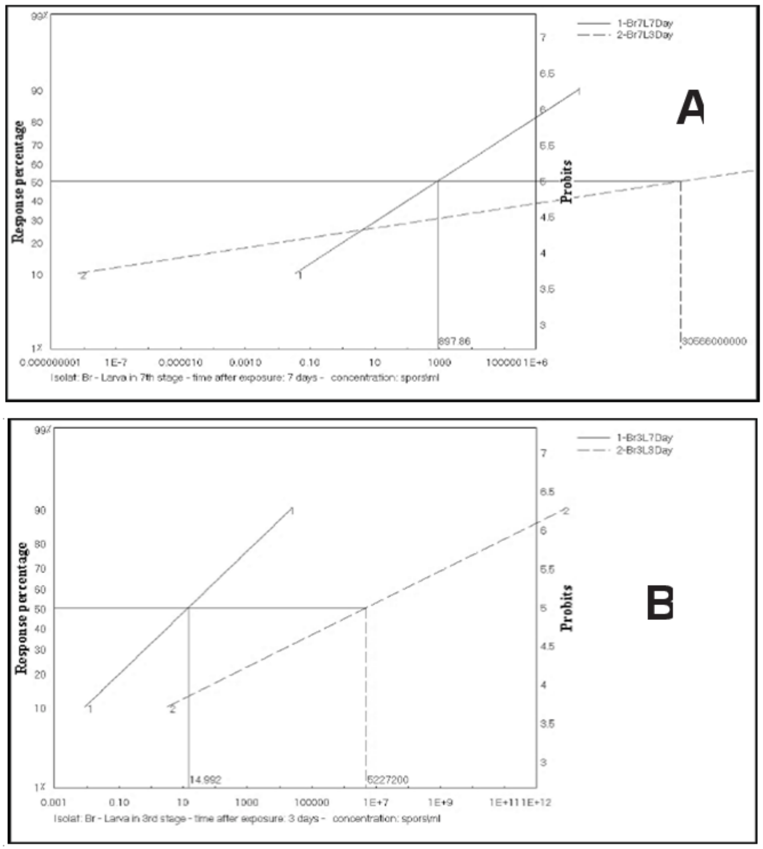


Figure 2. Propit values and the half-killer concentration of the three tested concentrations of *B. bassiana* (10^4, 10^6, 10^8 spores mL^{-1}) of Br isolates on the third age L3 (B) and the last L7 (A) for red palm succulence larvae. Averages followed by the same character in each column make no significant difference between them at the 1% probability level.

The effect of *B. bassiana* on larvae was demonstrated by observing a change in the behavior of diseased insects where very slow movement was observed and then stopped feeding and with the appearance of some necrotic spots on the body of larvae indicating the places where the spores of the fungus penetrated the larval body. As the injury progressed, the larvae completely stopped moving and died, the body contracted and became almost rigid and fossilized, and the fungal growths emerged outside the larva's body (Figure 3). When growth is not observed on the surface of the body, the presence of the fungi is confirmed by dissecting the body and detecting the presence of mycelium within the larvae's body. The results also showed a decrease in the effect of *B. bassiana* on larvae with age, indicating that large phases are less sensitive to infection than younger phases, and may be due to the fact that larvae at the last age are larger and need higher doses of pathogenic fungi to reach death than at the early ages.

Figure 3. Appearance of *B. bassiana* on red palm weevil.

The effect of Br isolate at the adults of RPW

The results in Table 4 show a clear effect of *B. bassiana* on the adults of RPW with higher concentration than lower once, with mortality beginning on the fourth day in the trial and the percentages of death (34, 42, 60%) of the three used concentrations (10^4, 10^6, 10^8 spores mL^{-1}) respectively, with a LD_{50} concentration of 5×10^6 spores mL^{-1} without significant differences between them as compared to the control. After 8 days of fungi treatment, this percentage increased to 46, 80, 93%, respectively, with a significant difference between the lowest concentration (10^4 spores mL^{-1}) and the highest two concentrations (10^6 and 10^8 spores mL^{-1}), between which there was no significant difference, with the deadly LD_{50} on the eighth day of treatment 1×10^4 spores mL^{-1} (Figure 4). On the 14^{th} day of the experiment, the mortality rate was 54, 83, 100% at the same spore concentrations, respectively. This is a case reduction of daily LD_{50} to 7×10^3 spores mL^{-1} (Figure 4). These results were in agreement with previous studies where the use of isolation from naturally infected *B. bassiana* led to 100% death on adults after 14 days of experimentation, making this isolation promising in order to complete the study of its impact on RPW. The results also showed that the effect of *B. bassiana* was relatively low at the beginning of the experiment and then increased after a week of fungi treatment (Table 4). The symptoms of fungi infection were that the adults became highly mobile at the beginning of the injury and randomly moved and then began to slow down after a day to become almost non-existent within a few days and then stopped moving permanently and died, the innate growths were observed on the body of the dead insect between his throats as well as between the joints of the argel and in the oral hose area (Table 4). When not watching fungal growths the individuals of RPW was cut to make sure the fungi were within the body, and part of the insect's body was taken and placed on the PDA culture to monitor the growth of fungi in the dishes and to ensure that the cause of the insect's death was the infection of the pathogenic fungi.

Table 4. The LD_{50} of *B. bassiana* isolate (Br) on the third stage L3 and the last L7 for the larvae on the RPW and its deadly LD_{50}.

Treatment	Percentage of death of larvae L3		Percentage of death of larvae L7	
	after 3 days	after 7 days	after 3 days	after 7 days
1×10^8	60.20 c	100 b	42.70 b	90.22 c
1×10^6	44.64 c	100 b	38.90 b	87.90 c
1×10^4	28.38 b	85.88 b	32.27 b	58.45 b
Control	0.0 a	0.0 a	0.0 a	0.0 a
	5×10^6 spore mL^{-1}	**0.15×10^2 spore mL^{-1}**	**3×10^{10} spore mL^{-1}**	**1×10^3 spore mL^{-1}**

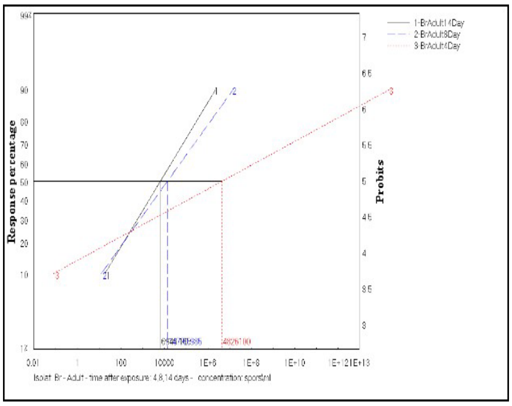


Figure 4. Propit and the deadly LD_{50} concentration of the three isolation tested compositions (Br) were assessed on the adult phase of the red palm weevil. Averages followed by the same character in each column make no moral difference between them at the 1% probability level.

CONCLUSIONS

All tested *B. bassiana* isolates used in this study showed effectiveness in killing larvae and adults of red palm weevils with clear differences in disease capacity. Br isolate of *B. bassiana* isolated from the natural infected RPW is more effective than those isolated from the soil or from other host insects. Br isolation is recommended for the biological control of the RPW as part of integrated insect control programs on the Syrian coast.

Literature cited

Deadman, M.L., Azam, K.M., Ravzi, S.A., and Kaakeh, W. (2001). Preliminary investigation into the biological control of the red palm weevil using *Beauveria bassiana*. Paper presented at: 2nd Int Conf – Date Palm (Al-Ain, UAE).

Dembilio, O., Llácer, E., Martínez de Altube, M.M., and Jacas, J.A. (2010). Field efficacy of imidacloprid and *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in *Phoenix canariensis.* Pest Manag Sci *66* (*4*), 365–370 https://doi.org/10.1002/ps.1882. PubMed

El-Akad, S., El-Banna, A., Abd El-Wahab, S., Abd El-Aal, E., and Amin, S. (2016). Effect of *Beauveria bassiana* and *Metarhizium anisoplae* on some biological aspect of *Pectinophora gossypiella* (Saunders) (*Lepidoptera*: *Gelechiidae*. Acad J Biol Sci *9* (*3*), 31–39.

El Husseini, M.M. (2019). Efficacy of the fungus *Beauveria bassiana* (Balsamo) Vuillemin on the red palm weevil *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*) larvae and adults under laboratory conditions. Egypt. J. Biol. Pest Control *29* (*1*), 58 https://doi.org/10.1186/s41938-019-0155-3.

Gindin, G., Levski, S., Glazer, I., and Soroker, V. (2006). Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus.* Phytoparasitica *34* (*4*), 370–379 https://doi.org/10.1007/BF02981024.

Güerri-Agulló, B., Gómez-Vidal, S., Asensio, L., Barranco, P., and Lopez-Llorca, L.V. (2010). Infection of the red palm weevil (*Rhynchophorus ferrugineus*) by the entomopathogenic fungus *Beauveria bassiana*: a SEM study. Microsc

Res Tech *73* (*7*), 714–725. PubMed

Güerri-Agulló, B., López-Follana, R., Asensio, L., Barranco, P., and Lopez-Llorca, L.V. (2011). Use of a solid formulation of *Beauveria bassiana* for bio-control of the red palm weevil (*Rhynchophorus ferrugineus* (*Coleoptera*: *Dryophthoridae*)) under field conditions in SE Spain. Fla. Entomol. *94* (*4*), 737–747 https://doi.org/10.1653/024.094.0402.

Llácer, E., Martínez de Altube, M.M., and Jacas, J.A. (2009). Evaluation of the efficacy of *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil, *Rhynchophorus ferrugineus*, in *Phoenix canariensis.* BioControl *54* (*4*), 559–565 https://doi.org/10.1007/s10526-008-9208-3.

Lo Verde, G., Torta, L., Mondello, V., Caldarella, C.G., Burruano, S., and Caleca, V. (2015). Pathogenicity bioassays of isolates of *Beauveria bassiana* on *Rhynchophorus ferrugineus.* Pest Manag Sci *71* (*2*), 323–328 https://doi.org/10.1002/ps.3852. PubMed

Ricaño, J., Güerri-Agulló, B., Serna-Sarriás, M.J., Rubio-Llorca, G., Asensio, L., Barranco, P., and Lopez-Llorca, L.V. (2013). Evaluation of the pathogenicity of multiple isolates of *Beauveria bassiana* (*Hypocreales*: *Clavicipitaceae*) on *Rhynchophorus ferrugineus* (*Coleoptera*: *Dryophthoridae*) for the assessment of a solid formulation under simulated field conditions. Fla. Entomol. *96* (*4*), 1311–1324 https://doi.org/10.1653/024.096.0410.

Saleem, M.A., Qayyum, M.A., Ali, M., Amin, M., Tayyab, M., and Maqsood, S. (2019). Effect of sub-lethal doses of *Beauveria bassiana* and Nitenpyram on the development of red palm weevil *Rhynchophorus ferrugineus* (Olivier). Pak. J. Zool. *51* (*2*), 556–565 https://doi.org/10.17582/journal.pjz/2019.51.2.559.565.

Sewify, G.H., and Fouad, S.H. (2006). Integrated control of red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). Agric Sci Mansoura Univ *31* (*4*), 2415–1426.

Sewify, G.H., Belal, M.H., and Saeed, M.Q. (2014). Using pheromone mass-trapping and the entomopathogenic fungus *Beauveria bassiana* in IPM programs forcontrolling the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). Egypt. J. Biol. Pest Control *24* (*1*), 197–202.

Yasin, M., Wakil, W., Ghazanfar, M.U., Qayyum, M.A., Tahir, M., and Bedford, G.O. (2019). Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Entomol. Res. *49* (*1*), 3–12 https://doi.org/10.1111/1748-5967.12260.

Development of novel biological treatment for palms against the red palm weevil (*Rhynchophorus ferrugineus*) using RNAi

D. Shalitin[1,a], N. Grimberg[1], I. Dodek[1], N. Mogilner[2], H. Caduri[2] and M. Bar-Joseph[3]

[1]PlantArc Bio Ltd., Giva'tChen, Israel; [2]Gadot Agro Ltd., Giva't Brenner, Israel; [3]ARO-Volcani Center, Bet Dagan, Israel.

Abstract

Insect pests cost billions of dollars in the form of crop losses and insecticides, and farmers face an ever-present threat of insecticide resistance, fueling a continual search for alternative pest-control strategies. Thus, there is a long-felt need for environmentally friendly methods for controlling or eradicating insect infestation on or in plants, i.e., methods that are selective, environmentally inert, non-persistent, and biodegradable, that fit well into pest resistance management schemes. RNA interference (RNAi) is a process utilizing endogenous cellular pathways, whereby an interfering RNA molecule that is specific for a target gene sequence results in the degradation of the mRNA encoded. The red palm weevil (RPW; *Rhynchophorus ferrugineus*) is a wide spread pest considered as a major threat for dates and other palm tree species in the Middle East, Mediterranean, and around the word. The RPW larvae excavate holes in the trunk of the tree, feed from it and eventually causing the death of the palm host. Although some available pesticides are used to control the infestation, the synthetic pesticides had failed so far to stop the infestation and the use of such chemicals has detrimental effects on the food safety and the environment. Here, we present an alternative RNAi biological treatment for palms based on a double-stranded RNA (ds02288) sequence of the protea some sub unit beta type-1 (PSMB1). ds02288 showed high efficacy against RPW larvae in feeding assays and was able to prevent and reduce infestation in treated date-palm trees in field trials. Our product is currently pending registration in Israel as a new bio-pesticide for the management of RPW in date-palm trees.

Keywords: red palm weevil, RPW, RNA interference, RNAi, double stranded RNA, dsRNA, Proteasome sub unit beta type-1, PSMB1

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is of utmost important fruit crop throughout the arid regions, of the Middle East, North Africa and Arabian Peninsula. The date fruits are not only highly esteemed as an essential component of daily nutrition but also highly valued for their health and medicinal qualities. Date production in these areas, are under serious threat by the red palm weevil (RPW) (*Rhynchophorus ferruginous* (Olivier); *Coleoptera*: *Curculionidae*). The RPW is a highly invasive beetle infesting palm trees (Faleiro, 2006; Kehat,1999). The adult beetles are relatively large, ranging between 2 and 4 cm long, and are usually a rusty red color. The weevil larvae excavate holes in the trunk of palm trees up to a meter long, thereby weakening and eventually killing the host plant. As a result, the weevil is considered a major pest in palm plantations, including coconut palms, date palms and oil palms (Pugliese et al., 2017).

The control of RPWs relies on the use of synthetic pesticides and remains a challenging problem. First because the toxic pesticides deteriorate environmental quality and endanger biological diversity. Furthermore, they are also not sufficiently effective to manage RPWs and interfere with the acceptance of date fruits as health components (Abo-El-Saad et al., 2012, 2013; Llácer et al., 2010).

[a]E-mail: dror@plantarcbio.com

As a result, there was an intense need to implement novel, less harming pest management measures for RPW control (Petrick et al., 2013; San Miguel and Scott, 2016).

The present report provides evidence for the possible application of a novel approach for RPW control based on RNA interference (RNAi). RNAi based pest control is a genetic sequence specific method (not involving genetic modification, GMO technology) of suppressing a targeted gene's expression. By selecting RPW sequences, from essential genes responsible for the insect's viability, we were able to cause up to 100% mortality to RPW larvae without adversely affecting non-target species (Wang et al., 2013; Gordon and Waterhouse, 2007; Price and Gatehouse, 2008). Both feeding assays and field trials in trees show great opportunity of using this novel method as a general pest control in date orchards and for ornamental purposes. Results using the RNAi technology for RPW control are presented.

MATERIALS AND METHODS

In the whole experiment a synthetic dsRNA was used. The dsRNA for the feeding assays were provided by Eupheria Biotech GmbH (Dresden, Germany). The dsRNA for the field trials were provided by Genolution Inc. (Seoul, Korea). Infestation levels of palm trees were monitored by IoTree® sensor system (Agrint, Rockville, USA).

Dose response feeding assay

RPW larvae (1-2.5 cm in length) were grown in a plastic 250-mL cup-like container (a single larvae in each container) and fed on crushed sugarcane stem. Synthesized dsRNA molecules of each of the indicated sequences were dissolved in 1 mL of ddH_2O and subsequently added to 5 g crushed sugarcane in a total of 1 µg per RPW larvae treatment (5-7 repeats per treatment). The containers were then placed in a dark humid location at a temperature of 25-30°C. The viability and change in mobility of the larvae was evaluated 3 times weekly (every 2-3 days) for 14 days following the treatment. The dsRNA sequences are 200-300 bp in length and are corresponding to mRNA sequences of RPW proteasomal genes: ds02288 – proteasome subunit beta type-1 (PSMB1); ds04581 – proteasome non-ATPase regulatory subunit 6 (RPN6); ds00295 – proteasome subunit alpha type-6 (PSMA6); ds00507 – proteasome subunit alpha type-1 (PSMA1); ds06181 – proteasome subunit beta type-2 (PSMB2). To determine optimal dose, the percentage of larvae death obtained using different doses of the dsRNA was also tested. The negative control used is a 300-bp dsRNA of green fluorescent protein (GFP).

Formulation feeding assay

To examine the efficacy of ds02288 in a formulation, designated for trees treatment, on larvae mortality rates by oral ingestion, RPW larvae (~2-3 cm in length) were grown in a plastic 250 mL cup-like container (a single larvae in each container) and fed on crushed sugarcane stem. Synthesized ds02288 (200 bp) molecules in 2 mL of liquid formulation or in ddH_2O added to 5 g crushed sugarcane per RPW larvae treatment, 20 repeats per treatment (Table 1). The containers were then placed in a dark humid location at a temperature of 25-30°C.

Table 1. Treatments used for larvae formulation feeding assay.

Treatment	ds02288 (µg mL^{-1})	Formulation[a]
A_10	10	+
A_1	1	+
A_0	0	+
B_0	0	-
B_10	10	-
B_1	1	-
B_0.1	0.1	-

[a]Samples in formulation A indicated in (+) and samples in ddH_2O indicated in (-).

The viability and change in mobility of the larvae was evaluated daily for 16 days following the treatment. Formulation or ddH_2O without ds02288 were used as a negative control.

Canary palm field trial

The field trial was conducted on grown Canary palm trees (*Phoenix canariensis*) at the southern region of Israel. The application of ds02288 formulation is performed by direct injection to the trunk and by crown irrigation of the palm tree. During the trial, infestation levels are monitored by IoTree® sensor system. Twenty-four monitored trees in a field were selected for the experiment. The trees were divided into two groups (12 trees per group) of dsRNA treated and control trees. In each group the number of infested and healthy trees (according to the sensors) were similar. Each tree was treated with 18 mL by injection (6 mL per cannula were injected at 3 bark positions) and 10 L were irrigated onto the tree crown. In each treatment a total of 20 mg were applied (10 mg by injection and 10 mg by crown irrigation) or water for control trees.

RPW infection trial

The inoculation trial was conducted on offshoots of 'Medjool' date trees (*Phoenix dactylifera*), ~2 m high, with a single cannula (injection hole). The trees were planted in the soil under a net house. A total of 36 trees were divided into five groups. Seven trees per treatment and eight trees at the control group. The trees were treated with two doses of ds02288 (7 and 17 mg in 6 mL). During the trial and at two time points, adult RPW beetles (~100 beetles) were spread in the net house to allow infestation. To promote the infestation, the bark of the trees was slightly injured (Figure 1A). Infestation levels were monitored by IoTree® sensor system. The treatment and infestation events are presented in Table 2. Treatments are indicated by (+) and an asterisk (*) above the month (M) indicates an inoculation event. During the trial several trees were dissected and searched for indication of RPW larvae activity to evaluate their infestation levels.

Movement of dsRNA in palm trees

A mature frond of an untreated 'Medjool' date was cut and placed in a tube containing 5.2 µg of ds02288 in 7.5 mL water. The tubes were sealed with para-film to ensure all water is absorbed by transpiration. After 48 h all water was absorbed. The petiole and rachis above the soaked area was cut to small pieces and frozen at -80°C. The pieces were then crushed and squeezed to extract the sap. Total RNA was isolated from 200 µL of palm leaves sap with Bio Tri RNA (Bio-Lab, Israel) and cDNA was synthesized using Maxima H minus RT enzyme (Thermo Fisher Scientific, USA) according to manufacturer's instructions, using random hexamer primers. Real Time qPCR (RT-qPCR) reactions were performed on AriMax (Agilent, USA) machine using Fast SYBR Green Master Mix (Applied Biosystem, USA) using 1 µL synthesized cDNA under cycling conditions following manufacture instructions with two sets of specific primers designed to detect ds02288. Non-specific product and primer dimer formations were ruled out by melting curve consisting of a single peak. dsRNA relative quantity was calculated relative to non-treated control tissue.

Table 2. Treatment and infestation events.

Treatment	ds02288 (mg)			*				*		
		M1	M2	M3	M4	M5	M6	M7	M8	M9
A_0	0	+	+		+	+		+	+	
A_7	7	+	+		+	+		+	+	
A_17	17	+	+		+	+		+	+	
B_7	7	+			+			+		
B17	17	+			+			+		

An asterisk (*) above the month (M) indicates an inoculation event.

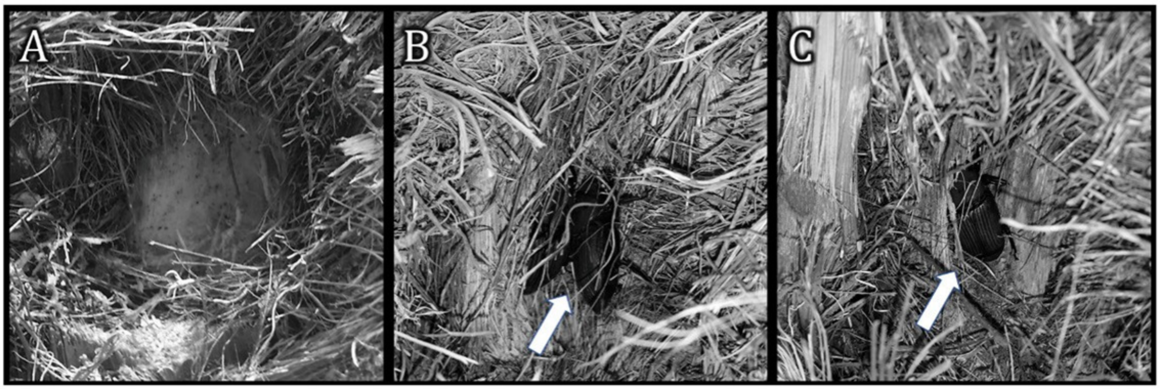


Figure 1. Injured bark of 'Medjool' date trees (*Phoenix dactylifera*) that were part of the RPW infection trial. Date trees were injured to promote infestation of the trees. A) fresh injury; B-C) adult beetles settle in the injured bark (arrow) 1-2 days following release in the contained net house.

RESULTS AND DISCUSSION

Dose response feeding assay

We tested several dsRNA molecules of RPW proteasomal genes as an efficient RNAi target. The RPW larvae mortality rates obtained are shown in Figure 2. The negative control used is a 300 bp dsRNA of green fluorescent protein (dsEGFP). As seen in Figure 2, all five dsRNAs tested caused a significant increase in larvae mortality from 10 DPT and exceeding 75% at 14 DPT (75% for ds00507, 85.7% for ds00295 and ds04581). Noteworthy, the ds00295 targeting the PSMA6 showed a marked increase in the number of dead larvae 10 DPT and the ds02288 targeting the PSMB1 and ds06181 targeting the PSMB2 showed a marked increase in the number of dead larvae 12 DPT.

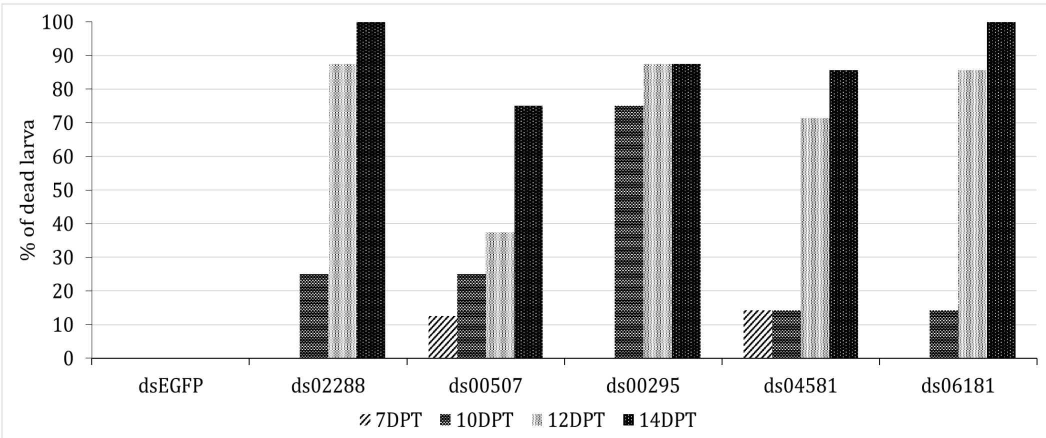


Figure 2. Feeding assays comparing different targets in the RPW, showing mortality rates presented as percentage of dead RPW larvae, days post treatment (DPT). RPW larvae were grown in plastic cups and fed on crushed sugarcane stem mixed with 1 µg of dsRNA (200-300 bp). The dsRNA molecules targeting RPW genes are as follows: ds02288 – proteasome subunit beta type-1 (PSMB1); ds04581 – proteasome non-ATPase regulatory subunit 6 (RPN6); ds00295 – proteasome subunit alpha type-6 (PSMA6); ds00507 – proteasome subunit alpha type-1 (PSMA1); ds06181 – proteasome subunit beta type-2 (PSMB2); dsEGFP that was used as negative control is a 300-bp dsRNA of green fluorescent protein.

To further test the efficacy of ds02288 and ds00295, the above-described experiment was repeated using a combination of the two dsRNAs. As seen from Figure 3, about 55% of the larvae were found dead at 7 DPT, 70% 10 DPT and all larvae were dead 14 DPT. These results were similar to those obtained in the two individual separated treatments only with a faster response time of 7 DPT vs. 10 DPT of ds00295 alone. Both ds02288 and ds00295 exceeded the 85% at 12 DPT and reached 100% at 14 DPT similar to the combined treatment.

To determine optimal dose, the percentage of larvae death obtained using different doses of the ds02288 were tested. As seen from Figure 4. All larvae were found dead 14 DPT using as little as 0.1 µg of ds02288 per treatment.

Formulation feeding assay

We measured the mortality rates of RPW larvae treated by oral ingestion of ds02288 in formulation and in ddH_2O at different concentrations. All treatments exhibited high efficacies reaching 100% at 15 DPT where the control treatments reached only 5 and 15% at the same time for larvae treated with formulation and with ddH_2O, respectively (Figure 5). The overall performance of all treatments is similar where mortality had increased from 7 DPT and reached its peak at 11-15 DPT. Treatment A_10 (10 µg ds02288 in formulation) showed early response time where mortality increased at 5 DPT (7 DPT for the rest of the treatments) but reached 100% mortality at 12 DPT, like the corresponding dose in ddH_2O.

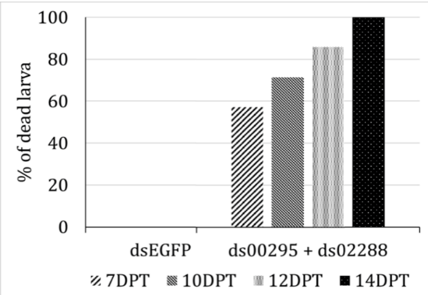


Figure 3. Feeding assays of using two different dsRNAs simultaneously, showing mortality rates as percentage of dead RPW larvae, days post treatment (DPT). RPW larvae were grown in a plastic cup and fed on crushed sugarcane stem mixed with a mix of two dsRNA (1 µg each). The dsRNA molecules targeting RPW genes: ds02288-proteasome subunit beta type-1 (PSMB1) and ds00295-proteasome subunit alpha type-6 (PSMA6). The negative control used is a 300 bp dsRNA of green fluorescent protein (EGFP).

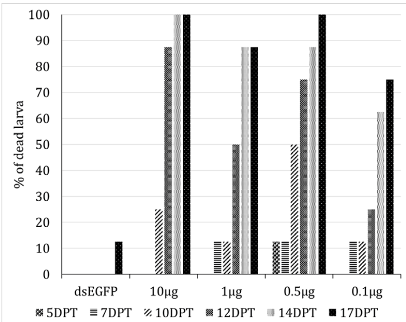


Figure 4. Measuring the efficacy of mortality rates in feeding assays as percent of dead RPW larvae days post treatment (DPT) in the dose response. RPW larvae were grown in a plastic cup and fed on crushed sugarcane stem mixed with dsRNA (0.1-10 µg). The ds02288 molecule targeting RPW proteasome subunit beta type-1 (PSMB1) gene. The negative control used is a 300-bp dsRNA of green fluorescent protein (EGFP).

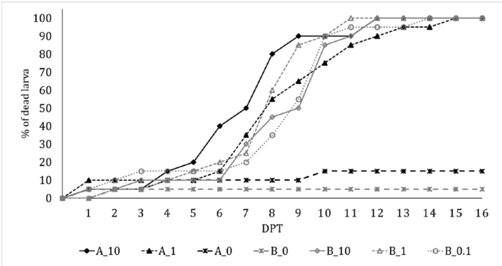


Figure 5. Feeding assays for formulations and dose response. Presented as mortality rates as percent of dead RPW larvae measured days post treatment (DPT). RPW larvae were grown in a plastic cup and fed on crushed sugarcane stem mixed with dsRNA (0.1, 1 or 10 µg) in formulation ('A') or in ddH_2O ('B'). The ds02288 targeting RPW proteasome subunit beta type-1 (PSMB1) gene was used. The negative control used is a formulation only or ddH_2O without dsRNA ('A_0' and 'B_0', respectively).

Canary palm field trial

The infestation levels of 24 Canary palm trees treated with ds02288 or water are monitored by Io Tree® sensor system. Infestation levels were collected at the end of each calendar month, indicated as (M). The IoTree® sensor system tracks the infestation levels by detecting the movement of the larvae in the tree and reports according to changes in the activity levels of the larvae. Since the activity levels of the larvae differs in each season the infestation levels are evaluated according to the past equivalent period. At the beginning of the trial the control group consisted of 10 healthy trees and 2 infested (Table 3). At the peak period of the infestation (M1-4) the control group had 8 healthy trees and 4 infested. At the equivalent period of M13-16, the control group showed similar levels at the end of the trial with 8 healthy trees and 4 infested. When analyzing each tree individually (results not shown) the increase of infected trees is due to 2 healthy trees found as infected at M4 and M9 suggesting a fresh infestation occurred during the trial. These two trees had collapsed during the trial due to high infestations leading to tree death (at M9 and M10). The two control infested trees at the beginning of the trial were still infested at M16 (one is in an intermediate level and was not counted as infected but displayed high infestation levels throughout the trial). The fourth infested tree was detected as infested during the peak period at M1. At the beginning of the trial the treated group consisted of 9 healthy trees and 3 infested. At the peak period of the infestation the treated group had 6 healthy trees and 6 infested. At the equivalent period of M13-16, the treated trees group showed a remarkable recovery level when finishing the trial with 11 healthy trees and only a single tree infested. When analyzing each tree individually (results not shown) all the infected trees at the beginning of the trial found as healthy at its end (M16) and a single tree infected at the peak period (M3) had remained infected at M16.

Table 3. Monthly infestation status of Canary palm trees.

Month		M0	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16
Control	Healthy	10	8	8	9	8	9	11	11	10	10	8	8	9	9	9	8	8
	Infested	2	4	4	3	4	3	1	1	2	2	4	4	3	3	3	4	4
ds02288	Healthy	9	8	7	6	7	6	7	7	8	9	11	12	12	11	12	11	11
	Infested	3	4	5	6	5	6	5	5	4	3	1	0	0	1	0	1	1

RPW intentional infestation trial

We tested the effect of pretreatment with ds02288 in date palms on the infection rates. Trees treated with ds02288 (once or twice) before introducing adult RPW beetles in a contained net-house to promote infection and the infestation rate were monitored with the IoTree® sensor system. Treatment "A" is an application at two consecutive months preceding a single month with no treatment. Treatment "B" is an application every three months. In both treatments two doses were tested (7 and 17 mg per application) (Table 2). The infestation levels measured at the end of each calendar month for each treatment presented in Figure 6. The results presented are from the time of the first exposure to the mature RPW (three months post first treatment). The control group (“A_0”; water treatment as treatment “A”) consisted of eight trees. Four trees were infested, two of them found to be highly infested one month after exposure. The severe infestation of the two trees had led to the death of the tree five and six month after the infestation was detected. At the low dose treatments (A_7 and B_7) a single tree had died and found to be infested by dissection of the bark. At group “B_7” (treatment every three months) a second tree found as infested at M8 (one month after the second exposure) but reported as clean the next month. At the high dose treatments (A_17 and B_17) a single tree had died in group B_17. No traces of RPW larvae activity were found in this tree that probably died from other reasons. Thus, no infested trees were found during the trial at the high dose treatments. The infestation rates of the control group (four out of eight) suggest an affective intentional infestation and the advancement of the normal RPW life cycle within these trees.

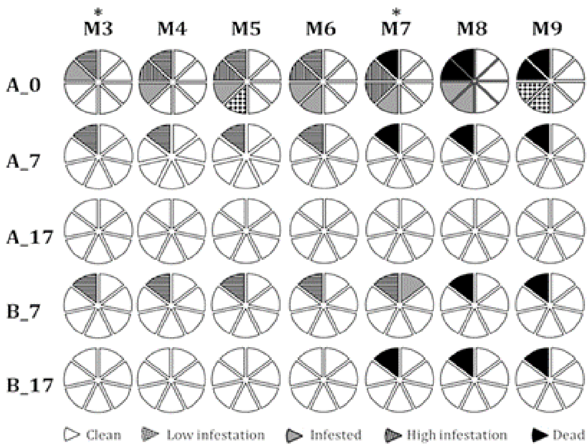


Figure 6. Infestation levels of offshoots of ‘Medjool’ date trees (*Phoenix dactylifera*) monitored during the RPW intentional infestation trial. A total of 36 trees were divided into five groups. Seven trees per treatment and eight trees at the control group. Two treatments regimes were applied: A) treatment at two consecutive months with a single month gap between; B) a single treatment every three months. Each treated tree was injected with ds0288 (7 or 17mg). The negative control used is a ddH_2O without dsRNA (‘A_0’). Each circle represents a treatment group for the corresponding month (each row represents a treatment) (each column is a monthly time point indicated by ‘M’). Each slice represents a single tree in the group. Infestation levels were monitored by IoTree® sensor system is represented by the filling color of each slice as indicated in the legend beneath. An asterisk (*) above the month (M) indicates an inoculation event.

As seen from Figure 1B-C, adult RPW beetles were found to settle in the injured tree bark. These observations, with the infestation rates of the control group (four out of eight), suggest an affective intentional infestation and the advancement of the normal RPW life cycle within these trees. When comparing the infestation levels of the treated trees vs. the control group we can conclude that the treatment with ds02288 had prevent the larvae to develop and further excavate into the tree causing its damage.

Movement of dsRNA in palm trees

RT-qPCR on cDNA synthesized from sap extract of treated fronds. Treated samples yielded relative quantity (ΔR) of 36.9 and 15.3 with primer set A and B, respectively. These results suggest that ds02288 was detected in leaves above the point of application after 2 days.

The RT-qPCR analysis of dsRNA levels, 48 h post-application shows that both amplicons were able to detect the presence of ds02288 (Figure 7) indicating that the molecule is stable in planta and is systemically translocated within the plant.

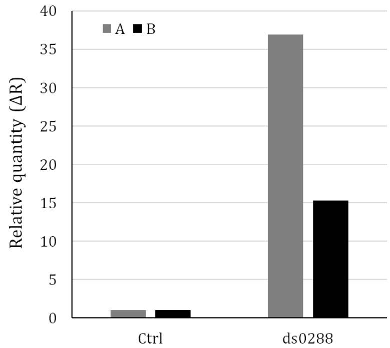


Figure 7. RT-qPCR profiling of ds02288 in distal tissue of dsRNA-treated palm leaves with two sets of primers (A and B). Higher Ct values of both dsRNA amplicons were found in treated fronds (ds02288) vs. untreated fronds (Ctrl) at 48 h post application.

CONCLUSIONS

In this study we attempted to establish an dsRNA molecule as an active ingredient of a biological treatment to prevent and try to eradicate the RPW infestation in palm trees. Several sequences of dsRNA targeting different proteasomal genes mRNA were tested for efficacy and dose response in a lab feeding bioassay (patent submitted). Of the five dsRNAs tested ds0288 (a sequence targeting the proteasome subunit beta type-1 (PSMB1) mRNA) was found with highest efficacy achieving 100% mortality rates 14 DPT, 17 DPT using dose of 10 and 0.5 µg, respectively, and 75% mortality 17 DPT using 0.1 µg dose. All sequences reached 75-87.5% mortality rates 14 DPT using dose of 10 µg. These results are exceeding similar publications of dsRNA applications tested on RPW larvae (Al-Ayedh et al., 2016) and correlates with similar work done on the Colorado potato beetle (CPB) that is currently in advance stages of product development (Rodrigues et al., 2021). In the reported case, injection into larvae of 1-5 mg of catalase 444 bp dsRNA resulted with similar mortality rates. From these results we conclude that dsRNA targeting proteasomal genes of the RPW are efficiently inducing a lethal RNAi response in the RPW larvae by oral ingestion. Same results were achieved when treating the RPW with ds02288 in soluble formulation to test the efficacy of a future product.

The effectivity of ds02288 to prevent and eradicate the RPW infestation in palm trees was tested in two trials on Canary palms in an open field and on 'Medjool' date trees in closed environment. At the field trial, a natural infestation occurred when the trees were in a highly infested area. The fast rise in infection levels at the beginning of the trial, suggest that the trial began at the peak of the infestation and the treatments were not sufficient to prevent the infestation at a short period of time prior to infection. Nevertheless, infected trees had recovered when treated with ds02288. At the net-house trial, the intentional exposure of the trees to adult RPW beetles resulted in high infestation. Here, a pretreatment had prevented the infestation of the trees supporting our assumption that the infestation observed at the open field trial initiated before the first treatment and did not leave sufficient time to prevent the infestation.

According to results in the various feeding assays and trials in trees of 'Medjool' and Canary, we conclude that the treatment with ds02288 exhibit high efficacy, is stable in the trees for at least several months with the targeted dsRNA and has great potential to become a product ready for use for the date and ornamental growers.

We managed to detect the movement of ds02288 in palm tissues by real-time PCR. These results confirm our hypothesis that ds02288 is stable in planta and is systemically translocated within the trees. These dsRNA molecules are probably the cause of larvae inability to grow and develop in the ds02288 treated trees, therefore prevent the damage they cause to the palm trees. These results are consistent with previous publications on the long-distance movement of RNA molecules in plants (Citovsky and Zambryski, 2000; Kehr and Kragler, 2018).

ACKNOWLEDGEMENTS

We want to thank Daniel Katz from Eden-Farm for supplying the larvae for some of the experiments and the RPW adults for the intentional infestation experiments. This work was supported by the Israel Innovation Authority (IAA), grant #69026.

Literature cited

Abo-El-Saad, M.M., Al-Abdan, S., and Bou-Khowh, I.A. (2012). In vivo toxicity of Beta-cyfluthrin insecticide against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier). J. Agric. Sci. Technol. *2*, 1322–1331.

Abo-El-Saad, M.M., El-Shafie, H.A., and Bou-Khowh, I.A. (2013). Toxicity of bio-insecticide, Abamectin, on red palm weevil, *Rhynchophorus ferrugineus* (Olivier). J. Agric. Sci. Technol. *2* (*4*), 107–115.

Al-Ayedh, H., Rizwan-Ul-Haq, M., Hussain, A., and Aljabr, A.M. (2016). Insecticidal potency of RNAi-based catalase knockdown in *Rhynchophorus ferrugineus* (Oliver) (*Coleoptera*: *curculionidae*). Pest Manag Sci *72* (*11*), 2118–2127 https://doi.org/10.1002/ps.4242. PubMed

Citovsky, V., and Zambryski, P. (2000). Systemic transport of RNA in plants. Trends Plant Sci *5* (*2*), 52–54 https://doi.org/10.1016/S1360-1385(99)01540-X. PubMed

Faleiro, J. (2006). A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Rhynchophoridae*) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci. *26* (*3*), 135–154.

Gordon, K.H., and Waterhouse, P.M. (2007). RNAi for insect-proof plants. Nat Biotechnol *25* (*11*), 1231–1232 https://doi.org/10.1038/nbt1107-1231. PubMed

Kehat, M. (1999). Threat to date palms in Israel, Jordan and the Palestinian Authority by the red palm weevil, *Rhynchophorus ferrugineus.* Phytoparasitica *27* (*3*), 241–242 https://doi.org/10.1007/BF02981465.

Kehr, J., and Kragler, F. (2018). Long distance RNA movement. New Phytol *218* (*1*), 29–40 https://doi.org/10.1111/nph.15025. PubMed

Llácer, E., Dembilio, O., and Jacas, J.A. (2010). Evaluation of the efficacy of an insecticidal paint based on chlorpyrifos and pyriproxyfen in a microencapsulated formulation against *Rhynchophorus ferrugineus* (*Coleoptera*: *curculionidae*). J Econ Entomol *103* (*2*), 402–408 https://doi.org/10.1603/EC09310. PubMed

Petrick, J.S., Brower-Toland, B., Jackson, A.L., and Kier, L.D. (2013). Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: a scientific review. Regul Toxicol Pharmacol *66* (*2*), 167–176 https://doi.org/10.1016/j.yrtph.2013.03.008. PubMed

Price, D.R., and Gatehouse, J.A. (2008). RNAi-mediated crop protection against insects. Trends Biotechnol *26* (*7*), 393–400 https://doi.org/10.1016/j.tibtech.2008.04.004. PubMed

Pugliese, M., Rettori, A.A., Martinis, R., Al-Rohily, K., Velate, S., Moideen, M.A., and Al-Maashi, A. (2017). Evaluation of the efficacy of insecticidal coatings based on teflutrin and chlorpyrifos against *Rhynchophorus ferrugineus.* Pest Manag Sci *73* (*8*), 1737–1742 https://doi.org/10.1002/ps.4527. PubMed

Rodrigues, T.B., Mishra, S.K., Sridharan, K., Barnes, E.R., Alyokhin, A., Tuttle, R., Kokulapalan, W., Garby, D., Skizim, N.J., Tang, Y.W., et al. (2021). First sprayable double-stranded RNA-based biopesticide product targets proteasome subunit Beta Type-5 in Colorado potato beetle (*Leptinotarsa decemlineata*). Front Plant Sci *12*, 728652 https://doi.org/10.3389/fpls.2021.728652. PubMed

San Miguel, K., and Scott, J.G. (2016). The next generation of insecticides: dsRNA is stable as a foliar-applied insecticide. Pest Manag Sci *72* (*4*), 801–809 https://doi.org/10.1002/ps.4056. PubMed

Wang, L., Zhang, X.W., Pan, L.L., Liu, W.F., Wang, D.P., Zhang, G.Y., Yin, Y.X., Yin, A., Jia, S.G., Yu, X.G., et al. (2013). A large-scale gene discovery for the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *curculionidae*). Insect Sci *20* (*6*), 689–702 https://doi.org/10.1111/j.1744-7917.2012.01561.x. PubMed

Feeding preference of the red palm weevil (*Rhynchophorus ferrugineus* Oliver) toward *Phoenix canariensis* Hortum ex Chabaud

M.A. Arif[1,2,a], P. Lo Bue[2], E. Peri[2], S. Colazza[2] and S. Guarino[3]

[1]Plant Protection Directorate, Ministry of Agriculture, Abu-Ghraib, Baghdad 10081, Iraq; [2]Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Palermo, Italy; [3]Institute of Biosciences and Bio-resources (IBBR), National Research Council of Italy (CNR), Palermo, Italy.

Abstract

***Rhynchophorus ferrugineus* Olivier (*Coleoptera: Curculionidae*), commonly called the red palm weevil (RPW), is one of the most severe palm pests of the tropics and Mediterranean countries. In these latter countries, this pest attacked mainly *Phoenix canariensis*, the most used ornamental palm for gardens and urban green spots. However, still is not understood if this preference for this host palm is determined by the attractiveness of its volatile organic compounds (VOCs) or to resistance/avoidance mechanisms present in the other palm species. In this study we investigate, in electro-physiological and behavioral experiment, the role of the *P. canariensis* VOCs in this host preference behavior versus other three palm species: *Chamaerops humilis*, *Trachycarpus fortunei* and *Washingtonia filifera*. In EAG recordings *P. canariensis* VOC extracts, elicited a stronger response on RPW antennae rather than solvent or *C. humilis* extract, while similar responses were recorded using *W. filifera* and *T. fortunei* extracts. In dual choice arena bioassays, females of RPW showed feeding preference for the *P. canariensis* in comparison with *T. fortunei* and *C. humilis*. Overall, the present findings suggest that this preference behavior is mediated by VOCs emitted.**

Keywords: EAG, bioassays, *Chamaerops humilis*, *Trachycarpus fortunei*, *Washingtonia filifera*

INTRODUCTION

Rhynchophorus ferrugineus Olivier (*Coleoptera: Curculionidae*), commonly called the red palm weevil (RPW), is one of the most severe palm pests of the tropical regions and Mediterranean countries (Murphy and Briscoe, 1999; Gindin et al., 2006; Guarino et al., 2013, Peri et al., 2017; Suma et al., 2017) (Figure 1). However, while in the Middle East, in India and in northern Africa the RPW attacks date, oil and coconut palms and is mainly an agricultural problem, in Europe it mainly affects ornamental palms. After its accidental introduction in the European countries of the Mediterranean basin, the RPW determined, over the years, a progressive impact on the green spots of urban environments (Ferry and Gomez, 2002; Kontodimas et al., 2006; Schimmenti et al., 2017; Soroker and Colazza, 2017). In these locations RPW attacked mainly *Phoenix canariensis* Hort. ex Chabaud, the Canary island palm. As this species is used for ornamental purposes (Noto and Romano, 1985), RPW became a serious pest of the urban, monumental and country landscape. In these environments, more rarely, other palm species have been infested by the weevil, such as *Phoenix dactylifera* L. (Faleiro, 2006), *Washingtonia* spp., *Trachycarpus fortunei* Wendel (Ju et al., 2011) and *Chamaerops humilis*, the latter the only native palm species of the Mediterranean basin (Longo et al., 2011) previously reported as resistant to *R. ferrugineus* (Barranco et al., 2000).

Observations conducted in the Autonomous Community of Valencia from 2004 to 2009, evidenced that *R. ferrugineus* killed about 20,000 palms the majority of them belonging to the species *P. canariensis* (Dembilio and Jacas, 2011). Similarly, other observations conducted from 2007 to 2010 in Sicily revealed that on a total of about 20,000 palms killed by the RPW, the 99.7% was belonging to the species *P. canariensis* while the remaining 0.3% belonged to

[a]E-mail: mokhtar.a.arif@gmail.com

other palm species (Peri et al., 2013). Raciti et al. (2013) observed the different palm species population reduction between the 2006 and 2012 in historical gardens of Sicily caused by RPW: Canary island palms were reduced of almost the 90%, *W. filifera* palms were reduced of the 4.19% while *T. fortunei* and *C. humilis* were only marginally attacked.

Figure 1. Red palm weevil (*Rhynchophorus ferrugineus* Oliver).

The higher activity of the RPW observed on *P. canariensis* could be determined by a stronger attraction of the Canary date palm, mediated by volatile compounds, or by antibiosis or avoidance mechanism performed by the other palms that in this way become somewhat resistant (Cangelosi et al., 2015). RPW was observed to strongly respond to "palm esters" in EAG studies (Guarino et al., 2013), however, these compounds have not been behaviorally proved to be attractive used alone but as co-attractant with the aggregation pheromone, as they are produced in fermenting tissues rather than in sane healthy tissues. To date the RPW response to palm volatiles has been evidence only for these fermenting compounds that in fact have been exploited positively to enhance the captures in the traps. However, the possible role of volatiles produced from healthy palms are to be clarified. It is also to be stated that olfactory cues that are detected from insect for host plant findings are rarely made by a taxonomically characteristic compound but rather, in many cases, insects recognize plants by detecting blends of ubiquitous compounds (Birkett et al., 2004; Bruce et al., 2005; Bruce and Pickett, 2011; Guarino et al., 2018). So it is likely that the RPW may use particular blend mixtures of compounds produced by the different palm species or rather than a specific compound to orient and recognize the host.

Objectives of our study were to address the RPW antennal response to the VOCs emitted from potential host palm species and if in choice conditions, RPW prefer to feed on *P. canariensis* palms tissues rather than *C. humilis*, *W. filifera* or *T. fortunei*. In this research, the following questions have been addressed: are the volatiles of *P. canariensis* more evidently perceived from the RPW adults rather than the other palm volatiles? Do the RPW individuals prefer to feed on of *P. canariensis* rather than on other palm tissues?

MATERIALS AND METHODS

Insects

RPW adults used for bioassays were collected from captures in pyramidal Picusan® pheromone traps (Sansan, Spain) and from cut infested Canary Island palms, located in urban areas in Palermo, Italy. The insects were sexed and kept separated in groups of up to 50 individuals each. Weevil groups were maintained in environmentally controlled rooms (25±1°C, 70±10% RH, photo period 14:10 L:D), in plastic cages (20×20×40 cm) with two 5-cm-diameter mesh-covered holes, and fed with 'Golden Delicious' apples until used for experiments.

Plants

Plants of *P. canariensis*, *T. fortunei*, *W. filifera* and *C. humilis*, of two years of age, of approximately 1 m height, were recovered from nursery plantation from Marsala (Italy) and kept in glass house until used for the experiments. Plants were kept in pots of 40 cm of diameter, and irrigated two time per week until used for the experiments.

EAG puff tests

Palm VOCs from *P. canariensis*, *T. fortunei*, *W. filifera* and *C. humilis* were tested on RPW antennae in EAG puff tests.

In order to collect palm volatiles from the different species, VOC collections were conducted in all plexiglas chambers of 110×50×50 cm where palms were placed individually. Humidified and charcoal filtered air was drawn through the chambers (flow 0.3 L min^{-1}) for 24 h. The volatiles were trapped in glass collectors (6 mm ID) filled with 70 mg of 18-35 mesh Porapak Q (Supelco, Bellefonte, PA) held in place by glass wool plugs. The VOC collections were carried out close to a window to provide a source of natural daylight, and two fluorescent light bulbs (Lival, 220V-11 W, Finland) controlled by a timer located above aeration apparatus to provide supplementary light source with a 16L:8D photo period. Collectors were prepared a few minutes before the start of a collection, then, at the end of the aeration period, were eluted with hexane (200 µL). For EAG puff test, a standard 1-µL aliquot of each palm extracted VOCs was pipetted onto a piece of filter paper (Whatman, n. 1), exposed to the air for 20 s to allow the solvent to evaporate, and then inserted into a glass Pasteur pipette. One µL of pure hexane was puffed as control. For the puff test, a stimulus-flow controller (model CS-05; Synthech; Hilversum, The Netherlands) was used to generate a 1.5-s stimulus at 1-min intervals, with a flow rate of 1.5 L min^{-1}. The RPW antenna was excised from the heads and was suspended above two silver wire electrodes using glass capillary tubes filled with 0.1 M KCl solution. The signals generated by the antennae were passed through a high-impedance amplifier (model IDAC-232; Synthech; Hilversum, The Netherlands) and recorded with the specialized software (EAG Pro, Synthech; Hilversum, The Netherlands). The sequence of the stimuli of each of the tested compounds and of control was randomized. Each one of the four extracts was tested on one randomly selected antenna per females (n=13).

Laboratory bioassays

In behavioral experiments the feeding activity of RPW females was assessed in dual choice tests using *P. canariensis* versus *W. filifera*, *T. fortunei* and *C. humilis*. These experiments were carried out using cages (size 30×10×8 cm) made by zinc-sheet with a bottom made by metal net (holes size 3.35 mm^2) that allows the females RPW to feed in the substrates placed below. The cage was provided with a cap with metal net for ventilation (holes size 1.00 mm^2). Underneath the cage bottom, at the two opposite sides, two palm slices (slice deep 1±0.1 cm; surface 14.15±1 cm^2) one of *P. canariensis* and one from the three tested palm species were placed as substrate for female feeding. Only the apical portion of each plant (i.e., first 25 cm from the meristematic apex) was used for the experiments. The substrates were sandwiched in two filter paper sheets (Whatman no. 1). Experiments lasted 24 h starting at 10:00 am and were carried out in dark condition in an environmentally controlled room at 25±1°C. RPW females were introduced in the cage individually and left free to feed in the *P. canariensis* or in the other tested palm substrates. To verify possible bias due to the structure of the used cages, blank bioassays were carried out using both palm slices of *P. canariensis* called "A" and "B". Females were starved for 24 h before the trials. Each one was tested only once. The number of holes bored during substrate probing and feeding was counted in the different substrates was counted at the end of the trial to estimate the RPW host feeding preference activity.

Statistical analysis

The responses of RPW antenna evoked in the two sexes by the different stimuli were analyzed using a repeat-measure ANOVA and statistically separated by Fisher's LSD test. To assess substrate preference for feeding activity of RPW adults, the number of feeding holes in the tested substrates for each replicate was counted, log transformed and compared by t-test for dependent samples (Statistica for Windows 10.0).

RESULTS

EAG puff test

Mean EAG responses evoked on RPW antennae by the different palm volatiles and from the solvent are shown in Figure 2. RPW females showed different responses to the palm volatiles and/or the solvent ($F_{4, 32}$=2.9272; P=0.036; ANOVA). In particular *P. canariensis* volatiles determined a stronger EAG responses compared to solvent (P=0.009) and to volatiles of *C. humilis* (P=0.035). *Trachycarpus fortunei* volatiles determined a stronger EAG responses compared to solvent (P=0.016), while compared to response determined from volatiles of *C. humilis* values were marginally non-significant (P=0.058).

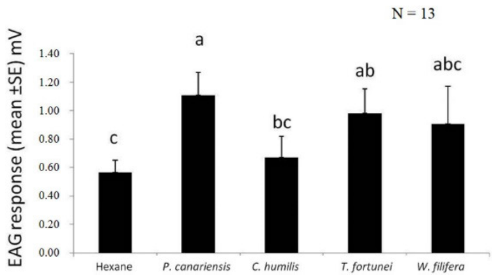


Figure 2. Mean (+SE) EAG responses of RPW antennae to puff test using 1 μL of the plant extracts tested. Columns labeled with a common letter do not differ significantly at P<0.05 (ANOVA followed by Fisher's LSD test).

No significant differences in the EAG responses were observed between the sexes ($F_{1, 8}$=0.39198; P=0.548; ANOVA). No treatment per sex interaction was observed ($F_{4, 32}$=1.2068; P=0.32708; ANOVA).

Laboratory bioassays

The results of the behavioral bioassays carried out in experimental cages are reported in Figure 3. Females RPWs prefer to feed on *P. canariensis* substrate in comparison with the *T. fortunei* substrate (t=2.10; P<0.05) and the *C. humilis* substrate (t=2.52; P<0.02). No statistical differences were observed in the feeding activity between the *P. canariensis* and *W. filifera* substrates (t=0.46; P=NS).

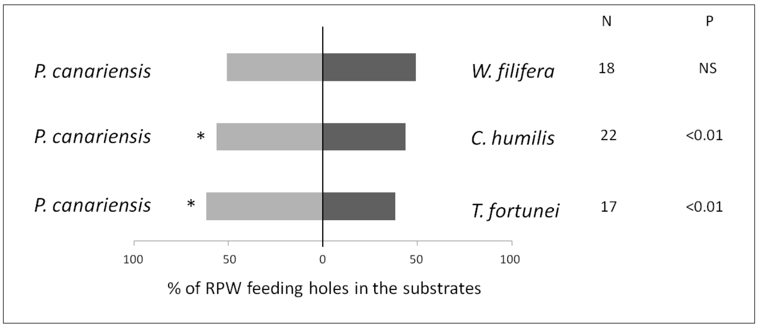


Figure 3. Percentages of feeding holes bored by female RPWs in the tested substrates (paired t-test).

DISCUSSION

The results described above provide evidence that *P. canariensis* is a preferred host for RPW, compared to *T. fortunei* end *C. humilis*, and that the VOCs emitted by the plants mediate this preference response. On the contrary, no preference has been exhibited between *P.*

canariensis and *W. filifera*.

In particular, in EAG experiments, VOC crude extracts from *P. canariensis* elicited EAG response on RPW females indicating the presence of a higher concentration of EAG active compounds in the VOC emission of this species. This is the first report of EAG response of RPW females to VOCs emitted from whole healthy plants. Previous studies indicate the responsiveness of the RPW to the so-called palm esters (Guarino et al., 2013). However, these compounds result more strongly produced from wounded palm tissues. Previous studies evidenced the responsiveness of the RPW antennae to a broad range of compounds, some of them reported in the VOCs from freshly cut or fermenting tissues of *P. canariensis* (Guarino et al., 2013).

Our behavioral experiments indicate a feeding preference for the *P. canariensis* tissues rather than on *C. humilis* and *T. fortunei* ones while no preference response was observed comparing *P. canariensis* with *W. filifera*. This preference response of the RPW might be linked with the quality of the substrate as important factor influencing the fitness of this pest. For example, a previous study showed the role of the diet on the micro-biota of RPW digestive system (Montagna et al., 2015). This aspect is particularly important in consideration that, for wood borers such as the RPW, the symbioses with cellulose-degrading microorganisms appear to compensate for the inability to synthesize cellulases (Muhammad et al., 2017).

On the other hand, the possibility that the RPW adults prefer to avoid feeding in less suitable substrates cannot be underestimated, in fact Barranco et al. (2000) observed in forced infestation tests, that *C. humilis* produce a plant secretion that killed the larvae of RPW. Furthermore, Dembilio et al. (2009) asserted that *C. humilis* are resistant to *R. ferrugineus* by an antixenotic mechanism.

In accordance with our behavioral observations, Ju et al. (2011) reported that, based on population growth parameters, *P. canariensis* and *W. filifera* are the more suitable hosts plants for RPW rather than *T. fortunei* and other palms. However, other authors observed the presence of resistance in *W. filifera* toward the RPW based on antibiosis mechanism (Dembilio et al., 2009). In particular, a study carried out from Cangelosi et al. (2015) showed the presence of the filiferol, a chalconoid analog, with larvicidal activity, in *W. filifera* leaves and absent in other palm species such as *P. canariensis* and *P. dactylifera*. Although in our research we did not find host preference response between the *P. canariensis* and *W. filifera* substrate, this might be determined by the possibility that these defensive compounds could be located only at the base of the leaves and not elsewhere (Cangelosi et al., 2015).

The results obtained in such studies suggest that *P. canariensis* is the most suitable host palm for RPW. Our findings evidence a stronger antennal sensitivity to the Canary Island palm volatiles and a higher feeding activity exhibited on *P. canariensis* tissues, suggesting a role of the plant metabolites in this host finding and acceptance behavior of RPW.

Literature cited

Barranco, A., Martin, M., and Cabello, T. (2000). Host rank for *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) (Olivier, 1790) and host palm tree diameter. Boletin de Sanidad Vegetal Plagas *26* (*1*), 73–78.

Birkett, M.A., Agelopoulos, N., Jensen, K.M., Jespersen, J.B., Pickett, J.A., Prijs, H.J., Thomas, G., Trapman, J.J., Wadhams, L.J., and Woodcock, C.M. (2004). The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. Med Vet Entomol *18* (*4*), 313–322 https://doi.org/10.1111/j.0269-283X.2004.00528.x. PubMed

Bruce, T.J., and Pickett, J.A. (2011). Perception of plant volatile blends by herbivorous insects–finding the right mix. Phytochemistry *72* (*13*), 1605–1611 https://doi.org/10.1016/j.phytochem.2011.04.011. PubMed

Bruce, T.J., Wadhams, L.J., and Woodcock, C.M. (2005). Insect host location: a volatile situation. Trends Plant Sci *10* (*6*), 269–274 https://doi.org/10.1016/j.tplants.2005.04.003. PubMed

Cangelosi, B., Clematis, F., Monroy, F., Roversi, P.F., Troiano, R., Curir, P., and Lanzotti, V. (2015). Filiferol, a chalconoid analogue from *Washingtonia filifera* possibly involved in the defence against the Red Palm Weevil *Rhynchophorus ferrugineus* Olivier. Phytochemistry *115*, 216–221 https://doi.org/10.1016/j.phytochem.2015.02.008. PubMed

Dembilio, O., and Jacas, J.A. (2011). Basic bio-ecological parameters of the invasive red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*), in *Phoenix canariensis* under Mediterranean climate. Bull Entomol Res *101* (*2*), 153–163 https://doi.org/10.1017/S0007485310000283. PubMed

Dembilio, Ó., Jacas, J.A., and Llácer, E. (2009). Are the palms *Washingtonia filifera* and *Chamaerops humilis* suitable hosts for the red palm weevil, *Rhynchophorus ferrugineus* (*Col. Curculionidae*)? J. Appl. Entomol. *133* (*7*), 565–567 https://doi.org/10.1111/j.1439-0418.2009.01385.x.

Faleiro, J. (2006). A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Rhynchophoridae*) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci. *26* (*3*), 135–154.

Ferry, M., and Gomez, S. (2002). The red palm weevil in the Mediterranean area. Palms *46* (*4*), 172–178.

Gindin, G., Levski, S., Glazer, I., and Soroker, V. (2006). Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus.* Phytoparasitica *34* (*4*), 370–379 https://doi.org/10.1007/BF02981024.

Guarino, S., Peri, E., Lo Bue, P., Germanà, M.P., Colazza, S., Anshelevich, L., Ravid, U., and Soroker, V. (2013). Assessment of synthetic chemicals for disruption of *Rhynchophorus ferrugineus* response to attractant-baited traps in an urban environment. Phytoparasitica *41* (*1*), 79–88 https://doi.org/10.1007/s12600-012-0266-9.

Guarino, S., Arif, M.A., Millar, J.G., Colazza, S., and Peri, E. (2018). Volatile unsaturated hydrocarbons emitted by seedlings of *Brassica* species provide host location cues to *Bagrada hilaris*. PLoS One *13* (*12*), e0209870 https://doi.org/10.1371/journal.pone.0209870. PubMed

Ju, R.-T., Wang, F., Wan, F.-H., and Li, B. (2011). Effect of host plants on development and reproduction of *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). J. Pest Sci. *84* (*1*), 33–39 https://doi.org/10.1007/s10340-010-0323-4.

Kontodimas, D., Milonas, P., Vassiliou, V., Thymakis, N., and Economou, D. (2006). The occurrence of *Rhynchophorus ferrugineus* in Greece and Cyprus and the risk against the native Greek palm tree *Phoenix theophrasti.* Entomol. Hell. *16*, 11–15 https://doi.org/10.12681/eh.11621.

Longo, S., Anderson, P., Smith, T., Stanley, J., and Inserra, R. (2011). New palm hosts for the red palm weevil, *Rhynchophorus ferrugineus*, in Sicily. Palms *55* (*1*), 15–20.

Montagna, M., Chouaia, B., Mazza, G., Prosdocimi, E.M., Crotti, E., Mereghetti, V., Vacchini, V., Giorgi, A., De Biase, A., Longo, S., et al. (2015). Effects of the diet on the microbiota of the red palm weevil (*Coleoptera*: *Dryophthoridae*). PLoS One *10* (*1*), e0117439 https://doi.org/10.1371/journal.pone.0117439. PubMed

Muhammad, A., Fang, Y., Hou, Y., and Shi, Z. (2017). The gut entomotype of red palm weevil *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Dryophthoridae*) and their effect on host nutrition metabolism. Front Microbiol *8*, 2291 https://doi.org/10.3389/fmicb.2017.02291. PubMed

Murphy, S. and Briscoe, B. (1999). The red palm weevil as an alien invasive: biology and the prospects for biological control as a component of IPM. Bio-Control News and Information *20*, 35N–46N.

Noto, G., and Romano, D. (1985). Palms in the urban environment in the southern latitudes of Italy. The Scientific Management of Vegetation in the Urban Environment *195*, 91–97.

Peri, E., Colazza, S., Guarino, S., Suma, P., Pergola, A.l., and Longo, S. (2013). The red palm weevil in Sicily: the introduction and spread of an invasive alien species. Paper presented at: Colloque Méditerranéen sur les Ravageurs des Palmiers (Nice, France: Association Française de Protection des Plantes (AFPP)).

Peri, E., Rochat, D., Belusic, G., Ilic, M., Soroker, V., Barkan, S., Guarino, S., Lo Bue, P., and Colazza, S. (2017). *Rhynchophorus ferrugineus*: behaviour, ecology, and communication. Handbook of Major Palm Pests: Biology and Management, V. Soroker, and S. Colazzo, eds. (Wiley), p.105–130.

Raciti, E., Conti, F., Cerrella, D.C., Morabito, M., Malfitana, S., and Romano, D. (2013). Palm species potentially resistant to red palm weevil attacks in sites of eastern Sicily heavily infested. Paper presented at: Colloque Méditerranéen sur les Ravageurs des Palmiers (Nice, France: Association Française de Protection des Plantes (AFPP)).

Schimmenti, E., Borsellino, V., Ingrassia, G., Peri, E., Farina, V., and Guarino, S. (2017). Urban landscape evolution as a consequence of an invasive pest: the case of a small Sicilian town. Landsc. Online *52*, 1–16 https://doi.org/10.3097/LO.201752.

Soroker, V., and Colazza, S. (2017). Handbook of Major Palm Pests: Biology and Management (John Wiley & Sons).

Suma, P., Peri, E., La Pergola, A., Soroker, V., Dembilio, O., Riolo, P., and Nardi, S. (2017). Action Programs for *Rhynchophorus ferrugineus* and *Paysandisia archon*. Handbook of Major Palm Pests: Biology and Management, V. Soroker, and S. Colazzo, eds. (Wiley), p.280–299.

Curative treatment of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in infested date palms using an innovative fumigation technique

S.R. Al-Ballaa[a]

Infectious Diseases and Medical Microbiology, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Abstract

The red palm weevil (RPW) *Rhynchophorus ferrugineus* Olivier (*Coleoptera: Curculionidae*) is a key pest of date palm *Phoenix dactylifera* L. in the Near-East and North Africa region. RPW infested date palms respond to curative chemical treatments if detected and judiciously treated in the early stage of attack. However, the currently used curative treatments, involve either excessive tissue removal of the palm around the infested palm section (mechanical sanitization) making the palm weak and vulnerable to toppling, injecting insecticide into the infested palms which often does not kill all the stages of the pest within the palm, calling for repeated applications or fumigating the infested palm section with phosphine gas which is also not always effective possibly due to inadequate dose and escape of the gas. A new fumigation technique using a specially designed air tight fumigation sleeve to ensure complete entrapment of the fumigant gas or liquid was devised and field tested on (463) infested dates palms in various stages of attack using either aluminum phosphide tablets (3 g) or one of commonly used insecticides that has fumigant action. The trials were conducted in Al-Qassim, Kingdom of Saudi Arabia, during 2017-2020. The technique was tested in both young date palms (9-12 years old) as well in offshoots (4 years old) through a series of field trials to optimize the dose, duration of treatment. Results revealed that 100% mortality of the larval, pupal and adult stages of the pest inside the infested palm could be achieved within 5 days due to the fumigant action of either of the following treatments: 15 tablets (3 g) of aluminum phosphine gas, 120 mL of Deltamethrin 10% EC, 150 mL of Chlorpyriphos 48% EC, 150 mL of Marqiuse™ (combination of Phoxim 15%, Cypermethrin 5% and Monosultap 20%), 150 mL of Malathion 57% EC, 150 mL of Diazinon 60% EC or 250 mL Cypermethrin 25% EC. The method offers a simple and curative treatment of RPW infested palms and is currently the main treatment method used in Saudi Arabia and many other regions.

Keywords: *Rhynchophorus ferrugineus*, Saudi Arabia, curative treatment, fumigation, insecticides, aluminum phosphide, mechanical injection, date palm

INTRODUCTION

The red palm weevil (RPW) *Rhynchophorus ferrugineus* Olivier (*Coleoptera: Curculionidae*) is a key pest of date palm *Phoenix dactylifera* L. (Faleiro, 2006). The pest is reported to attack 40 palm species in diverse agro-ecosystems worldwide (Giblin-Davis et al., 2013) and likely to expand its geographical range (Fiaboe et al., 2012).

RPW is an internal tissue borer that is difficult to detect and treat. Palms in the late stage of attack exhibit extensive tissue damage due to larval feeding. Such palms harbour several overlapping generations of RPW and are beyond any curative treatment and have to be eradicated. RPW infested date palms respond to curative chemical treatments if detected and judiciously treated in the early stage of attack (Abraham et al., 1998). However, the currently used curative treatments, involve either excessive tissue removal of the palm around the infested palm section (mechanical sanitization) often making the palm weak and vulnerable to toppling, injecting insecticide into the infested palms which does not always kill all the

[a]E-mail: sballaa@gmail.com

stages of the pest within the palm, calling for repeated applications, or fumigating the infested palm section with phosphine gas which is also not always effective due to inadequate dose and escape of the gas. Although, treatment of RPW infested date palms using aluminum phosphide is widely practiced in UAE and the state of Bahrain, there is no information on the dose and duration of treatment using aluminum phosphide to safely and effectively treat RPW infested date palms.

In this study, treatment of RPW infested date palms using a new fumigation technique with either aluminum phosphide tablets or one of six commonly available insecticides that are known to passes fumigant action (Chlorpyriphos, Cypermethrin, Marqiuse™, Deltamethrin, Malathion and Diazinon) was extensively evaluated in the Kingdom of Saudi Arabia with an objective to standardise the dose and duration of treatment for the safe and effective use of each of them. The protocols adopted and results obtained are presented below.

MATERIALS AND METHODS

A series of Laboratory and field experiments were conducted by Rasheed Mohammed Al Ballaa and Munira Mohammed Al-Hothaili Endowment fund (May the Mercy of Allah be upon them) in Al-Qassim region of Saudi Arabia between 2017 and 2020 with an aim to develop an effective, low-cost and easy to apply curative treatment method for control of RPW in infested palm trees using aluminum phosphide or one of six commonly available insecticides.

The fumigant action of those insecticides against larval and adult stages of RPW was first assessed for mortality in the laboratory to gauge the pronounced fumigant action of different insecticides. Here, 50 mL of concentrated insecticide was placed in an open container inside a 1000-mL air tight glass cylinder in which adult insects and larvae were placed on the floor of the cylinder subsequent to which the cylinder was closed.

The insects were observed every 15 min and the time to death was recorded. Only insecticides that resulted in 100% mortality of adult weevils within 3 h were used in further field trials (Figure 1).

Figure 1. Preliminary laboratory assays to assess the fumigant action of common insecticides and acetone.

The field trials were carried out in 463 infested date palms in different stages of attack by RPW using one of those insecticide or aluminum phosphide (3 g)

The trials were based on the hypothesis that “RPW needs oxygen to survive, air reaches RPW stages within the palm tree through the feeding tunnels cavities, the tunnels are connected to outside air and can be used to deliver fumigants”. Keeping this in view a new fumigation protocol involving treatment of infested date palms with aluminum phosphide tablets (3 g, 50-60% a.i.) or any of the insecticide indicated above that ensure complete entrapment of fumigant gas was devised and field tested in 463 infested palms in various stages, the results are reported in this study. The material and apparatus used to assemble a gas entrapment chamber on the infested palm is depicted in Figure 2.

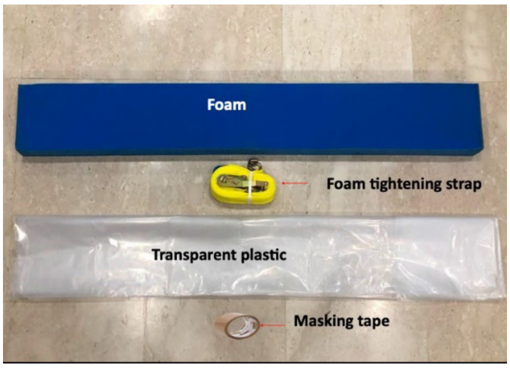


Figure 2. Material and apparatus used to assemble a gas entrapment cylinder/chamber on the RPW infested palm.

Entrapment of the phosphine gas and the fumes of other insecticides around the infested site in the palm tree (usually the trunk) was ensured by securing a plastic wrap (4 m long, 2 m wide, 150 microns thick) on two foam pieces (toluene diisocyanate foam (2×0.3×0.15 m) pressure 40) encircling the palm above and below the infested site. The foam pieces were secured around the palm trunk initially by using an adhesive tape. In case of using aluminum phosphide, the tablets were placed directly on the palm trunk around the infested site as per the treatment schedule mentioned in Table 1. In case of using insecticides of a measured quantity of each insecticide was diluted with water to make a 3-L treatment solution that was sprayed in the infested part of the trunk, about 1 m long, between the two foam pieces as per the treatment schedule mentioned in Table 2. Subsequently, the plastic wrap was fastened to the foam at both the ends using masking tape, which was also used to seal both the longitudinal ends of the plastic wrap then the tightening strap was applied on the middle of the width of the two foams and tightened to the maximum limit using the mechanical tightening apparatus (Figure 3), the detailed treatment protocol is presented in Table 1.

Figure 3. Preparing RPW infested palms for fumigation treatment in the field. A) Securing foam pieces around the short-pruned palm trunk using adhesive tape; B) Placing the aluminum phosphide tablet on the trunk (in case of using aluminum phosphide treatment method); C) Infested palm site of the palm trunk between the two foam pieces sprayed with insecticide treatments (in case of using insecticides); D) Plastic wrap fastened to the foam at both the ends using adhesive tape and the tightening straps are applied; E) Bottom of the plastic sheet compacted with soil at ground level forming an air tight sleeve.

The technique was tested in both young date palms (9-12 years old) as well in offshoots (4 years old), through a series of field trials to optimize the number of applications, dose, duration of treatment and type of wrapping to entrap the gas.

The treatments protocols tested for aluminum phosphide tables are presented in Table

2 while that for the 6 insecticide used are presented in Table 3.

Table 1. Protocol adopted to treat RPW infested date palms with aluminum phosphid.

Grass/weeds around the trunk of the palm were removed
The trunk of the palm was pruned very short to have maximum 5 cm frond base using a manual or automatic saw so as to remove any palm parts that may puncture the plastic Note: There is no need to clean the site of the infestation on the palm and remove the insect stages
Two pieces of foam were carefully installed around the trunk of the palm so that the first was at a level of about 1.25 m above entry site of the pest and the second at a level of about 1.25 m below entry site or at the level of the soil surface if the infestation site was low, and held the foam in place using the adhesive masking tape
Aluminum phosphide tablets were placed around the trunk of the palm as per the treatment schedule presented above in Table 1 near the entry site (aluminum phosphide tablets usually start emitting toxic phosphine gas not less than 1 h after exposure to air)
In case of using insecticides a measured quantity of each insecticide was diluted with water to make a 3-L treatment solution Table 2 that was sprayed on the infested part of the trunk, about 1 m long, between the two foam pieces
The plastic sheet was placed neatly and quickly around the trunk of the palm so that it became two continuous layers of plastic around the trunk and was fixed in place using the adhesive masking tape
The tightening belt (strap) was placed on the plastic sheet on the middle of the width of the foam. The belt was then tightened so that the tightening strength reaches maximum to prevent phosphine gas from escaping
The tightening belt was also placed around the plastic sheet on the middle of bottom foam and the belt was tightened in the manner described above
Soil was placed above the bottom of plastic sheet and the soil was compacted
On completion of the treatment duration the plastic sheet was removed quietly and carefully after removing the tightening belts from the top and bottom. The plastic sheet and belt can be used again several times provided it is not punctured

Table 2. Aluminum phosphide treatment imposed in RPW infested date palms.

Treatment	Number of palms treated	Severity of infestation	Distance of infestation from ground (cm)	Average age of treated palm (years)
T1	100	Mild to severe	40-90	12
T2	50	Mild to severe	40-80	12
T3	50	Mild to severe	40-80	12
T4	25	Mild to severe	40-80	12
T5	50	Mild to severe	10-60	6
T6	10	Mild to severe	40-80	12
T7	10	Mild to severe	40-80	12

T1: 20 Tablets for 10 days applied in 3 splits of 10, 5 and 5 at 1,3 and 6 days, respectively (transparent plastic wrap); T2: 15 tablets for 10 days (transparent plastic wrap); T3: 15 tablets for 5 days (transparent plastic wrap); T4: 10 tablets for 10 days (transparent plastic wrap); T5: 10 tablets for 5 days (black plastic wrap); T6: 5 tablets for 10 days (transparent plastic wrap); T7: control (no treatment).

Table 3. Insecticide treatments tested for fumigant action against *R. ferrugineus* infested date palms.

No.	Treatment[a]
T1	Chlorpyriphos 48% EC, 60 mL for 10 days
T2	Chlorpyriphos 48% EC, 120 mL for 10 days
T3	Chlorpyriphos 48% EC, 150 mL for 10 days
T4	Chlorpyriphos 48% EC, 150 mL for 5 days
T5	Cypermethrin 25% EC, 150 mL for 10 days
T6	Cypermethrin 25% EC, 250 mL for 10 days
T7	Cypermethrin 25% EC, 250 mL for 5 days
T8	Marqiuse™ 120 mL for 10 days
T9	Marqiuse™ 150 mL for 10 days
T10	Marqiuse™ 250 mL for 10 days
T11	Marqiuse™ 150 mL for 5 days
T12	Deltamethrin 10% EC,30 mL for 10days
T13	Deltamethrin 10% EC, 60 mL for10 days
T14	Deltamethrin 10% EC, 90 mL for 10 days
T15	Deltamethrin 10% EC, 90 mL for 5 days
T16	Deltamethrin 10% EC, 120 mL for 5 days
T17	Malathion 57% EC, 120 mLl for 10 days
T18	Malathion 57% EC, 150 mL for 10 days
T19	Malathion 57% EC, 150 mL for 5 days
T20	Diazinon 60% EC, 120 mL for 10 days
T21	Diazinon 60% EC, 150 mL for 10 days
T22	Diazinon 60% EC, 150 mL for 5 days
T23	Control: Deltamethrin 10% EC, 90 mL for 10 days without cover
T24	Control: No insecticide for 10 days

[a]Each insecticide was diluted with water to make a 3-L treatment solution.

The above treatments were administered by staff using protective gear including, hand gloves, nasal mask, eye goggles and safety shoes.

In treatment T5 for using aluminum phosphide tablets (Table 2), black plastic wrap was used to assess its impact on treatment dose and duration.

Upon completion of the treatment duration, the plastic enclosure was removed and all treated palms were manually scrapped to remove all dead palm tissue and count the dead and live stages of the pest including larvae, pupae and adults. Data on pest mortality (%) were subjected to statistical analysis (ANOVA). Phosphine gas levels (ppm) were also measured inside the gas entrapment cylinder on the palm and also outside the treated palm between 6 to 96 h after treatment using (gas alert extreme), a portable phosphine gas detector. Results of the study are presented and discussed below.

RESULTS AND DISCUSSION

Results presented in Table 4 show that the treatment means were highly significant ($p<0.0001$) indicating that aluminum phosphide treatment of RPW infested palm trees using the innovative fumigation technique is very effective in killing all the hidden stages of the pest when 15 tablets were used for 5 days. However, what is required is to determine the minimum dose for the shortest possible time to obtain 100% mortality. Although the first three treatments resulted in 100% mortality of all the three stages of the pest, either the dose or the duration were on the higher side. In an effort to reduce the number of tablets used (dose/palm) and duration to treat an infested palm,10 aluminum phosphide tablets for 10 days in transparent plastic wrap (T4) was not satisfactory as this resulted in several live stages of the pest. Also, in T6 (5 tablets for 10 days with transparent plastic wrap), live stages of the pest were detected. However, in T5, when 10 tablets were used for 5 days in black plastic wrap, 100% mortality of the larval, pupal and adult stages was obtained (Table 4; Figure 4). It can be inferred that the black plastic wrap made the difference resulting in 100% mortality probably due to the longer half-life for phosphine gas in absence of exposure to light, allowing for higher phosphine gas level maintained for longer time.

Table 4. Mortality of different stages of RPW in date palm treated with aluminum phosphide.

Treatment name	% Mortality[a] Adults	Larvae	Pupae	Number of insect stages dead/alive
T1	100.00 A	100.00 A	100.00 A	5215/0
T2	100.00 A	100.00 A	100.00 A	2007/0
T3	100.00 A	100.00 A	100.00 A	2115/0
T4	94.00 B	99.50 B	96.00 B	787/8
T5	100.00 A	100.00 A	100.00 A	2185/0
T6	76.55 C	67.02 B	100.00 A	543/108
T7	0.00 D	0.00 C	0.00 C	0/100
p-value	<0.0001	<0.0001	<0.0001	
CV (%)	7.59	5.53	6	

T1: 20 Tablets for 10 days applied in 3 splits of 10, 5 and 5 at 1,3 and 6 days, respectively (transparent plastic wrap); T2: 15 tablets for 10 days (transparent plastic wrap); T3: 15 tablets for 5 days (transparent plastic wrap); T4: 10 tablets for 10 days (transparent plastic wrap); T5: 10 tablets for 5 days (black plastic wrap); T6: 5 tablets for 10 days (transparent plastic wrap); T7: control (no treatment).

[a]Means with at least one letter common are not statistically significant using Duncan's multiple range test.

Results presented in Table 5 for the different fumigant insecticides reveal that the treatment means were highly significant ($p<0.001$ for larva and adult and $p<0.05$ for pupa) indicating that the chemicals tested for treating RPW infested palms were very effective in killing the hidden stages of the pest. Furthermore, 100% mortality of the larva, pupa and adult stages of the pest inside the infested palm could be achieved within 5 days due to the fumigant action of either 120 mL Delta methrin 10% EC or 150 mL Chlorpyriphos 48% EC or 150 mL Marqiuse™ or 150 mL Malathion 57% EC or 150 mL Diazinon 60% EC or 250 mL Cypermethrin 25% EC. Lowering the dose resulted in delaying mortality to 10 days in case of Chlorpyriphos (120 mL) and Delta methrin (60 mL).

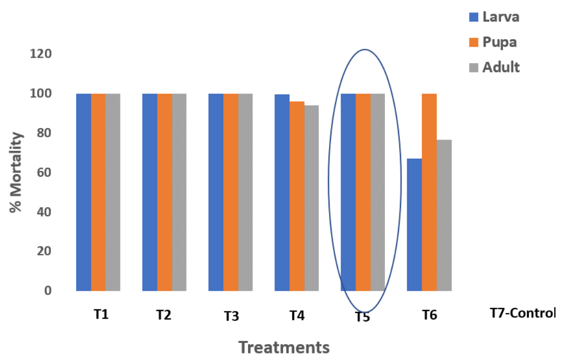


Figure 4. Mortality (%) of RPW in infested palms treated with aluminum phosphide. T1: 20 Tablets for 10 days applied in 3 splits of 10, 5 and 5 at 1,3 and 6 days, respectively (transparent plastic wrap); T2: 15 tablets for 10 days (transparent plastic wrap); T3: 15 tablets for 5 days (transparent plastic wrap); T4: 10 tablets for 10 days (transparent plastic wrap); T5: 10 tablets for 5 days (black plastic wrap); T6: 5 tablets for 10 days (transparent plastic wrap); T7: control (no treatment).

Table 5. Mortality of *R. ferrugineus* in infested date palms treated with different fumigant insecticides.

No.	Treatment[a]	No. of palms treated	Mortality %		
			Larva	Pupa	Adult
T1	Chlorpyriphos 48% EC, 60 mL for 10 days	10	95.00a	100.00a	100.00a
T2	Chlorpyriphos 48% EC, 120 mL for 10 days	10	100.00a	100.00a	100.00a
T3	Chlorpyriphos 48% EC, 150 mL for 10 days	5	100.00a	-**	100.00a
T4	Chlorpyriphos 48% EC, 150 mL for 5 days	5	100.00a	100.00a	100.00a
T5	Cypermethrin 25% EC, 150 mL for 10 days	10	100.00a	100.00a	99.38a
T6	Cypermethrin 25% EC, 250 mL for 10 days	10	100.00a	100.00a	100.00a
T7	Cypermethrin 25% EC, 250 mL for 5 days	5	100.00a	100.00a	100.00a
T8	Marqiuse™ 120 mL for 10 days	5	98.43a	100.00a	98.00a
T9	Marqiuse™ 150 mL for 10 days	10	100.00a	100.00a	100.00a
T10	Marqiuse™ 250 mL for 10 days	5	100.00a	100.00a	100.00a
T11	Marqiuse™ 150 mL for 5 days	5	100.00a	100.00a	100.00a
T12	Deltamethrin 10% EC,30 mL for 10 days	13	98.94a	100.00a	97.14a
T13	Deltamethrin 10% EC, 60 mL for10 days	10	100.00a	100.00a	100.00a
T14	Deltamethrin 10% EC, 90 mL for 10 days	10	100.00a	100.00a	100.00a
T15	Deltamethrin 10% EC, 90 mL for 5 days	5	98.26a	-	50.00b
T16	Deltamethrin 10% EC, 120 mL for 5 days	5	100.00a	-	100.00a
T17	Malathion 57% EC, 120 mL for 10 days	5	93.33a	-	66.67b
T18	Malathion 57% EC, 150 mL for 10 days	10	100.00a	100.00a	100.00a
T19	Malathion 57% EC, 150 mL for 5 days	5	100.00a	100.00a	100.00a
T20	Diazinon 60% EC, 120 mL for 10 days	5	87.43a	-	66.67b
T21	Diazinon 60% EC, 150 mL for 10 days	10	100.00a	100.00a	100.00a
T22	Diazinon 60% EC, 150 mL for 5 days		100.00a	100.00a	100.00a
T23	Control: Deltamethrin 10% EC, 90 mL for 10 days without cover	5	6.67b	0.00c	16.67c
T24	Control: no insecticide for 10 days	5	0.00b	0.00c	0.00c
P value			<0.01	<0.05	<0.01

[a]Each insecticide was diluted with water to make a 3L treatment solution.

- pupal stage missing.

Values followed by the same letter in the same column are not significantly different at the P level indicated for each column.

Fumigants can often provide effective, economic control where other forms of pest control are not feasible due to the unique characteristics and the great adaptability of the fumigation technique (Bond, 1984). 1-2 aluminum phosphide tablets have been used to treat RPW infested coconut and date palm (Lakshmanan et al., 1972; Subba Rao et al., 1973; Vidhyasagar et al., 2000). However, there are no data on the duration of treatment and the precise protocol adopted to ensure that there is no escape of phosphine gas after treatment. Furthermore, inadequate sealing of the infested site on the palms results in the escape of the phosphine gas. As in some GCC countries, in Saudi Arabia too farmers apply the fumigant aluminum phosphide to date palms by simply wrapping plastic sheet around the palm trunk and sealing the top and bottom of the sheath by mud or moist sandy soil, which is often inadequate (Almansoori et al., 2015; Al-Ayedh and Al-Jber, 2019). El-Shafie (2019) indicated the possibility of using curative treatment with aluminum phosphide for date palm borers including RPW, long horn beetle, *Jebusaea hammerschmidti* and rhinoceros beetle, *Oryctes* spp. and recommended more field work and research were needed to improve fumigation techniques of phosphine gas and elucidate its phytotoxicity to date palm before being recommended for the management of date palm borers. Recently an effective quarantine protocol, with exposure period of 72 h at 25°C using ECO2FUME (EF) with phosphine concentration of 1500 ppm has been developed for date palm offshoots against coleopteran internal tissue borers, no phytotoxicity to date palm trees were observed in that study. Fumigation of RPW infested date palms with aluminum phosphide is often considered hazardous and challenging task due to the escape of poisonous phosphine gas into the surroundings. Our studies using a portable phosphine gas detector (Gas Alert Extreme) revealed the detection of high levels of phosphine gas outside the plastic wrap for up to 36 h after treatment in infested palms treated by the old method, this finding may contribute to the limited effectiveness of the old method. In palms treated by the method developed in this study no phosphine gas was detected outside the palm, while therapeutic levels of phosphine gas were detected inside the plastic wrap up to 7 days after treatment ensuring complete mortality of all pest stages.

Inappropriate treatments with aluminum phosphide could lead to enhanced levels of resistance. Studies carried out in Pakistan recorded high resistance ratios (RRs) ranging from 63- to79-fold for phosphine (Wakil et al., 2018). This could lead to reduced effectiveness of the chemical. Use of the insecticides tested has the advantage over the use of aluminum phosphide for being less hazardous and that the protective effect of the insecticides against new infestation remain long after the end of treatment.

CONCLUSIONS

The results of this study offers a curative treatment of RPW in mild-severely infested date palms by the fumigant action of aluminum phosphide tablet and 6 other commonly available insecticides (Chlorpyriphos, Cypermethrin, Marqiuse, Deltamethrin, Malathion and Diazinon) using a specially designed air tight fumigation sleeve placed around the infested section of the palm trunk. This technique also offers a more effective and less damaging alternative to the mechanical sanitization and injection techniques currently used to treat RPW infested palms, and should replace them.

ACKNOWLEDGEMENTS

This study was funded by Rasheed Al Ballaa and Munira Al Hothaili Endowment Fund. Valuable comments and support by Dr. J.R. Faleiro, Food and Agriculture Organization of the U.N. Goa, India and support provided for statistical analysis by Dr. Bappa Das, Scientist, Agricultural Meteorology, ICAR-CCARI, Goa, India his highly appreciated.

Literature cited

Abraham, V.A., Al-Shuaibi, M.A., Faleiro, J.R., Abozuhairah, R.A., and Vidyasagar, P.S.P.V. (1998). An integrated management approach for red palm weevil, *Rhynchophorus ferrugineus* Oliv., a key pest of date palm in the Middle East. Sultan Qaboos Uni. J. Sci. Res. Agric. Sci. (Melb.) *3* (*1*), 77–83 https://doi.org/10.24200/jams.vol3iss1pp77-83.

Al-Ayedh, H.Y., and Al-Jber, A.M. (2019). Controversial aspects about red date palm weevil. Arab Journal of Plant Protection *37* (*2*), 153–155 https://doi.org/10.22268/AJPP-037.2.153155.

Almansoori, T.A., Al-Khalifa, M.A., and Mohamed, A.M.A. (2015). Date palm status and perspective in Bahrain. In Date Palm Genetic Resources and Utilization, Vol. 2: Asia and Europe, J.M. Al-Khayri, S.M. Jain, and D. Johnson, eds. (Dordrecht: Springer Science + Business Media).

Bond, E.J. (1984). Manual of Fumigation for Insect Control. FAO Plant Production and Protection Paper No. 54 (Rome: FAO).

El-Shafie, H.A.F. (2019). The use of phosphine as curative treatment against date palm borers. Outlooks Pest Manag. *30* (*5*), 204–207 https://doi.org/10.1564/v30_oct_04.

Faleiro, J.R. (2006). A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Rhynchophoridae*) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci. *26*, 135–154.

Fiaboe, K.K.M., Peterson, A.T., Kairo, M.T.K., and Roda, A.L. (2012). Predicting the potential worldwide distribution of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) using ecological niche modeling. Fla. Entomol. *95* (*3*), 659–673 https://doi.org/10.1653/024.095.0317.

Giblin-Davis, R.M., Faleiro, J.R., Jacas, J.A., Peña, J.E., and Vidyasagar, P.S.P.V. (2013). *Coleoptera*: biology and management of the red palm weevil, *Rhynchophorus ferrugineus*. In Potential Invasive Pests of Agricultural Crop Species, J.E. Peña, ed. (UK: CABI Wallingford), p.1–34.

Lakshmanan, P.L., Subba Rao, P.V., and Subramaniam, T.R. (1972). A note on the control of the coconut red palm weevil, *Rhynchophorus ferrugineus* with certain new chemicals. Madras Agric. J. *59*, 638–639.

Subba Rao, P.V., Subramaniam, T.R., and Abraham, E.V. (1973). Control of red palm weevil on coconut. J. Plant. Crops

1 (*1&2*), 26–27.

Vidhyasagar, P.S.P.V., Al-Saihati, A.A., Al-Mohanna, O.E., Subbei, A.I., and Abdul Mohsin, A.M. (2000). Management of red palm weevil *Rhynchophorus ferrugineus* Olivier, a serious pest of date palm in Al-Qatif, Kingdom of Saudi Arabia. J. Plant. Crops *28* (*1*), 35–43.

Wakil, W., Yasin, M., Qayyum, M.A., Ghazanfar, M.U., Al-Sadi, A.M., Bedford, G.O., and Kwon, Y.J. (2018). Resistance to commonly used insecticides and phosphine fumigant in red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in Pakistan. PLoS One *13* (*7*), e0192628 https://doi.org/10.1371/journal.pone.0192628. PubMed

A novel approach of nanoemulsion formulations of metarhizium anisopliae to control *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera: Dryophthoridae*) in Malaysia

A.Z. Abdulqader[1,2,a], D.B. Omar[1], R.M. Awang[1], A.M. Ahmed[3], Q. Ali[4], Y. Islam[5], M.H. Saleem[5]

[1]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia; [2]Department of Plant Protection, College of Agriculture, University of Basrah, Basrah, Iraq; [3]Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University Tando Jam, Sindh, Pakistan; [4]Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing, China; [5]College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China.

Abstract

In this article, we provided current information on developing, use of new nano bio-insecticide of *Metarhizium anisopliae* as components of integrated pest management strategies for control of RPW. The effect of Agnique PG9116 at 1% concentration gave 87.5% germination while at 5% surfactant Emereen1604 gave 70% conidia germination. Sunflower and glycerin gave 49.13 and 44.13% growth rate at 1% concentration, respectively. Palm oil at 5% concentration showed a 56.88% growth rate. At 10% concentration of oils, soy bean and glycerin gave 48.63 and 45.38% growth rate, respectively. Four ternary phase diagram systems were constructed. All the formulations were stable under centrifuge, storage at 26±1°C, and 54±1°C for two weeks. Three formulations were in the range below <100 nm sizes, indicating that the formulations were in the category of the nanoemulsion. The zeta potential of the formulations ranged between -7.22 to -39.06 mV, the surface tension ranged from 32.03 to 37.90 mN m^{-1}, and the viscosity ranged from 2.40 to 28.8 mPas. The formulation coded as E1604 gave the LT_{50} of 4.90 days while the water suspension gave LT_{50} of 6 days. On adults, the LT_{50} was 2.20 days while the water suspension was 5 days. The E1604 recorded 100% cumulative mortality after 6 and 4 days on larvae and adults, respectively. Effect of oil nano-formulations on the conidia germination on the cuticle of *R. ferrugineus* was also observed, and after 20 h, the E1604 showed 55% germination compared to water suspension of 49.8%. The formulation E1604 showed the most extended germ tube of 41.34 µm and full penetration while the water suspension gave 5.28 µm length of a germ tube. The oil nano-emulsion of *M. anisopliae* conidia shows good potential for the sustainable control of both adults and larvae of *R. ferrugineus*.

Keyword: nanoemulsion, *M. anisopliae*, *R. ferrugineus*, conidia, SEM

INTRODUCTION

The red palm weevil is a destructive phytophagous insect, infesting explicitly palm trees. It is a challenging problem around the globe due to its harmful feeding habits within palm trees and further threats to ornamental and other date palm species (Navarro-Llopis et al., 2015). The RPW rapidly spread to date palm-growing countries during the past two decades. It affects more than 20 palm species (Barranco et al., 2000). The studies show red palm weevil as a pest of palm trees, especially the economically significant coconut, *C. nucifera*, and the sago, *Metroxylon sagu* (Idris et al., 2014). In the recent decade, red palm weevil (RPW) *R. ferrugineus* has become a noxious pest of coconut and date palm trees in most Asian countries.

The widely used control methods for this aggressive pest are chemical insecticides such as Phosmet, Imidacloprid, Diazinon, and Phosphine (Llácer et al., 2010; Llácer and Jacas,

[a]E-mail: ali.zachi@uobasrah.edu.iq

2010). Non-judicious and over-reliance on pesticides have resulted in resistance, resurgence, and outbreaks of new insect pest species. There has been a continuous ongoing endeavor to reduce harmful effects of these pesticides, either by the development of more targeted compounds that exhibit fewer side effects, abandon of highly hazardous chemicals, or by the development of non-chemical methods of pest management (Gill and Garg, 2014). The functional utilization of biological agents as pesticides have efficiently delivered satisfactory results in reducing the incidences of insect pests, weeds, and diseases for many years. Several entomopathogenic agents have been documented (Gindin et al., 2006).

Metarhizium anisopliae Metchnikoff (*Hypocreales: Clavicipitaceae*) is a fungal pathogen that causes disease in different arthropod pests, and it can be employed as a biological control candidate to control this severe pest. An introduction of *M. anisopliae* has displayed a great ability to control this pest and was found to be the main pathogen against it through using the proper formulation and application method (Francardi et al., 2013). It is also suggested that the fungi could spread infecting healthy insects of *R. ferrugineus* by horizontal transmission as the insect is highly promiscuous and live in aggregation (Francardi et al., 2013). It possesses a high rate of germination and is responsible for the production of various enzymes that may result in insect toxins but is not infectious or toxic to mammals(Kurouski et al., 2012). Although entomopathogenics play a vital part in pest management, they are still facing many challenges about improving the efficiency of these insect-microbial pesticides formulation, shelf life, and compatibility with the environment. Therefore, improving the conidial formulation of *M. anisopliae* is an indispensable step to optimize its biological control strategy. It has been well documented that the oil in water emulsion enhances the efficacy of entomopathogens against various insects in controlled and uncontrolled conditions (Polar et al., 2005).

The oil formulation of the Citoweet oil enhanced the fungal virulence on *Eurygaster integriceps* (Sedighi et al., 2013). Also, the oil formulations are useful in strengthening the transfer of conidia to the areas of the thinner cuticle (Ibrahim et al., 1999). Similarly, these bio fungicides ensure the more exceptional ability of adherence to the host body and the combination of oil and conidia further assists in protecting the fungus against fast dehydration in low-humidity environments, high temperature (Hueston et al., 1995) and ultraviolet radiation (Bateman and Alves, 2000). The preference thus has been given to the use of oil-based formulations fungi that are effective in controlling various arthropods in laboratory and field conditions (Batta, 2003). The oil-based formulation of *Beauveria bassiana* has reported little effect on RPW (Abdel-Samad et al., 2011) in comparison with *M. anisopliae* that shows the highest efficacy against RPW larvae and adults (Francardi et al., 2013, 2016).

The oil-based formulations of *M. anisopliae* were more effective than aqueous suspensions against eggs, larvae, and engorged females of *Rhipicephalus microplus* (Camargo et al., 2012). The mycopesticide containing *M. anisopliae* has been developed worldwide to control numerous insect pests including *R. ferrugineus*. However, there is a lack of information on the *M. anisopliae* formulated as an oil emulsion for the control of RPW (Francardi et al., 2016). Therefore, this study was designed to expand the knowledge on a specific entomopathogenic fungus *M. anisopliae*, and to examine a novel approach in preparing oil nano-emulsions formulations as a microbial biopesticide and observe the biological efficiency of the prepared and tested formulations against RPW.

MATERIALS AND METHODS

Oils and surfactants

Four different surfactants were used in this study, as mentioned in Table 1 with their supplying source. Seven oils such as canola oil, corn oil, glycerin oil, palm oil, sunflower oil, sesame oil, and soy bean oil were also used as carriers. The water was obtained from the ultra-purification system. Agnique PG9116, Emereen 1604, Tensiofix 96DB10, and Tensiofix 96B8 were used as the surfactant phase. The conidia of *M. anisopliae* were used as an active ingredient.

Table 1. Ingredients used to observe the effects of the surfactants and oils.

No.	Compounds	Trade names	Classes supplier/company
1	Alkyl polyglycoside	Agnique PG9116	Nonionic Surfactant Congis Olechmicals (M) Sdn.
2	Castor Oil Ethoxylate 40 EO	Emereen 1604	Nonionic Surfactant Emery Olechmicals Malaysia
3	N/A	Tensiofix DB08	Nonionic Surfactant Tensiofix Agrochemical Belgium
4	N/A	Tensiofix-96 DB10	Nonionic Surfactant Tensiofix Agrochemical Belgium
5	N/A	Canola	Oil Local Market, Malaysia
6	N/A	Corn	Oil Local Market, Malaysia
7	Propanetriol	Glycerin	Oil Qrec quality reagent chemical product
8	N/A	Palm	Oil Local Market, Malaysia
9	N/A	Safflower	Oil Local Market, Malaysia
10	N/A	Sesame	Oil Local Market, Malaysia
11	N/A	Soybean	Oil Local Market, Malaysia
12	Conidia	N/A	Active ingredient

Effect of oils and surfactants on the conidial viability of *M. anisopliae*

The direct plating technique was used to determine the conidia viability of different surfactants. The procedure of Sandrin et al. (2003) was followed regarding the selection of different compositions. Thus, three compositions of oils and the surfactants (1, 5, and 10%) were used and mixed with 10^6 spores mL^{-1} fungal suspension (Sandrin et al., 2003). From the prepared solution, 200 µL was sprayed in a petri dish (60×15 mm) containing Ypss medium. The pH was adjusted to 7±1 and incubated at 26±1°C. The evaluation of germination was assessed after 24 h and the conidia germination was examined using a microscope at 100× magnification and 200 conidia were counted from each plate. The conidia were considered germinated successfully; when the length of the germ tube was as long as conidia width.

Effect of the surfactants on the larvae and adults of *R. ferrugineus*

In selecting the larvae of *R. ferrugineus* for a present experiment, the instruction given by Dotaona et al. (2015) was followed in this experiment. The effect of the surfactants on the larvae and adults has been determined by preparing three different concentrations of surfactants such as 1, 5, and 10%. Both larvae and adults were collected from the larvae rearing boxes and then shifted into the new boxes containing sugarcane and sugar solution. Approximately 3 mL of the surfactant solution was sprayed on each treatment and incubated for ten days under laboratory-controlled conditions 26±1°C, 70±5% RH. The mortality of insects was monitored during this period. The experiment was carried out with a total of 16 larvae per treatment, and each treatment was replicated four times. However, the control treatment was sprayed with sterilized distilled water, as suggested by previous researchers (Gindin et al., 2006; Mwamburi et al., 2015).

Effect of the oils on the larvae and adults of *R. ferrugineus*

Seven oil (Table 1) were used to observe their effect on the *R. ferrugineus* larvae and adults the procedure was determined as described earlier.

Preparation of oil nanoemulsion formulation

Several parameters have been chosen to prepare the oil nanoemulsion of *M. anisopliae* conidia, as mentioned below in detail.

Miscibility test pre-formulation

The selection of the ingredients was made based on the miscibility among the oil, surfactants, and water to prepare the oil emulsion (Table 2). Two mL of each ingredient mixture was mixed then vortexed for a proper time to determine the miscibility. A similar procedure was repeated four times. The miscibility standards were based on optical transparency and phase transition of the mixture.

Table 2. Surfactants, glycerin and water grouped in different combinations for phase diagram construction.

Group	Surfactant	Oil phase	Aqueous phase
1	Agnique PG9116	Canola	Water
2	Agnique PG9116	Corn	Water
3	Agnique PG9116	Glycerin	Water
4	Agnique PG9116	Palm	Water
5	Agnique PG9116	Sunflower	Water
6	Agnique PG9116	Sesame	Water
7	Agnique PG9116	Soybean	Water
8	Emereen 1604	Canola	Water
9	Emereen 1604	Corn	Water
10	Emereen 1604	Glycerin	Water
11	Emereen 1604	Palm	Water
12	Emereen 1604	Sunflower	Water
13	Emereen 1604	Sesame	Water
14	Emereen 1604	Soybean	Water
15	Tensiofix 96 B08	Canola	Water
16	Tensiofix 96 B08	Corn	Water
17	Tensiofix 96 B08	Glycerin	Water
18	Tensiofix 96 B08	Palm	Water
19	Tensiofix 96 B08	Sunflower	Water
20	Tensiofix 96 B08	Sesame	Water
21	Tensiofix 96 B08	Soybean	Water
22	Tensiofix 96 DB10	Canola	Water
23	Tensiofix 96 DB10	Corn	Water
24	Tensiofix 96 DB10	Glycerin	Water
25	Tensiofix 96 DB10	Palm	Water
26	Tensiofix 96 DB10	Sunflower	Water
27	Tensiofix 96 DB10	Sesame	Water
28	Tensiofix 96 DB10	Soybean	Water

Construction of ternary phase diagram

A mixture of oil, surfactants, and water was used to construct the ternary phase diagrams by the aqueous titration method (Shafiq et al., 2007). Oil phase and surfactant were mixed at the weight ratios (w/w). Appropriate amounts of surfactant and carrier according to the ratio were weighed for a total of 0.5 g into a 7-mL tube the mixture was vortex to achieve equilibrium. The mixture in which the emulsion phase formed was used to determine the phase domains, including isotropic, monophase, and phase or triphase. Each sample was assessed visually for spontaneous emulsification based on clarity, stability, and transparency. The observation of samples and experimental measurements were performed at room temperature of 26±1°C. All the phases diagram systems were characterized according to their ratios (oil, surfactant, and water).

Characterization of oil nano-emulsion formulation

1. Stability of formulations under centrifugation.

The procedure prescribed by Baboota et al. (2007) and Shafiq et al. (2007) was used in the centrifugation test. The selected formulations were centrifuged at 3500 rpm for 30 min. The formulations were kept at room temperature (26±1°C) without any phase separation for not less than two days.

2. Stability of oil emulsion formulations under storage conditions.

The formulations that passed the centrifuged test were subjected to the storage test at room temperature of 26±1°C for three months and under high temperature of 54±1°C for two weeks (Roland et al., 2003).

3. Particle size and zeta potential measurements of emulsion.

The particle size of the emulsion and zeta potential of the formulation samples were executed by a Zeta Sizer Nano-ZS (Malvern, UK). A total of 16 samples of emulsions were analyzed; each sample of one emulsion was run four times, as described by Rodrigues et al. (2014). The analyses were carried out in the completely automatic mode with three replicates, and each sample was analyzed three time.

4. Surface tension measurement of emulsions.

A tension meter (Tensiometer K6: Model KRUSS, UK) was used to measure the surface tension of the emulsion samples. The technique that followed in this test was the Du Nüoy ring immersing method (Green et al., 2003).

5. Viscosity measurement of emulsions.

The viscosity of the emulsion formulations was determined by using approximately 20 mL of the formulation was filled into the sample container of the viscometer (Model RheolabQC). The procedure that followed in this test was described by Roland et al. (2003). Each run was repeated four times.

6. Toxicity of the oil nano-emulsion formulations against *R. ferrugineus*.

The nano-emulsion formulations have investigated the effectiveness against *R. ferrugineus*, and eight different nano-emulsion formulations were used (Table 2). The insects were removed from the feeding boxes before spray with *M. anisopliae* nano-emulsion formulations. The formulations were prepared with a concentration of 10^7 spores mL^{-1}. The mortality of the treated larvae and adults was monitored after every two days for 18 days (Gindin et al., 2006; Alves et al., 2002). The experiment was conducted with four treatments, and each treatment was replicated four times, with a total of 40 adults for each treatment, and an equal number of males and females (5♂+5♀) were used. The control (water suspension 10^7) treatment was performed with four replicates with a total of 40 adults, and an equal number of males and females (5♂+5♀) were used.

Infection process of *M. anisopliae* to the larvae and adults of *R. ferrugineus*

Random specimens were chosen from the toxicity experiment for observation with LEO 1455 VP SEM attached with EDX scanning electron microscope. After 20 h of spraying the specimens were processed to SEM procedure (Moino et al., 2002).

Statistical analysis

The bioassay was conducted in a complete randomized design (CRD). Median lethal time of the larvae and adults, LT_{25}, $_{50}$, and $_{75}$ for Ag9116, B08, B10, and E1604, formulations were determined using probit analysis was computed using SAS version 9.4.

RESULTS AND DISCUSSION

Effect of surfactants and oils on *R. ferrugineus* larvae and adults

The results in (Tables 3) showed that there was no effect of the surfactants and oils against the RPW larvae as well as adults at three prepared concentrations of 1, 5, and 10%.

Table 3. Effect of surfactants and oils on *R. ferrugineus* larvae and the adults after exposure to three different concentrations.

Surfactants/oils	Stage of insect/concentration					
	Larvae			Adults		
	1%	5%	10%	1%	5%	10%
Agnique PG9116	No	No	No	No	No	No
Emereen 1604	No	No	No	No	No	No
Tensiofix DB08	No	No	No	No	No	No
Tensiofix96 DB10	No	No	No	No	No	No
Canola	No	No	No	No	No	No
Corn	No	No	No	No	No	No
Glycerin	No	No	No	No	No	No
Palm	No	No	No	No	No	No
Safflower	No	No	No	No	No	No
Sesame	No	No	No	No	No	No
Soybean	No	No	No	No	No	No

Table 4. Viability of *M. anisopliae* conidia in different oils.

Oils	Germination (%)/compositions (%)		
	Mean ± SE (1%)	Mean ± SE (5%)	Mean ± SE (10%)
Canola	11.25±1.47	7.50±1.67	12.88±2.62
Corn	11.75±2.21	8.38±1.39	11.50±2.57
Glycerin	44.13±8.66	11.75±1.64	45.38±3.20
Palm oil	35.25±3.45	56.88±4.27	39.50±2.38
Sesame	11.25±2.01	8.13±1.56	6.88±0.58d
Soybean	36.75±3.44	36.00±4.85	48.63±4.72
Sunflower	49.13±2.08	38.25±2.46	43.25±3.17

Miscibility test of the inert ingredients

The results obtained from the miscibility test between oil, surfactants, and water were classified into three classes (transparent with one phase, gel, and turbid). The obtained results are shown in Table 5. The surfactants Agnique PG 9116 and Emereen 1604 were miscible with glycerin. The surfactants Tensiofix 96 DB08 and Tensiofix 96 DB10 were turbid.

Table 5. Miscibility test between oils and surfactants used based on spontaneous emulsification.

Surfactant	Canola	Corn	Glycerin	Palm	Soybean	Sunflower	Sesame
	Water						
Agnique PG9116	***	***	✓	***	***	***	***
Emereen 1604	****	m	✓	***	****	****	****
Tensiofix 96 DB08	****	***	**	***	***	***	***
Tensiofix 96 DB10	***	***	*	****	****	****	****

*Turbid; **Gel; *** 2 Phases; **** Cream ✓ Transparent with one phase.

Thermo stability test of nanoemulsion formulations

All the formulations were observed stable and homogenized because no phase separation was detected under the temperature of 26±1°C and 54±1°C for three months and 14 days' storage, respectively (Table 6). The main purpose of this experiment was to find a thermodynamically stable and dilutable nanoemulsion system of glycerin with minimum surfactant percentage that could improve its solubility, stability, and shelf life.

Table 6. Stability test of nano-emulsion formulation with centrifugation at a storage temperature of 26 and 54°C.

No.	Formulation	Stability under centrifuge	Stability under 26±1°C	Stability under 54±1°C
1	Ag9116	✓	✓	✓
2	E1604	✓	✓	✓
3	B08	✓	✓	✓
4	B10	✓	✓	✓

Ternary phase diagram of nano-emulsion system study

The phase diagram of Agnique PG9116/glycerin/water system provided the largest one phase region of 83% (Figure 1A) that showed the system consists of one phase nano-emulsion in all compositions of surfactant and the oil. Meanwhile, the smallest one phase region of 25% was noted in the phase diagram of Tensiofix DB08/glycerin/water system. The large isotropic region of the phase diagram obtained from Agnique PG9116 exposed that it was easily dissolved due to Alkyl polyglycoside surfactant which was miscible in glycerin and water. A ternary phase diagram for Emereen 1604/glycerin/water system provided a 79.5% isotropic region of the system (Figure 1B). The isotropic region obtained in this system when the percentage of Emereen 1604 was 10-78% W/W, the percentage of glycerin was 10-90% w/w and the composition of water was 23-88% W/W, respectively. In Figure 1C a ternary phase diagram has been presented for the Tensiofix DB08/glycerin/water system which provided 58% isotropic region of the system. However, the separation into two phases occurred when water composition was 10.23% W/W of the system. Nano-emulsion gel area was observed when water composition was 12.43% W/W. The phase diagram system shown in Figure 1D presents the phase diagram for Tensiofix DB 10/glycerin/water system. This system provided 25% isotropic region, 5% two phases, and nano emulsion gel area was 10% of the system. The separation into two phases occurred when water composition was 10.19-27.56% W/W. Nano-emulsion gel area occurred when water composition was 27.56 and 38.17% W/W.

Particle size and zeta potential

The result indicated that there was a significant difference was observed ($p<0.05$) between the formulations. The results (Table 7) of particle size analysis showed that three formulations out of four were in the range below <100 nm except formulation Ag9116 with a range of 102.10 nm. The smallest particle size was detected in formulation W70 with 19.53 nm. E1604 and B08 were with a reading of 19.94, and 22.79 nm, respectively. The zeta potential of the formulations was ranged between -7.22 to -39.06 mV. However, formulation Ag9116 -39.06 was the highest value as compared to other formulations. Meanwhile, formulation B08 obtained the lowest zeta value -7.22 mV.

Surface tension measurement of emulsions

The results regarding the values of surface tension for the formulations tested are shown in (Table 8). All the formulations showed significantly lower surface tension in the range of 32.03 to 41.83 mN m^{-1} as compared to the control (water) treatment (≈70.20 mN m^{-1}) at the room temperature of 26±1°C. Formulation Term1284 showed the highest surface tension of 41.83 mN m^{-1} as compared to other formulations and the lowest surface tension was at the formulation of Ag 9116 with 32.03 mN m^{-1}.

Viscosity measurements of emulsions

The low viscosity characteristics of the surfactant will allow the droplet to spread more on the leaf surface. Viscosity measurements showed that all the formulations gave low viscosity, the values ranged from 2.40 to 28.8 mPas. The formulations Ag9116 and B08 presented the lowest viscosity values with 2.40 mPas and the highest value obtained by formulation B10 with 28.8 mPas (Table 8).

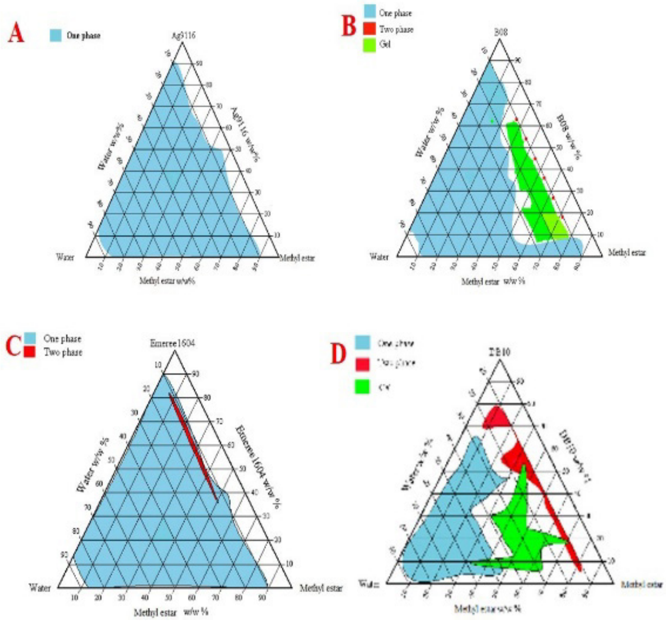


Figure 1. A) Phase diagram of Agnique PG9116/glycerin/water system presents one-phase region of 83%; B) Phase diagram of Emeree1604/glycerin/water system shows 79.5% isotropic region; C) Phase diagram of Tensiofix DB08/glycerin/water system presented 58% isotropic region and 12% nano gel; D) Phase diagram of Tensiofix DB10/glycerin/water system 25% isotropic region, 5% 2 ph and 10% nano gel.

Table 7. Mean particle size and zeta potential of the formulations.

No.	Formulation	Particle size (nm)	Zeta potential (mV)
1	Ag9116	102.10a	-39.06a
2	E1604	19.94e	-25.80b
3	B08	22.79e	-7.22d
4	B10	69.81c	-18.16c

Table 8. Surface tension values of nano-emulsion.

No.	Formulation	Surface tension (mN m^{-1})	Viscosity value (mPas)
1	Ag9116	32.03	2.40
2	E1604	37.90	6.00
3	B08	36.70	2.40
4	B10	34.10	28.8

Toxicity of the oil nano-emulsion formulations against *R. ferrugineus*

The results in Tables 9 and 10 show the analysis of probit of larvae and adults of *R. ferrugineus* after exposure to the formulated conidia of nano-emulsions of Ag 9116, E1604, B08, B10, and unformulated conidia (control). The highest mortality in larvae treatments was at the formulation W1604 which gave at LT_{20}, LT_{50}, and LT_{75}, 3.20, 4.90 and 6 days, respectively. Whereby the larvae were exposed to a water suspension of *M. anisopliae* conidia was less effective against the larvae and gave at LT_{20}, LT_{50}, and LT_{75}, 6, 6 and 8 days, respectively. However, in the adults test the rapid death was recorded among all formulations that indicated LT_{20}>2 days, LT_{50}=2.20 and LT_{75}=3.80 days at formulation E1604. Meanwhile, in unformulated conidia the death was at LT_{20}=3.80, LT_{50}=5 and LT_{75}=6.20 days, respectively. The results showed that the formulation E1604 showed the best effectiveness against the larvae and adults of *R. ferrugineus* because when the time decreased the mortality was increased. The effect of formulations on the conidia germination appeared after 20 h of the application on the cuticle of *R. ferrugineus* (Table 11). It was observed that the formulation E1604 showed the lowest effectiveness on the conidia activity with germination percentage of 55.80%. The formation was achieved after 20 h in the longest germ tube (41.34 µm) and full penetration in the cubicle of the *R. ferrugineus* among all the formulations. However, the control treatment achieved 49.80% conidia germination, and the formation was achieved after 20 h in the shortest germ tube with 5.28 µm and full penetration in the cuticle of *R. ferrugineus.*

Table 9. Mortality time analysis of *R. ferrugineus* larvae after exposure to four different nano-emulsion conidial formulations 10^7 conidia mL^{-1}.

Formulations	b ± SE	χ^2	LT_{50} day 25	LT_{50} day 50	LT_{50} day 75	95% fiducial limits
Ag9116	0.25±0.04	34.64	10	12.40	15	0.17-0.34
E1604	0.61±0.12	24.22	3.20	4.90	6	0.37-0.86
B08	0.19±0.04	15.80	13	m	m	0.09-0.28
B10	0.20±0.04	20.49	12	15.40	m	0.11-0.29
Control	0.32±0.05	35.59	4	6	8	0.21-0.42

b = slope of regression line; SE = Standard error; χ^2 = computed chi-square to indicate goodness-of fit regression line; LT_{50} = median lethal time in days; m = need more time than 18 days.

Table 10. Mortality time analysis of *R. ferrugineus* adults after exposure to four different nano-emulsion conidial formulations 10^7 conidia mL^{-1}.

Formulations	b ± SE	χ^2	LT_{50} day 25	LT_{50} day 50	LT_{50} day 75	95% fiducial limits
Ag9116	0.12±0.03	12	8.20	13.60	18	0.05-0.20
E1604	0.41±0.15	7.25	L	2.20	3.80	0.11-0.72
B08	0.13±0.06	4.66	m	15	m	0.01-0.25
B10	0.18±0.06	7.28	m	18	m	0.04-0.31
Control	0.57±0.14	16.59	3.80	5	6.20	0.29-0.85

b = slope of regression line; SE = standard error; χ^2 = computed chi-square to indicate goodness-of fit regression line; LT_{50} = median lethal time in days; L = less than 2 days needed; m = need more than 18 days.

The results obtained from SEM study showed that the formulations enhanced the germination and produce the germ tube in comparison with the control treatment. Besides that, the fungus germ tubes were well preserved in the specimens. Thus, it was observed that the conidia were cabbala to adhere to all the body parts and body region with stronger cuticle such as the sheath wings and the head which was less responsive to alterations comparing with joining parts. There was a significant difference between the formulations in adults Figure 2 (1-5) and larvae Figure 3 (1-5) treatments, respectively.

Table 11. Germ tube of *M. anisopliae* characterization after 20 h of treatment with the nano-emulsion formulations.

Formulations	% Germination of conidia after 20 h	Formation after 20 h	Germ tube length after 20 h µm	Penetration after 20 h
Ag9116	45.10	Formation	6.20	No penetration
E1604	55.80	Formation	41.34	Full penetration
B10	No germination	No formation	n	No penetration
B08	No germination	No formation	n	No penetration
Control	45.90	Formation	5.28	Full penetration

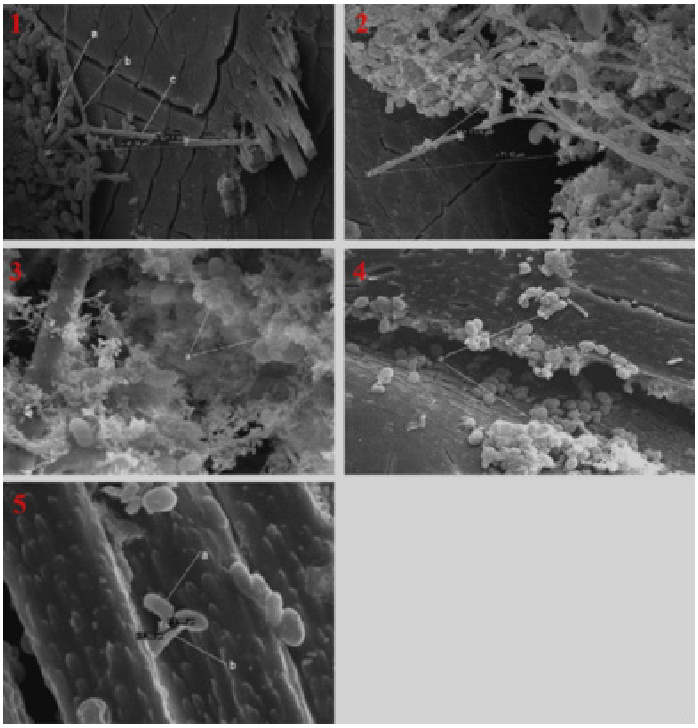


Figure 2. 1) SEM micrograph of the treatment adult by the formulation of 1604; a) germinated conidia; b) germ tube; c) germination of a germ tube into the cuticle of *R. ferrugineus* growth of germ tube after 20 h; 2) SEM micrograph of the treatment adult by the formulation Ag 9116; a) the germinated conidia; b) growth of the germ tube after 20 h; 3) SEM micrograph of the adult treatment by the formulation of B08; a) conidia of *M. anisopliae* not germinated; b) conidia adhered to the seta after 20 h; 4) SEM micrograph of the treatment adult by the formulation of B10; a) conidia of *M. anisopliae* not germinated after 20 h; 5) SEM micrograph of control treatment adult; a) germinated conidia of *M. anisopliae*; b) germ tube penetrates the adult cuticle of *R. ferrugineus* after 20 h.

Furthermore, it was noticed that a thickening of the edge of the germ-tube describing the development of appressoria was recognized during the penetration by the fungus. *M. anisopliae* was often found developing locally dense hyphal colonies at the cuticle surface of *R. ferrugineus* as shown in Figure 4 in the adults' treatment and was shown in Figure 5 at larva. Each conidium normally performed only one germ tube. Nevertheless, there was a difference in its length, and earlier to appressoria formation some conidia performed long germ-tubes.

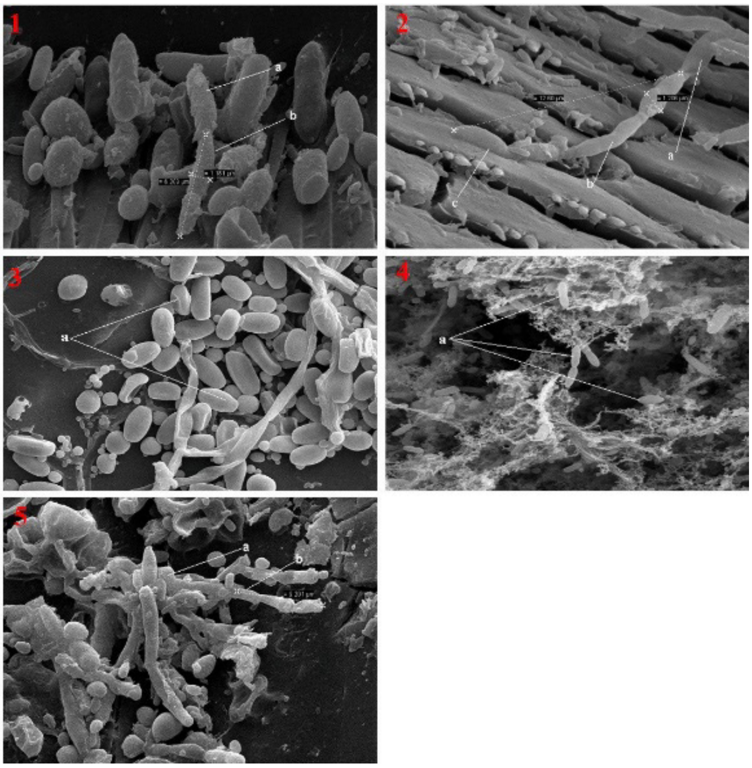


Figure 3. 1) SEM micrograph of the larva treated by the formulation Ag 9116; a) the germinated conidia; b) growth of the germ tube after 20 h; 2) SEM micrograph of the larva treated by the formulation 1604; a) adhered germinated conidia; b) germ tube with full formation; c) germ tube penetration the cuticle after 20 h; 3) SEM micrograph of the larval treatment by the formulation of B08; a) conidia of *M. anisopliae* not germinated after 20 h; 4) SEM micrograph of the larval treatment by the formulation of DB10; a) conidia of *M. anisopliae* not germinated after 20 h; 5) SEM micrograph of the larva treated by water suspension (control treatment); a) the germinated conidia; b) growth of the germ tube after 20 h.

Figure 4. Toxicity of the best four formulations on *R. ferrugineus* adult after applied the oil nanoformulation of *M. anisopliae* conidia (A) healthy adult of R. ferrugineus before the treatment, (B) infected adult of *R. ferrugineus* with *M. anisopliae* after the treatment.

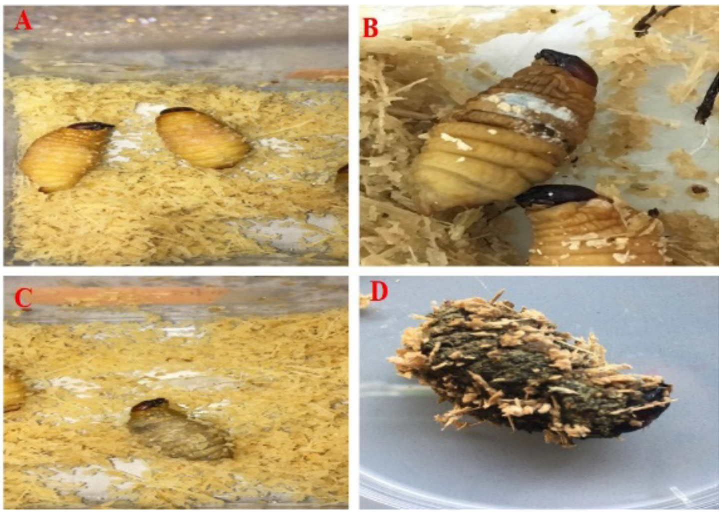


Figure 5 Toxicity of the best four formulations on *R. ferrugineus* larvae (A) healthy larvae before treatment with *M. anisopliae* oil nanoformulation (B) changing the color to dark (C) appear the mycelium out of the body (D) covering the larva body with *M. anisopliae* conidia.

The results of the present study showed that the ingredients (oils and surfactants) used to prepare and formulate oil nano-emulsion of *M. anisopliae* were non-toxic for *M. anisopliae* conidia. Many studies mentioned that non-ionic surfactants are considered harmless and less toxic to living organisms such as insects, plants, and animals (Jin et al., 2008; Azeem et al., 2009). Azeem et al. (2009) suggested that nonionic surfactants be chosen as they are less toxic than anionic and cationic surfactants. Moreover, the results suggest that the oils effectively enhanced the conidia germination and it could act as synergists for improving the efficiency of *M. anisopliae* (Azeem et al., 2009). The increase in the rate of germinated conidia occurred because the glycerin was non-inhibitory oil and may supply the necessary nutrients or provide effective surroundings to keep the conidia (Becker et al., 2019). Ibrahim et al. (1999) investigated that the conidia of *M. anisopliae* formulated in oils frequently germinated over the surface of insect and plant cuticles contrasted with aqueous suspension (Ibrahim et al., 1999). In another study, dry conidia of *M. robertsii* and *M. pingshaense* were formulated in emulsifiable oil, earlier blending with water increased the germination of the conidia to 21% compared to the non-formulated conidia (Xavier-Santos et al., 2011).

The findings of the ternary phase diagram system presented a large isotropic region. These results may be due to the use of nonionic surfactants, the physico-chemical properties of surfactants possess the ability to mix oil with water by decreasing the inter facial tension between water and oil (Sharma et al., 2016.) The surfactant composition affects the stabilization and droplets size of the emulsion (Bernardi et al., 2011). The best weight ratios of oil and surfactant were determined by their miscibility with water. These results are in line with Bouchemal et al. (2004) and Azeem et al. (2009) who stated that the nano-emulsion requires a minimum surfactant ratio of 20% in the formulation of the nano-emulsion (Azeem et al., 2009; Bouchemal et al., 2004). Stability tests are needed due to their predictive capacity as formulations are subjected to situations designed to promote changes that may happen under market conditions (Ribeiro et al., 2015).

Thermodynamic stability presents long shelf life to the nano-emulsion formulation as compared to conventional emulsions (Azeem et al., 2009). Since particle size interferes with coalescence and flocculation determination of zeta potential and particle size is the necessary method that can be employed to evaluate the stability of the colloidal system (Ribeiro et al., 2015). The particle size for the four formulations remained in the range of nanoscale with 200 nm in size that characterizes a nano-emulsion. Morales et al. (2003) found that the nano-emulsion droplet sizes increased by reducing surfactant/water ratios at fixed oil percentage or by increasing oil/water percentage at fixed surfactant. Tadros (2006) stated that if the particle size of an emulsion is less than 80 nm, it gets superior characteristics compared to conventionally sized emulsions including optical transparency, high colloidal stability, and a large inter facial area to volume ratio (Tadros, 2006). Wooster et al. (2008) found that the particle size of the emulsion decreases with the increase of surfactants percentage.

Besides, the measurement of zeta potential provides evidence of the inherent stability of the nano-emulsion system. Shanmugam and Ashokkumar (2014) reported that the value of zeta potential between -25 to -30 mV is already enough to produce an energy boundary between the particles by avoiding unnecessary coalescence. The appearance of negative charge in zeta potential values suggests the presence of anionic particles on the surface of these emulsion droplets. The surface tensions detected were considerably similar for the formulations. The lower surface tension in oil nano-emulsion formulations is advantageous for the improvement of spreading, wetting, and penetration of the oil nano-emulsion (Tadros, 2006). The lower surface tension value allows the insecticide droplet particles to penetrate and spread evenly on the leaf surface with smaller contact angles during applications. The decrease in the surface tensile value of all the formulations in this study describes the reaction of surfactant and oil in reducing the surface tension of the formulations. It was noted that the centrifugation did not influence phase separation and cracking in all the formulations. Azeem et al. (2009) stated that the solubility of the surfactant with oil is a significant factor in preparing nano-emulsion formulations. Bouchemal et al. (2004) reported that surfactant and oil miscibility could give a primary indicator of the possibility of nano-emulsion formation.

The results showed dilution prepared the solubilization capability of the concentrates with water, which shows this system is highly proper for effective agrochemical delivery. To manage the formulation decision, perception regarding spray droplet and the propagule of entomopathogenic interaction with the host surface could assist. If particles adhere to the host surface, the droplet must be capable of wetting the host surface. Generally, for a solid to be wet by liquid the surface tension of the liquid must be lower than the surface energy of the solid. Therefore, any prepared formulations must have low surface tension for successful results. Dynamic surface tension is necessary for spray applications because through the spraying the droplet-forming process, new droplet surfaces are continually being formed and the surfactant must disperse to the surface to reduce the surface tension (Jackson et al., 2010). Du et al. (2016) reported the wetting, spreading, and penetrating could be increased by the low surface tension of the whole system. The oil viscosity, the surfactant hydrophilic-lipophilic (HLB), and miscibility with water represent the critical parameters in determining the quality of the final nano-emulsion achieved through processes of spontaneous emulsification. Similarly, Bouchemal et al. (2004) stated that the combination of the oils and surfactants phase is highly imperative to obtain and to characterize the spontaneous nano-emulsion formulations. Furthermore, if the miscibility between surfactants, oil mixture, and water is good the kinetics of spontaneous emulsification are greatly expressed.

The results confirmed the materials used to prepare nano-emulsions formulations were not toxic for *R. ferruginous* in different concentrations. Despite this, the mixture of surfactants and oils with *M. anisopliae* achieved high toxicity under lab conditions against RPW. Due to, the effect of environmental factors on entomopathogenic fungi the formulation of the entomopathogenic is a good technique to overcome this issue (Ment et al., 2010). In the present study, nano-emulsion formulations of *M. anisopliae* conidia were found to cause disease and death of *R. ferrugineus* larvae and adults. There are many mechanisms of *M. anisopliae* response of the insect death in this regard. Butt et al. (2013) reported that one of the potential mechanisms that may ultimately influence the mortality of the host is the activation of apoptotic pathways (involving caspase enzymes) by releasing proteases through the conidia of the fungus. Moreover, one of the hypotheses suggested that the mechanism of killing of *M. anisopliae* is to produce a family of destruxin, which could be responsible for the paralysis and death in the insect (Hinaje et al., 2002).

The mechanism of *M. anisopliae* begins with spore adhesion, germination, and penetration of the germ tube through the insect cuticle. Similarly, Moino et al. (2002) and Güerri-Agulló et al. (2010) did a SEM study of *B. bassiana* and *M. anisopliae* infected with termite *H. tenuis* and *R. ferrugineus* which showed adhesion and penetration structures of these fungi. The other studies also described that *M. anisopliae* conidia germinated within 72 h post-infection and penetration of germ tube occurred 48-72 h post-infection (Moino et al., 2002). The fungus typically invades the insect through areas that are not strongly hard, such as the inter segmental joints, mouth parts, and also spiracles as these regions provide conditions of high humidity which are convenient for germination and development of the fungus (Butt et al., 2013). Similarly, the results of the recent study showed that the fungus invaded the insect through the rostrum parts. Kershaw et al. (1999) studied the mode of action of destruxins in the pathogenicity of *M. anisopliae* for three insect species. They suggested that at least two possible virulence strategies existed between their isolates such as 1) a toxin strategy in which the *M. anisopliae* has limited growth in the insect hemolymph and produces destruxins in satisfactory quantity to kill the host; 2) a growth strategy in which an isolate of *M. anisopliae* presents plentiful growth in the hemolymph which creates a disruption of homeostasis and starvation leading to insect death. Both of these strategies may exist with the fungus examination in the present study. The low toxicity of the glycerine oil, the Emereen 1604, Tensiofix 96DB10, Tensiofix 96DB08 Agnique PG9116 against *R. ferrugineus* larvae and adults in the present study because it is short-chain oil (Becker et al., 2019). Moreover, many studies reported the nonionic surfactants are low toxicity to mammals and plants (Setya et al., 2014). The oil could improve the infections as they carry inoculum to parts of the insect body parts more conducive for germination and infection than happens with water applications of conidia in Tween (Ibrahim et al.,1999). Similarly, it was observed that the mortality occurred after 48 h in all the treatments because the conidia needed 48 h to recover from unfavorable effects caused by some formulations and provided high germination rates (Alves et al., 2002).Leemon and Jonsson (2008) investigated 31 *M. anisopliae* isolates, and they found that an application of oil formulation with 3×10^8 spores mL^{-1} caused 100% mortality after two days in comparison to water suspension at the same concentration spores needed five days with similar results. Several studies have been previously carried out regarding the infection patterns and histopathology of *M. anisopliae* in chosen economic insects, but there is still lacking in documenting the histopathology of *M. anisopliae* in *R. ferrugineus*.

However, the present study revealed that conidia of *M. anisopliae* firmly adhere to the surface of the cuticle of *R. ferrugineus* larvae and adults. Conidia of *M. anisopliae* have been reported adhering to the cuticle of the termite *H. tenuis* and were less adhesion in the hard cuticle parts such as the head of various insects (Moino et al., 2002; Boruah et al., 2016). Arruda et al. (2005) observed that most *M. anisopliae* conidia adhered to the cuticle of the host after 24 h of post-infection and the infection happened by the germ tube and formation of appressoria. Camargo et al. (2014) observed the oil formulations of *M. anisopliae* caused a reduction of 50% of *Rhipicephalus microplus* tick within 21 days. Besides, the oil-based formulations at 10, 15 and 20% enhanced the efficacy of *M. anisopliae*, and the fungal oil formulations tested were more efficient than the water suspension (Camargo et al., 2012). It has been reported that *M. anisopliae* produces a family of proteases enzymes and toxins (destruxin), a positive coloration was found between the destruxin and the speed power of killing (Kershaw et al., 1999). Such information regarding destruxins has been reported as virulence determinants in the pathogenicity of *M. anisopliae* against several insect hosts.

CONCLUSIONS

There was no effect of the surfactants and oils against the RPW larvae as well as adults at concentrations of 1, 5, and 10% was observed. The rapid death observed among all the formulations with larvae and adults test was done at Equation E1604. It showed the best effectiveness of formulation E1604 against the larvae and adults of *R. ferrugineus*. The effect of formulations E1604 on the conidia germination appeared after 20 h of the application on the cuticle of *R. ferrugineus* that represented the lowest effectiveness on the conidia activity with the highest germination percentage. The adults *R. ferrugineus* were highly susceptible to *M. anisopliae* nano-formulations as compared to adults that displayed a maximum mortality percentage in a shorter time. Thus the present is quite useful in which speed of invasion of the conidia of *M. anisopliae* in different nano-emulsions formulations by observing conidia germination and penetrate the cuticle by formation appressoria using SEM was successfully observed. This work has also provided data that suggests that a formulation based on an oil nano-emulsion will promote fungal effectiveness and is more suitable for use against *R. ferrugineus*. Based on the information it could be said that *M. anisopliae* is a potential bio-control agent that can be formulated and use as an important component in the biological control of *R. ferrugineus*.

Literature cited

Abdel-Samad, S.S.M., Mahmoud, B.A., and Abbas, M.S.T. (2011). Evaluation of the fungus, *Beauveria bassiana* (Bals.) Vuill as a bio-control agent against the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*: *Curculionidae*). Pest Control. *21*, 125–129.

Alves, R.T., Bateman, R.P., Gunn, J., Prior, C., and Leather, S.R. (2002). Effects of different formulations on viability and medium-term storage of *Metarhizium anisopliae* conidia. Neotrop. Entomol. *31* (*1*), 91–99 https://doi.org/10.1590/S1519-566X2002000100013.

Arruda, W., Lübeck, I., Schrank, A., and Vainstein, M.H. (2005). Morphological alterations of *Metarhizium anisopliae* during penetration of *Boophilus microplus* ticks. Exp Appl Acarol *37* (*3-4*), 231–244 https://doi.org/10.1007/s10493-005-3818-6. PubMed

Azeem, A., Rizwan, M., Ahmad, F.J., Iqbal, Z., Khar, R.K., Aqil, M., and Talegaonkar, S. (2009). Nanoemulsion components screening and selection: a technical note. AAPS PharmSciTech *10* (*1*), 69–76 https://doi.org/10.1208/s12249-008-9178-x. PubMed

Baboota, S., Shakeel, F., Ahuja, A., Ali, J., and Shafiq, S. (2007). Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta Pharm *57* (*3*), 315–332 https://doi.org/10.2478/v10007-007-0025-5. PubMed

Barranco, A., Martfn, M.M., and Cabello, T. (2000). Host rank for *Rhynchophorus ferrugineus* (Olivier, 1790) and host palm tree diameter (*Coleoptera*: *Curculionidae*). Bol. Sanid. Veg., Plagas *26* (*1*), 73–78.

Bateman, R.P., and Alves, R.T. (2000). Delivery systems for mycoinsecticides using oil-based formulations. Asp. Appl. Biol. *57*, 163–170.

Batta, Y.A. (2003). Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (*Deuteromycotina*: *Hyphomycetes*). Crop Prot. *22* (*2*), 415–422 https://doi.org/10.1016/S0261-2194(02)00200-4.

Becker, L.C., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G., Jr., Shank, R.C., Slaga, T.J., Snyder, P.W., et al. (2019). Safety assessment of glycerin as used in cosmetics. Int J Toxicol *38* (*3_suppl*), 6S–22S https://doi.org/10.1177/1091581819883820. PubMed

Bernardi, D.S., Pereira, T.A., Maciel, N.R., Bortoloto, J., Viera, G.S., Oliveira, G.C., and Rocha-Filho, P.A. (2011). Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J Nanobiotechnology *9* (*1*), 44 https://doi.org/10.1186/1477-3155-9-44. PubMed

Boruah, S., Dutta, P., Pegu, J., Kaushik, H., Gogoi, N., Puzari, K.C., and Hazarika, G.N. (2016). SEM study on morphological changes in *Metarhizium anisopliae* infected *Aphis craccivora* Koch. J. Biol. Control *30* (*1*), 29–33 https://doi.org/10.18641/jbc/30/1/85022.

Bouchemal, K., Briançon, S., Perrier, E., and Fessi, H. (2004). Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. Int J Pharm *280* (*1-2*), 241–251 https://doi.org/10.1016/j.ijpharm.2004.05.016. PubMed

Butt, T.M., Greenfield, B.P.J., Greig, C., Maffeis, T.G.G., Taylor, J.W.D., Piasecka, J., Dudley, E., Abdulla, A., Dubovskiy, I.M., Garrido-Jurado, I., et al. (2013). *Metarhizium anisopliae* pathogenesis of mosquito larvae: a verdict of accidental death. PLoS One *8* (*12*), e81686 https://doi.org/10.1371/journal.pone.0081686. PubMed

Camargo, M.G., Golo, P.S., Angelo, I.C., Perinotto, W.M.S., Sá, F.A., Quinelato, S., and Bittencourt, V.R.E.P. (2012). Effect of oil-based formulations of acaripathogenic fungi to control *Rhipicephalus microplus* ticks under laboratory conditions. Vet Parasitol *188* (*1-2*), 140–147 https://doi.org/10.1016/j.vetpar.2012.03.012. PubMed

Camargo, M.G., Marciano, A.F., Sá, F.A., Perinotto, W.M.S., Quinelato, S., Gôlo, P.S., Angelo, I.C., Prata, M.C.A., and Bittencourt, V.R.E.P. (2014). Commercial formulation of *Metarhizium anisopliae* for the control of *Rhipicephalus microplus* in a pen study. Vet Parasitol *205* (*1-2*), 271–276 https://doi.org/10.1016/j.vetpar.2014.07.011. PubMed

Dotaona, R., Wilson, B.A.L., Stevens, M.M., Holloway, J., and Ash, G.J. (2015). Screening of tropical isolates of *Metarhizium anisopliae* (*Hypocreales*: *Clavicipitaceae*) for virulence to the sweet potato weevil, *Cylas formicarius* (*Coleoptera*: *Brentidae*). Int. J. Trop. Insect Sci. *35* (*4*), 153–163 https://doi.org/10.1017/S1742758415000211.

Du, Z., Wang, C., Tai, X., Wang, G., and Liu, X. (2016). Optimization and characterization of biocompatible oil-in-water nanoemulsion for pesticide delivery. ACS Sustain. Chem.& Eng. *4* (*3*), 983–991 https://doi.org/10.1021/acssuschemeng.5b01058.

Francardi, V., Benvenuti, C., Barzanti, G.P., and Roversi, P.F. (2013). Autocontamination trap with entomopathogenic fungi: a possible strategy in the control of *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera Curculionidae*). Redia (Firenze) *96*, 57–67.

Francardi, V., Benvenuti, C., Barzanti, G.P., and Roversi, P.F. (2016). *Metarhizium anisopliae* biopesticides and fungus isolates: control efficacy against *Rhynchophorus ferrugineus* (Olivier)(*Coleoptera Dryophthoridae*) on different contamination substrata. Redia (Firenze) *98*, 25–29.

Gill, H.K., and Garg, H. (2014). Pesticide: environmental impacts and management strategies. Pestic. Asp. *8*, 187.

Gindin, G., Levski, S., Glazer, I., and Soroker, V. (2006). Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus.* Phytoparasitica *34* (*4*), 370–379 https://doi.org/10.1007/BF02981024.

Green, D.L., Lin, J.S., Lam, Y.-F., Hu, M.Z., Schaefer, D.W., and Harris, M.T. (2003). Size, volume fraction, and nucleation of Stober silica nanoparticles. J Colloid Interface Sci *266* (*2*), 346–358 https://doi.org/10.1016/S0021-9797(03)00610-6. PubMed

Güerri-Agulló, B., Gómez-Vidal, S., Asensio, L., Barranco, P., and Lopez-Llorca, L.V. (2010). Infection of the red palm weevil (*Rhynchophorus ferrugineus*) by the entomopathogenic fungus *Beauveria bassiana*: a SEM study. Microsc Res Tech *73* (*7*), 714–725. PubMed

Hinaje, M., Ford, M., Banting, L., Arkle, S., and Khambay, B. (2002). An investigation of the ionophoric characteristics of destruxin A. Arch Biochem Biophys *405* (*1*), 73–77 https://doi.org/10.1016/S0003-9861(02)00275-8. PubMed

Hueston, W.J., Applegate, J.A., Mansfield, C.J., King, D.E., and McClaflin, R.R. (1995). Practice variations between family physicians and obstetricians in the management of low-risk pregnancies. J Fam Pract *40* (*4*), 345–351 https://doi.org/10.1146/annurev-phyto-073009. PubMed

Ibrahim, L., Butt, T.M., Beckett, A., and Clark, S.J. (1999). The germination of oil-formulated conidia of the insect pathogen, *Metarhizium anisopliae.* Mycol. Res. *103* (*7*), 901–907 https://doi.org/10.1017/S0953756298007849.

Idris, A.B., Mokhtaruddin, H., Zazali, C., Nurul Wahida, O., Yaakop, S., and Hazmi, I.R. (2014). The potential of red palm weevil infesting and destroying oil palm industry in Malaysia. Planter *90*, 329–335.

Jackson, M.A., Dunlap, C.A., and Jaronski, S.T. (2010). Ecological considerations in producing and formulating fungal entomopathogens for use in insect bio-control. BioControl *55* (*1*), 129–145 https://doi.org/10.1007/s10526-009-9240-y.

Jin, X., Streett, D.A., Dunlap, C.A., and Lyn, M.E. (2008). Application of hydrophilic–lipophilic balance (HLB) number to optimize a compatible non-ionic surfactant for dried aerial conidia of *Beauveria bassiana.* Biol. Control *46* (*2*), 226–233 https://doi.org/10.1016/j.biocontrol.2008.03.008.

Kershaw, M.J., Moorhouse, E.R., Bateman, R., Reynolds, S.E., and Charnley, A.K. (1999). The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insect. J Invertebr Pathol *74* (*3*), 213–223 https://doi.org/10.1006/jipa.1999.4884. PubMed

Kurouski, D., Washington, J., Ozbil, M., Prabhakar, R., Shekhtman, A., and Lednev, I.K. (2012). Disulfide bridges remain intact while native insulin converts into amyloid fibrils. PLoS One *7* (*6*), e36989 https://doi.org/10.1371/journal.pone.0036989. PubMed

Leemon, D.M., and Jonsson, N.N. (2008). Laboratory studies on Australian isolates of *Metarhizium anisopliae* as a biopesticide for the cattle tick *Boophilus microplus.* J Invertebr Pathol *97* (*1*), 40–49 https://doi.org/10.1016/j.jip.2007.07.006. PubMed

Llácer, E., and Jacas, J.A. (2010). Efficacy of phosphine as a fumigant against *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in palms. Span. J. Agric. Res. *8* (*3*), 775–779 https://doi.org/10.5424/sjar/2010083-1278.

Llácer, E., Dembilio, O., and Jacas, J.A. (2010). Evaluation of the efficacy of an insecticidal paint based on chlorpyrifos and pyriproxyfen in a microencapsulated formulation against *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). J Econ Entomol *103* (*2*), 402–408 https://doi.org/10.1603/EC09310. PubMed

Ment, D., Gindin, G., Glazer, I., Perl, S., Elad, D., and Samish, M. (2010). The effect of temperature and relative humidity on the formation of *Metarhizium anisopliae* chlamydospores in tick eggs. Fungal Biol *114* (*1*), 49–56 https://doi.org/10.1016/j.mycres.2009.10.005. PubMed

Moino, A., Jr., Alves, S.B., Lopes, R.B., Neves, P.M.O.J., Pereira, R.M., and Vieira, S.A. (2002). External development of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in the subterranean termite *Heterotermes tenuis.* Sci. Agric. *59* (*2*), 267–273 https://doi.org/10.1590/S0103-90162002000200010.

Morales, D., Gutiérrez, J.M., García-Celma, M.J., and Solans, Y.C. (2003). A study of the relation between bicontinuous microemulsions and oil/water nano-emulsion formation. Langmuir *19* (*18*), 7196–7200 https://doi.org/10.1021/la0300737.

Mwamburi, L.A., Laing, M.D., and Miller, R.M. (2015). Effect of surfactants and temperature on germination and vegetative growth of *Beauveria bassiana.* Braz J Microbiol *46* (*1*), 67–74 https://doi.org/10.1590/S1517-838246120131077. PubMed

Navarro-Llopis, V., Ayala, I., Sanchis, J., Primo, J., and Moya, P. (2015). Field efficacy of a *Metarhizium anisopliae*-based attractant–contaminant device to control *Ceratitis capitata* (*Diptera*: *tephritidae*). J Econ Entomol *108* (*4*), 1570–1578 https://doi.org/10.1093/jee/tov157. PubMed

Polar, P., Kairo, M.T.K., Moore, D., Pegram, R., and John, S.-A. (2005). Comparison of water, oils and emulsifiable adjuvant oils as formulating agents for *Metarhizium anisopliae* for use in control of *Boophilus microplus.* Mycopathologia *160* (*2*), 151–157 https://doi.org/10.1007/s11046-005-0120-4. PubMed

Ribeiro, R.C. de A., Barreto, S.M.A.G., Ostrosky, E.A., da Rocha-Filho, P.A., Veríssimo, L.M., and Ferrari, M. (2015). Production and characterization of cosmetic nanoemulsions containing *Opuntia ficus-indica* (L.) Mill extract as moisturizing agent. Molecules *20* (*2*), 2492–2509 https://doi.org/10.3390/molecules20022492. PubMed

Rodrigues, E. da C.R., Ferreira, A.M., Vilhena, J.C.E., Almeida, F.B., Cruz, R.A.S., Florentino, A.C., Souto, R.N.P., Carvalho, J.C.T., and Fernandes, C.P. (2014). Development of a larvicidal nanoemulsion with copaiba (*Copaifera duckei*) oleoresin. Rev. Bras. Farmacogn. *24* (*6*), 699–705 https://doi.org/10.1016/j.bjp.2014.10.013.

Roland, I., Piel, G., Delattre, L., and Evrard, B. (2003). Systematic characterization of oil-in-water emulsions for formulation design. Int J Pharm *263* (*1-2*), 85–94 https://doi.org/10.1016/S0378-5173(03)00364-8. PubMed

Sandrin, T.R., TeBeest, D.O., and Weidemann, G.J. (2003). Soy bean and sunflower oils increase the infectivity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* to northern jointvetch. Biol. Control *26* (*3*), 244–252 https://doi.org/10.1016/S1049-9644(02)00156-1.

Sedighi, N., Abbasipour, H., Askary, H., and Sheikhi Gorjan, A. (2013). Effect of oil suspended conidia of *Metarhizium anisopliae* var. *major* on mortality of the sunn pest, *Eurygaster integriceps* Puton (*Hemiptera*: *scutelleridae*). Arch. Phytopathol. Pflanzenschutz *46* (*2*), 128–140 https://doi.org/10.1080/03235408.2012.735083.

Setya, S., Talegaonkar, S., and Razdan, B.K. (2014). Nanoemulsions: formulation methods and stability aspects. World J. Pharm. Pharm. Sci. *3*, 2214–2228.

Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F.J., Khar, R.K., and Ali, M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm *66* (*2*), 227–243 https://doi.org/10.1016/j.ejpb.2006.10.014. PubMed

Shanmugam, A., and Ashokkumar, M. (2014). Ultrasonic preparation of stable flax seed oil emulsions in dairy systems – physico chemical characterization. Food Hydrocoll. *39*, 151–162 https://doi.org/10.1016/j.foodhyd.2014.01.006.

Sharma, S., Byrne, H., and O'Kennedy, R.J. (2016). Antibodies and antibody-derived analytical biosensors. Essays Biochem *60* (*1*), 9–18 https://doi.org/10.1042/EBC20150002. PubMed

Tadros, T.F. (2006). Applied Surfactants: Principles and Applications (John Wiley & Sons).

Wooster, T.J., Golding, M., and Sanguansri, P. (2008). Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir *24* (*22*), 12758–12765 https://doi.org/10.1021/la801685v. PubMed

Xavier-Santos, S., Lopes, R.B., and Faria, M. (2011). Emulsifiable oils protect *Metarhizium robertsii* and *Metarhizium pingshaense* conidia from imbibitional damage. Biol. Control *59* (*2*), 261–267 https://doi.org/10.1016/j.biocontrol.2011.08.003.

The red palm weevil *Rhynchophorus ferrugineus* in the omics and post-genomic eras

N. Montagné[1], A. Pain[2], B. Antony[3] and E. Jacquin-Joly[1,a]

[1]INRAE, Sorbonne Université, CNRS, IRD, UPEC, Université Paris Diderot, Institute of Ecology and Environmental Sciences of Paris, iEES-Paris, 78000 Versailles, France; [2]King Abdullah University of Science and Technology (KAUST), BESE Division, Thuwal, Jeddah 23955-6900, Saudi Arabia; [3]King Saud University, Department of Plant Protection, Chair of Date Palm Research, College of Food and Agricultural Sciences, Riyadh 11451, Saudi Arabia.

Abstract

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) is palm trees' most destructive and invasive insect pest. However, the molecular bases of its ecology and behavior are still poorly understood. Recently, valuable transcriptomic and genomic resources have been cumulated on this species, with the objective to understand important traits of the life of the RPW. These omics resources led to the annotation of numerous genes involved in important biological functions, such as immunity, detoxification, digestion, and chemo reception. In particular, the identification of the principal chemosensory gene families coupled to functional studies led to start deciphering the molecular bases of the RPW chemosensation, especially its pheromone communication. This mini-review describes these recent advances, focusing on olfactory detection, and highlights how they could be used to optimize the RPW control.

Keywords: red palm weevil, date palm, genome, transcriptome, chemosensory receptors, functional genomics

INTRODUCTION

Palm tree cultivation is threatened by the global expansion of an invasive pest, the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Figure 1). This *Coleoptera* is the most destructive and invasive insect pest of palm trees worldwide. Originating from South Asia, the RPW has spread through the Middle East, Africa, and the whole Mediterranean area since 1980. The phenomenal expansion and global spread of the RPW in almost all dominant palm tree-growing countries over the last three decades, as a consequence of the commercial exchange of palm trees worldwide, has recently resulted in its attainment of category-1 pest status (EPPO, 2019).

Figure 1. Adults (left) and larvae (right) of the red palm weevil *Rhynchophorus ferrugineus*. Photo credit: E. Jacquin-Joly, in Saudi Arabia, December 2021.

[a]E-mail: emmanuelle.joly@inrae.fr

The RPW lives in a microhabitat where different stages live inside the tree trunk. The RPW synchronizes mass gathering on the palm tree for feeding and mating, using a male-produced aggregation pheromone composed of two methyl-branched compounds (4RS,5RS)-4-methyl-nonan-5-ol (ferrugineol) and 4(RS)-methylnonan-5-one (ferrugineone) (Oehlschlager, 2016). The larvae bore soft tissues such as the tree crown, the base of the petioles, the trunks of young palms that lead to tissue rotting, and palm tree waning. The RPW Infested palms often die if not detected early and treated with insecticides.

The RPW control mainly relies on the use of systemic insecticides. Still, eco-friendly management techniques, such as the use of aggregation pheromone traps with or without palm tissues or kairomones in integrated pest management strategies, have emerged (Faleiro, 2006; Al-Dosary et al., 2016). Pheromone traps are also used to monitor populations and new potential invasions.

Despite the enormous economic losses worldwide due to the RPW, the molecular bases of its ecology and behavior are still poorly understood. Recently, valuable transcriptomic and genomic resources have been cumulated on this species and functional genomics tools are now available. This mini-review describes these recent advances, focusing on olfactory detection, and highlights how they could be used to optimize the RPW control.

RPW TRANSCRIPTOMES

As valuable resources to understand the RPW important traits of life, different transcriptomes from different tissues and developmental stages, including embryos, have been generated via assembly of Roche 454 or Illumina sequences (Wang et al., 2013; Yan et al., 2015; Yin et al., 2015; Antony et al., 2016; Yang et al., 2020; Zhang et al., 2020; Rasool et al., 2021). This led to the description of a plethora of transcripts from different families, such as neuropeptides and G-protein coupled receptors (Zhang et al., 2020) and reproduction control genes (Rasool et al., 2021). According to chemoreception, antennal transcriptome sequencing allowed the identification of chemosensory receptors. In insects, chemoreception relies on three families of chemosensory receptors, the odorant receptors (ORs) involved in volatile detection at a distance or close range, gustatory receptors (GRs) involved in detection at contact, and ionotropic receptors (IRs) involved in olfaction and taste (Robertson, 2019). While IRs are relatively conserved between insect species, ORs and GRs are highly divergent between and within species, with only 20-40% identity in amino acid sequences. This results from the fast evolution of these gene families via active gene birth and death (Engsontia et al., 2014; Robertson, 2019). The first RPW antennal transcriptome identified 18 ORs, 9 GRs and 9 IRs (Table 1). The second one greatly improved the chemosensory receptor repertoires, identifying 76 ORs, 15 GRs and 10 IRs (Table 1).

Table 1. Numbers of chemosensory receptors identified in the RPW transcriptomes and genomes.

	Yan et al., 2015	Antony et al., 2016	Hazzoury et al., 2020	Dias et al., 2021	Engsontia and Satasook, 2021
ORs	18	76	80	76	Not searched
GRs	9	15	Not searched	15	50 (encoding 65 proteins)
IRs	9	10	Not searched	10	Not searched

THE RPW GENOME

As additional resources, two genomes have been published recently (Hazzouri et al., 2020; Dias et al., 2021). The first genome was assembled from a combination of paired-end Illumina, Oxford Nanopore long reads, 10X Genomics data, and synteny analysis (Hazzouri et al., 2020). The assembly had a scaffold N50 of ~60 Mb, and the genome size was estimated at around 720 Mb. Analyses of this first genome and comparison with other beetle species revealed expansion of gene families important in insect response to environmental conditions, in detoxification of secondary metabolites and insecticide resistance (such as glutathione S-transferase, P450, Rdl channel), and immunity (such as secretin), that may reflect RPW adaptation. According to chemoreception, annotation led to the identification of 80 ORs (Table 1). Latter, the repertoire of GRs has been annotated in this genome, leading to the description of 50 GR genes encoding 65 GRs (Engsontia and Satasook, 2021) (Table 1). IRs have not been searched.

The second genome used 10× Genomics linked-read sequencing to produce a haplotype-resolved diploid genome assembled from a single RPW individual. The genome size was estimated at 590 Mb, thus smaller than the previous one, with a scaffold N50 of over 470 kb. In fact, the second genome analysis (Dias et al., 2021) evidenced that the previously reported RPW genome hybrid assembly contained a large proportion of artificially duplicated sequences due to the scaffolding of multiple haplotypes into a single haploid representation of the genome, and most gene expansions could not be confirmed. According to chemoreception, the second genome annotation identified 76 ORs, 10 IRs and 15 GRs (Table 1), as found in the antennal transcriptome (Antony et al., 2016). The number of GRs is far lower than the 50 GRs identified in the first genome. However, in view of the heterozygosity of the first genome, one could expect that 50 is an overestimated GR number.

FUNCTIONAL GENOMICS TOOLS DEVELOPED FOR THE RPW AND APPLICATION TO CHEMOSENSORY GENES

The transcriptomes and genomes described above constitute valuable resources and open up the route for further gene functional characterization. Tools to functionally characterize encoded proteins include loss-of-function experiments, via gene expression knock-down (e.g., via RNA interference, RNAi) or gene knockout (e.g., via CRIS- PR-Cas9 gene editing), and heterologous expression of the gene of interest in vitro (e.g., in cultured cells, *Xenopus* oocytes) or in vivo (e.g., in a host organism) (Montagné et al., 2021).

CRISPR-Cas9 is not yet developed for the RPW, but RNAi functions well in this insect, and several genes have been knocked down especially chemosensory genes. Orco, the co-receptor necessary for insect OR functioning (Larsson et al., 2004), was the first RPW OR to be silenced via RNAi (Soffan et al., 2016) (Table 2). As expected, injection of double-stranded RNA (dsRNA) from Orco into RPW pupae reduced Orco expression. This led to the failure of odorant detection, including pheromone detection, as confirmed through olfacto meter studies and electroan-tennography (EAG) assays. Odorant-binding proteins (OBPs) are also important proteins for odorant detection within the antennae. They are secreted proteins proposed to bind and transport odorants to the ORs (Pelosi et al., 2006). Silencing of one antennal-specific OBP, named RferOBP1768 (Table 2), significantly disrupted pheromone communication, suggesting that this OBP is involved in capturing and transporting the pheromone to ORs (Antony et al., 2018). RNAi has also been applied to ORs highly expressed in the antennae, and changes in pheromone detection have been assessed using electrophysiological recordings (Antony et al., 2021). This study revealed that the silencing of one particular OR, RferOR1 (Table 2), led to impaired pheromone detection, suggesting that this OR is involved in pheromone recognition. This hypothesis has been further confirmed using functional expression of this OR in a heterologous system, the so-called *Drosophila* “empty neuron” system (Kurtovic et al., 2007). In this system, an exogenous OR is expressed in *Drosophila* olfactory sensory neurons devoid of their own OR, and these neurons are screened with odorants using electrophysiology. When challenged with the RPW aggregation pheromone, pheromones from other weevils, and structurally related compounds, RferOR1 responded with high frequency and relative specificity to ferrugineol and ferrugineone, confirming that RferOR1 is a pheromone recep- tor (Antony et al., 2021). Another RPW OR that was found to be antennae-biased expressed (RferOR6) has been functionally characterized via expression, together with Orco, in *Xenopus* oocytes coupled to two-electrode voltage clamp (Ji et al., 2021) (Table 2). This OR could detect alpha-pinene, a non-palm plant volatile compound that has been shown to induce repellent behavior in the RPW.

Table 2. RPW chemosensory genes functionally characterized, methods used, and function.

References	Soffan et al., 2016	Antony et al., 2018	Antony et al., 2021	Ji et al., 2021
Genes	RferOrco	RferOBP1768	RferOR1	RferOR6
Methods	RNAi	RNAi	Heterologous expression in *Drosophila* neurons	Heterologous expression in *Xenopus* oocytes
Function	Chemoreception	Aggregation pheromone	Aggregation pheromone	alpha-pinene

PERSPECTIVES IN RPW CONTROL

Chemosensation is vital for the RPW to detect palm and non-host plant volatiles, orient toward a good food source, and detect the presence of conspecifics for aggregation and mating. Interfering with the mechanisms of odorant detection is thus a promising way to manipulate the RPW behavior. It has been recently proposed that insect OBPs and ORs could represent interesting targets for the design of molecules able to disrupt their correct functioning (Caballero-Vidal et al., 2020, 2021). Identifying and characterizing OBPs and ORs involved in key behavior is the first step in such perspectives. As exemplified with RferOR6 (Ji et al., 2021), studying ORs and identifying their odorant ligands also help identify new attractants or repellents.

Another approach may include silencing key OBPs and ORs, through host-induced gene silencing or spray-induced gene silencing techniques (Hernández-Soto and Chacón-Cerdas, 2021). Future development of CRISPR-Cas9 in the RPW will also allow conducting gene-drive approaches. In this view, the genomic data will be instrumental in designing an RNA guide and correctly estimating possible off-targets.

Last, the identification of OBPs and ORs detecting key RPW cues (like the pheromone) or volatile indicators of palm infestation (like herbivore-induced plant volatiles) opens up the development of OBP or OR-based biosensors (Bohbot and Vernick, 2020) for the early detection of RPWs in the field.

Literature cited

Al-Dosary, N.M.N., Al-Dobai, S., and Faleiro, J.R. (2016). Review on the management of red palm weevil *Rhynchophorus ferrugineus* Olivier in date palm *Phoenix dactylifera* L. Emir. J. Food Agric. *28* (*1*), 34 https://doi.org/10.9755/ejfa.2015-10-897.

Antony, B., Soffan, A., Jakše, J., Abdelazim, M.M., Aldosari, S.A., Aldawood, A.S., and Pain, A. (2016). Identification of the genes involved in odorant reception and detection in the palm weevil *Rhynchophorus ferrugineus*, an important quarantine pest, by antennal transcriptome analysis. BMC Genomics *17* (*1*), 69 https://doi.org/10.1186/s12864-016-2362-6. PubMed

Antony, B., Johny, J., and Aldosari, S.A. (2018). Silencing the odorant binding protein RferOBP1768 reduces the strong preference of palm weevil for the major aggregation pheromone compound ferrugineol. Front Physiol *9*, 252 https://doi.org/10.3389/fphys.2018.00252. PubMed

Antony, B., Johny, J., Montagné, N., Jacquin-Joly, E., Capoduro, R., Cali, K., Persaud, K., Al-Saleh, M.A., and Pain, A. (2021). Pheromone receptor of the globally invasive quarantine pest of the palm tree, the red palm weevil (*Rhynchophorus ferrugineus*). Mol Ecol *30* (*9*), 2025–2039 https://doi.org/10.1111/mec.15874. PubMed

Bohbot, J.D., and Vernick, S. (2020). The emergence of insect odorant receptor-based biosensors. Biosensors (Basel) *10* (*3*), 26 https://doi.org/10.3390/bios10030026. PubMed

Caballero-Vidal, G., Bouysset, C., Grunig, H., Fiorucci, S., Montagné, N., Golebiowski, J., and Jacquin-Joly, E. (2020). Machine learning decodes chemical features to identify novel agonists of a moth odorant receptor. Sci Rep *10* (*1*), 1655 https://doi.org/10.1038/s41598-020-58564-9. PubMed

Caballero-Vidal, G., Bouysset, C., Gévar, J., Mbouzid, H., Nara, C., Delaroche, J., Golebiowski, J., Montagné, N., Fiorucci, S., and Jacquin-Joly, E. (2021). Reverse chemical ecology in a moth: machine learning on odorant receptors identifies new behaviorally active agonists. Cell Mol Life Sci *78* (*19-20*), 6593–6603 https://doi.org/10.1007/s00018-021-03919-2. PubMed

Dias, G.B., Altammami, M.A., El-Shafie, H.A.F., Alhoshani, F.M., Al-Fageeh, M.B., Bergman, C.M., and Manee, M.M. (2021). Haplotype-resolved genome assembly enables gene discovery in the red palm weevil *Rhynchophorus ferrugineus.* Sci Rep *11* (*1*), 9987 https://doi.org/10.1038/s41598-021-89091-w. PubMed

Engsontia, P., and Satasook, C. (2021). Genome-wide identification of the gustatory receptor gene family of the invasive pest, red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790). Insects *12* (*7*), 611 https://doi.org/10.3390/insects12070611. PubMed

Engsontia, P., Sangket, U., Chotigeat, W., and Satasook, C. (2014). Molecular evolution of the odorant and gustatory receptor genes in lepidopteran insects: implications for their adaptation and speciation. J Mol Evol *79* (*1-2*), 21–39 https://doi.org/10.1007/s00239-014-9633-0. PubMed

EPPO. (2019). EPPO A1 and A2 Lists of Pests Recommended for Regulation as Quarantine Pests (European union: EPPO Global Database), https://gdeppoint/taxon/RHYCFE/documents.

Faleiro, J. (2006). A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Rhynchophoridae*) in coconut and date palm during the last one hundred years. Int. J. Trop. Insect Sci. *26*, 135–154.

Hazzouri, K.M., Sudalaimuthuasari, N., Kundu, B., Nelson, D., Al-Deeb, M.A., Le Mansour, A., Spencer, J.J., Desplan, C., and Amiri, K.M.A. (2020). The genome of pest *Rhynchophorus ferrugineus* reveals gene families important at the plant-beetle interface. Commun Biol *3* (*1*), 323 https://doi.org/10.1038/s42003-020-1060-8. PubMed

Hernández-Soto, A., and Chacón-Cerdas, R. (2021). RNAi crop protection advances. Int J Mol Sci *22* (*22*), 12148 https://doi.org/10.3390/ijms222212148. PubMed

Ji, T., Xu, Z., Jia, Q., Wang, G., and Hou, Y. (2021). Non-palm plant volatile alpha-pinene is detected by antenna-biased expressed odorant receptor 6 in the *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). Front Physiol *12*, 701545 https://doi.org/10.3389/fphys.2021.701545. PubMed

Kurtovic, A., Widmer, A., and Dickson, B.J. (2007). A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. Nature *446* (*7135*), 542–546 https://doi.org/10.1038/nature05672. PubMed

Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., and Vosshall, L.B. (2004). Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron *43* (*5*), 703–714 https://doi.org/10.1016/j.neuron.2004.08.019. PubMed

Montagné, M., Wanner, K.W., and Jacquin-Joly, E. (2021). Olfactory genomics within the *Lepidoptera*. In Insect Pheromone Biochemistry and Molecular Biology, 2nd edn, G.J. Blomquist, and R.G. Vogt, eds. (Academic Press), p.469–505.

Oehlschlager, A.C. (2016). Palm weevil pheromones - discovery and use. J Chem Ecol *42* (*7*), 617–630 https://doi.org/10.1007/s10886-016-0720-0. PubMed

Pelosi, P., Zhou, J.J., Ban, L.P., and Calvello, M. (2006). Soluble proteins in insect chemical communication. Cell Mol Life Sci *63* (*14*), 1658–1676 https://doi.org/10.1007/s00018-005-5607-0. PubMed

Rasool, K.G., Mehmood, K., Husain, M., Tufail, M., Alwaneen, W.S., and Aldawood, A.S. (2021). De novo transcriptome analysis and identification of reproduction control genes from the red palm weevil *Rhynchophorus ferrugineus.* PLoS One *16* (*5*), e0251278 https://doi.org/10.1371/journal.pone.0251278. PubMed

Robertson, H.M. (2019). Molecular evolution of the major arthropod chemoreceptor gene families. Annu Rev Entomol *64* (*1*), 227–242 https://doi.org/10.1146/annurev-ento-020117-043322. PubMed

Soffan, A., Antony, B., Abdelazim, M., Shukla, P., Witjaksono, W., Aldosari, S.A., and Aldawood, A.S. (2016). Silencing the olfactory co-receptor RferOrco reduces the response to pheromones in the red palm weevil, *Rhynchophorus ferrugineus.* PLoS One *11* (*9*), e0162203 https://doi.org/10.1371/journal.pone.0162203. PubMed

Wang, L., Zhang, X.W., Pan, L.L., Liu, W.F., Wang, D.P., Zhang, G.Y., Yin, Y.X., Yin, A., Jia, S.G., Yu, X.G., et al. (2013). A large-scale gene discovery for the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). Insect Sci *20* (*6*), 689–702 https://doi.org/10.1111/j.1744-7917.2012.01561.x. PubMed

Yan, W., Liu, L., Qin, W.Q., Li, C.X., and Peng, Z.Q. (2015). Transcriptomic identification of chemoreceptor genes in the red palm weevil *Rhynchophorus ferrugineus.* Genet Mol Res *14* (*3*), 7469–7480 https://doi.org/10.4238/2015.July.3.23. PubMed

Yang, H., Xu, D., Zhuo, Z., Hu, J., and Lu, B. (2020). SMRT sequencing of the full-length transcriptome of the *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). PeerJ *8*, e9133 https://doi.org/10.7717/peerj.9133. PubMed

Yin, A., Pan, L., Zhang, X., Wang, L., Yin, Y., Jia, S., Liu, W., Xin, C., Liu, K., Yu, X., et al. (2015). Transcriptomic study of the red palm weevil *Rhynchophorus ferrugineus* embryogenesis. Insect Sci *22* (*1*), 65–82 https://doi.org/10.1111/1744-7917.12092. PubMed

Zhang, H., Bai, J., Huang, S., Liu, H., Lin, J., and Hou, Y. (2020). Neuropeptides and G-protein coupled receptors (GPCRs) in the red palm weevil *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *dryophthoridae*). Front Physiol *11*, 159 https://doi.org/10.3389/fphys.2020.00159. PubMed

Temporal presence of adults red palm weevil *Rhynchophorus ferrugineus* Oliv. in Safwan-Basra Governorate south of Iraq

J.K. Mohammed[1,a], S.A. Hasan[1], B.Y. Mahdi[2] and H.J. Hamid[2]

[1]Plant Protection Directorate, Ministry of Agriculture, Baghdad, Iraq; [2]Basrah Department of Plant Protection, Ministry of Agriculture, Baghdad, Iraq.

Abstract

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) is considered as a dangerous pest infesting date palm causing serious damage to palm trees in the world. In Iraq, infestation of this pest was recorded for the first time in Safwan, Basrah Governorate south of Iraq during October 2015. The present study was conducted in date palm groves in Safwan region of Basra in Iraq during 2017-2020. The main objective of the study was to investigate the seasonal distribution of RPW population in the region by using aggregation pheromone traps placed in date palm orchards. Results indicated that RPW has three peaks on date palm trees throughout the year 2017, the weevils appeared during the second week of march and continued till the end of the year. The highest number of weevils captured was 13, 13 and 19 adults biweekly in May, October and November, respectively. Two peaks were recorded in 2018-2020.The highest rate of adults catches was 41 adults bi-weekly during the end of April, and 31 adults bi-weekly in end of October in 2018. In 2019 and 2020, the weevils appeared in January. The highest number of adults was 35 adults bi-weekly at the end of April and 31 adults bi-weekly in the second week of November in 2019. While in 2020, the highest number of adults captured was 25 adults bi-weekly in the second week of April and 33 adult bi-weekly in the second week of November. Results also indicated there were different relations between some climate factors (such as temperature, relative humidity and wind speed) and population activity of *R. ferrugineus* during 2017-2020. It was concluded that the pheromone traps were effective in capturing adults and monitoring population of RPWs, and climatic factors play an important role on the abundance of this pest in the study region.

Keywords: population, aggregation pheromone, traps, climate factors, palm orchards

INTRODUCTION

The red palm weevil *Rhynchophorus ferrugineus* Oliv. is considered one of extremely destructive serious pests attacking all types of palm trees worldwide and this pest is native in South and Southeast Asia (Al-Dosary et al., 2016; Azmi et al., 2017; Manzoor et al.,2020) which is now widely spreading in most date palm growing countries in the Middle East, Europe, North Africa, USA, South America and Australia (Aldawood and Rasool, 2011; Al-Shawaf et al., 2013). In Iraq, RPW infestation was first recorded in Safwan, Basrah Governorate, south of Iraq during October 2015 (Aletby, 2016). Management protocol of RPW decreased the population of the invasive pest preventing the spread of insects from first infested areas to other date palm growing Governorates in the country (Alderawii et al., 2020). The adult females of *R. ferrugineus* are attracted to wounded, damaged, or dying palms and lay their eggs in protected parts in palm sheaths and stems of young date palm trees less than 20 years old being most preferred hosts (Hunsberger et al., 2000; Faleiro et al., 2012). The attack of *R. ferrugineus* occurs in the tree crown or the upper part of the stem, including the base of petioles (Faleiro, 2006). The infestation of RPW is very difficult to detect in the early stages because the pest completes most of life cycle stages inside palm tree trunks. In high

[a]E-mail: jasim_aljanabi1968@yahoo.com

infestations levels all life stages of RPW from the overlapping generations can be found inside the palm trunks eventually leading to kill the palm tree (Avand, 1996; Justin et al., 2008).

Control of RPW is very difficult due to the concealed nature of the life-cycle of the pest. Integrated pest management (IPM) strategy has been applied successfully to deal with the RPW problem (El-Mergawy and Ajlan, 2011). During the last decades all efforts to control *R. ferrugineus* in Arab countries was applied by adopting an old integrated pest management (IPM) strategy, focusing on the use of traditional insecticides, modified cultural practices and recently pheromone traps (Kaakeh, 2006).

Pheromone traps have been widely used in controlling RPW as a main component of an integrated pest management regime. The use of aggregation pheromones has been reported as an effective tool for monitoring and mass trapping of the weevil adults in the field (Abd-Allah and Al-Khatri, 2005; Abuagla and Al-Deeb, 2012).

Environmental parameters (a biotic factor) influence the biology, ecology and population dynamics of organisms including insects. The *R. ferrugineus* seasonal activity, mating, growth and the host finding are fluctuating throughout the year depending on climatic conditions. Thus, it is important to understand the characteristics of the climate and environmental parameters such as rainfall, light intensity, temperature, humidity, etc., which play a critical role in the implementation of pheromone mass trappings of RPWs (Dembilio and Jacas, 2011). The present work was conducted to investigate temporal presence of RPW, *R. ferrugineus*, population on a date palm plantation by using pheromone traps during 2017-2020 and to study the effect of some weather factors on weevil catches in traps.

MATERIALS AND METHODS

Study sites

The work was carried out in Safwan region, Basra Governorate south of Iraq (30.132873N, 47.720521E) during 2017-2020. Pheromone traps (30 pheromone traps) were distributed in the investigated 30 orchards chosen to be the field of study; the number of palm trees ranged between 100 and 200 trees orchard^{-1} and they were approximately 12 years old.

Pheromone traps

The local pheromone traps were designed in plant protection directorate/department of diagnosis of agricultural pest by using a 10-L plastic bucket with four circular holes of 4 cm diameter near the upper edge of side walls and three holes were made in the cover of trap. The outer surface of the trap was covered with rough jute fabric to help the adults of red palm weevil to climb up and enter inside the trap through the holes. The traps were cleaned and the water was changed every two weeks to maintain better performance of each trap by avoiding the growth of fungi or algae on the water surface and avoid escaping of the adult. Each trap contained a dispenser of the *R. ferrugineus* aggregation pheromone [4-methyl-5-nonanol and 4-methyl-5-nonanone] with the trade name RHYFER produced by Alpha Scents, Inc., USA. The pheromone lure hung on cover center of the trap in inner side by a wire. The pheromone lure was replaced every two weeks in summer and every one month in the winter. Traps were placed in the ground down to the level of 15 cm except 5 cm above the ground to facilitate entrance of *R. ferrugineus* adults. The distance between traps was at least 100 m each trap was 4 m away from date palm trees. Traps were set under the shade of the plant canopy and not exposed to direct sunlight in order to obtain a sustainable and uniform release of the chemical lure into the environment and minimize water evaporation. The insects in the traps were collected, counted and recorded every two weeks.

Effect of some climate factors on the activity of red palm weevil

The influence of weather factors such as average daily mean temperature(ATAvg°C), relative humidity(RHAvg%) and wind speed (WSAvg K/H) were studied from January 2017 to December 2020 and were calculated for each two weeks to find the relation between the numbers of captured RPWs and the variations of each intended climatic factor. The weather information was obtained from the Iraqi Agro Meteorological Network, Ministry of

Agriculture.

Statistical analysis

Simple correlation and multiple regression of average daily mean temperature, relative humidity and wind speed and the population activity of RPW adults during 2017-2020 were undertaken and subjected to statistical analysis of computer (GenStat 12th edition) program. The simple correlation coefficient "r" for the relationship between each weather factor and weevils' population was then calculated.

RESULTS AND DISCUSSION

Population presence of *R. ferrugineus* on date palm plantation during (2017-2020)

The results of this study confirm that beginning, peak and the average number of *R. ferrugineus* caught in the traps were varied according to the season and climatic factors of surrounding environment.

Results of the first year (2017) showed that the weevils were not present in January to February and were observed in the second week of March and increased gradually. An obvious fluctuation with three peaks of insects number caught in the traps were recorded during this year. The first peak was recorded in end of May with an average of 13 adults traps^{-1} biweekly when an average of temperatures, relative humidity and wind speed were 33.38°C, 15.53% and 13.71 km h^{-1}, respectively, and the second peak observed in middle of August with 12 adults trap^{-1} biweekly when an average of temperatures, relative humidity and wind speed were 40.023°C, 17.53% and 8.92 km h^{-1}, respectively. While the third peak occurred in the end of November with 19 adults trap^{-1} biweekly when an average of temperatures, relative humidity and wind speed were 17.23°C, 50.1% and 7.92 km h^{-1}, respectively (Figure 1).

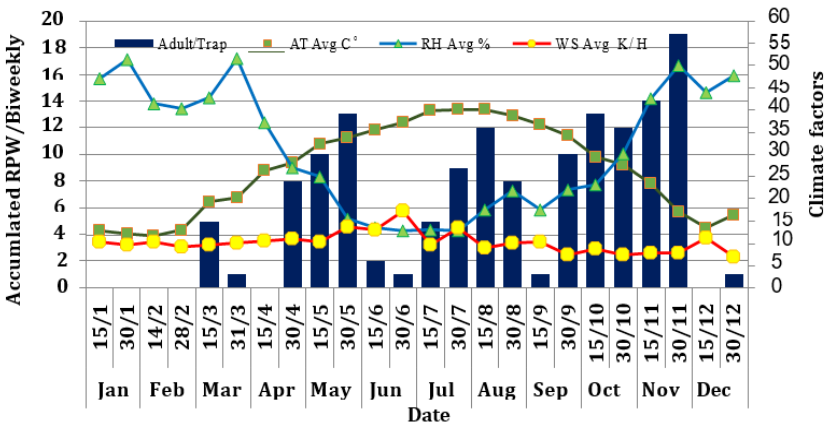


Figure 1. Temporal presence of RPW in relation to climate factors in Safwan-Basra during 2017.

Data presented in Figure 2 show that the adult RPW were found during the entire year of 2018 and started to increase drastically to reach the first peak with 41 adults trap^{-1} biweekly at the end of April when average temperature, relative humidity and wind speed were 25.75°C, 40.69% and 11.16 km h^{-1}, respectively. Then the population presence was decreased with a noticeable fluctuation till the end of September. The second peak occurred in October when the highest number of adults captured was 31 adults trap^{-1} biweekly in the end of October when average of temperature, relative humidity and wind speed were 25.07°C, 49.75% and7.2 km h^{-1}, respectively. The number of adults captured decreased to reach 4 adults trap^{-1} biweekly in the end of December when average temperature, relative humidity and wind speed were 12.98°C, 68.15% and 7.63 km h^{-1}, respectively.

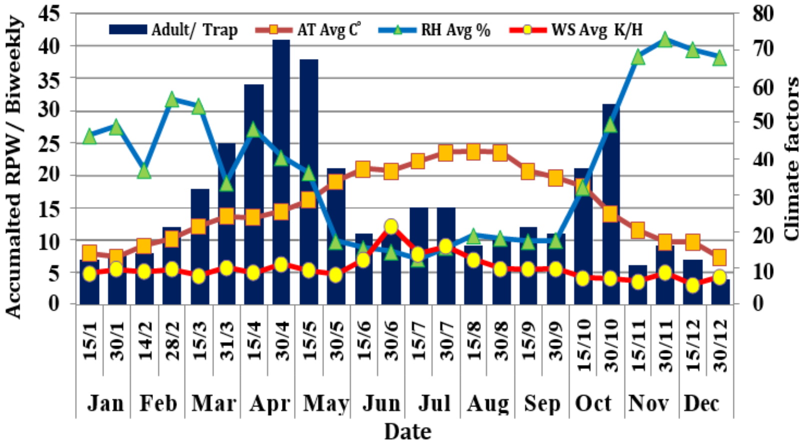


Figure 2. Temporal presence RPW in relation to climate factors in Safwan-Basra during 2018.

In 2019 and 2020, seasonal presence of *R. ferrugineus* population showed an approximately similar trend of abundance as in the 2018 recording two peaks for each year. The adult weevils appeared in January in low number and increased gradually in numbers. In 2019 the highest value of the first peak was 35 adults traps^{-1} biweekly recorded at the end of April when average temperature, relative humidity and wind speed were 23.1°C, 38.22% and 9.75 km h^{-1}, respectively. While in the second peak the highest number of adults captured was 31 adults trap^{-1} biweekly recorded in the second week of November when average temperature, relative humidity and wind speed were 21.36°C, 47.72% and 7.02 km h^{-1}, respectively. While in 2020, the first peak was recorded in the second week of April with highest number of adults of 25 adults trap^{-1} biweekly with an average temperature, relative humidity and wind speed of 26.1°C, 37.9% and 6.12 km h^{-1}, respectively. In the second peak the highest number of adults captured was 33 adults trap^{-1} biweekly recorded in the second week of November with an average temperature, relative humidity and wind speed of 23.1°C, 55.13% and 7.88 km h^{-1}, respectively (Figures 3 and 4).

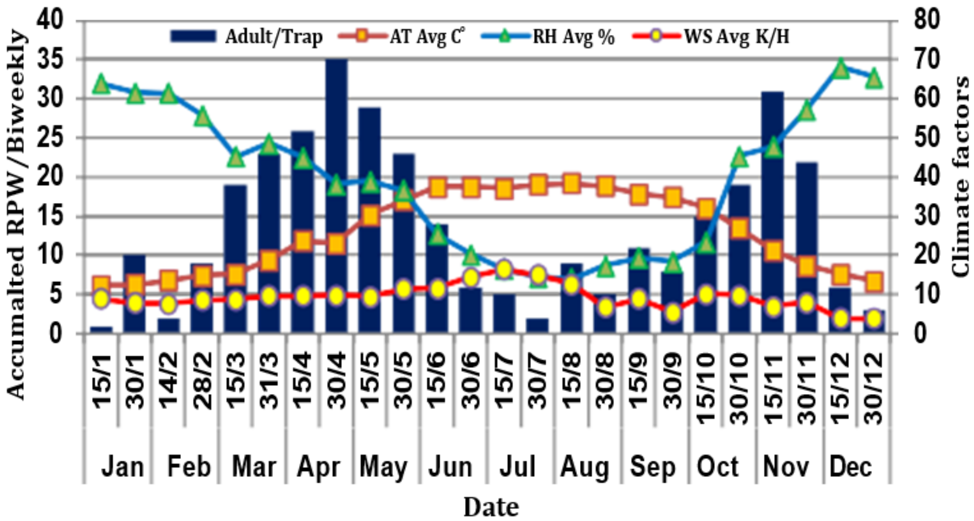


Figure 3. Temporal presence of RPW in relation to climate factors in Safwan-Basra during 2019.

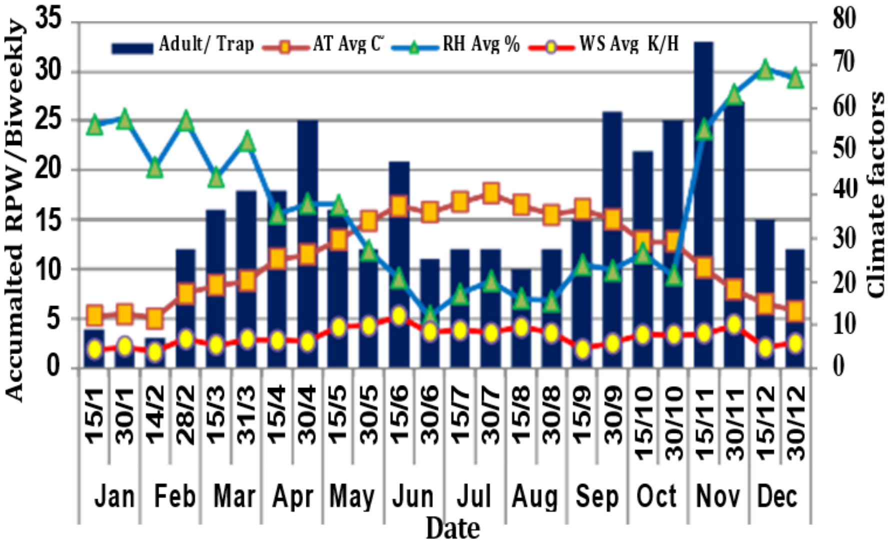


Figure 4. Temporal presence of RPW in relation to climate factors in Safwan-Basra during 2020.

Results also indicated that the most weevils activity occurred when surrounding weather temperature ranged between 20 and 40°C, 15-55% RH and wind speed of 1.5-3.7 m s^{-1}. Two major peaks were observed during the whole year. Increased weather temperature and wind speed along with decreased humidity had negative effect on flight activity of RPW. The results of this study in agreement with previous studies findings of Vidyasagar et al. (2000) detected two weevil activity peaks, during April-May and much smaller second peak was observed during October-November in the Kingdom of Saudi Arabia. In the United Arab Emirates Abbas et al. (2006) reported that the population fluctuation of *R. ferrugineus* captured in traps using the aggregation pheromone during 2000 and 2001, increased gradually from January to reach its peak between March-May. Other studies indicated that weevils of *R. ferrugineus* were found in pheromone traps during the entire year of two successive seasons 2007-2008 and 2008-2009. Two flight peaks were recorded during both years, the first peak was recorded in September and the second peak occurred in March. In a later study, El-Bokl et al. (2015) noticed two major population peaks in Egypt, the first peak reached its maximum in March, while the second peak started in September and reached its maximum in October. Metwally and Basheer (2019) found that population dynamics of RPW were different and the number of generations varied during the seasons 2014 and 2015. There were two peaks of swarming activity on date palm trees throughout the first year, and three peaks throughout the second year.

The correlation between weather factors and population activity of RPW adults during 2017-2020

1. Effect of daily mean temperature.

Results showed that the decrease in the average numbers of adults RPW caught by pheromone traps coincided with the decrease and increase in the maximum and minimum temperatures below 20 and 10°C, respectively, in the beginning and end of the all years under study.

The obtained results presented in Figures 5-8 show the relation between daily mean temperature and the population abundance of RPW were quadratic curve with significant correlation coefficient (r) in year 2017, 2018,2019 and 2020 (r value = 0.504, 0.735, 0.859 and 0.656, respectively).

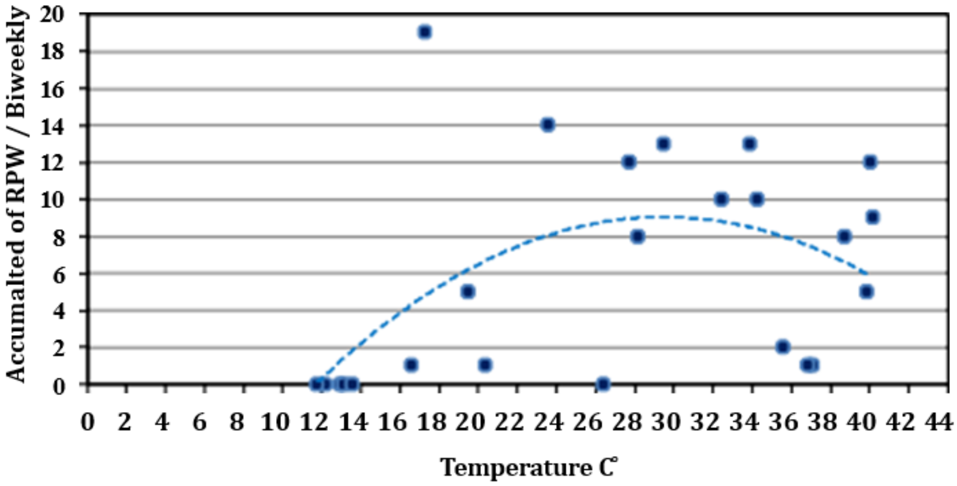


Figure 5. The correlation between number of RPW and average temperature during 2017.

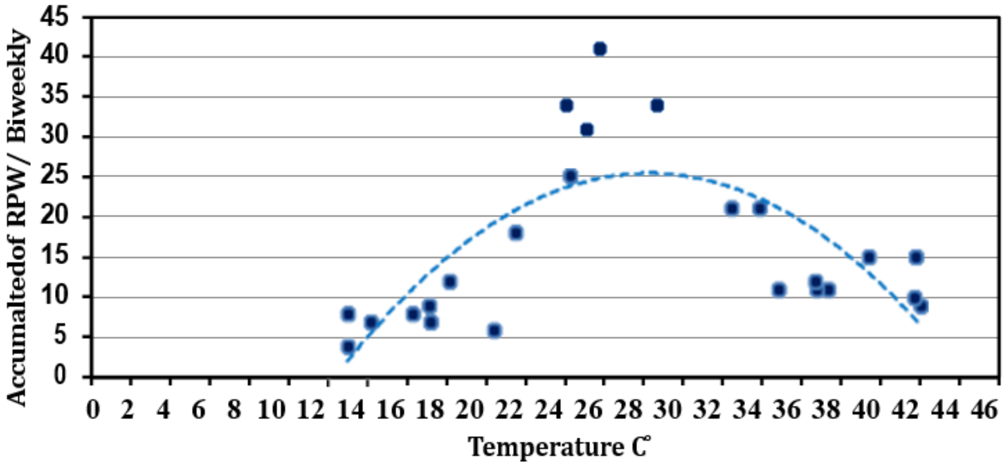


Figure 6. The correlation between number of RPW and average temperature during 2018.

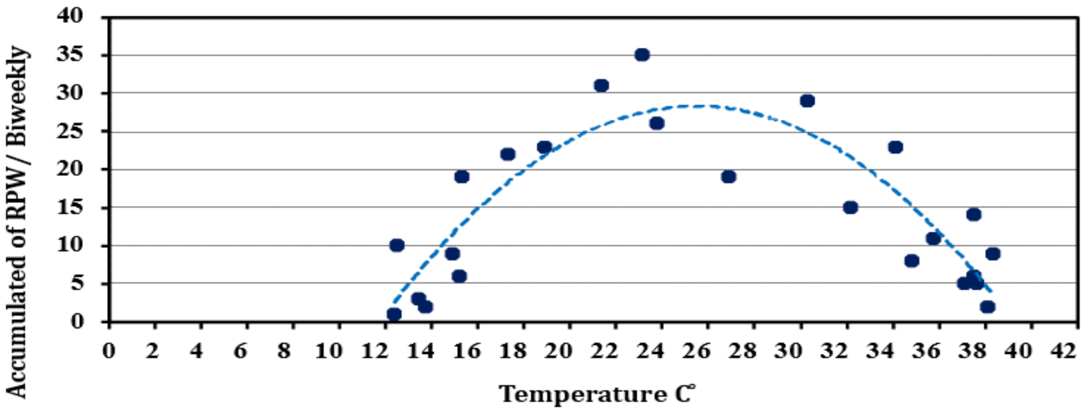


Figure 7. The correlation between number of RPW and average temperature during 2019.

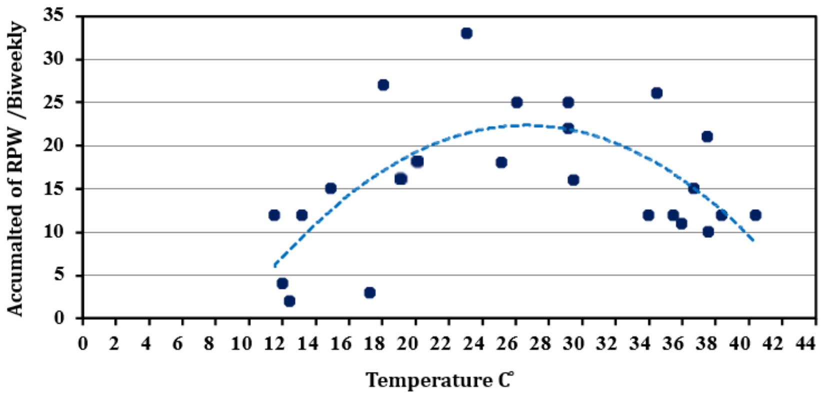


Figure 8. The correlation between number of RPW and average temperature during 2020.

2. Effect of daily average relative humidity.

The relative humidity had different correlation with the population abundance of the RPW adults during in 2017-2020. There was significant correlation in 2018 and 2019 (r value = -0.508 and 0.865, respectively). On the other hand daily mean RH% had no significant correlation in 2017 and 2020 (r value = 0.291 and 0.202, respectively) (Figures 9-12).

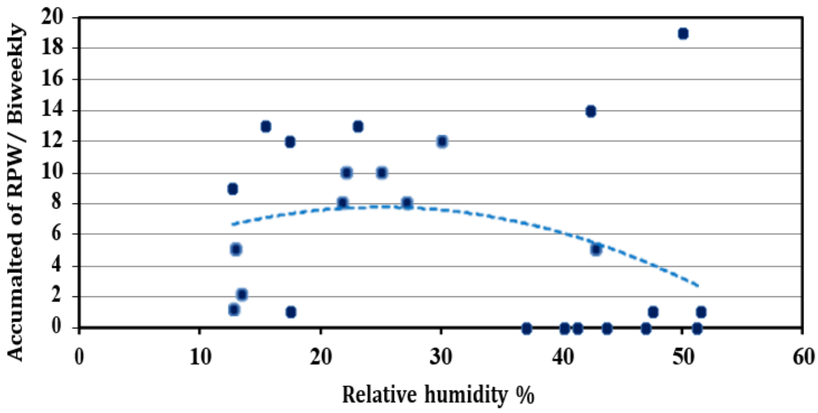


Figure 9. The correlation between number of RPW and average relative humidity during 2017.

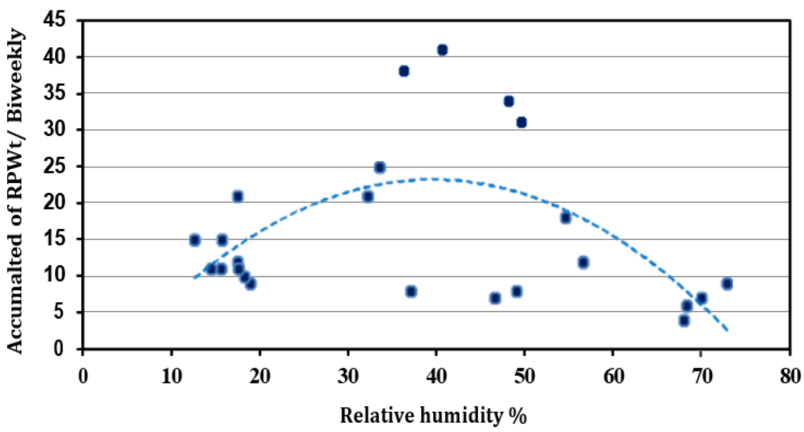


Figure 10. The correlation between number of RPW and average relative humidity during 2018.

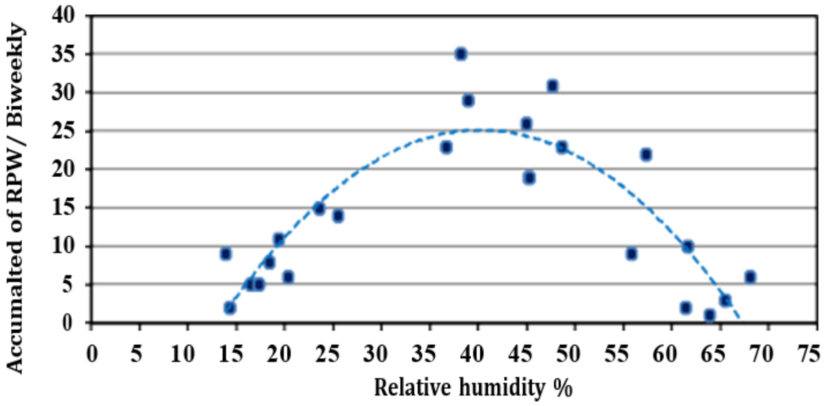


Figure 11. The correlation between number of RPW and average relative humidity during 2019.

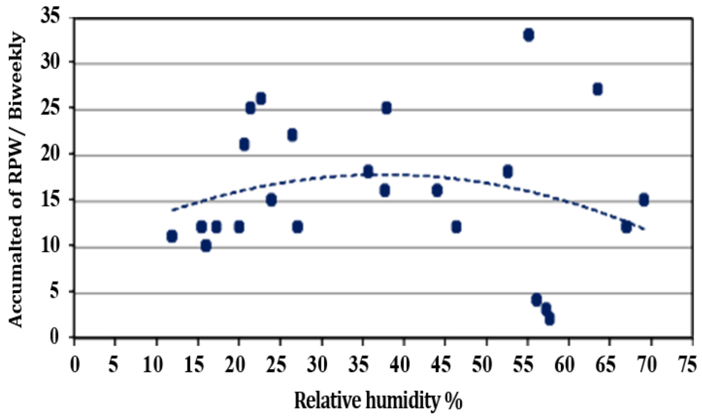


Figure 12. The correlation between number of RPW and average relative humidity during 2020.

3. Effect of daily average wind speed.

The wind speed had different correlation with the population abundance of the RPW adults during in 2017-2020. There was significant correlation in 2019 and 2020 (r value = 0.545 and 0.429, respectively). However, there was no significant correlation between daily mean wind speed and RPW in 2017 and 2018 (r value = 0.343 and 0.284, respectively) (Figures 13-16).

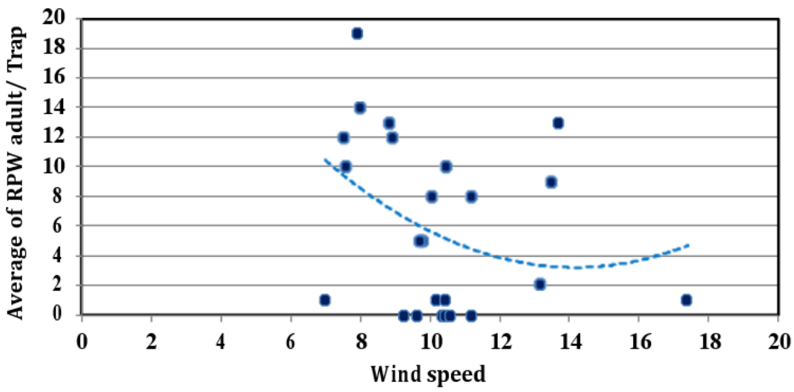


Figure 13. The correlation between adult of RPW and wind speed during 2017.

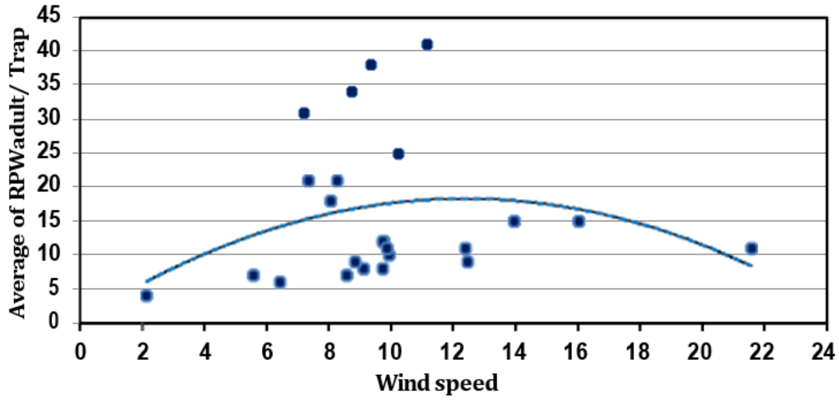


Figure 14. The correlation between adult of RPW and wind speed during 2018.

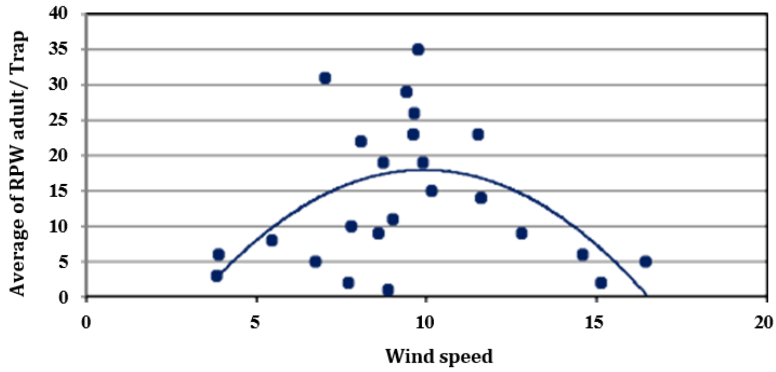


Figure 15. The correlation between average of RPW and wind speed during 2019.

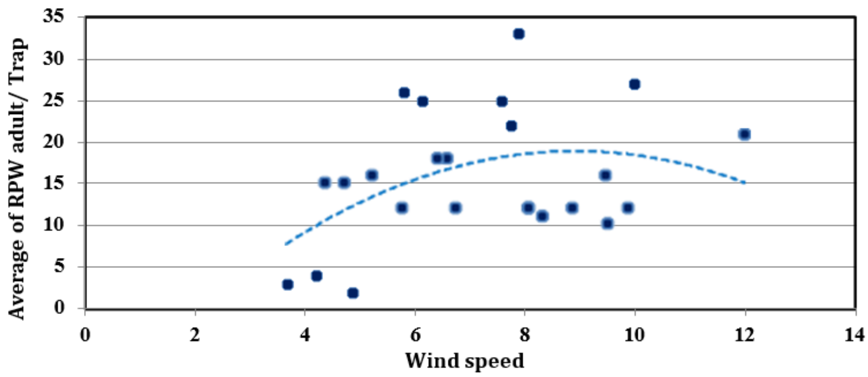


Figure 16. The correlation between adult of RPW and wind speed during 2020.

Previous studies Faleiro (2005) indicated that maximum temperature and rainfall had a significant impact on the weevil activity in India. Abd-Allah and Al-Khatri (2005) reported that there is an effect of the climatic conditions such as maximum and minimum temperature and the relative humidity on the population fluctuation of RPW. Later studies by Hussain et al. (2016) found different effects of daily mean temperature and relative humidity on the population activity of *R. ferrugineous* in Baharia Oases during 2013-2014. Other studies by Metwally and Basheer (2019) showed that there was a positive and negative correlation between weather factors (mean daily temperature and daily relative humidity) and the population abundance of RPW during 2014-2015.

CONCLUSIONS

- Results of the present study showed that red palm weevil is a noxious invasive pest in Iraq. The presence of the weevil still in the Safwan region/Basra south of Iraq. The insect was present throughout the year, beginning, peak and the average number of insects caught in the traps were varied during the seasons of study;
- The traps showed two peaks of the population were recorded during each year of study except 2017 when three peaks were observed;
- Climatic factors had an important impact on the presence of RPW pest. However, more studies are still needed to investigate the influence of other climatic factors such as wind storms, dust storms, along with surrounding habitat and type of vegetation on the spread RPW. Environmental studies are essential for controlling the pest and prevent its movement to other regions.
- The agricultural practices for date palm trees especially pruning operation to remove the palm dry leaves, leaf bases, fiber, spines and high offshoots should be carried out in the low activity period of the RPW insect and need to implement precautionary measures that follow the pruning process, which prevent attracted pest to date palm trees.

ACKNOWLEDGEMENTS

We thank Dr. Abdul-Sattar Aref for critical comments on this paper. We also thank Dr. Waleed Al-Shafie and Mr. Emad Alani from Iraqi Agro Meteorological Network, Ministry of Agricultural for providing the weather information. We also thank all colleagues in Basra Department of Plant Protection for their assistance in the research.

Literature cited

Abbas, M.S.T., Hanounik, S.B., Shahdad, A.S., and Al-Bagham, S.A. (2006). Aggregation pheromone traps, a major component of IPM strategy for the red palm weevil, *Rhynchophorus ferrugineus* in date palms (*Coleoptera*: *curculionidae*). J. Pest Sci. *79* (*2*), 69–73 https://doi.org/10.1007/s10340-005-0113-6.

Abd-Allah, F.F., and Al-Khatri, S.A. (2005). Pheromone, kairomone and food bait on attracting adults of red palm weevil *Rhynchophorus ferrugineus* in the Sultanate of Oman in date palm plantations. Egypt. J. Agric. Res. *83*, 169–177.

Abuagla, A.M., and Al-Deeb, M.A. (2012). Effect of bait quantity and trap color on the trapping efficacy of the pheromone trap for the red palm weevil, *Rhynchophorus ferrugineus*. J Insect Sci *12* (*1*), 120 https://doi.org/10.1673/031.012.12002. PubMed

Al-Dosary, N.M., Al-Dobai, S., and Faleiro, J.R. (2016). Review on the management of red palm weevil *Rhynchophorus ferrugineus* Olivier in date palm *Phoenix dactylifera* L. Emir. J. Food Agric. *28* (*1*), 34–44 https://doi.org/10.9755/ejfa.2015-10-897.

Al-Shawaf, A., Al-Shagag, A., Al-Bagshi, M., Al-Saroj, S., Al-Bather, S., Al-Dandan, A., Abdallah, A., and Faleiro, J.R. (2013). A quarantine protocol against red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleptera*: *Curculiondae*) in date palm. J. Plant Prot. Res. *53* (*4*), 409–415 https://doi.org/10.2478/jppr-2013-0061.

Aldawood, A.S., and Rasool, K.G. (2011). Rearing optimization of red palm weevil: *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) on date palm: *Phoenix dactylifera.* Fla. Entomol. *94* (*4*), 756–760 https://doi.org/10.1653/024.094.0404.

Alderawii, M.M., Alyousuf, A.A., Hasan, S.A., Mohammed, J.K., Jappar, H.A., and Paudyal, S. (2020). An Evaluation of invasive pest, red palm weevil *Rhynchophorus ferrugineus* (Olivier, 1790) (*Coleoptera*, *Curculionidae*) population in Iraq. Bull. Iraq Nat. Hist. Mus. *16* (*2*), 203–218 https://doi.org/10.26842/binhm.7.2020.16.2.0203.

Aletby, M.A.A.W. (2016). First record of red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) on the date palm, *Phoenix dactylifera* in Basrah, Iraq. Basrah Journal of Agricultural Sciences *29* (*1*), 1–6 https://doi.org/10.33762/bagrs.2016.120920.

Avand, F.A. (1996). The biology of red palm weevil, *Rhynchophorus ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*) in Saravan region (Sistan and Balouchistan Province, Iran). Appl. Entomol. Phytopathol. *63*, 16–18.

Azmi, A., Lian, C.J., Zakeri, H.A., Yusuf, N., Omar, W.B.W., Wai, Y.K., and Husasin, M. (2017). The red palm weevil, *Rhynchophorus ferrugineus*: current issues and challenges in Malaysia. Oil Palm Bul. *74*, 17–24.

Dembilio, O., and Jacas, J.A. (2011). Basic bio-ecological parameters of the invasive red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*), in *Phoenix canariensis* under Mediterranean climate. Bull Entomol Res *101*

(*2*), 153–163 https://doi.org/10.1017/S0007485310000283. PubMed

El-Bokl, M.M., Sallam, A.R.M., Abdallah, G.A., and Gabr, B.M. (2015). Efficacy of aggregation pheromone in trapping red palm weevil (*Rhynchophorus ferrugineus* Olivier) infested date palms in Damietta, Egypt. Egypt. Acad. J. Biolog. Sci. *7* (*1*), 51–59 https://doi.org/10.21608/eajbsc.2015.13702.

El-Mergawy, R., and Ajlan, A. (2011). Red palm weevil, *Rhynchophorus ferrugineus* (Olivier): economic importance, biology, biogeography, and integrated pest management. J. Agric. Sci. Technol. A *1* (*1*), 1–23.

Faleiro, J.R. (2005). Pheromone technology for the management of red palm weevil *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*: *Curculionidae*) a key pest of coconut. Technical Bulletin ICAR Research Complex for Goa. *4*, 40.

Faleiro, J. (2006). Insight into the management of red palm weevil *Rhyncophorus ferrugineus* Olivier: based on experiences on coconut in India and date palm in Saudi Arabia. In I Jorn. Int. sobre el Picudo Rojo de las Palmeras (Valencia, Spain: Fundación Agroalimed), p.33–57.

Faleiro, J.R., Abdullah, B.A., El-Bellaj, M., Al-Ajlan, A.M., and Oihabi, A. (2012). Threat of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) to date palm plantations in North Africa. Arab J. Plant Prot. *30*, 274–280.

Hunsberger, A., Giblin-Davis, R., and Weissling, T. (2000). Symptoms and population dynamics of *Rhynchophorus cruentatus* (*Coleoptera*: *Curculionidae*) in Canary Island date palms. Fla. Entomol. *83* (*3*), 290–303 https://doi.org/10.2307/3496348.

Hussain, A.E., Elsharabasy, S.F., Megahed, M.M.M., and Abd Elmagid, A.R.M. (2016). Population abundance of the red palm weevil *Rhynchophorus ferrugineus* (Oliv.) adults on date palm plantations in Baharia Oases Giza Governorate-Egypt. J. Plant Prot. and Path. Mansoura Univ. *7* (*10*), 649–654 https://doi.org/10.21608/jppp.2016.52097.

Justin, C.G.L., Leelamathi, M., Thangaselvabai, T., and Johnson, S.B.N. (2008). Bioecology and management of the red palm weevil, *Rhynchophorus ferrugineus* Oliv. on coconut. a review. Agric. Rev. (Karnal) *29*, 117–124.

Kaakeh, W. (2006). Toxicity of imidacloprid to developmental stages of *Rhynchophorus ferrugineus* (*Curculionidae*: *Coleoptera*): laboratory and field tests. Crop Prot. *25* (*5*), 432–439 https://doi.org/10.1016/j.cropro.2005.07.006.

Manzoor, M., Ahmad, J.N., Ahmad, S.J., Naqvi, S.A., Rasheed, R., and Haider, M.S. (2020). Population dynamics, abundance and infestation of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in different geographical regions of date palm in Pakistan. Pak. J. Agric. Sci. *57* (*2*), 381–391.

Metwally, H.A.A., and Basheer, A.M. (2019). the behaviour and activity of the red palm weevil *Rhynchophorus ferrugineus* throughout the year under Baharia Oasis conditions, Egypt. Middle East J. Agric. Res. *8* (*3*), 797–807.

Vidyasagar, P., Hagi, M., Abozuhairah, R., Al Mohanna, O., and Al-Saihati, A. (2000). Impact of mass pheromone trapping on red palm weevil: adult population and infestation level in date palm gardens of Saudi Arabia. Planter *76*, 347–335.

Bibliography of date palm pests and lessons learned to manage RPW

M. El-Said El-Zemaity[a]

Plant Protection Department, Faculty of Agriculture, Ain shams University, Cairo, Egypt.

Abstract

Establishing a bibliography of the main palm pests in the Arab region, especially the red palm weevil (RPW), is a very important approach. The bibliography includes a comprehensive inventory of all independent and arranged data sources for information related to the pest. Such a bibliography will have a role in assisting workers, technicians, and officials responsible for developing pest management programmes in general and date palm pests in particular. Also, it will be a useful aid to researchers, extension agents, agricultural producers, and industry, as well as government officials involved in pest management. Since bibliographies are important keys as sources of information characterized by saving effort, time, and costs, they will help researchers to complete their research faster, more comprehensively, more accurately, and more efficiently. This paper will focus on an analytical study of a prepared bibliography for the RPW *Rhynchophorus ferrugineus* Oliver (*Coleoptera: Curculionidae*), as it is now one of the most dangerous palm pests in our Arab region. The study started by compiling 621 articles from different sources, including publications in scientific journals, proceedings of scientific meetings, specialized books, scientific theses, internet, specialized databases, the Arab Scientific Community Organization list of published research on palm pests, and others. The scientific papers were classified into 18 major research areas and 28 sub-fields. Data extracted from such bibliography indicate that there is a real need for more efforts and research to: strengthen the role of some of the basic components in IPM programmes such as threshold level and natural control; enhancing the adoption of biotechnology products, and some other potential components; carry out more molecular investigations to provide a better understanding of the insect's behavior and the interaction between its immune system and biological control agents; encouraging researches on evaluating the current RPW management programs in light of the local conditions of each Arab country.

Keywords: date palm pests, red palm weevil, IPM programmes

INTRODUCTION

The word bibliography has become an expressive term well-established in use by all conferences, specialized organizations, and researchers in the field of libraries and information in the Arab world. It is expected that the bibliographic list for each pest includes a comprehensive inventory of all independent and arranged data sources for information related to the main palm pests in the Arab region, especially the red palm weevil RPW, according to the known scientific bases. It is expected that this approach will have a role in assisting workers, technicians and officials responsible for developing pest management programs in general and date palm pests in particular. Establishing this bibliography as a specialized source of information will undoubtedly be relied upon in program planning, implementation and evaluation. The bibliography will also be a useful aid to researchers, extension agents, agricultural producers, industry and government officials involved in pest management. Since bibliographies are important keys as sources of information characterized by saving effort, time and costs, they will help researchers to complete their research faster,

[a]E-mail: mselzemaity@hotmail.com

more comprehensively, more accurately and more efficiently, and it is expected to play an important role in:

1. Facilitating the process of gathering information for pest management managers and the scientific researcher, in terms of obtaining the sources for the subject of his research through all the extensions he desires, temporally, spatially, linguistically and objectively;
2. Helping the researcher to choose and select the sources he desires, as well as guiding him to sources he did not think of;
3. Enable the researcher to verify certain information and work on completing or correcting it;
4. Providing the existing sites and databases with audited information, and encouraging the establishment of specialized databases by the parties interested in the problems of date palm pests;
5. Encouraging and disseminating expert systems for the management of date palm pests, and making them easily available to benefit date producers and growers in solving the pest problems facing them.

The RPW, *Rhynchophorus ferrugineus* (Oliv.) is a dangerous pest that infects palm trees, especially the coconut and date palm. As an invasive pest, it was established in recent decades in most Arab countries and many other regions at the global level. It is now one of the most dangerous pests threatening the date palm, which occupies great importance and interest to palm growers throughout the Arab world. Because of its economic importance, it has attracted special interest to researchers from all over the world, which has led to the emergence of a huge number of scientific literature and publications on various aspects of the pest, which has made researchers and documentaries feel an urgent need for a bibliography of the pest. In fact, efforts in this direction began with an attempt in 1984 by Divakaran Pillai, who at that time included a list of 48 publications about RPW on the coconut palm. In 2000, Thajudin published a bibliography titled "An Annotated Bibliography on *Rhynchophorus ferrugineus* – a Pest of Coconut". After that Thajudin and Mohan noted that there are a large number of publications that had subsequently accumulated on the pest, and that there was a need to update the bibliography. So they compiled the literature on the red palm weevil, and published as bibliography by CPCRI in 2013. The last bibliography included 518 publications, mostly about the biology of the insect, its life aspects, and its control on coconuts (https://krishi.icar.gov.in/jspui/bitstream/123456789/9080/1/RPW%20Bibliography.pdf). With the invasion of the pest and its establishment in the date palm regions, the scientific literature focused on its management on date palms. Accordingly, the current compiled bibliography deals with the published literature on the pest on date palm without ignoring the valuable researches published on coconut so that the picture is complete for those interested. The objective of the present work is to update a bibliography includes the recently published articles until 2020 about RPW on date palm.

ESTABLISHING THE BIBLIOGRAPHY

The work began by compiling 621 articles from different sources, including publications in scientific journals, proceedings of scientific meetings, specialized books, scientific theses, internet, specialized databases, the Arab Scientific Community Organization list of published research on palm pests, Grafiati Bibliography on the topic RPW, EPPO Data Sheet on *Rhynchophorus ferrugineus* and others. The compiling articles were classified into 18 major research areas and 28 sub-fields as shown in Tables 1 and 2. To facilitate the review of the research directions of the collected publications, they will be addressed as follows:

- Comprehensive knowledge;
- Monitoring, sampling, detection and the economic threshold;
- Natural control;
- Management practices;
    1. Proactive practices;
    2. Active practices;
    2.1 Agricultural and mechanical methods;

2.2 Biological control and biopesticides;
2.3 Chemical control;
2.4 Pheromones and attractants;
2.5 Genetic control, sterilization and irradiation;
2.6 Information and communication technologies.

Table 1. Compiling articles published about comprehensive knowledge and basic IPM components needed for management of RPW.

Field of research	No.	%
1. Identification/diagnosis/symptoms/damage	12	4.22
2. Biological and ecological aspects/molecular biology	59	
2.1 Life cycle and factors affecting the insect development	2	20.77
2.2 Seasonal activity/population dynamics	1	
2.3 Longevity, fecundity and fertility	7	
3. Other principle information	81	
3.1 Morphology	6	
3.2 Physiology	20	
3.3 Behavior	13	28.52
3.4 Biochemistry	7	
3.5 Defense mechanism	1	
3.6 Rearing	19	
3.7 Host plants	14	
4. Distribution/threat/infestations	59	20.77
5. General aspects	11	3.87
6. Survey/field monitoring/scouting	4	1.40
7. Detection	41	
7.1 Smell by dogs	2	14.43
7.2 Bioacoustic/acoustic methods	14	
7.3. Signal processing technology	6	
8. Sampling and action levels/area-wide management (operation)	2	0.70
9. Natural controls	15	
9.1 Entomophagous enemies	4	5.28
9.2 Entomopathogenic microorganisms	11	
Total	**284**	**100**

Table 2. Compiling articles published about management practices and control methods of RPW.

Field of research	No.	%
10. Control/IPM/management practices	55	16.32
11. Preventive strategy/regulation and legislative interventions	11	3.26
11.1 Quarantine	3	
11.2 Regional campaigns	1	
12. Agricultural and mechanical methods	3	0.89
13. Resistant cultivars (plants)/transgenic date palms	8	2.37
14 Biological control/bio-insecticides	96	28.48
14.1 Natural enemies	6	
14.2 Entomopathogenic nematodes	41	
14.3 Entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*)	27	
14.4 Entomopathogenic bacterium (*Bacillus thuringiensis*)	4	
15. Chemical control (insecticides)	46	13.64
15.1 Trunk injection	6	
15.2 Fumigation	3	
15.3 Root feeding	1	
16. Pheromone traps/attractants/adult trapping	92	27.29
16.1 Natural or synthetic attractants	4	
16.2 Aggregation pheromone	13	
16.3 Pheromone lures	6	
17 Genetic control/sterilization/irradiation/sterile male technique	21	6.23
18. Information and communication technologies	5	1.48
Total	**337**	**100**

REVIEW OF OBTAINED DATA

Comprehensive knowledge

The number of articles related to the basic aspects of the red palm weevil RPW that were included in the prepared bibliography reached 222 papers (Table 1), including: 12 papers of identification, diagnosis, symptoms of infestation and damage caused by this dangerous insect to the date palm; 59 papers on biological and ecological aspects including molecular biology, life cycle and factors affecting development and growth (two papers), seasonal activity and population dynamics (one paper), lifespan and fertility (7 papers); 81 papers for other background information, including morphological studies (6), physiological and defense mechanisms (21), behavioral (13), biochemical (7), rearing (19) and host plants (14); 59 papers of the occurrence of infestation and the geographical distribution of the insect; 11 research on different general aspects, such as controversial aspects about the red palm weevil, the species associated with the insect, especially the mite and the nematode, and larval gut microbes.

Monitoring, sampling, detection and the economic threshold

The number of papers that were concerned with the process of monitoring, sampling and detecting the insect and proposing the levels or threshold of economic intervention reached 51, including 41 on methods of detection and early detection by using non-traditional methods such as the dogs' sense of smell (two papers), audio/acoustic methods (14), and modern technologies such as signal processing technology (6 papers). These data show that there is a clear scarcity of research related to proposing and determining the appropriate economic intervention threshold, and this may be due to the nature of the insect and the difficulty of detecting its early infestation and stages. Alternatively, only one study suggested relying on what is known as area-wide management/incidence rate at which intervention is required (Faleiro and Ashok Kumar, 2008). Another study suggested a district-level sequential sampling plan for area-wide management of RPW (Faleiro et al., 2010).

Natural control

The bibliographic list included 4 papers on the natural enemies of the insect, the most recent of which was an overview of the natural enemies of the palm weevil *Rhynchophorus*, with a focus on *R. ferrugineus* (Mazza et al., 2014). Figure 1 shows a comparison between the % numbers of articles published about comprehensive knowledge and basic IPM components needed for management of RPW.

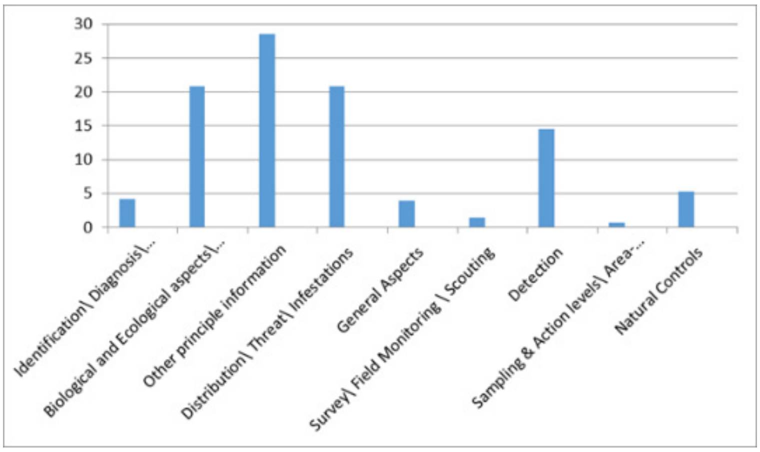


Figure 1. Comparison between the % numbers of articles published about comprehensive knowledge and basic IPM components needed for management of RPW.

Management practices

Papers that dealt with the practices and methods of controlling the RPW, including studies on preventive methods to avoid infestation with the insect (proactive practices), and studies related to the effectiveness and efficiency of treatment methods or based on IPM tactics (effective practices), and the total of these studies amounted to 55 papers.

1. Proactive practices.

The research that dealt with the preventive strategy, regulatory and legislative interventions amounted to 11 papers, including quarantine measures (3 papers) on the quarantine protocol and one paper on problems and challenges facing quarantine measures. It is noted that there was no research on health measures and the role of extension campaigns in measures to avoid weevil infestation.

2. Active practices

Agricultural and mechanical methods.

The bibliographic list included only two papers on agricultural methods and one investigation on mechanical measures, and two other papers on the importance of field operations to reduce the spread of RPW on date palms (Salah, 2019) and a case study of the effect of farming practices on the infestation of red palm weevil on date palms (Sallam et al., 2012), while the number of studies that dealt with resistant cultivars and genetically modified palms to resist the pest and other biological techniques (8 papers).

Biological control and biopesticides.

The investigations that dealt with biological methods amounted to 96 papers, of which 6 were about natural enemies, and the rest dealt with the bio-agents or its formulations that are pathogenic to insects, including 41 about pathogenic nematodes, 27 about pathogenic fungi, especially *Beauveria bassiana*, and *Metarhizium anisopliae*, 4 researches on pathogenic bacteria all using *Bacillus thuringiensis*. Often, biopesticides investigations focused on the use of plant extracts such as: neem seed extract black pepper, cashew apple extracts, as well as active substances from natural extracts such as camphene. It is striking that the investigations that focused on the role of specific natural enemies of predators and parasitoids and their use on the applied scale is very rare, and this may be because the red palm weevil is an invasive pest to many of the locations in which it was established due to the appropriate environmental conditions and the absence of such enemies.

Chemical control.

The investigations that concerned with the use of different insecticides amounted to 46 papers, of which 6 were using the stem injection method, 3 using the fumigation method, and only one research using root feeding. These investigations were concerned with evaluating the effectiveness of different old groups of insecticides (e.g., phosphorous, carbamates, mineral oils and inorganic salts, etc.) and relatively new (e.g., amidacloprid, abamectin, emamectin benzoate) against adults and other stages of the red palm weevil under laboratory and field conditions, and there is some investigations which dealt with the status or development of resistance of RPW field populations to the action of insecticides (Al-Ayedh et al., 2016; Wakil et al., 2018), or the factors affecting the efficiency of insecticides and their histopathological effects on the insect.

Pheromones and attractants.

The most investigated potential components researches dealt with pheromone traps, attractants and adult traps, with 92 investigations, of which 4 are about natural or synthetic attractants, 13 are about aggregation pheromone, and 6 are about pheromone lures. This research dealt with different aspects of the use of pheromone traps in managing RPW in terms of: pheromone type, efficiency, longevity of pheromone, improvement of trap contents and placement in terms of trap position and density relative to area, the effect of trap size and color on the numbers that are caught and traps equipped with pheromone bait. As for the attraction with food baits, it included investigations on bait-free attraction and killing technology, the efficiency of different types of natural and synthetic baits, and the periodic replacement of food baits.

Genetic control, sterilization and irradiation.

The bibliographic list included 21 papers on genetic control methods, sterilization and irradiation, as well as the method of male sterilization. These papers dealt with determining the basics and doses of gamma irradiation, the effect of irradiation processes on some morphological and biological aspects, insect ovaries and mating competitiveness. Also, dealt with the use of some radioactive isotopes in male sterilization.

Information and communication technologies ICT

The number of researches that dealt with the applications of ICT in the management of the red palm weevil reached 5, including 3 papers on GIS (Fajardo et al., 2017; Massoud et al., 2011, 2012), and one paper on each of the Internet of Things (Koubaa et al., 2019) and image processing technologies. Figure 2 shows comparison between the % numbers of articles published about management practices and control methods of RPW.

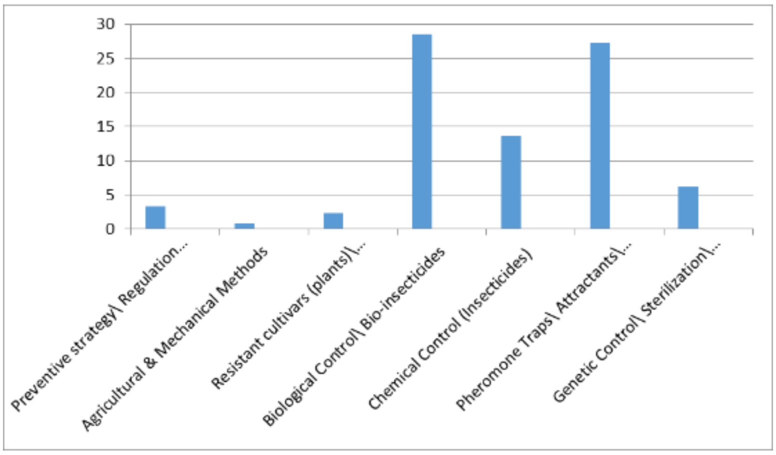


Figure 2. Comparison between the % numbers of articles published about management practices and control methods of RPW.

CONCLUSIONS

Data extracted from such bibliography indicate that there is a real need for more efforts and research to: strengthen the role of some of the basic components in IPM programs such as threshold level and natural control; enhancing the adoption of biotechnology products, and some other potential components; carry out more molecular investigations to provide a better understanding of the insect's behavior and the interaction between its immune system and biological control agents; encouraging researches on developing resistance of RPW to commonly used insecticides, using ICT tools especially GIS, evaluating the current RPW management programs in light of the local conditions of each Arab country.

Literature cited

Al-Ayedh, H., Hussain, A., Rizwan-ul-Haq, M., and Al-Jabr, A.M. (2016). Status of insecticide resistance in field collected populations of *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *curculionidae*). Int. J. Agric. Biol. *18* (*1*), 103–110 https://doi.org/10.17957/IJAB/15.0070.

Fajardo, M., Guerra, J.A., Barroso, L., Morales, M., and Martín, R. (2017). Use of GIS (geographical information system) for data analysis in a *Rhynchophorus ferrugineus* eradication program. Paper presented at: Scientific Consultation and High-Level Meeting on Red Palm Weevil Management (Rome, Italy: FAO and CIHEAM).

Faleiro, J.R., and Ashok Kumar, J. (2008). A rapid decision sampling plan for implementing area-wide management of the red palm weevil, *Rhynchophorus ferrugineus*, in coconut plantations of India. J Insect Sci *8* (*1*), 15 https://doi.org/10.1673/031.008.1501. PubMed

Faleiro, J.R., Abdallah, A.B., Kumar, J.A., Shagagh, A., and Alabdan, S. (2010). Sequential sampling plan for area-wide management of *Rhynchophorus ferrugineus* (Olivier) in date palm plantations of Saudi Arabia. Int. J. Trop. Insect Sci. *30* (*3*), 145–153 https://doi.org/10.1017/S1742758410000226.

Koubaa, A., Aldawood, A., Saeed, B., Hadid, A., Ahmed, M., Saad, A., Alkhouja, H., and Alkanhal, M. (2019). Smart Palm: an IoT Framework for Red Palm Weevil Early Detection. https://www.researchgate.net/publication/336230429_Smart_Palm_An_IoT_Framework_for_Red_Palm_Weevil_Early_Detection.

Massoud, M.A.E., Faleiro, J.R., El-Saad, M.A., and Sultan, E. (2011). Geographic information system used for assessing the activity of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the date palm oasis of Al-Hassa, Saudi Arabia. J. Plant Prot. Res. *51* (*3*), 234–239 https://doi.org/10.2478/v10045-011-0039-3.

Massoud, M.A., Sallam, A.A., Faleiro, J.R., and Al Abdan, S. (2012). Geographic information system-based study to ascertain the spatial and temporal spread of red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in date plantations. Int. J. Trop. Insect Sci. *32* (*2*), 108–115 https://doi.org/10.1017/S174275841200015X.

Mazza, G., Francardi, V., Simoni, S., Benvenuti, C., Cervo, R., Faleiro, J.R., Llácer, E., Longo, S., Nannelli, R., Tarasco, E., and Roversi, P.F. (2014). An overview on the natural enemies of *Rhynchophorus* palm weevils, with focus on *R. ferrugineus.* Biol. Control *77*, 83–92 https://doi.org/10.1016/j.biocontrol.2014.06.010.

Salah, M.B. (2019). Importance of field operations for reducing red palm weevil (RPW) infestation on date palm. Arab Journal of Plant Protection *37* (*2*), 159–162 https://doi.org/10.22268/AJPP-037.2.159162.

Sallam, A.A., El-Shafie, H.A.F., and Al-Abdan, S. (2012). Influence of farming practices on infestation by red palm weevil *Rhynchophorus ferrugineus* (Olivier) in date palm: a case study. Int. Res. J. Agric. Sci. Soil Sci. *2* (*8*), 370–376.

Wakil, W., Yasin, M., Qayyum, M.A., Ghazanfar, M.U., Al-Sadi, A.M., Bedford, G.O., and Kwon, Y.J. (2018). Resistance to commonly used insecticides and phosphine fumigant in red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in Pakistan. PLoS One *13* (*7*), e0192628 https://doi.org/10.1371/journal.pone.0192628. PubMed

Efforts of researchers to manage the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera: Curculionidae*) in the Arab world: a bibliography

M.A. Amro[a], A.M. Amro[b] and A.M. Amro

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

Abstract

Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), is one of the most invasive pest species that causes serious damage to date palm trees. RPW causes massive economic losses in the date palm production worldwide. Infested palm trees show visible signs when the infestation is more advanced. Early detection is a useful tool to eradicate and control RPW successfully. Since *R. ferrugineus* was first recognized as a threat to date palm production in the mid-1980s for the Arabian Gulf countries, entomology scientists have deployed several methods to control this destructive insect pest. These include studying the biology, ecology and adult trapping of this pest. Integrated pest management (IPM) that included biological and chemical control methods has been used by more than 400 Arab researchers and clarified in more than 300 manuscript presented in this investigation.

Keywords: red palm weevil *Rhynchophorus ferrugineus* (Olivier), management, Arab world, bibliography

INTRODUCTION

Red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera: Curculionidae*) early described as a harmful insect on the date palm tree in 1917 (Brand, 1917). It was first discovered in the Gulf Region (KSA) in the mid-1980s by Al-Abdulmohsin (1987). It spread rapidly to several date producing countries through infested planting material that is mainly transported for ornamental gardening (Faleiro et al., 2012) and is now reported from several Asian, African, European and American countries (Al-Dosary et al., 2016). In recent years, *R. ferrugineus* has been the most destructive insect of palm plantations throughout the world (Bertone et al., 2010), and FAO has designated it as a category-1 pest on date palm in the Middle-East. Losses in global production of dates have been estimated at 30% due to the plant diseases and pests (FAOSTAT, 2013). The annual loss in the Gulf region of the Middle-East due to eradication of severely infested palms has been estimated to range from US$ 1.74 to 8.69 million at 1 and 5% infestation, respectively (El-Sabea et al., 2009). Therefore, the present investigation was initiated to clarify the efforts of researchers to manage the red palm weevil *R. ferrugineus* in the Arab world throughout the last 4 decades (1980s-2020s).

Sources of the reviewed articles: an extensive bibliography about the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) was established by the writer. Publications dealing with the biological and ecological studies (survey, seasonal abundance and adult trapping) in addition to the integrated pest management (IPM) that included biological and chemical control methods has been collected. Taxonomy of (RPW) was in special interest. Journals, bulletins, conferences, approved theses, dissertations and internet sites were the main source of information.

The present bibliography includes the following subjects: 1) adult trapping (pheromone traps); 2) biological control; 3) biological studies; 4) chemical control; 5) ecological studies; 6) essential oils; 7) feeding; 8) first record; 9) general; 10) host plant resistance; 11) IPM; 12) non-chemical control; 13) physiological studies; 14) relative susceptibility; 15) review; 16)

[a]E-mail: moamro1953@yahoo.com

[b]E-mail: raoof_amro@yahoo.com

seasonal abundance; 17) toxicological studies; 18) taxonomy.

DATA ANALYSIS

Among 314 collected research articles that contained 18 subjects, data presented in (Table 1) and illustrated in (Figure 1), clarify that biological control, adult trapping and chemical control of the RPW ranked the first between entomology researchers' attention with an average of 15.31, 12.42 and 12.10%, respectively. The remaining scope of study percentages were clarified in Figure 1. On the other hand, participation rate of each Arab country was clarified in Figure 2. It is clear that Egyptian researchers make the biggest effort to manage *R. ferrugineus* by the available methods and followed by Saudi Arabian and United Arab Emirate researchers by 37.26, 34.39 and 13.69%, respectively. Except of the unmentioned resources the remaining researchers' efforts in the Arab countries constituted 5.74%.

Table 1. Annotated list of management efforts of *R. ferrugineus* in different Arabian countries.

Scope of study	Area of study	Author(s) and year
Adult trapping (pheromone traps)	Egypt	Abbas et al. (2016); Abbas and Mesallam (2013); Abdel-Moety et al. (2012); Abd El-Wahab et al. (2021); Al-Elimi et al. (2000); El-Bokl et al. (2015)
	KSA	Abraham et al. (2001); Ajlan and Abdulsalam (2000, 2005); Ajlan et al. (1995); Al-Saroj et al. (2017); Faleiro et al. (2011); Hoddle et al. (2013); Vidyasagar et al. (2000a, 2016)
	Oman	Abdallah and Al-Khatri (2000b); Abdallah and Al-Khatri (2005, 2016)
	UAE	Al-Saoud (2004, 2007, 2009a, b, 2010a, b, 2011a, b, 2012, 2013, 2014, 2015, 2017); Al-Saoud and Ajlan (2013); Al-Saoud et al. (2010); Hanounik et al. (2000c)
	Unmentioned	Abuaglala and Al-Deeb (2012); Al-Shagag et al. (2008); Faleiro et al. (1998, 1999, 2000)
Biological control	Egypt	Abd-Elgawad (1998); Abdel-Razek (2016); Abdel-Razek et al. (2004); Abdel-Rahman and Abdel-Raheem (2018); Abdel-Raheem et al. (2019); Abdel-Salam et al. (2016a, b); Abdel-Samad et al. (2011); Abdullah (2009b); Alfazairy (2004); Atwa and Hegazi (2016); El-Bishry et al. (2000); El-Garhy (1996); Gomaa (2006); Hajjar et al. (2015); Khamiss and Abdel Badeea (2013); Ragaei et al. (2009); Ragaei and Sabry (2013, 2016); Sabbour (2006, 2013a, b); Sabbour and Solieman (2014); Salama and Abd-Elgawad (2001, 2010); Salama et al. (2004); Saleh et al. (2010); Salem et al. (2012); Sewify et al. (2009, 2014)
	KSA	Aldafer et al. (1998); Aldossary et al. (2009); Alsuhaibani et al. (2001); Hussain et al. (2013a, 2015); Saleh and Alheji (2003); Saleh et al. (2004, 2011)
	UAE	Abbas and Hanounik (1999); Abbas et al. (2000); Abbas et al.(2001a,b,c); Elawad et al. (2007a,b); El-Safty et al. (2007, 2009, 2010, 2011)
	Unmentioned	Al-Bagshi et al. (2013).
Biological studies	Egypt	El-Ezaby (1997b); El-Saadany et al. (2007b); Ibrahim et al. (2011); Kamel et al. (2005); Mahmoud et al. (2011); Monzer and El-Rahman (2003); Salama et al. (2002); Shamseldean (2002)
	KSA	Abraham et al. (1999); Aldawood and Rasool (2011); Al-Dawsary (2012); Al-Dawsary (2013a, b, 2014); Al-Dhafer (1997); Al-Dosary et al. (2010); Alanazi et al. (2020); Alshammari et al. (2021); Khiyami and Alyamani (2008); Mozib and El-Shafie (2013); Sadder et al. (2016); Sabit et al. (2021)
	UAE	Kaakeh (2005); Kaakeh et al. (2001d)
	Unmentioned	Abdel-Azim et al. (2012); Al-Deeb et al. (2011); Al Mohanna et al. (2000); El-Muhanna et al. (2000)

Table 1. Continued.

Scope of study	Area of study	Author(s) and year
Chemical control	Egypt	Abdel-Salam et al. (2016a, b, 2014); Abdullah (2009c); Abdulsalam et al. (2001); Alfazairy (2011); Belal et al. (2012); El-Ezaby(1997a); El-Bokl et al. (2010); El Husseini (2019); El-Sebay (2004a, b); El-Zemaity et al. (2010); Ghonim et al. (2003); Girgis et al. (2002); Reda and Abdel-Salam (2014); Saleh et al. (1996); Shamseldean and Abd-Elgawad (1994); Shamseldean and Atwa (2004); Shaarawi et al. (2000); Shawir et al. (2014)
	KSA	Ajlan et al. (2000); Al-Ayedh et al. (2015); Al-Ayedh et al. (2016); Aldawood et al. (2013); Al-Dhafar and Al-Qahtani (2012); Al-Rajhy et al. (2005); Al-Shawaf et al. (2010); El-Saeid and Al-Dosari (2010)
	Oman	Abdallah and Al-Khatri (2000a); Azam and Razvi (2001); Azam et al. (2000)
	UAE	Al-Wahshi et al. (2017); Hanounik (1998); Hanounik et al. (2000a, b)
	Tunisia	Habib et al. (2017).
	Unmentioned	Abo-El-Saad et al. (2011, 2012)
Ecological studies	Egypt	Abbas and Al Nasser (2012); El-Sebay (2003); El-Sharabasy (2010); Hassan et al. (2011); Monzer and Al-Elimi (2002); Salama et al. (2009); Salem and Reda (2015); Sharshir et al. (2007)
	KSA	Aldryhim and Al-Bukiri (2003); Aldryhim and Khalil (2003); El-Faki et al. (2015); Hoddle et al. (2015); Kurdi et al. (2021); Massoud et al. (2011, 2012); Mehmood et al. (2017)
	Unmentioned	El-Mergawy et al. (2011a, b); El-Sabea et al. (2009)
Essential oils	Egypt	Abdel-Raheem et al. (2020); Abdullah and Nassar (2005)
	KSA	Nassar and Abdulah (2001); Sharaby and Al-Dosary (2007); Shukla et al. (2012)
Feeding	Egypt	Alfazairy et al. (2003); El-Saadany et al. (2007a); El-Sebay et al. (2003); El-Zoghby and Abdel-Hameid (2018); Salama and Abdel-Razek (2002)
	KSA	Al-Ayedh (2011); El-Shafie et al. (2013); Rasool et al. (2014, 2015, 2017); Sharaby and Al-Dhafar (2013)
	UAE	Kaakeh et al. (2001b)
First record	Egypt	Saleh (1992)
	KSA	Al-Abdulmohsin (1987)
	Libya	Al-Eryan et al. (2010)
	Palestine	Samara (2017)
	Tunisia	Chebbi (2011)
General	Egypt	Abbas (2000, 2013); Abdel-Moniem (2016); Salama and Abd-Elgawad (2003); Saleh and Gouhar (1993); Salem et al. (2012, 2016); Salem (2015); Zayed (2008)
	Bahrain	Hamdi (1998)
	Jordan	Al Antary et al. (2015)
	KSA	Aldawood (2017); Al-Hudaib et al. (2007, 2008, 2017); Alkhazal et al. (2009); Al-Saqer (2012); Al-Saqer and Hassan (2011a, b); Al-Shawaf et al. (2012, 2017); Hassan and Al-Saqer (2012)
	Maghreb region	Faleiro et al. (2012)
	Qatar	Abdulla (1997)
	UAE	Kaakeh et al. (2001a); Khalifa et al. (2007)
	Unmentioned	Ajlan (2008); Al-Khatri (2004); Al-Manie and Alkanhal (2007)
Host plant resistance	Egypt	Abbas and El-Sebay (2013); Abd-Elgawad (1996); Abd-Elgawad (2000); Abdel-Megeed et al. (2004)

Table 1. Continued.

Scope of study	Area of study	Author(s) and year
IPM	Egypt	Abbas (2005); Hussein (1998); Sewify et al. (2010); Sewify and Fouad (2006)
	KSA	Abozuhairah et al. (1996); Abraham et al. (1998, 2000); Abraham and Vidyasagar (1992); Al-Anazi (2017); Aldawood (2016); Ali-Bob (2019); El-Shafie et al. (2011); Faleiro (2005); Hussain et al. (2013b); Mukhtar et al. (2011); Vidyasagar et al. (2000b)
	Libya	Al-Eryan et al. (2014)
	UAE	Abbas (2010); Abbas et al. (2006); El-Ezaby et al. (1998a, b); Kaakeh (2000); Kaakeh et al. (2001c)
	Unmentioned	El-Mergawy and Al-Ajlan (2011)
Non-chemical control	Egypt	Abdullah (2009a); Ahmed et al. (2015); Al-Bartya and Hamzab (2015); Salama and Ismail (2007)
	KSA	Al-Ayedh and Rasool (2010b); Farooq et al. (2015); Fetoh (2011); Nassar and Abdulah (2001)
	Unmentioned	Abo-El-Saad et al. (2013); Bream et al. (2001); Hamad and El Faith (2004); Faleiro et al. (2019)
Physiological studies	Egypt	El-Naggar et al. (2010); Salama and Saker (2002); Shaarawi (2001)
	Bahrain	Bannari et al. (2017)
	KSA	Abdally (2007); Abdally et al. (2010); Abdel-Banat et al. (2017); Abd El-Fattah et al. (2021); Al-Ayedh et al. (2006); Al-Ayedh and Rasool (2010a); Al-Dhafar and Sharaby (2012); Al-Dosary and El-Tahir (2012); Al-Jabr and Abo-El-Saad (2008); Aljabr et al. (2014); Antony et al. (2017); Sharaby and Al-Dosary (2014); Tufail et al. (2017).
	Unmentioned	Alzahrani et al. (2013); El-Mergawy et al. (2011c, d)
Relative susceptibility	Egypt	Abdel-Moniem (2016); Hussain et al. (2016a, b); Ibrahim (2010); Merghem (2011); Mesallam et al. (2009); Mogahed (2011, 2010)
	KSA	Al-Ayedh (2008); Al-Nujiban et al. (2015); Hussain et al. (2016a, b)
	Unmentioned	Faleiro et al. (2014); Al-Bagshi et al. (2013, 2008)
Review	KSA	Al-Dosary et al. (2016)
Seasonal abundance	Egypt	El Sebay et al. (2010); Abulyazid et al. (2002); El-Lakwah et al. (2011); Saleh et al. (2012)
	Oman	Abdallah and Al-Khatri (2003); Al-Khatri and Abd-Allah (2003); Azam et al. (2001)
	KSA	Al-Ayedh and Aldryhim (2017); Faleiro et al. (2010); Mohammed et al. (2021)
	Yemen	Al-Amodi et al. (2018)
Toxicological studies	UAE	Kaakeh (2006)
Taxonomy	Egypt	Ragaei (2010); Salama and Aziz (2002, 2001)
	KSA	Sharaby and Al-Dosary (2006)

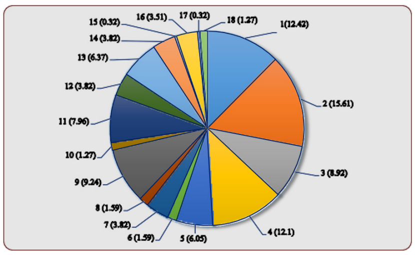


Figure 1. Scope of study percentages: 1) adult trapping (pheromone traps); 2) biological control; 3) biological studies; 4) chemical control; 5) ecological studies; 6) essential oils; 7) feeding; 8) first record; 9) general; 10) host plant resistance; 11) IPM; 12) non-chemical control; 13) physiological studies; 14) relative susceptibility; 15) review; 16) seasonal abundance; 17) toxicological studies; 18) taxonomy.

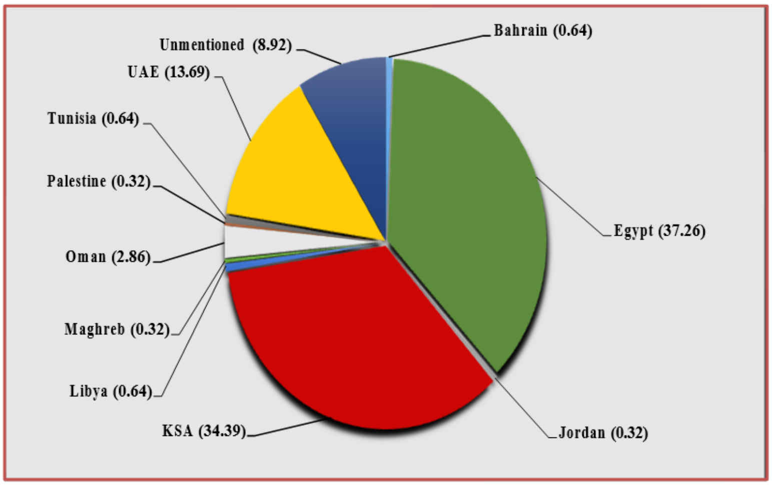


Figure 2. Participation rate/country: 1) Bahrain; 2) Egypt; 3) Jordan; 4) KSA; 5) Libya; 6) Maghreb region; 7) Oman; 8) Palestine; 9) Tunisia; 10) UAE; 11) unmentioned.

ACKNOWLEDGEMENTS

The authors wishes to express their sincere appreciation and gratitude to Prof. Dr. Farouk Abdel-Kawi Abdel-Galil, Professor of Economic Entomology and Biological Control, Plant Protection Department, Faculty of Agriculture, Assiut University for support and many helpful suggestions, valuable assistance and constructive criticism during the preparation of this manuscript. Sincere thanks extended to Dr. Mervat A.B. Mahmoud, Zoology Department, Faculty of Science, south Valley University for her great helping and revision of this bibliography.

Literature cited

Abbas, M.K. (2000). Studies on the red palm weevil. M.Sc. thesis (Fac. Agric., Zagazig Univ.).

Abbas, M.K. (2005). Integrated management for controlling red palm weevil. Ph.D. thesis (Fac. Agric., Ain-Shams Univ.).

Abbas, M.S.T. (2010). IPM of the red palm weevil, *Rhynchophorus Ferrugineus*. In Integrated Management of Arthropod Pests and Insect Borne Diseases. Integrated Management of Plant Pests and Diseases, Vol. 5, A. Ciancio, and K. Mukerji, eds. (Dordrecht: Springer), https://doi.org/10.1007/978-90-481-8606-8_9.

Abbas, M.K. (2013). Evaluation methods for red palm weevil control in Egypt during (1992-2010). Paper presented at: AFPP – Palm Pest Mediterranean Conference (Nice, France).

Abbas, M.K., and Al-Nasser, A.S. (2012). Ecological studies and evaluation of some aggregation pheromone types with measuring the potential of female reproductive system in red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Nat. Sicil. *10* (*12*), 188–193.

Abbas, M.K., and El-Sebay, M.Y. (2013). Studies on sugar cane susceptibility for infestation with red palm weevil, *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*). Paper presented at: AFPP – Palm Pest Mediterranean Conference (Nice, France).

Abbas, M.S.T., and Hanounik, S.B. (1999). Pathogenicity of entomopathogenic nematodes to red palm weevil, *Rhynchophorus ferrugineus*. Int. J. Nematol. *9*, 84–86.

Abbas, M.K., and Mesallam, T. (2013). Evaluation of different aggregation pheromones technique used for trapping red palm weevil *Rhynchophorus ferrugineus*. J. Appl. Sci. Res. *9*, 5223–5228.

Abbas, M.S.T., Hanounik, S.B., Mousa, S.A., and Al-Bagham, S.H. (2000). Soil application of entomopathogenic nematodes as a new approach for controlling *Rhynchophorus ferrugineus* on date palm. Int. J. Nematol. *10*, 215–218.

Abbas, M.S.T., Hanounik, S.B., Mousa, S.A., and Awash, S.A. (2001a). Isolation of entomopathogenic nematodes from Ras Al-Khaima and Al-Fugaira Emirates (UAE). Egypt. J. Biol. Pest Cont. *11* (*1/2*), 191.

Abbas, M.S.T., Hanounik, S.B., Mousa, S.A., and Mansour, M.I. (2001b). On the pathogenicity of *Steinernema abbasi* and *Heterorhabditis indicus* isolated from adult *Rhynchophorus ferrugineus* (*Coleoptera*). Int. J. Nematol. *11* (*1*), 69–72.

Abbas, M.S.T., Saleh, M.M.E., and Akil, A.M. (2001c). Laboratory and field evaluation of pathogenicity of entomopathogenic nematodes to the red palm weevil, *Rhynchophorus ferrugineus.* Anz. Schädlkd. *74* (*6*), 167–168 https://doi.org/10.1046/j.1439-0280.2001.d01-1.x.

Abbas, M.S.T., Hanounik, S.B., Shahdad, A.S., and Al-Bagham, S.A. (2006). Aggregation pheromone traps as a major component of an IPM strategy for the red palm weevil, *Rhynchophorus ferrugineus* in date palms. J. Pest Sci. *79* (*2*), 69–73 https://doi.org/10.1007/s10340-005-0113-6.

Abbas, M.K., El-Banna, A.M., Ibrahim, T.M., and Sorour, H.A. (2016). Evaluation of trap types and synthetic attractants on *Rhynchophorus ferrugineus* Oliv. by aggregation pheromone traps. Paper presented at: 2nd Int. Conf. for Date Palm (ICDP) (Qassim, KSA).

Abd-Elgawad, M.M.M. (1996). The Indian red palm weevil: modernization of the methods for the pest management. Agric. Dev. Arab Homeland *15*, 36–45.

Abd-Elgawad, M.M.M. (1998). Experimental Design and Analysis of Biological Statistics in the Biological Control of Date Palm Red Weevils Stem Borers and Grubs (Khartoum, Sudan: AOAD Press).

Abd-Elgawad, M.M.M. (2000). The palm weevil: integrated control and new aspects. Nat. Res. Center News *94*, 75–77.

Abdallah, F.F., and Al-Khatri, S.A. (2000a). The effectiveness of trunk injection and fumigation for control of the red palm weevil, *Rhynchophorus ferrugineus* in date palm. J. Plant Protect. Tropics *13*, 17–21.

Abdallah, F.F., and Al-Khatri, S.A. (2000b). Efficacy of different attractant traps on red palm weevil, *Rhynchophorus ferrugineus*. Paper presented at: 1st Workshop on Control of Red Palm Weevil (Oman).

Abdallah, F.F., and Al-Khatri, S.A. (2003). Seasonal fluctuation of *Rhynchoporus ferrugineus* (Oliv.) (*Coleoptera* - *Curculionidae*) in the Sultanate of Oman. Paper presented at: Int. Conf. on Date Palm (Kingdom of Saudi Arabia: King Saud Univ.).

Abdallah, F.F., and Al-Khatri, S.A. (2005). The effect of pheromone, kairomone and food baits on attracting adults of red palm weevil, *Rhynchophorus ferrugineus* in Sultanate of Oman. Egypt. J. Agric. Res. *83*, 169–177.

Abdallah, F.F., and Al-Khatri, S.A. (2016). Comparison study between pheromone traps of AOAD and recommended traps in Oman on red palm weevil in date palm plantation. Paper presented at: 2nd Int. Conf. for Date Palm (ICDP) (Qassim, KSA).

Abdally, M.H. (2007). In vitro studies of the agglutination activity of the haemolymph and midgut extracts from larvae and adults the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), in Al-Hasa District, Saudi Arabia. Abstract presented at: Fourth Symposium on Date Palm in Saudi Arabia.

Abdally, M.H., Abo-Elsaad, M.M., Al-Shaggag, A.A., Al-Bagshy, M.M., and Al-Shawaf, A.A. (2010). Detection of insect immunity substances (lectins) in the midgut extracts from larvae and adult red palm weevil *Rhynchophorus ferrugineus* (Olivier) in Al-Ahsa, Saudi Arabia. Pak J Biol Sci *13* (*5*), 223–228 https://doi.org/10.3923/pjbs.2010.223.228. PubMed

Abdel-Azim, M.M., Vidyasagar, P.S.P.V., Aldosari, S.A., and Mumtaz, R. (2012). Impact of mating frequency on fecundity, fertility and longevity of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *curuclionidae*). J. Agric. Sci. and Technol. SA *2* (*4*), 520–528.

Abdel-Banat, B.M.A., Al-Shafie, H.A.F., and Alhudaib, K.A. (2017). Molecular characterization of genes for molting enzyme from red palm weevil, *Rhynchophorus ferrugineus*. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Abdel-Megeed, M.E., Zedan, H.A., and El-Saadany, G.B. (2004). The Integrated Management for Controlling Pests of Date Palm (Egypt: Kenza Group Pub.), pp.483 (in Arabic).

Abdel-Moety, E.M., Lotfy, H.M., and Rostom, Y. (2012). Trace determination of red palm weevil, *Rhynchophorus ferrugineus*, pheromone at trapping locations under Egyptian climate. Int. J. Agric. and Food Sci. *2* (*2*), 44–50.

Abdel-Moniem, A.S.H. (2016). The red palm weevil, *Rhynchophorus ferrugineus* Olivier, as edible insects for food and feed a case study in Egypt. Abstract presented at: 2nd Int. Conf. for Date Palm (ICDP) (Qassim, KSA).

Abdel-Moniem, A.S.H., El-Kholy, M., Mohanny, K.M., and Salem, S.A. (2014). Susceptibility of some fresh date palm varieties to infestation by *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*) in relation physic chemical composition. Int. J. Geology Agric. and Environ. Sci. *2* (*4*), 1–3.

Abdel-Raheem, M.A., Al Ghamdi, H.A., and Reyad, N.F. (2019). Virulence of fungal spores and silver nanoparticles from entomopathogenic fungi on the red palm weevil, *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*). Egypt. J. Biol. Pest Cont. *29*.

Abdel-Raheem, M.A., Al Ghamdi, H.A., and Reyad, N.F. (2020). Nano essential oils against the red palm weevil, *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *curculionidae*). Entomol. Res. *50* (*5*), 215–220 https://doi.org/10.1111/1748-5967.12428.

Abdel-Rahman, I.E., and Abdel-Raheem, M.A. (2018). Using entomopathogenic fungi as bio agents control on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). J. Entomol. Zool. Stud. *6* (*6*), 387–390.

Abdel-Razek, A.S. (2016). Impact of entomopathogenic bacterial symbiont, *Photorhabdus luminescens*, and *Bacillus thuringiensis* subsp. *tenebrionis* on management of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in Egypt. Abstract presented at: 2nd Int. Conf. for Date Palm (ICDP) (Qassim, KSA).

Abdel-Razek, A.S., Kamel, K.E., and Salama, H.S. (2004). Biochemical effects of the nematode-bacteria complex on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *curculionidae*). Arch. Phytopathol. Pflanzenschutz *37* (*3*), 205–214 https://doi.org/10.1080/0323540042000218754.

Abdel-Salam, A.H., El-Bana, A.A., and El-Rehewy, E.H. (2014). Evaluation of some insecticides on infestation of red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *curculionidae*). J. Plant Prot. and Path. Mansoura Univ. *5* (*5*), 567–571 https://doi.org/10.21608/jppp.2014.87962.

Abdel-Salam, A.H., El-Bana, A.A., and El-Rehewy, E.H. (2016a). The predacious efficiency of *Labidura riparia pallas* reared on red palm weevil, *Rhynchophorus ferrugineus* (Olivier), immature stages (*Coleoptera*: *Curculionidae*). Abstract presented at: 2nd Int. Conf. for Date Palm (ICDP) (Qassim, KSA).

Abdel-Samad, S.S.M., Mahmoud, B.A., and Abbas, M.S.T. (2011). Evaluation of the fungus, *Beauveria bassiana* (Bals.) Vuill as a bio-control agent against the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*: *Curculionidae*). Egypt. J. Biol. Pest Cont. *21* (*2*), 125–129.

Abdel-Salam, S.A., Alzahrani, A.M., Elmenshawy, O.M., and Abdel-Moneim, A.M. (2016b). Spinosad induces antioxidative response and ultrastructure changes in males of red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *dryophthorinae*). J. Insect Sci. *16* (*1*), 106 https://doi.org/10.1093/jisesa/iew089.

Abdulla, S. (1997). Report on present status of red palm weevil in Qatar. Paper presented at: 1st Meeting of Steering Committee of the Project of Biological Control of Red Palm Weevil (Riyadh, Saudi Arabia).

Abdullah, M.A.R. (2009a). Identification of the biological active compounds of two natural extracts for the control of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (*Coleoptera* - *Curculionidae*). Egypt. Acad. J. Biol. Sci. *2* (*2*), 35–44 https://doi.org/10.21608/eajbsa.2009.15427.

Abdullah, M.A.R. (2009b). Biological control of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) by the parasitoid mite, *Rhynchopolipus rhynchophori* (Ewing) (*Acarina*: *Podapolipidae*). J Egypt Soc Parasitol *39* (*2*), 679–686. PubMed

Abdullah, M.A.R. (2009c). Toxicological and histopathological studies of *Boxus chinensis* oil and Precocene II on larvae of the red palm weevil *Rynchophorus ferrugineus* (Oliver) (*Coleoptera*: *Curculionidae*). Egypt. Acad. J. Biol. Sci. *2* (*2*), 45–54 https://doi.org/10.21608/eajbsa.2009.15428.

Abdullah, M.A.R., and Nassar, M.I. (2005). Assessment of *Boxus chinensis* oil and Precocene II for control of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (*Coleoptera* - *Curculionidae*) and the palm beetle *Pseudophilus testaceous* (Gahan) (*Coleoptera* - *Cerambycidae*). J. Entomol. *2* (*1*), 1–8.

Abdulsalam, K.S., Shawi, M.S., Abo-El-Saad, M.M., Rezk, M.A., and Ajlan, A.M. (2001). Regent (fipronil) as a candidate insecticide to control red palm weevil, *Rhynchophorus ferrigineus* (Olivier). Ann. Agric. Sci., Ain Shams Univ. Cairo. *46* (*2*), 841–849.

Abo-El-Saad, M.M., Elshafie, H.A., Faleiro, J.R., and Bou-Khowh, I.A. (2011).Toxicity evaluation of certain insecticides against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), under laboratory conditions. Paper presented at: ESA Annual Meeting.

Abo-El-Saad, M.M., Al-Abdan, S.A., and Bou-Khowh, I.A. (2012). In vivo toxicity of beta-cyfluthrin insecticide against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier). J. Agric. Sci. Technol. A *2* (*12*), 1322–1331.

Abo-El-Saad, M.M., Elshafie, H.A., and Bou-Khowh, I.A. (2013). Toxicity of bioinsecticide, Abamectin, on red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Int. J. Agric. Sci. Res. (Chennai) *4*, 107–115.

Abozuhairah, R.A., Vidyasagar, P.S.P.V., and Abraham, V.A. (1996). Integrated management of red palm weevil, *Rhynchophorus ferrugineus* in date palm plantations of the Kingdom of Saudi Arabia. Paper presented at: 20th Int. Cong. Entomol. (Firenze, Italy).

Abraham, V.A., and Vidyasagar, P.S.P.V. (1992). Strategy for control of red palm weevil of date palm in the Kingdom of Saudi Arabia. Consultancy report submitted to the Ministry of Agriculture and Water, Kingdom of Saudi Arabia. pp.36.

Abraham, V.A., Al-Shuaibi, M.A., Faleiro, J.R., Abozuhairah, R.A., and Vidyasagar, P.S.P.V. (1998). An integrated management approach for RPW, a key pest of date palm in the Middle East. Agric. Sci. (Melb.) *3*, 77–83.

Abraham, V.A., Faleiro, J.R., Kumar, T.P., and Al-Shuaibi, M.A. (1999). Sex ratio of red palm weevil *Rhynchophorus ferrugineus* Olivier captured from date plantation of Saudi Arabia using pheromone traps. Indian J. Entomol. *61* (*2*), 201–204.

Abraham, V.A., Faleiro, J.R., Al-Shuaibi, M.A., and Kumar, T.P. (2000). A strategy to manage red palm weevil *Rhynchophorus ferrugineus* Oliv. in date palm *Phoenix dactylifera*. Its successful implementation in Al-Hassa, Kingdom of Saudi Arabia. Pestology. *24* (*12*), 23–30.

Abraham, V.A., Faleiro, J.R., Al-Shuaibi, M.A., and Alabdan, S. (2001). Status of pheromone trap captured female red palm weevil from date gardens in Saudi Arabia. J. Trop. Agric. *39*, 197–199.

Abuaglala, A.M., and Al-Deeb, M.A. (2012). Effect of bait quantity and trap color on the trapping efficacy of the pheromone trap for the red palm weevil, *Rhynchophorus ferrugineus.* J Insect Sci *12* (*1*), 120 https://doi.org/10.1673/031.012.12002. PubMed

Abulyazid, I., Kamel, I.K.E., Sharawi, F.A., and El-Bermawi, S. (2002). Comparison between different populations of red palm weevils *Rhynchophorus* species using RAPD-PCR. J. Egypt. German. Soc. Zool. *38*, 1–15.

Ahmed, F.A., Hussein, K.T., and Gad, M.I. (2015). Biological activity of four plant oils, against the red palm weevil, *Rhynchophorus ferrugineus* (Oliver), (*Coleoptera*: *curculionidae*). J. Biosci. Appl. Res. *1* (*5*), 213–222 https://doi.org/10.21608/jbaar.2015.106030.

Ajlan, A.M. (2008). Red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). In Encyclopedia of Entomology, 18, J.L. Capinera, ed. (Springer Science, New York), p.3127–3130.

Ajlan, A.M., and Abdulsalam, K.S. (2000). Efficiency of pheromone traps for controlling the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*), under Saudi Arabia conditions. Bull. Entomol. Soc. Egypt. Economic Series. *27*, 109–120.

Ajlan, A.M., and Abdulsalam, K.S. (2005). Efficacy of two pheromone types on red palm weevil, *Rhynchophorus ferrugineus*, under Saudi Arabian conditions. Indian J. Plant Prot. *33* (*2*), 220–222.

Ajlan, A.M., Abraham, V.A., and Vidyasagar, P.S.P.V. (1995). Use of pheromone traps for controlling the red palm weevil in the Kingdom of Saudi Arabia. Abstract presented at: 16th Annual Meeting of the Saudi Biological Society on Biological Natural Resources in Kingdom of Saudi Arabia (Riyadh).

Ajlan, A.M., Shawir, M.S., Abo-Elsaad, M.M., and Rezk, M.A. (2000). Laboratory evaluation of certain organophosphorus insecticides against the red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Scientific J. King Faisal Univ. Basic and Applied Sciences *1*, 119–130.

Al-Abdulmohsin, A.M. (1987). First record of red date palm weevil in Saudi Arabia. Arab World Agriculture. *3*, 15–16.

Al-Amodi, M.O., Baekabah, A.M., Alsaqir, S.R., Easukul, M.M., and Dahlus, S.S. (2018). Survey on red palm weevil (*Rhynchophorus ferrugineus* Olivier, *Coleoptera*, *Curculionidae*) on date palm in some infected areas in Wadi Hadhramout. Hadhramout University Journal of Natural & Applied Sciences *15* (*1*), 51–58.

Al-Anazi, N.A. (2017). Current situation of controlling the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) from farmer's perspective in Jubbah village, Hail region northern Saudi Arabia. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Al Antary, T.M., Al-Khawaldeh, M.M., and Ateyyat, M.A. (2015). Economic importance of date palm *Phoenix dactylifera* L. (*Liliopsida*: *Arecales*: *Arecaceae*) pests in Jordan Valley. Braz. J. Biol. Sci. *2* (*3*), 121–134.

Al-Ayedh, H.Y. (2008). Evaluation of date palm cultivars for rearing the red date palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *curculionidae*). Fla. Entomol. *91* (*3*), 353–358 https://doi.org/10.1653/0015-4040(2008)91[353:EODPCF]2.0.CO;2.

Al-Ayedh, H.Y. (2011). Evaluating a semi-synthetic diet for rearing the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *curculionidae*). Int. J. Trop. Insect Sci. *31* (*1–2*), 20–28 https://doi.org/10.1017/S1742758411000063.

Al-Ayedh, H.Y., and Aldryhim, Y.N. (2017). Diel flight activity patterns of the red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) as monitored by smart traps. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Al-Ayedh, H.Y., and Rasool, K.G. (2010a). Determination of the optimum sterilizing radiation dose for control of the red date palm weevil *Rhynchophorus ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*). Crop Prot. *29* (*12*), 1377–1380 https://doi.org/10.1016/j.cropro.2010.06.019.

Al-Ayedh, H.Y., and Rasool, K. (2010b). Sex ratio and the role of mild relative humidity in mating behaviour of red date palm weevil *Rhynchophorus ferrugineus*. (*Coleoptera*: *Curculionidae*) gamma irradiated adults. J. Appl. Entomol. *134* (*2*), 157–162 https://doi.org/10.1111/j.1439-0418.2009.01460.x.

Al-Ayedh, H.Y., Alswailem, A.M., Shair, O., and Al Jabr, A.M. (2006). Evaluation of phylogenetic relationship between three phenotypically different forms of red date palm weevil *Rhynchophorus ferrugineus* Oliv. using PCR-based RAPD technique. Arch. Phytopathol. Pflanzenschutz *39* (*4*), 303–309 https://doi.org/10.1080/03235400500222461.

Al-Ayedh, H.Y., Hussain, A., Rizwan-Ul-Haq, M., and Al-Jabr, A.M. (2015). Status of insecticide resistance in field-collected populations of *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). Int. J. Agric. Biol. *18* (*1*), 103–110 https://doi.org/10.17957/IJAB/15.0070.

Al-Ayedh, H., Rizwan-Ul-Haq, M., Hussain, A., and Aljabr, A.M. (2016). Insecticidal potency of RNAi-based catalase knockdown in *Rhynchophorus ferrugineus* (Oliver) (*Coleoptera*: *Curculionidae*). Pest Manag Sci *72* (*11*), 2118–2127 https://doi.org/10.1002/ps.4242. PubMed

Al-Bagshi, M., Al-Shagag, A., Al-Saroj, S., Salim, A.S., Al-Shawaf, A.M., Al-Dandan, A.M., Al-Suleiman, Y., Al-Abdallah, E., and Ben Abdallah, A. (2013). Oviposition preference of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) to date palm cultivars. Pest Manage. Hortic. Ecosyst. *19* (*1*), 108–112.

Al-Bagshi, M., Al-Saroj, S., Ben Abdallah, A., and Faleiro, J.R. (2008). Extent of antixenosis in date palm for egg laying by red palm weevil *Rhyncophorus ferrugineus*. Indian J. Plant Prot. *36* (*2*), 292–293.

Al-Bartya, A.M.F., and Hamzab, R.Z. (2015). Larvicidal, antioxidant activities and perturbation of transminases activities of titanium dioxide nanoparticles synthesized using *Moringa oleifera* leaves extract against the red palm weevil (*Rhynchophorus ferrugineus*). Eur. J. Pharm. Med. Res. *2* (*6*), 49–54.

Al-Dawsary, M.M.S. (2012). Functional role of neuropeptide leucokininII in growth inhibition of red palm weevil *Rhynchophorus ferrugineus*. J. Agric. Sci. Technol. A *2* (*8*), 988–996.

Al-Dawsary, M.M.S. (2013a). Quantitative changes in protein and cholesterol in haemolymph of the red palm weevil *Rhynchophorus ferrugineus* after treatment Leucokinin II. J. Agric. Sci. Technol. A *3* (*2*), 140–145.

Al-Dawsary, M.M.S. (2013b). Sensory receptors on abdominal and thorax segments in male and female red palm weevil *Rhynchophorus ferrugineus.* Agric. Biol. J. N. Am. *4* (*1*), 23–32 https://doi.org/10.5251/abjna.2013.4.1.23.32.

Al-Dawsary, M.M.S. (2014). Functional compounds from the integument of adult red palm weevil *Rhynchophorus ferrugineus.* Saudi J Biol Sci *21* (*3*), 275–279 https://doi.org/10.1016/j.sjbs.2013.10.003. PubMed

Al-Deeb, M.A., Muzaffar, S., Abuagla, A.M., and Sharif, E.M. (2011). Distribution and abundance of phoretic mites (*Astigmata*, *Mesostigmata*) on *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). Fla. Entomol. *94* (*4*), 748–755 https://doi.org/10.1653/024.094.0403.

Al-Dhafar, Z.M., and Al-Qahtani, A.M. (2012). Mites associated with the red palm weevil, *Rhynchophorus ferrugineus* Oliver in Saudi Arabia with a description of a new species. Acarines *6* (*1*), 3–6 https://doi.org/10.21608/ajesa.2012.163616.

Al-Dhafar, Z.M., and Sharaby, A. (2012). Effect of zinc sulfate against the red palm weevil *Rhynchophorus ferrugineus* with reference to their histological changes on the larval midgut and adult reproductive system. J. Agric. Sci. Technol. A *2* (*7*), 888–900.

Al-Dhafer, H.M.H. (1997). Biological and morphological characteristics of red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Curculionidae*: *Coleoptera*) in Saudi Arabia. M.Sc. thesis (Fac. Agric., King Saud Univ.).

Al-Dosary, M.M., and El-Tahir, K.E.H. (2012). Pharmacological action of integument extracts of red palm weevil, *Rhynchophorus ferrugineus* on albino mice. Agric. Biol. J. N. Am. *3* (*2*), 49–56 https://doi.org/10.5251/abjna.2012.3.2.49.56.

Al-Dosary, M.M., Al-Bekairi, A.M., and Moursy, E.B. (2010). Morphology of the egg shell and the developing embryo of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver). Saudi J Biol Sci *17* (*2*), 177–183 https://doi.org/10.1016/j.sjbs.2010.02.012. PubMed

Al-Dosary, N.M.N., Al-Dobai, S., and Faleiro, J.R. (2016). Review on the management of red palm weevil *Rhynchophorus ferrugineus* Olivier in date palm *Phoenix dactylifera* L. Emir. J. Food Agric. *28* (*1*), 34–44 https://doi.org/10.9755/ejfa.2015-10-897.

Al-Elimi, M.H., Monzer, H.A., and El-Sebay, Y. (2000). Field and chemical evaluation of some aggregation pheromone lures of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.), (*Coleoptera*: *Curculionidae*). Paper presented at: 1st Conf. of Central Agric. Pesticide Lab.(Egypt).

Al-Eryan, M.A.S., El-Ghariani, I.M., Massry, H.A.A., Agleyo, H.A., and Mohammed, S.A. (2010). First record of the red palm weevil, *Rhynchophorus ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*) in Libya. Paper presented at: Int. Joint Conf. on Date Palm (Abu Dhabi, United Arab Emirates).

Al-Eryan, M.A.S., El-Ghariani, I.M., Ismail, S.S., Massry, H.A.A., Al-Taeeb, S.A., and Raheel, S. (2014). Management protocol depending on both pheromones and burning palms for the red palm weevil *Rhynchophorus ferrugineus*

Oliv. (*Coleoptera*: *Curculionidae*) recently recorded in Libya. Entomol. Ornithol. Herpetol. *3* (*1*), 1–3.

Al-Hudaib, K., Ajlan, A., and Al-Abdulsalam, K. (2007). The first worldwide website of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), (*Coleoptera*: *Curullionidae*). Abstract presented at: Fourth Symposium on Date Palm in Saudi Arabia.

Al-Hudaib, K., Ajlan, A.M., Ben Abdullah, A., and Faleiro, J.R. (2008). Date palm farming practices in relation to red palm weevil, *Rhynchophorus ferrugineus* (Olivier) infestation in the Gulf region of the Middle East. Paper presented at: Tele-conference in the Plant-Insect Ecosystems Symposium at the Annual Meeting of the Entomological Society of America (Reno, USA).

Al-Hudaib, K., Ajlan, A., and Faleiro, J. (2017). Genetic diversity among *Rhynchophorus ferrugineus* populations from Saudi Arabia and India. Scientific Journal of King Faisal University *19* (*1*), 35–42.

Al-Jabr, A.M., and Abo-El-Saad, M. (2008). A putative serine protease from larval midgut of red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*): partial purification and biochemical characterization. Am. J. Environ. Sci. *4* (*6*), 595–601.

Al-Khatri, S.A. (2004). Date palm pests and their control. Paper presented at: Date Palm Regional Workshop on Ecosystem-Based IPM for Date Palm in Gulf Countries (Al-Ain, UAE).

Al-Khatri, S.A., and Abd-Allah, F.F. (2003). Seasonal fluctuation of *Rhynchophorus ferrugineus* in Sultanate of Oman. Paper presented at: International Conference on Date Palm (King Saud University).

Al-Manie, M.A., and Alkanhal, M.I. (2007). Acoustic detection of the red date palm weevil. World Acad. Sci. Eng. Technol. *1* (*2*), 337–340.

Al Mohanna, O.E., Hanounik, S.B., Hegazy, G., and Salem, M. (2000). Biology of the red palm weevil *Rhynchophorus ferrugineus* (Oliv.). Paper presented at: First Workshop on Control of Date Palm Weevil (Kingdom of Saudi Arabia: King Faisal University).

Al-Nujiban, A.A., Aldosari, S.A., Al Suhaibani, A.M., Abdel-Azim, M.M., Ibrahim, S.M.M., and Shukla, P. (2015). Effect of date palm cultivar on fecundity and development of *Rhynchophorus ferrugineus*. Bull. Insectol. *68* (*2*), 199–206.

Al-Rajhy, D.H., Hussein, H.I., and Al-Shawaf, A.M.A. (2005). Insecticidal activity of carbaryl and its mixture with piperonylbutoxide against red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Curculionidae*: *Coleoptera*) and their effects on acetylcholinesterase activity. Pak. J. Biol. Sci. *8*, 679–682 https://doi.org/10.3923/pjbs.2005.679.682.

Al-Saoud, A.H. (2004). The role of aggregation pheromones in integrated control of red palm weevil *Rhynchophorus ferrugineus* Oliver (*Coleoptera*: *Curculioinidae*:). Paper presented at: Date Palm Regional Workshop on Ecosystem Based IPM for Date Palm in the Gulf Countries (Al Ain, UAE).

Al-Saoud, A.H. (2007). Importance of date fruits in red palm weevil, *Rhynchophorus ferrugineus* aggregation pheromone traps. Acta Hortic. *736*, 405–413 https://doi.org/10.17660/ActaHortic.2007.736.37.

Al-Saoud, A.H. (2009a). Effect of red palm weevil, *Rhynchophorous ferriouginous* Olivier (*Coleoptera*: *Curculionidae*) aggregation pheromone traps contains on the number of capture weevils. Damascus Univ. J. Agric. Sci. *25* (*1*), 151–175.

Al-Saoud, A.H. (2009b). The role of kairomone in red palm weevil, *Rhynchophorous ferriouginous* Olivier (*Coleoptera*: *Curculionidae*) aggregation pheromone traps. Damascus Univ. J. Agric. Sci. *25* (*2*), 125–144.

Al-Saoud, A.H. (2010a). Effect of red palm weevil, *Rhynchophorous ferriouginous* Olivier (*Coleoptera*: *Curculionidae*) aggregation pheromone traps, height and colors on the number of captured weevils. Acta Hortic. *882*, 419–429 https://doi.org/10.17660/ActaHortic.2010.882.47.

Al-Saoud, A.H. (2010b). Investment optimization of (RPW) *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) aggregation pheromone traps in United Arab Emirates. Paper presented at: Red Palm Weevil, the Challenge (Riyadh, Kingdom of Saudi Arabia: SABIC, Saudi Basic Industries Corporation).

Al-Saoud, A.H. (2011a). Effect of red palm weevil, *Rhynchophorous ferrugineus* Olivier (*Coleoptera*: *Curculionidae*) aggregation pheromone traps sites on the number of captured weevils. Damascus Univ. J. Agric. Sci. *27* (*2*), 77–97.

Al-Saoud, A.H. (2011b). Comparative effectiveness of four food baits in aggregation pheromone traps on red palm weevil *Rhynchophorous ferrugineus* Olivier. Arab J. Plant Protect. *29* (*1*), 83–89.

Al-Saoud, A.H. (2012). Role of aggregation pheromone traps in the management of red palm weevil. Paper presented at: III Workshop & Int. Training Program on Red Palm Weevil Management (Riyadh, Kingdom of Saudi Arabia: King Saud Univ.).

Al-Saoud, A.H. (2013). Effect of ethyl acetate and trap color on weevil captures in red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) pheromone traps. Int. J. Trop. Insect Sci. *33* (*3*), 202–206 https://doi.org/10.1017/S1742758413000167.

Al-Saoud, A.H. (2014). Factors affecting the efficacy of ethyl acetate in the red palm weevil aggregation pheromone traps. Paper presented at: 5th International Date Palm Conference (Abu Dhabi).

Al-Saoud, A.H. (2015). Effect of ethyl acetate on the number of red palm weevil *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*) captured in dark red and yellow aggregation pheromone traps. Syrian J. Agric. Res. *2* (*1*), 128–140.

Al-Saoud, A.H. (2017). Effect of stirring of water and dates in the red palm weevil aggregation pheromone trap on the adults captured in UAE. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Al-Saoud, A.H., and Ajlan, A. (2013). Effect of date fruits quantity on the numbers of red palm weevil, *Rhynchophorus ferrugineus* (Olivier), captured in aggregation pheromone traps. Agric. Biol. J. N. Am. *4* (*4*), 496–503 https://doi.org/10.5251/abjna.2013.4.4.496.503.

Al-Saoud, A.H., Al-Deeb, M.A., and Murchie, A.K. (2010). Effect of color on the trapping effectiveness of red palm weevil pheromone traps. J. Entomol. *7* (*1*), 54–59 https://doi.org/10.3923/je.2010.54.59.

Al-Saqer, S.M. (2012). A reliable identification system for red palm weevil. Am. J. Appl. Sci. *9* (*8*), 1150–1157.

Al-Saqer, S.M., and Hassan, G.M. (2011a). Artificial neural networks based red palm weevil (*Rynchophorus ferrugineous*, Olivier) recognition system. Am. J. Agric. Biol. Sci. *6* (*3*), 356–364 https://doi.org/10.3844/ajabssp.2011.356.364.

Al-Saqer, S.M., and Hassan, G.M. (2011b). Red palm weevil (*Rynchophorus ferrugineous*, Olivier) recognition by image processing techniques. Am. J. Agric. Biol. Sci. *6* (*3*), 365–376 https://doi.org/10.3844/ajabssp.2011.365.376.

Al-Saroj, S., Al-Abdallah, E., Al-Shawaf, A., Al-Dandan, A., Al-Abdullah, I., Al-Shagag, A., Al-Fehaid, Y., Abdallah, A.B., and Faleiro, J.R. (2017). Efficacy of bait free pheromone trap (ElectrapTM) for management of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). Pest Manage. Hortic. Ecosyst. *23* (*1*), 55–59.

Al-Shagag, A., Al-Abbad, A.H., Al-Dandan, A.M., Ben Abdallah, A., and Faleiro, J.R. (2008). Enhancing trapping efficiency of red palm weevil pheromone traps with ethyl acetate. Indian J. Plant Prot. *36*, 310–311.

Al-Shawaf, A.M., Al-Shagag, A.A., Al-Bagshi, M.M., Al-Saroj, S.A., Al-Badr, S.M., Al-Dandan, A.M., and Ben Abdallah, A. (2010). Toxicity of some insecticides against red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). Indian J. Plant Prot. *38* (*1*), 13–16.

Al-Shawaf, A.M., Al-Abdan, S., Al-Abbad, A.H., Ben Abdallah, A., and Faleiro, J.R. (2012). Validating area-wide management of *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in date plantation of Al-Hassa. Indian J. Plant Prot. *40* (*4*), 255–259.

Al-Shawaf, A.M., Al-Shagag, A., Al-Bagshi, M., Al-Saroj, S., Al-Bather, S., and Al-Dandan, A.M. (2017). A quarantine protocol against red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleptera*: *Curculiondae*) in date palm. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Al-Wahshi, K., Jaddou, M., AlHosani, I., Al-Sammarraie, A., Farag, M., Beziou, A., Al-Kaabi, A., and Al-Nuaimi, A. (2017). Determining the toxicity of the common used pesticides on red palm weevil. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Alanazi, N.A., Sulieman, A.M., Alshammari, N.I., Al-Azmi, M., Aziz, A., Elbadri, G.A., and Stephenson, S.L. (2020). Fungi related with the red palm weevil (*Rhynchophorus ferrugineus*) in the Hail Area. Northern Saudi Arabia. Biosci. Biotech. Res. Commun. *13* (*4*), 2233–2239 https://doi.org/10.21786/bbrc/13.4/91.

Aldafer, H.A., Alahmadi, A.Z., and Alsuhaibani, A.M. (1998). Biological studies on *Rhynchophorus ferrugineus* in Saudi Arabia. Research Bulletin *75* (Agric. Res. Centre, King Saud University), p.5–30.

Aldawood, A.S. (2016). Integrated Research Programs for Date Palm Pest Management: New Initiatives and Future Perspectives (AIP Publishing), http://scitation.aip.org/ content/aip/proceeding/aipcp.

Aldawood, A.S. (2017). An overview of red palm weevil management: Current status and future challenges. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Aldawood, A.S., and Rasool, K.G. (2011). Rearing optimization of red palm weevil: *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) on date palm: *Phoenix dactylifera.* Fla. Entomol. *94* (*4*), 756–760 https://doi.org/10.1653/024.094.0404.

Aldawood, A.N., Alsagan, F., Altuwariq, H., Almuteri, A., and Rasool, K. (2013). Red palm weevil chemical treatments on date palms in Saudi Arabia: results of extensive experimentations. Paper presented at: AFPP - Colloque Méditerranéen sur les Ravageurs des Palmiers (Nice, France).

Aldossary, A.A., Shehata, S.T., Hegazy, G., Salem, M.A., and Faiza, M.A.M. (2009). Assessment of the entomopathogenic fungus Beauveria bassiana Saudi Arabian isolate (B–SA3) against the developmental stages of the red palm weevil, *Rhynochophorus ferrungineus* (Oliv.). J. Agric. Sci., Ain Shams Univ., Cairo *17* (*1*), 227–237.

Aldryhim, Y., and Al-Bukiri, S. (2003). Effect of irrigation on within-grove distribution of red palm weevil *Rhynchophorous ferrugineus.* Agricultural and Marine Sciences *8* (*1*), 47–49 https://doi.org/10.24200/jams.vol8iss1pp47-49.

Aldryhim, Y., and Khalil, A. (2003). Effect of humidity and soil type on survival and behaviour of red palm weevil *Rhynchophorus ferrugineus* (Oliv.) adults. Agric. and Marine Sci. *8* (*2*), 87–90.

Alfazairy, A.A. (2004). Notes on the survival capacity of two naturally occurring entomopathogens of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). Egypt. J. Biol. Pest Cont. *14* (*2*), 423.

Alfazairy, A.A. (2011). A simple feeding diet for evaluating the efficacy of microbial or chemical control agents against larval stage of the curculionid red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Egypt. J. Biol. Pest Cont. *21* (*1*), 101.

Alfazairy, A.A., El-Minshawy, A.M., Karam, H.H., and Hendi, R. (2003). An easy and cheap feeding diet of vegetable origin for rearing the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), (*Coleoptera*: *Curculionidae*). Paper presented at: First Int. Egyptian-Romanian Conf. (Zagazig).

Ali-Bob, M. (2019). Management of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) using sustainable options in Saudi Arabia. Arab Journal of Plant Protection *37* (*2*), 163–169 https://doi.org/10.22268/AJPP-037.2.163169.

Aljabr, A.M., Rizwan-ul-Haq, M., Hussain, A., Al-Mubarak, A.I., and Al-Ayied, H.Y. (2014). Establishing midgut cell culture from *Rhynchophorus ferrugineus* (Olivier) and toxicity assessment against ten different insecticides. In Vitro Cell Dev Biol Anim *50* (*4*), 296–303 https://doi.org/10.1007/s11626-013-9694-1. PubMed

Alkhazal, M.H., Youssef, L.A., Abdel-Wahed, M.S., Kassab, A.S., and Saleh, M.M.E. (2009). Factors affecting infestation pattern of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) in date palm farms in Qatif, Saudi Arabia. J. Agric. Sci. Ain Shams Univ. Cairo *17* (*1*), 177–183.

Alshammari, N., Alazmi, M., Alanazi, N.A., Sulieman, A.E., Veettil, V.N., and Ponce-Alonso, M. (2021). A comparative study on the microbial communities of *Rhynchophorus ferrugineus* (red palm weevil) infected and healthy palm trees. Arab. J. Sci. Eng. *47*, 6735–6746.

Alsuhaibani, A.M., Aldafer, H.M., and Alahmadi, A.Z. (2001). Behavioural and biological studies on the red palm weevil, *Rhynchophorus ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*) in Riyadh, Saudi Arabia. Res. Bull. (Int. Comm. Northwest Atl. Fish.) *107*, 5–30.

Alzahrani, A.M., Abdelsalam, S.A., Elmenshawy, O.M., and Abdel-Moneim, A.M. (2013). Ultrastructural characteristics of spermiogenesis in *Rhynchophorus ferrugineus* (*Coleoptera*: *curculionidae*). Fla. Entomol. *96* (*4*), 1463–1469 https://doi.org/10.1653/024.096.0426.

Antony, B., Johny, J., Soffan, A., and Aldosari, S.A. (2017). Pheromone communication disruption through olfactory gene silencing a novel approach for controlling red palm weevil, *Rhynchophorus ferrugineus*. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Atwa, A.A., and Hegazi, E.M. (2016). Curative trials by some entomopathogenic nematodes against the red palm weevil infestation. Paper presented at: 2nd Int. Conf. for Date Palm (ICDP) (Qassim, KSA).

Azam, K.M., and Razvi, S.A. (2001).Control of red palm weevil, *Rhynchophorus ferrugineus* Oliver using prophylactic spraying of date palms and trunk injection. Paper presented at: 2nd Int. Conf. on Date Palm (Al Ain, UAE).

Azam, K.M., Razvi, S.A., and Al-Mahmuli, I. (2000). Management of red date palm weevil, *Rhynchophorus ferrugineus* Oliver on date palm by prophylactic measures. Paper presented at: First Workshop on Control of Date Palm Red Weevil (KSA: King Faisal University, Date Palm Research Center).

Azam, K.M., Razvi, S.A., and Al-Mahmuli, I. (2001). Survey of red palm weevil (*Rhynchophorus ferrugineus* Olivier) infestation in date palm in Oman. Paper presented at: 2nd Int. Conf. on Date Palms (Al-Ain, UAE).

Bannari, A., Mohamed, A.M.A., and El-Battay, A. (2017). Bio-physiological stress indicators retrieval and statistical analysis for red palm weevil attack detection using high spatial resolution satellite data. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Belal, M.H., Swelam, E.S., and Mohamed, M.A. (2012). Evaluation of eight insecticides belonging to four different groups for control of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). Ann. Agric. Sci. Moshtohor *50*, 235–242.

Bertone, C., Michalak, P.S., and Roda, A. (2010). New Pest Response Guidelines, Red Palm Weevil (*Rhynchophorus ferrugineus*). http://www. aphis. usda. gov/import export/plants.

Brand, E. (1917). Coconut red weevil. Some facts and fallacies. Trop. Agric. Mag. Ceylon Agric. Soc. *49* (*1*), 22–24.

Bream, A.S., Ghoneim, K.S., Tanani, M.A., and Nassar, M.M. (2001). Evaluation of the plant extracts, Azadirachtin and Jojoba oil, on the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curulionidae*). Paper presented at: II Int. Conf. Date Palms (Al-Ain, UAE: Fac. Agric.).

Chebbi, H. (2011). First record of *Rhynchophorus ferrugineus* on *Phoenix canariensis* in Tunisia. Tunisian J. Plant Prot. *6*, 149–153.

El-Bishry, H.M., El-Sebaey, Y., and Al-Elimi, M.H. (2000). Impact of environment in date palm infested with *Rhynchophorus ferrugineus* on five entomopathogenic nematodes. Int. J. Nematol. *10*, 75–80.

El-Bokl, M.M., Baker, R.F.A., El-Gammal, H.L., and Mahmoud, M.Z. (2010). Biological and histopathological effects of some insecticidal agents against red palm weevil *Rhynchophorus ferrugineus.* Acad. J. Biolog. Sci. *1* (*1*), 7–22 https://doi.org/10.21608/eajbsd.2010.14151.

El-Bokl, M.M., Sallam, A.M., Abdallah, G.A., and Gabr, B.M. (2015). Efficacy of aggregation pheromone in trapping red palm weevil (*Rhynchophorus ferrugineus* Olivier) infested date palms in Damietta, Egypt. Egypt. Acad. J. Biol. Sci. Physiology & Molecular Biology *7* (*1*), 51–59 https://doi.org/10.21608/eajbsc.2015.13702.

El-Ezaby, F.A. (1997a). Injection as a method to control the red Indian date palm weevil, *Rhynchophorus ferrugineus.* Arab Journal Plant Protection *15* (*1*), 31–38.

El-Ezaby, F.A. (1997b). A biological in-vitro study on the Red Indian date palm weevil, *Rhynchophorus ferrugineus*. Arab J. Plant Prot. *15* (*2*), 84–87.

El-Ezaby, F.A., Khalifa, O., and El-Assal, A. (1998a). Integrated pest management for the control of red palm weevil, *Rhynchophorus ferrugineus* Oliv. in the United Arab Emirates, Eastern region, Al-Ain. Paper presented at: First Int. Conf. on Date Palm (Al-Ain, UAE).

El-Ezaby, F.A., Khalifa, O., El-Assal, A., Rahman, A.M., and Al-Sharif, A.A. (1998b). Integrated pest management for the control of red palm weevil in the UAE Eastern region, Al-Ain. Paper presented at: First Int. Conf. on Date Palms (Al-Ain, UAE).

El-Faki, M.S., El-Shafie, H.A.F., and Al-Hajhoj, M.B.R. (2015). Potentials for early detection of red palm weevil (*Coleoptera*: *Curculionidae*) infested date palm (*Arecaceae*) using temperature differentials. Can. Entomol. *10* (*51*), 1–7.

El-Fattah, A.Y.A., El-Wahab, A.S.A., Jamal, Z.A., and El-Helaly, A.A. (2021). Histopathological studies of red palm weevil *Rhynchophorus ferrugineus*, (Olivier) larvae and adults to evaluate certain nano pesticides. Braz J Biol *81* (*1*), 195–201 https://doi.org/10.1590/1519-6984.227621. PubMed

El-Garhy, M.E. (1996). Field evaluation of aggregation pheromone on the red palm weevil, *Rhynchophorus ferrugineus* in Egypt. Paper presented at: Brighton Crop Protect. Conf. Pest and Diseases (Brighton, UK: British Crop Protection Council).

El Husseini, M. (2019). Efficacy of the fungus *Beauveria bassiana* (Balsamo) Vuillemin on the red palm weevil *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*) larvae and adults under laboratory conditions. Egypt. J. Biol. Pest Control *29* (*1*), 58 https://doi.org/10.1186/s41938-019-0155-3.

El-Lakwah, F.A.M., El-Banna, A.A., El-Hosary, R.A., and El-Shafei, W.K.M. (2011). Population dynamics of the red palm weevil (*Rhynchophorus ferrugineus* (Oliv.) on date palm plantations in 6th October Governorate. Egypt. J. Agric. Res. *89* (*3*), 1105–1118 https://doi.org/10.21608/ejar.2011.177676.

El-Mergawy, R.A.A.M., and Al-Ajlan, A.M. (2011). Red palm weevil, *Rhynchophorus ferrugineus* (Olivier): economic importance, biology, biogeography and integrated pest management. J. Agric. Sci. Technol. *1*, 1–23.

El-Mergawy, R.A.A.M., Al-Ajlan, A.M., Abdallah, N.A., Nasr, M.I., and Silvain, J.F. (2011a). Determination of different geographical populations of *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) using RAPD-PCR. J. Agric. Biol. *13* (*2*), 227–232.

El-Mergawy, R.A.A.M., Al-Ajlan, A.M., Abdalla, N., Vassiliou, V., Capdevielle-Dulac, C., Kontodimas, D.C., Silvain, J.F., and Nasr, M.I. (2011b). Preliminary study on geographical variation of cytochrome b gene and ITS2-rDNA among populations of *Rhynchophorus ferrugineus*. J. Agric. Sci. Technol. B *1*, 189–197.

El-Mergawy, R.A.A.M., Al-Ajlan, A.M., Abdallah, N.A., Nasr, M.I., and Silvain, J.F. (2011c). Genetic comparison among *Rhynchophorus ferrugineus* and four *Rhynchophorus* species. J. Agric. Sci. Technol. B *1*, 610–615.

El-Mergawy, R.A.A.M., Faure, N., Nasr, M.I., Avand-Faghih, A., Rochat, D., and Silvain, J.F. (2011d). Mitochondrial genetic variation and invasion history of red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*), in Middle-East and Mediterranean basin. Int. J. Agric. Biol. *13* (*5*), 631–637.

El-Muhanna, O., Hanounik, S.B., Hegazy, G., and Salem, M. (2000). Biology of the red date palm weevil Rhynchophorus ferrugineus Oliv. Paper presented at: First Workshop on Control of Date Palm Red Weevil (Kingdom of Saudi Arabia: Ministry of Higher Education, King Faisal University, Date Palm Research Center).

El-Naggar, S.E.M., Mohamed, H.F., and Mahmoud, E.A. (2010). Studies on the morphology and histology of the ovary of red palm weevil female irradiated with gamma rays. J. Asia Pac. Entomol. *13* (*1*), 9–16 https://doi.org/10.

1016/j.aspen.2009.08.004.

El-Saadany, G.T., Abdel-Megeed, M.L., El-Sebay, Y., and Kamal, M. (2007a). Food suitability for the red palm weevil *Rhynchophorus ferrugineus* (Oliv.). Abstract presented at: Fourth Symposium on Date Palm (Saudi Arabia).

El-Saadany, G.T., Abdel-Megeed, M.L., El-Sebay, Y., and Kamal, M. (2007b). The estimated number and duration of the red palm weevil *Rhynchophorus ferrugineus* (Oliv.) generation based on degree-days. Abstract presented at: Fourth Symposium on Date Palm (Saudi Arabia).

El-Sabea, A.M.R., Faleiro, J.R., and Abo El Saad, M.M. (2009). The threat of red palm weevil *Rhynchophorus ferrugineus* to date plantations of the Gulf region of the Middle East: an economic perspective. Outlooks Pest Manag. *20* (*3*), 131–134 https://doi.org/10.1564/20jun11.

El-Saeid, M.H., and Al-Dosari, S.A. (2010). Monitoring of pesticide residues in Riyadh dates by SFE, MSE, SFC, and GC techniques. Arab. J. Chem. *3* (*3*), 179–186 https://doi.org/10.1016/j.arabjc.2010.04.007.

El-Safty, R., Awash, S.A., Al-Amiri, A.M., Shadad, A.S., Al-Bathra, A.H., and Mousa, S.A. (2007). Biological control of red palm weevil, *Rhynchophorus ferrugineus* by the entomopathogenic fungus, *Beauveria bassiana* in United Arab Emirates. Abstract presented at: 3rd International Date Palm Conference (Abu Dhabi, United Arab Emirates).

El-Safty, R., Awash, S.A., Al-Bagham, S.H., Shahdad, A.S., and Al-Bathra, A.H. (2009). Pathogenicity of the fungus, *Beauveria bassiana* to the red palm weevil, *Rhynchophorus ferrugineus* under laboratory and field conditions. Egypt. J. Biol. Pest Cont. *19* (*1*), 81–85.

El-Safty, R., Al Bgham, S., Al-Awash, S., Shahdad, A., and Al Bathra, A. (2010). A study on a trap for autodissemination of the entomopathogenic fungus *Beauveria bassiana* by red palm weevil adults in date palm plantations. J. Basic Appl. Mycol. *1*, 61–65.

El-Safty, R., Al Bgham, S., Al-Awash, S., Shahdad, A., and Al Bathra, A. (2011). A trap for auto-dissemination of the entomopathogenic fungus *Beauveria bassiana* by red palm weevil adults in date palm plantations. Egypt. J. Biol. Pest Cont. *21* (*2*), 271–276.

El-Sebay, Y. (2003). Ecological studies on the red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in Egypt. Egypt. J. Agric. Res. *81* (*2*), 523–529 https://doi.org/10.21608/ejar.2003.276553.

El-Sebay, Y. (2004a). Control of red palm weevil, *Rhynchophorus ferrugineus* in Egypt. Egypt. J. Agric. Res. *82* (*4*), 1581–1589 https://doi.org/10.21608/ejar.2004.275295.

El-Sebay, Y. (2004b). Field evaluation of certain insecticides against red palm weevil, *Rhynchophorus ferrugineus* in Egypt. Egypt. J. Agric. Res. *82*, 1591–1599.

El-Sebay, Y., Abdel-Lattef, M.K., and Makhlouf, T.M. (2003). Laboratory rearing of red palm weevil *Rhynchophorus ferrugineus* Olv. (*Coleoptera*: *Curculionidae*) on artificial diet. Egypt. J. Agric. Res. *81* (*2*), 551–554 https://doi.org/10.21608/ejar.2003.276562.

El-Sebay, Y., Abbass, M., and El-Shezly, M. (2010). Seasonal abundance and population trends of red palm weevil, *Rhynchophorus ferrugineus* Oliver (*Coleoptera*: *Curculionidae*). J. Plant Protect. Pathol. Mansoura Univ. *1* (*8*), 577–583 https://doi.org/10.21608/jppp.2010.86896.

El-Shafie, H.A.F., Faleiro, J.R., Al-Abbad, A.H., Stoltman, L., and Mafra-Neto, A. (2011). Bait-free attract and kill technology (HookTM RPW) to suppress red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in date palm. Fla. Entomol. *94* (*4*), 774–778 https://doi.org/10.1653/024.094.0407.

El-Shafie, H.A.F., Faleiro, J.R., Abo-El-Saad, M.M., and Aleid, S.M. (2013). A meridic diet for laboratory rearing of red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). Sci. Res. Essays *8*, 1924–1932.

El-Sharabasy, H.M. (2010). A survey of mite species associated with the red palm weevil, *Rhyncophorus ferrugineus* (Olivier) in Egypt. Egypt. Biol. Pest Cont. *20* (*1*), 67–70.

El-Wahab, A.S.A., El-Fattah, A.Y.A., El-Shafei, W.K.M., and Helaly, A.A.E. (2021). Efficacy of aggregation nano gel pheromone traps on the catchability of *Rhynchophorus ferrugineus* (Olivier) in Egypt. Braz J Biol *81* (*2*), 452–460 https://doi.org/10.1590/1519-6984.231808. PubMed

El-Zemaity, M.S., Abdel-Megeed, M.I., Abdul-Wahed, M.S., and Reda, A.A. (2010). Factors influencing the effectiveness of certain novel insecticides against red palm weevil. Acta Hortic. *882*, 923–927 https://doi.org/10.17660/ActaHortic.2010.882.107.

El-Zoghby, I.R.M., and Abdel-Hameid, N.F. (2018). Rearing of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) on different natural diets. Paper presented at: 4th International Conference on Biotechnology Applications in Agriculture (ICBAA) (Moshtohor and Hurghada: Benha University).

Elawad, S.A., Mousa, S.A., Shahbad, A.S., Alawaash, S.A., and Alamiri, A.M.A. (2007a). Efficacy of entomopathogenic nematodes against red palm weevil in UAE. Acta Hortic. *736*, 415–420 https://doi.org/10.17660/ActaHortic.2007.736.38.

Elawad, S.A., Mousa, S.A., Shahbad, A.S., Alawaash, S.A., and Alamiri, A.M.A. (2007b). Potential of entomopathogenic nematodes against the red palm weevil in United Arab Emirates. Pak. J. Nematol. *25* (*1*), 5–13.

Faleiro, J.R. (2005). Insight into the management of red palm weevil *Rhynchophorus ferrugineus* Olivier: based on experiences on coconut in India and date palm in Saudi Arabia. In I Jornada Internacional sobre el Picudo Rojo de las Palmeras(Fundación Agroalimed), p.35–57.

Faleiro, J.R., Abraham, V.A., and Al-Shuaibi, M.A. (1998). Role of pheromone trapping in the management of red palm weevil. Indian Concr. J. *29* (*5*), 1–3.

Faleiro, J.R., Al-Shuaibi, M.A., Abraham, V.A., and Kumar, T.P. (1999). A technique to assess the longevity of the pheromone (Ferrolure) used in trapping the date red palm weevil *Rhynchophorus ferrugineus* Oliv. Sultan Qabus Univ. J. Agric. Sci. Res. *4* (*1*), 5–9.

Faleiro, J.R., Abraham, V.A., Noudi, N.A.L., Shuaibi, M.A., and Kumar, T.P. (2000). Field evaluation of red palm weevil, *Rhynchophorus ferruginous* Oliv. Pheromone (Ferrugineol) lures. Indian J. Entomol. *62* (*4*), 427–433.

Faleiro, J.R., Abdallah, A.B., Kumar, J.A., Abdallah, S., and Alabdan, S. (2010). Sequential sampling plan for area-wide management of *Rhynchophorus ferrugineus* (Olivier) in date palm plantations of Saudi Arabia. Int. J. Trop. Insect Sci. *30* (*3*), 145–153 https://doi.org/10.1017/S1742758410000226.

Faleiro, J.R., El-Saad, M.A., and Al-Abbad, A.H. (2011). Pheromone trap density to mass trap *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*/*Rhynchophoridae*/*Dryophoridae*) in date plantations of Saudi Arabia. Int. J. Trop. Insect Sci. *31* (*1-2*), 75–77 https://doi.org/10.1017/S1742758411000099.

Faleiro, J.R., Abdullah, A.B., El-Bellaj, M., Al Ajlan, A.M., and Oihabi, A. (2012). Threat of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) to date palm plantations in North Africa. Arab J. Plant Prot. *30*, 274–280.

Faleiro, J.R., El-Shafie, H.A.F., Ajlan, A.M., and Sallam, A.A. (2014). Screening date palm cultivars for resistance to red palm weevil *Rhynchophorus ferrugineus* (*Coleptera*: *Curculiondae*). Fla. Entomol. *97* (*4*), 1529–1536 https://doi.org/10.1653/024.097.0427.

Faleiro, J.R., Al-Shawaf, A.M., El-Shafie, H.A.F., and Pai Raikar, S. (2019). Studies on service free semiochemical mediated technologies to control red palm weevil *Rhynchophorus ferrugineus* Olivier based on trials in Saudi Arabia and India. Arab Journal of Plant Protection *37* (*2*), 136–142.

FAOSTAT. (2013). Food and Agricultural Commodities Production. http://www.faostat3.fao.org/download/Q/QC/E.

Farooq, W.A., Rasool, K.G., Tawfik, W., and Aldawood, A.S. (2015). Application of laser induced breakdown spectroscopy in early detection of red palm weevil: (*Rhynchophorus ferrugineus*) infestation in date palm. Plasma Sci. Technol. *17* (*11*), 948–952 https://doi.org/10.1088/1009-0630/17/11/11.

Fetoh, B.A. (2011). Latent effects of gamma radiation on certain biological aspects of the red palm weevil (*Rhynchophorus ferrugineus* Olivier) as a new control technology. Agric. Technol. Thail. *7* (*4*), 1169–1175.

Ghonim, K.S., Al-Dali, A.G., and Abdel-Ghaffar, A.A. (2003). Effectiveness of lufenuron (CGA-184699) and Diofenolan (CGA-59205) on the general body metabolism the Red Palm Weevil, *Rhynchophorus ferrugineus* (*Curculionidae*: *Coleoptera*). Pak. J. Biol. Sci. *6* (*13*), 1125–1129 https://doi.org/10.3923/pjbs.2003.1125.1129.

Girgis, G.N., Batt, A.M., Okil, A.M., Haggag, S.M., and Abdel Azim, M.M. (2002). Evaluation of trunk injection methods for the control of red palm weevil, *Rhynchophorus ferrugineus* (Oliver) in date palm trees in Egypt. Paper presented at: 2nd International Conference (Cairo, Egypt: Plant Protection Institute).

Gomaa, W.O. (2006). Three mites' species associated with the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.), in Egypt. Bull. Fac. Agric. Cairo Univ. *57* (*3*), 543–548.

Habib, D.M., Mouna, N., and Wiem, H. (2017). Red palm weevil *Rhynchophorus ferrugineus* chemical treatments applied on ornamental palms in Tunisia: results of extensive experiments. Int. J. Agric. Innov. Res. *5* (*6*), 2319–1473.

Hajjar, M.J., Ajlan, A.M., and Al-Ahmad, M.H. (2015). New approach of *Beauveria bassiana* to control the red palm weevil (*Coleoptera*: *Curculionidae*) by trapping technique. J Econ Entomol *108* (*2*), 425–432 https://doi.org/10.1093/jee/tou055. PubMed

Hamad, S.A.S., and El Faith, M.M. (2004). Endoscope: a potential diagnostic tool for red palm weevil infestation. Paper presented at: Date Palm Regional Workshop on Ecosystem-Based IPM for Date Palm in Gulf Countries.

Hamdi, A.N. (1998). Report on present status of red palm weevil and date palm borers in Bahrain. Bulletin of Arab Organization for Agriculture Development. Agric. Dev. *1*, 51–55.

Hanounik, S.B. (1998). Steinernematids and heterorhabditids as biological control agents for the red palm weevil (*Rhynchophorus ferrugineus* Oliv.). Sultan Qabus University Journal for Scientific Research. Agric. Sci. (Melb.) *3*, 95–102.

Hanounik, S.B., Hegazy, G., Abbas, M.S.T., Salem, M., Saleh, M.M.E., Mansour, M.I., El Muhanna, O., Bgham, S.A.I., Abuzuhaira, R., Awash, S., and Shambia, A. (2000a). Biological control of *Rhynchophorus ferrugineus* (Oliv.) as a major component of IPM. Paper presented at: First Workshop on Control of Date Palm Weevil (Kingdom of Saudi Arabia: King Faisal University).

Hanounik, S.B., Saleh, M.M.E., Abzuhairah, R.A., Alheji, M., Al Dhahir, H., and Al Garrash, Z.H. (2000b). Efficacy of entomopathogenic nematodes with antidesicants in controlling the red palm weevil, *Rhynchophorus ferrugineus* on date palm trees. Int. J. Nematol. *10* (*2*), 131–134.

Hanounik, S.B., Salem, M., Hegazy, G., Al Mohanna, O.E., Al Hegi, M., and Al Zahir, H. (2000c). Development of a new food-baited aggregation pheromone/kairomone trapping system for the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.). Paper presented at: First workshop on Control of Date Palm Weevil (Kingdom of Saudi Arabia: King Faisal University).

Hassan, G.M., and Al-Saqer, S.M. (2012). Support vector machine based red palm weevil (*Rynchophorus ferrugineous*, Olivier) recognition system. Am. J. Agric. Biol. Sci. *7* (*1*), 36–42 https://doi.org/10.3844/ajabssp.2012.36.42.

Hassan, M.F., Nasr, A.K., Allam, S.F., Tana, H.A., and Mahmoud, R.A. (2011). Biodiversity and seasonal fluctuation of mite families associated with the red palm weevil, *Rhynchophorus ferrugineus* Oliver (*Coleoptera*: *Curculionidae*) in Egypt. Egypt. J. Biol. Pest Cont. *21* (*2*), 317–323.

Hoddle, M.S., Al-Abbad, A.H., El-Shafie, H.A.F., Faleiro, J.R., Sallam, A.A., and Hoddle, C.D. (2013). Assessing the impact of pheromone trapping, pesticide applications, and eradication of infested date palms for *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) management in Al Ghowaybah, Saudi Arabia. Crop Prot. *53*, 152–160 https://doi.org/10.1016/j.cropro.2013.07.010.

Hoddle, M.S., Hoddle, C.D., Faleiro, J.R., El-Shafie, H.A.F., Jeske, D.R., and Sallam, A.A. (2015). How far can the red palm weevil (*Coleoptera*: *Curculionidae*) fly? Computerized flight mill studies with field-captured weevils. J Econ Entomol *108* (*6*), 2599–2609 https://doi.org/10.1093/jee/tov240. PubMed

Hussain, A., Rizwan-ul-Haq, M., and Al-Jabr, A.M. (2013a). Red palm weevil: understanding the fungal disease mechanism and host defense. In Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education, A. Méndez-Vilas, ed. (Formatex Research Center), p.1278–1286.

Hussain, A., Rizwan-ul-Haq, M., Al-Jabr, A.M., and Al-Ayedh, H.Y. (2013b). Managing invasive populations of red palm weevil: a worldwide perspective. J. Food Agric. Environ. *11*, 456–463.

Hussain, A., Rizwan-ul-Haq, M., Al-Ayedh, H., Ahmed, S., and Al-Jabr, A.M. (2015). Effect of *Beauveria bassiana* infection on the feeding performance and antioxidant defence of red palm weevil, *Rhynchophorus ferrugineus* Olivier (*Curculionidae*: *Coleoptera*). BioControl *60* (*6*), 849–859 https://doi.org/10.1007/s10526-015-9682-3.

Hussain, A.E., El-Ansari, M.K., and Zahra, A.A.A. (2016a). Susceptibility of different date palm cultivars to *R. ferrugineus* infestation in Egypt. Paper presented at: 2nd Conf. for Date Palm (ICDP) (Qassim, KSA).

Hussain, A., Rizwan-Ul-Haq, M., Al-Ayedh, H., and AlJabr, A.M. (2016b). Susceptibility and immune defence mechanisms of *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) against entomopathogenic fungal infections. Int J Mol Sci *17* (*9*), 1518 https://doi.org/10.3390/ijms17091518. PubMed

Hussein, K.M.A. (1998). Biological, ecological and control studies on red palm weevil, *Rhynchophorus ferrugineus* in Sharkia and Ismailia Governorates, Egypt. M.Sc. thesis (Fac. Agric., Zagazig Univ.).

Ibrahim, T.M.I. (2010). Effect of different date palm varieties on some biological aspects of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) and its control. Ph.D. thesis (Fac. Agric., Zagazig Univ.).

Ibrahim, A.M.A., Sewify, G.H., Moursy, E.B., and Hassan, J.M. (2011). Microbial profile from gut of red palm weevil *Rhynchophorus ferrugineus* Olivier (*Curculionidae*: *Coleoptera*). Egypt. J. Biotechnol. *37*, 9.

Kaakeh, W. (2000). The use of synthetic pheromones in integrated pest management program. Emirates J. Agric. Sci. *12*, 1–32.

Kaakeh, W. (2005). Longevity, fecundity, and fertility of the red palm weevil, *Rhynchophorus ferrugineus* Olivier (*Coleoptera*: *Curculionidae*) on natural and artificial diets. Emirates J. Agric. Sci. *17* (*1*), 23–33.

Kaakeh, W. (2006). Toxicity of imidacloprid to developmental stages of *Rhynchophorus ferrugineus* (*Curculionidae*: *Coleoptera*): laboratory and field tests. Crop Prot. *25* (*5*), 432–439 https://doi.org/10.1016/j.cropro.2005.07.006.

Kaakeh, W., Aboul-Nour, M.M., and Khamis, A.A. (2001a). The Red Palm Weevil: the Most Destructive Agricultural Pest (UAE: United Arab Emirates University Printing Press), pp.160.

Kaakeh, W., Aboul-Nour, M.M., and Khamis, A.A. (2001b). Mass rearing of the red palm weevil, *Rhynchophorus ferrugineus* Oliv., on sugar cane and artificial diets for laboratory studies. Paper presented at: Second International Conference on Date Palm (Al-Ain, UAE).

Kaakeh, W., El-Ezaby, F., Aboul-Nour, M.M., and Khamis, A.A. (2001c). Management of the red palm weevil by a pheromone/food-based trapping system. Paper presented at: Second Int. Conf. on Date Palms (Al-Ain, UAE).

Kaakeh, W., Khamis, A.A., and Aboul-Nour, M.M. (2001d). Life parameters of the red palm weevil, *Rhynchophorus ferrugineus* Oliv., on sugar cane and artificial diet. Paper presented at: Second International Conference on Date Palm (Al-Ain, UAE).

Kamel, K.E., Mohamed, M.I., and Shaarawi, F.A. (2005). Histology and ultrastructure of the ovary of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). J. Egypt. Ger. Soc. Zool. *47E*, 87–105.

Khalifa, O., El-Assal, A.H., El-Ezaby, F.A.A., Murse, M.A., Al-Nuaimi, S.M., and Al-Zehli, N.S. (2007). Database for infestation of date palm by red palm weevil, *Rhynchophorus ferrugineus* in UAE and Oman. Paper presented at: 3rd International Date Palm Conference (Abou Dhabi, United Arab Emirates).

Khamiss, O., and Abdel Badeea, A. (2013). Initiation, characterization and karyotyping of a new cell line from red palm weevil *Rhynchophorus ferrugineus* adapted at 27°C. Paper presented at: AFPP – Palm Pest Mediterranean Conference (Nice, France).

Khiyami, M., and Alyamani, E. (2008). Aerobic and facultative anaerobic bacteria from gut of red palm weevil (*Rhynchophorus ferrugineus*). Afr. J. Biotechnol. *7* (*10*), 1432–1437.

Kurdi, H., Al-Aldawsari, A., Al-Turaiki, I., and Aldawood, A.S. (2021). Early detection of red palm weevil, *Rhynchophorus ferrugineus* (Olivier), infestation using data mining. Plants (Basel) *10* (*1*), 95 https://doi.org/10.3390/plants10010095. PubMed

Mahmoud, M.M.A., Ismail, I.M., Amin, M.K., Fayed, A.H., and Mostafa, S.A. (2011). Isolation and identification of novel local isolates of *Bacillus thuringiensis* active against red palm weevil (RPW). Egypt. J. Genet. Cytol. *40* (*2*), 337–350 https://doi.org/10.21608/ejgc.2011.10796.

Massoud, M.A.E., Faleiro, J.R., El-Saad, M.A., and Sultan, E. (2011). Geographic information system used for assessing the activity of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the date palm oasis of Al-Hassa, Saudi Arabia. J. Plant Protec. Res. *51* (*3*), 234–239 https://doi.org/10.2478/v10045-011-0039-3.

Massoud, M.A.E., Sallam, A.A., Faleiro, J.R., and Al-Abdan, S. (2012). Geographic information system-based study to ascertain the spatial and temporal spread of red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in date plantations. Int. J. Trop. Insect Sci. *32* (*2*), 108–115 https://doi.org/10.1017/S174275841200015X.

Mehmood, K., Tufail, M., Husain, M., Rasool, K.G., and Aldawood, A.S. (2017). Transcriptome analysis of fat body tissues from the adult red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*), a highly destructive pest of palm trees. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Merghem, A. (2011). Susceptibility of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) to the green muscardine fungus, *Metarhizium anisopliae* (Metsch.) in the laboratory and in palm trees orchards. Egypt. J. Biol. Pest Cont. *21* (*2*), 179–183.

Mesallam, T., Saleh, M.R.A., Ibrahem, S.S.M., and El-Sebay, Y.M.A. (2009). Some biological aspects of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) as influenced by rearing on certain palm varieties, temperature and relative humidity. Zagazig J. Agric. Res. *36* (*6*), 1110–1118.

Mogahed, M.I. (2010). Studies on host preference and its biological effects on the red palm weevil, *Rhynchophorus ferrugineus* Olivier in Egypt: the fruit trees. Arch. Phytopathol. Pflanzenschutz *43* (*10*), 949–956 https://doi.org/10.1080/03235400802176175.

Mogahed, M.I. (2011). Studies on host preference and its biological effects on the red palm weevil, *Rhynchophorus ferrugineus* Olivier in Egypt vegetable crops. Arch. Phytopathol. Pflanzenschutz *44* (*11*), 1053–1060 https://doi.org/10.1080/03235401003673028.

Mohammed, A.W., Alghamdi, K.M., and Mahyoub, J.A. (2021). The dynamic fluctuation of red palm weevil *Rhynchophorus ferrugineus* (Olivier) in Makkah Al-Mukarramah city. Biosci. Biotechnol. Res. Asia *18* (*1*), 85–97 https://doi.org/10.13005/bbra/2898.

Monzer, M.A., and Al-Elimi, M.H. (2002). Further investigation on the impact of the environment in date palm infested with *Rhynchophorus ferrugineus* on entomopathogenic nematodes: I. Preliminary identification of potent chemical volatiles. Egypt. J. Biol. Pest Cont *12* (*1*), 39–42.

Monzer, M.A., and El-Rahman, R.A. (2003). Effect on *Heterorhabditis indica* of substances occurring in decomposing palm tissues infested by *Rhynchophorus ferrugineus.* Nematology *5* (*5*), 647–652 https://doi.org/10.1163/156854103322746823.

Mozib, M.E., and El-Shafie, H.A. (2013). Effect of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) infestation on temperature profiles of date palm tree. J. Entomol. Nematol. *5* (*6*), 77–83 https://doi.org/10.5897/JEN2013.0081.

Mukhtar, M., Rasool, K.G., Parrella, M.P., Sheikh, Q.I., Pain, A., Lopez-llorca, L.V., Aldryhim, Y.N., Mankin, R.W., and Aldawood, A.S. (2011). New initiatives for management of red palm weevil threats to historical Arabian date palms. Fla. Entomol. *94* (*4*), 733–736 https://doi.org/10.1653/024.094.0401.

Nassar, M.M., and Abdulah, M.A. (2005). Assessment of *Bombex chinensis* oil and Precocene II for the control of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*: *Curculionidae*) and the palm beetle *Pseudopilus testaceous* (Gahan) (*Coleoptera*: *Cerambycidae*). J. Entomol. *2* (*1*), 1–8 https://doi.org/10.3923/je.2005.1.8.

Nassar, M.M., and Abdulah, M.A. (2001). Evaluation of azadirachtin for control of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (*Coleoptera*: *Curculionidae*). J. Egypt. German Soc. Zool. and Entomol. *36*, 163–173.

Ragaei, M. (2010). Wings of red palm weevil (RPW) and their behaviour as semiconductor. Acta Hortic. *882*, 1033–1043 https://doi.org/10.17660/ActaHortic.2010.882.120.

Ragaei, M., and Sabry, A.H. (2013). Red palm weevil, *Rhynchophorus ferrugineus* and harvest termites, *Anacanthotermes ochraceus* with the infrared receptors structures as a sensitive detector to hosts and shelters. J. Appl. Sci. Res. *9* (*1*), 1010–1014.

Ragaei, M., and Sabry, A.H. (2016). Role of photonic crystals in red palm weevil, *Rhynchophorus ferrugineus* Olivier coloration. Paper presented at: 2nd Int. Conf. for Date Palm (ICDP) (Qassim, KSA).

Ragaei, M., Gesraha, M.A., Mohamed, H., and El-Shishtawi, R. (2009). Symbiotic flagellated protozoa isolated from red palm weevil, *Rhynchophorus ferrugineus* (Olivier), (*Coleoptera*: *Curculionidae*). Aust. J. Basic Appl. Sci. *3* (*2*), 604–606.

Rasool, K.G., Khan, M.A., Aldawood, A.S., Tufail, M., Mukhtar, M., and Takeda, M. (2014). Optimization of protein isolation from date palm plants and its utilization in differential proteomics associated with red palm weevil infestation. Pak. J. Agric. Sci. *51* (*4*), 907–917.

Rasool, K.G., Khan, M.A., Aldawood, A.S., Tufail, M., Mukhtar, M., and Takeda, M. (2015). Identification of proteins modulated in the date palm stem infested with red palm weevil (*Rhynchophorus ferrugineus* Oliv.) using two dimensional differential gel electrophoresis and mass spectrometry. Int J Mol Sci *16* (*8*), 19326–19346 https://doi.org/10.3390/ijms160819326. PubMed

Rasool, K.G., Tufail, M., Husain, M., Mehmood, K., and Aldawood, A.S. (2017). Differential proteomic analysis of date palm leaves infested with the red palm weevil, *Rhynchophorus ferruginneus* (Oliv.) (*Coleoptera*: *Curculionidae*). Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Reda, A.R., and Abdel-Salam, A.M.E. (2014). Lab-field evaluation of some neem productions and chemical insecticides against red palm weevil, *Rhynchophorus ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*). Can. J. Plant Protect. *2* (*2*), 60–63.

Sabbour, M.M. (2006). Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* against the red palm weevil *Rhynchophorus ferrugineus* in Egypt. Phytoparasitica *34* (*4*), 370–379.

Sabbour, M.M. (2013a). Preliminary investigations into the biological control of red palm weevil *Rhynchophorus ferrugineus* by using *Beauveria bassiana* in Egypt. Emerging Issues in the Natural and Applied Sciences. *3* (*1*), 85–99.

Sabbour, M.M. (2013b). Evaluation of isolated entomopathogenic fungi against the red palm weevil *Rhynchophorus ferrugineus* in Egypt. Emerging Issues in the Natural and Applied Sciences. *3* (*1*), 111–125.

Sabbour, M.M., and Solieman, N.Y. (2014). Preliminary investigations into the biological control of red palm weevil *Rhynchophorus ferrugineus* by using three isolates of the fungus *Lecanicillium* (*Verticillium*) *lecanii* in Egypt. Int. J. Sci. Res. *3* (*8*), 2060–2066.

Sabit, H., Abdel-Ghany, S., Al-Dhafar, Z., Said, O.A., Ali Al-Saeed, J., Ahmed Alfehaid, Y., and Aly Osman, M. (2021). Molecular characterization and phylogenetic analysis of *Rhynchophorus ferrugineus* (Olivier) in Eastern Province, Saudi Arabia. Saudi J Biol Sci *28* (*10*), 5621–5630 https://doi.org/10.1016/j.sjbs.2021.05.078. PubMed

Sadder, M.T., Vidyasagar, P.S.P.V., Aldosari, S.A., Abdel-Azim, M.M., and Al-Doss, A.A. (2016). Phylogeny of red palm weevil (*Rhynchophorus ferrugineus*) based on ITS1 and ITS2. Orient. Insects *49* (*3–4*), 198–211.

Salama, H.S., and Abd-Elgawad, M.M. (2001). Isolation of heterorhabditid nematodes from palm tree planted areas and their implications in the red palm weevil control. J. Pest Sci. *74* (*2*), 43–45 https://doi.org/10.1111/j.1493-0280.2001.01010.x.

Salama, H.S., and Abd-Elgawad, M.M. (2003). Quarantine problems: an analytical approach with special reference to palm weevils and phytonematodes. Arch. Phytopathol. Pflanzenschutz *36* (*1*), 41–46 https://doi.org/10.1080/0323540031000080137.

Salama, H.S., and Abd-Elgawad, M.M. (2010). Spatial patterns of the red palm weevil and applied entomopathogenic nematode *Heterorhabditis bacteriophora.* Arch. Phytopathol. Pflanzenschutz *43* (*7*), 689–699 https://doi.org/10.

1080/03235400802144397.

Salama, H.S., and Abdel-Razek, A.S. (2002). Development of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), (*Coleoptera*: *Curculionidae*) on natural and synthetic diets. Anz. Schädlkd. *75* (*5*), 137–139 https://doi.org/10.1046/j.1472-8206.2002.02039.x.

Salama, H.S., and Aziz, S.E.A. (2001). Distribution of the sensillae of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*: *curculionidae*). Insect Sci. Appl. *34* (*2*), 179–188.

Salama, H.S., and Aziz, S.E.A. (2002). Sensillae distribution on the antennae and mouth parts of the larvae of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.), (*Coleoptera*: *Curculionidae*). Arch. Phytopathol. Pflanzenschutz *35* (*1*), 61–71.

Salama, H.S., and Ismail, I.A. (2007). Potential of certain natural extracts for the control of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver). Arch. Phytopathol. Pflanzenschutz *40* (*4*), 233–236 https://doi.org/10.1080/03235400500383669.

Salama, H.S., and Saker, M.M. (2002). DNA fingerprints of three different forms of the red palm weevil collected from Egyptian date palm orchards. Arch. Phytopathol. Pflanzenschutz *35* (*4*), 299–306 https://doi.org/10.1080/03235400216136.

Salama, H.S., Hamdy, M.K., and El-Din, M.M. (2002). The thermal constant for timing the emergence of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (*Coleoptera*, *Curculionidae*). Anz. Schädlkd. *75* (*1*), 26–29 https://doi.org/10.1046/j.1439-0280.2002.02004.x.

Salama, H.S., Foda, M.S., El-Bendary, M.A., and Abdel-Razek, A. (2004). Infection of red palm weevil, *Rhynchophorus ferrugineus*, by spore- forming bacilli indigenous to its natural habitat in Egypt. J. Pest Sci. *77* (*1*), 27–31 https://doi.org/10.1007/s10340-003-0023-4.

Salama, H.S., Zaki, F.N., and Abdel-Razek, A.S. (2009). Ecological and biological studies on the red palm weevil *Rhynchophorus ferrugineus* (Olivier). Arch. Phytopathol. Pflanzenschutz *42* (*4*), 392–399 https://doi.org/10.1080/03235400601121521.

Saleh, M.R.A. (1992). Red palm weevil *Rhynchophorus ferrugineus* Oliver is first recorded in Egypt and indeed in Africa continent. List No. 10634 Africa: Collection No. 22563 (London, UK: International Institute of Entomology).

Saleh, M.M.E., and Alheji, M. (2003). Biological control of red palm weevil with entomopathogenic nematodes in the Eastern province of Saudi Arabia. Egypt. J. Biol. Pest Cont. *13*, 55–59.

Saleh, M.R.A., and Gouhar, K.A. (1993). Red palm weevil attacking date palm trees in limited areas of Egypt at the present time and how can be eradicated from these areas. Report (Plant Protection Department, Faculty of Agriculture, Zagazig University), pp.20 (in Arabic).

Saleh, M.R.A., Omar, A.E., Ibrahim, A.E., Hassanein, S.S.M., and Hussein, K.M.A. (1996). An approach to chemical control of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) on the date palm in Sharkia and Ismailia Governorates, Egypt. Egypt. J. Appl. Sci. (Thailand) *11* (*4*), 250–260.

Saleh, M.M.E., Hegazy, G., Salem, M., Hanounik, S.B., Al Mohanna, O., and Alheji, M.A. (2004). Persistence of *Steinernema carpocapsae* (*Nematoda*: *Steinernematidae*) and *Beauveria bassiana* (*Deuteromycotina*: *Hyphomycetes*) in soil around date palm trunks and their effect on adults of *Rhynchophorus ferrugineus*. Egypt. J. Biol. Pest Cont. *14* (*1*), 141–145.

Saleh, M.M.E., Kassab, A.S., Abdelwahed, M.S., and Alkhazal, M.H. (2010). Semi-field and field evaluation of the role of entomopathogenic nematodes in the biological control of the red palm weevil *Rhynchophorus ferrugineus.* Acta Hortic. *882*, 407–412 https://doi.org/10.17660/ActaHortic.2010.882.45.

Saleh, M.M.E., Alheji, M.A., Alkhazal, M.H., Alferdan, H., and Darwish, A. (2011). Evaluation of *Steinernema* sp. SA a native isolate from Saudi Arabia for controlling adults of the red palm weevil. *Rhynchophorus ferrugineus* (Oliver). Egypt. J. Biol. Pest Cont. *21* (*2*), 277–282.

Saleh, M.M.E., Abdel-Monim, A.S.H., and El-Kholy, M.Y. (2012). Population of adults of the red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in date palm farms in Ismailia Governorate, Egypt. J. Am. Sci. *8* (*12*), 440–443.

Salem, S.A. (2015). Accuracy of trained dogs for early detection of red palm weevil, *Rhynchophorus ferrugieneus* Oliv. Infestations in date palm plantations. Swift J. Agric. Res. *1* (*1*), 1–4.

Salem, S.A., and Reda, A.S. (2015). The relationship between environmental factors and cultural practices and red palm weevil, *Rhynchophorus ferrugineus* Olivier infestation. Swift J. Agric. Res. *1* (*2*), 5–8.

Salem, M.S., Belal, M.H., Nour, M.E., and Sayed, E.A. (2012). Detection of a chemical marker from ovipositing females in *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculiondae*). Adv. Environ. Biol. *6* (*8*), 2280–2284.

Salem, S.A., Abd El-Salam, A.M.E., and El-Kholy, M.Y. (2016). Field evaluation of red palm weevil *Rhynchophorus* *ferrugineus* Oliv. (*Coleoptera*: *Curculionidae*) responses to its fermenting date tree volatiles. Int. J. Chemtech Res. *9* (*5*), 12–17.

Sallam, A.A., El-Shafie, H.A.F., and Al-Abdan, S. (2012). Influence of farming practices on infestation by red palm weevil *Rhynchophorus ferrugineus* (Olivier) in date palm: a case study. Int. Res. J. Agric. and Soil Sci. *2* (*8*), 370–376.

Samara, R. (2017). First record of endogenous bio-agent of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*) in Palestine. Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Sewify, G.H., and Fouad, S.H. (2006). Integrated control of red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*). Agric. Sci. Mansoura Univ. *31* (*4*), 2415–2426.

Sewify, G.H., Belal, M.H., and Awash, S.A. (2009). Use of the fungus, *Beauveria bassiana* for the biological control of the red palm weevil, *Rhynchophorus ferrugineus* Olivier. Egypt. J. Biol. Pest Cont. *19* (*2*), 157–163.

Sewify, G.H., Belal, M.H., and Saeed, M.Q. (2010). Using pheromone mass trapping within IPM programmes for controlling the red palm weevil, *Rhyncophorus ferrugineus* Olivier. Alex. J. Agric. Res. *55* (*2*), 1–9.

Sewify, G.H., Belal, M.H., and Saeed, M.Q. (2014). Using pheromone mass-trapping and the entomopathogenic fungus *Beauveria bassiana* in IPM programs for controlling the red palm weevil, *Rhyncophorus ferrugineus* Olivier (*Coleoptera*: *Rhynchophoridae*). Egypt. J. Biol. Pest Cont. *24* (*1*), 197–202.

Shaarawi, F.A. (2001). Ultrastructure of the midgut in the fourth larval instar of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (*Coleoptera*: *Curculionidae*). J. Egypt. Acad. Soc. Environ. Develop. *1*, 71–94.

Shaarawi, F.A., Kamel, K.E., and Al Qahtani, A.M. (2000). Toxicity and histopathologic effects of two plant extracts, as compared to an insecticide, on the midgut of the fourth instar larvae of the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (*Coleoptera*: *Curculionidae*). J. Union Arab Biol. *13A*, 491–503.

Shamseldean, M.M. (2002). Laboratory trials and field applications of Egyptian and foreign entomopathogenic nematodes used against the red palm weevil, *Rhynchophorus ferrugineus* Oliv. Int. J. Nematol. *14* (*1*), 44–55.

Shamseldean, M.M., and Abd-Elgawad, M.M. (1994). Laboratory evaluation of six Egyptian isolates of Heterorhabditis nematodes for control of the red palm weevil. Egypt. J. Appl. Sci. *9* (*3*), 670–679.

Shamseldean, M.M., and Atwa, A.A. (2004). Virulence of Egyptian steinernematid nematodes against the red palm weevil, *Rhynchophorus ferrugineus*. Egypt. J. Biol. Pest Cont. *14* (*1*), 135–140.

Sharaby, A.M., and Al-Dhafar, Z.M. (2013). Successful laboratory culture for the red palm weevil *Rhynchophorus ferrugineus* (*Colioptera*: *Curculionidae*) reared on semi-artificial diet. J. Basic Appl. Sci. Res. *3* (*5*), 1–7.

Sharaby, A.M., and Al-Dosary, M.M. (2006). Sense organs on the head of the red palm weevil adults *Rhynchophorus ferrugineus* Olive. (*Coleoptera*: *Curculionidae*) of Saudi Arabian strain. Bull. Entomol. Soc. Egypt *83*, 99–132.

Sharaby, A.M., and Al-Dosary, M.M. (2007). Natural oils and their terpenes for controlling for the red palm weevil *Rhynchophorus ferrugineus* in KSA. Abstract presented at: Fourth Symposium on Date Palm in Saudi Arabia.

Sharaby, A.M., and Al-Dosary, M.M. (2014). An electric air flow olfactometer and the olfactory response of *Rhynchophorous ferrugineus* weevil to some volatile compounds. J. Agric. Ecol. Res. Int. *1* (*1*), 40–50 https://doi.org/10.9734/JAERI/2014/11854.

Sharshir, F.A., El-Monsif, R., and El-Gertemi, S. (2007). Some ecological studies and natural enemies associated with the red palm weevil, *Rhynchophorus ferrugineus* (Oliver) on palm trees at Balteem, Kafr El-Sheikh Egypt. Abstract presented at: Fourth Symposium on Date Palm in Saudi Arabia.

Shawir, M.S., Abbassy, M.A., and Salem, Y.M. (2014). Laboratory evaluation of some insecticides against larval and adult stages of red palm weevil *Rhynchophorus ferrugineus* (Olivier). Alexandria Sci. Exchange J. *35* (*2*), 75–79.

Shukla, P., Vidyasagar, P.S.P.V., Aldosari, S.A., and Abdel-Azim, M. (2012). Antifeedant activity of three essential oils against the red palm weevil, *Rhynchophorus ferrugineus*. Bull. Insectol. *65* (*1*), 71–76.

Tufail, M., Mehmood, K., Husain, M., Rasool, K.G., and Aldawood, A.S. (2017). RNAi-based vitellogenin gene silencing: a promising approach for the control of red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Abstract presented at: 1st Int. Conf. Integrated Protection of Date Palms (Kingdom of Bahrain).

Vidyasagar, P.S.P.V., Mohammed, H.R.A., Abozuhairah, A.E., Mohanna, A.E., and Saihati, A.A. (2000a). Impact of mass pheromone trapping on red palm weevil: adult population and infestation level in date palm gardens of Saudi Arabia. Planter *76*, 347–355.

Vidyasagar, P.S.P.V., Saihati, A.A., Mohanna, O.A., Subbei, A.I., and Abdul Mohsin, A.M. (2000b). Management of red palm weevil *Rhynchophorus ferrugineus* Oliv., a serious pest of date palm in Al Qatif, Kingdom of Saudi Arabia. J. Plant. Crops *28*, 35–43.

Vidyasagar, P.S.P.V., Aldosari, S.A., Sultan, E.M., Al Saihati, A., and Khan, R.M. (2016). Efficiency of optimal pheromone trap density in management of red palm weevil, *Rhynchophorus ferrugineus* Olivier. Afr. J. Agric. Res. *11* (*12*), 1071–1078 https://doi.org/10.5897/AJAR2013.6817.

Zayed, T.M. (2008). Studies on IPM of red palm weevil, *Rynchphorus ferrugineus* (*Coleoptera*, *Curculionidae*) in El-Beheira Governorate, Egypt. Ph.D. thesis (Fac. Agric., Saba Basha, Alexandria Univ.).

Date palm tissue culture and the green Morocco plan

M. Ait Chitt[1,a,b] and R. Meziani[2,a]

[1]Domaine El Bassatine, Laboratoire de Biotechnologie Appliquée, BP 299, Meknès, Morocco; [2]Institut National de la Recherche Agronomique, CRRA-Errachidia, Laboratoire National de Culture des Tissus de Palmier Dattier, Avenue Moulay Ali Chérif, BP 2, Errachidia, Morocco.

Abstract

Date palm (*Phoenix dactylifera* L.) is a socio-economically, ecologically and agronomically important fruit tree in the Middle East and North Africa. In Morocco, millions of date palm trees have been destroyed over a century by bayoud, a severe wilt disease caused by *Fusarium oxysporum* f. sp. *albedinis*. The development of the date industry in Morocco cannot be done without increasing the number of palm trees dramatically and expanding the cultivation of this crop to new zones where the right soil, conditions and water resources are available. The entire sector needed also to be organized in order to increase the productivity, yield and quality of the dates upstream and the commercialization of the product downstream of the production chain. The Green Morocco Plan strategy is addressing these issues. The only way to offer large numbers of certified and top-quality date palm plants to fulfil the goals of this national program is by providing tissue culture plants. This communication shows the great impact that tissue culture technology holds on the development of the date palm sector in Morocco. Furthermore, it explains how the Green Morocco Plan is transforming Morocco from one of the biggest importers of dates to an exporting country.

Keywords: date palm, tissue culture, Green Morocco Plan, Green Generation Strategy

INTRODUCTION

Date palm is an important crop in Morocco and a strategy was put in place in order to develop this sector within the framework of a larger program on agriculture called the Green Morocco Plan (GMP). This communication is to present the strategy followed and the key role played by tissue culture in providing top quality plant material to fulfil the requirement for large plantations.

IMPORTANCE OF DATE PALM IN MOROCCO

The total number of date palms in the world reaches more than 150 million trees, represented by more than 5000 cultivars and distributed in more than 30 countries. Of which more than 100 million trees are grown in Arab countries (Sedra, 2013). The annual world production of dates is estimated at more than 9 million tons (FAOSTAT, 2020). The main date producing countries are Egypt, Iran, Algeria, Saudi Arabia and the United Arab Emirates, which hold more than 60% of the world production.

In Morocco, date palm plays a vital role in preserving biodiversity, protecting lands from desertification, creating favorable microclimates for agriculture, and providing a steady income to the inhabitants of the oases areas. This crop is grown mainly in areas on the southern flank of the Atlas Mountains, along rivers and around water points (Figure 1).

The total number of date palms in Morocco is around 6.9 million trees, which represents about 4% of the world population (MAPMDREF, 2010). The provinces of Ouarzazate (Draa valley), Errachidia (Tafilalet and Ziz valleys) and Tata (Bani) alone represent almost 90% of the national date palm population.

Regarding genetic resources, the Moroccan palm grove exhibits a high genetic diversity accounting for more than 453 genotypes (52.3% as known cultivars and 47.7% as seedlings

[a]Co-first authors, they contributed equally to this work.
[b]E-mail: aitchitt@domaines.co.ma; aitchitt@gmail.com

called khalts). However, among all the existing genotypes, 12 cultivars are the most appreciated by farmers, namely: ‘Mejhoul’, ‘Boufeggous’, ‘Jihel’, ‘Bouskri’, ‘Bousthammi Noir’, ‘Bouslikhène’, ‘Outokdime’, ‘Bouittob’, ‘Ahardane’, ‘Aguelid’, ‘Taabdount’ and ‘Aziza Bouzid’ (Sedra, 2015).

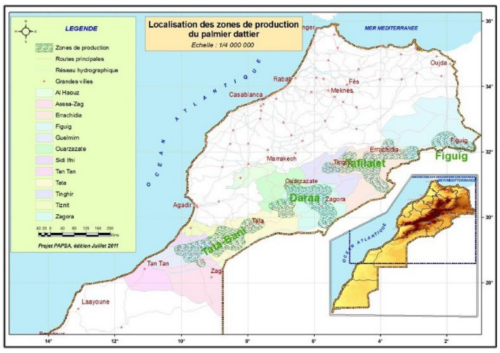


Figure 1. Zones where the main date palm groves are located.

At the end of the 19th century, Morocco was ranked 3rd among date-producing countries and had a prominent place in the foreign trade of dates thanks, in particular, to the quality of these dates. Unfortunately, after this long period of prosperity of the Moroccan palm groves, and from the beginning of the 20th century, the situation has completely declined and continues to deteriorate mainly because of the drought, and the bayoud disease.

Drought

Although date palm is considered to be one of the most adapted species to the conditions of arid and semi-arid zones, water nevertheless remains an essential factor for its development and its proliferation. In 1985 (dry year), production was only 12,000 t, whereas it was around 120,000 t in 1990 (wet year).

Bayoud disease

Although the causes of this degradation are multiple, bayoud remains, in the Moroccan context, the most damaging disease and the most studied in the literature. Bayoud is a vascular disease caused by the soil fungus *Fusarium oxysporum* f. sp. *albedinis* (Foa). Since its appearance toward the end of the 19th century, this disease has caused considerable damage to the Moroccan date palm sector, estimated at more than 10 million palm trees destroyed (Essarioui and Meziani, 2018). Bayoud was also the cause of an intense genetic erosion causing the disappearance of a number of good quality genotypes. The disease continues to spread in the traditional oases by destroying the best Moroccan cultivars (‘Mejhoul’, ‘Boufeggous’, ‘Bouskri’, etc.) (Sedra, 2015). In Morocco, bayoud has spread in the main palm groves of Darâa (Ouarzazate-Zagora), Ziz (Errachidia-Errfoud), Bani (Tata) and the oriental (Figuig). The disease also exists in smaller oases such as Akka, Fom Elhisn, Fom Zguid, Taghajijte and Guelmim (Essarioui and Meziani, 2018).

STRATEGY WITHIN THE GREEN MOROCCO PLAN (GMP)

Like other strategic sectors, the Green Morocco Plan (GMP) designed and monitored by the Ministry of Agriculture had designed date palm as a crop of high priority in arid regions. Indeed, this plan aims at the reconstitution and densification of existing palm groves and the creation of new modern plantations wherever the climate is suitable and water available. To achieve these objectives, an agreement was signed in 2010 between the Government and the inter-profession with an investment cost of 7.7 billion Dirhams (820 million dollars). The main objectives of this program were as follows:

- To develop the capacities of the country in terms of production of tissue culture plants;
- To rehabilitate and reconstitute the existing palm groves over a total area of 48,000

ha;
- To create new plantations outside the traditional palm groves, over an area of 17,000 ha;
- To reach a production of dates of around 160,000 t in 2020 compared to 90,000 t at the beginning of the program;
- To pack 70% of the production expected by 2020;
- To export 5,000 t of high-quality dates in 2020 compared to insignificant quantities exported at the beginning of the program.

In order to implement this program, the GMP was set to have 2 pillars (Figures 2 and 3):
- Pillar 1 concerns new zones with no previous plantation at all. The land is offered for rent to private investors with some important subsidies (35,000 dh ha^{-1} for the plants, 80% for the investment for irrigation system and other subsidies);
- Pillar 2 concerns the traditional oasis and small producers. These small producers are highly subsidized to encourage them for the plantation of new trees of date palm, especially clones selected by INRA for their resistance to bayoud disease. Tissue culture plants as well as the investment for the irrigation system is subsidized at 100% for any farmer with less than 5 ha.

Figure 2. Modern plantation (Pillar 1).

Figure 3. Traditional oasis (Pillar 2).

DATE PALM TISSUE CULTURE

The use of tissue culture techniques is by far the best way to rehabilitate the Moroccan oases areas. Date palm is propagated in vitro using two techniques, the somatic embryogenesis and organogenesis.

Somatic embryogenesis is the process by which somatic cells develop into embryos after a series of morphological and biochemical changes (Quiroz-Figueroa et al., 2006). This technique includes a sequence of steps, namely the induction of embryogenic calli, the formation of somatic embryos, the maturation of somatic embryos and their conversion into plantlets. In the date palm, the success of each of these steps depends on several factors, mainly the genotype, the type of explant and the growth regulators used (Fki et al., 2011).

In Morocco, this technique has been studied and optimized for several genotypes like 'Iklane' and 'Jihel' (El Hadrami et al., 1995), 'Jihel' and 'Bousthami Noir' (Zouine and El Hadrami, 2007), 'Boufeggouss' and 'Bouskri' (Abohatem et al., 2011), 'Mejhoul' (Mazri et al., 2018), 'Al-Fayda' (Mazri et al., 2019).

Organogenesis is the technique by which adventitious buds are formed directly on the explant. It comprises numerous steps: initiation of vegetative buds, bud multiplication, shoot elongation and rooting, then plantlet acclimatization (Figures 4 and 5).

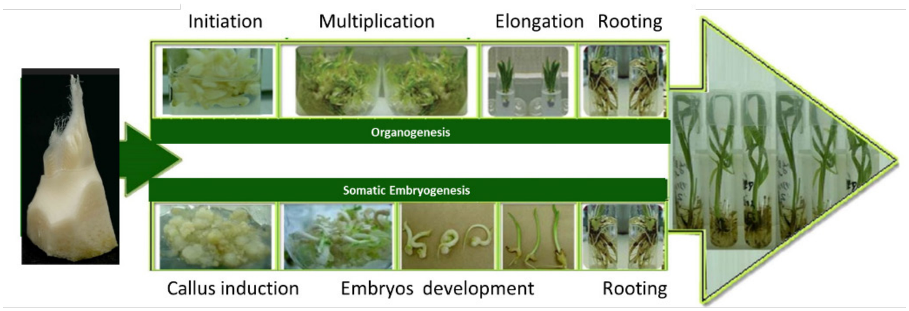


Figure 4. Organogenesis vs. somatic embrygenesis.

Figure 5. Acclimatized plantlets under shade house.

The first research on the development of this technique for the date palm was carried out at INRA-Morocco (Rhiss et al., 1979; Zaid and Tisserat, 1983; Beauchesne et al., 1986; Aitchitt, 1989; Anjarne and Zaid, 1993; Bouguerfaoui and Zaid, 1993; Anjarne et al., 1995; Mazri and Meziani, 2013, 2015; Meziani et al., 2015, 2016, 2019a, b). Organogenesis is the only technique used in Morocco for the commercial production of date palm plants. To date, millions of tissue culture plants have been produced using this propagation technique. The main genotypes propagated are 'Mejhoul', 'Bouffegous', 'Bousekri', 'Najda', 'Aziza Bouzid' and some selected clones and males. In order to solve certain problems related to tissue culture, particularly in terms of somaclonal variations and mixing cultivars, a rigorous traceability system and analyses by DNA markers have been adopted.

A certification scheme was established for the production of certified date palm plants through tissue culture. The Moroccan plants protection department (ONSSA) delivers certifications to the producing laboratories once they comply with the regulations (Figure 6).

Figure 6. Certified date palm ready to be planted in the field.

Situation of the date palm tissue culture in Morocco

To protect the new date palm groves from any diseases and quarantine pests, all the new plantations had to be planted using tissue culture derived plants. This situation created a high demand of plant material and new laboratories were established while only two excited at the beginning of the program. This industry comprises now 7 private and 2 government laboratories (Table 1).

Table 1. Date palm tissue culture laboratories in Morocco.

Laboratories	Status	Location
Laboratoire El Bassatine (Domaines Agricoles)	Private	Meknes
Issemghy Biotechnology	Private	Casablanca
Palmagro	Private	Agadir
Oasis Biotechnologie	Private	Errachidia
In vitro Palm Biotechnology	Private	Casablanca
Marrakech Date Palm Project	Private	Marrakech
Maghreb Palm	Private	Agadir
National date palm tissue culture laboratory INRA (PalmINRA)	Public	Errachidia
Date palm tissue culture laboratory INRA	Public	Marrakech

These laboratories are organized under an association of plant tissue culture producers and the government laboratories are providing technical assistance and starting plant material. During the period of the GMP (2010-2020), three million date palm trees have been produced and distributed to farmers. 67% of the new date palms groves are planted with the cultivar 'Mejhoul'. This cultivar originating in Morocco is widely demanded in the local and international markets. Male trees as pollinators represent 3% while 30% is composed of several other cultivars including resistant clones to the bayoud disease. These plants have been sold to the private sector establishing modern orchards as well as to small farmers in the traditional oasis.

To promote investment in the date sector, the government established incentives and important subsidies. The private sector is subsidized for tissue culture plants as well as for other investments such as irrigation systems, land preparation and machinery. The subsidy for such a project can reach up to 50% of the total investment.

NEW STRATEGY GREEN GENERATION

As part of the continued development of the date palm sector, and after the success of the GMP, Morocco has launched a new strategy under the name of " Green Generation 20-30" (GG). The GG strategy is based on two main concepts:

The first is to consider the human element as a priority in any development related to the agricultural sector, while the second relates to continuing the dynamism of agricultural development and focusing on its modernization. As well as creating a new generation of "young entrepreneurs" in the agricultural sector.

Within the framework of this strategy, it is planned to provide 5 million date palm trees produced in vitro to the continuing effort in establishing new date palm groves as well as replanting (densification) the traditional oasis. The private laboratories will provide half a million-date palm three every year.

CONCLUSIONS AND PERSPECTIVES

Planting more than 12,000 ha would not have been possible without the use of tissue culture. Furthermore, establishing new palm groves free from bayoud using the most demanded cultivar ('Mejhoul') would not have been possible either using conventional offshoots because of the risk of contamination by the bayoud disease.

'Mejhoul' used to represent only 3% among the rest of the date cultivars in Morocco. With the results of the GMP and the plantations to come within the GG, Morocco will become a major player in the international market for this cultivar.

Literature cited

Abohatem, M., Zouine, J., and El Hadrami, I. (2011). Low concentrations of BAP and high rate of subcultures improve the establishment and multiplication of somatic embryos in date palm suspension cultures by limiting oxidative browning associated with high levels of total phenols and peroxidase activities. Sci. Hortic. (Amsterdam) *130* (*1*), 344–348 https://doi.org/10.1016/j.scienta.2011.06.045.

Aitchitt, M. 1989. Multiplication du palmier dattier par organogenèse in vitro. Paper presented at: 2ème Séminaire Maghrébin sur la Culture In Vitro du Palmier Dattier. FAO/PNUD/RAB/88/024 (Marrakech).

Anjarne, M., and Zaid, A. (1993). Effets de certains équilibres hormonaux sur l'enracinement précoce des tissus du palmier dattier (*Phoenix dactylifera* L.). Al Awamia *82*, 197–210.

Anjarne, M., Bougerfaoui, M., Cheikh, R., and Aitchitt, M. (1995). Production de vitroplants de palmier dattier par la technique d'organogenèse in vitro: l'expérience marocaine. Paper presented at: Séminaire International sur la Culture du Palmier Dattier dans les Oasis des Pays Méditerranéens (Elche, Espagne).

Beauchesne, G., Zaid, A., and Rhiss, A. (1986). Meristematic potentialities of bottom of young leaves to rapidly propagate date palm. Paper presented at: Second Symposium on Date Palm (Saudi Arabia: King Faisal University).

Bouguerfaoui, M., and Zaid, A. (1993). Effet de la teneur du milieu de culture en ammoniaque sur la vitrification des tissus du palmier dattier cultivés in vitro. Al Awamia *82*, 177–196.

El Hadrami, I., Cheikh, R., and Baaziz, M. (1995). Somatic embryogenesis and plant regeneration from shoot-tip explants in *Phoenix dactylifera* L. Biol. Plant. *37* (*2*), 205–211 https://doi.org/10.1007/BF02913212.

Essarioui, A., and Meziani, R. (2018). Gestion de l'épiphytie du Bayoud dans les palmeraies marocaines. Agriculture du Maghreb *110*, 68–69.

FAOSTAT. (2020). http://faostat.fao.org (accessed April 18, 2022).

Fki, L., Masmoudi, R., Kriaâ, W., Mahjoub, A., Sghaier, B., Mzid, R., Mliki, A., Rival, A., and Drira, N. (2011). Date palm micro-propagation via somatic embryogenesis. In Date Palm Biotechnology, S.M. Jain, J.M. Al-Khayri, and D.V. Johnson, eds. (Dordrecht: Springer), p.47–68.

MAPMDREF. (2010). https://www.agriculture.gov.ma/fr/filiere/palmier-dattier (accessed April 18, 2022).

Mazri, M.A., and Meziani, R. (2013). An improved method for micro-propagation and regeneration of date palm (*Phoenix dactylifera* L.). J. Plant Biochem. Biotechnol. *22* (*2*), 176–184 https://doi.org/10.1007/s13562-012-0147-9.

Mazri, M.A., and Meziani, R. (2015). Micro-propagation of date palm: a review. Cell Dev. Biol. *4* (*3*), 160.

Mazri, M.A., Meziani, R., Belkoura, I., Mokhless, B., and Nour, S. (2018). A combined pathway of organogenesis and somatic embryogenesis for an efficient large-scale propagation in date palm (*Phoenix dactylifera* L.) cv. Mejhoul. 3Biotech *8*, 215.

Mazri, M.A., Meziani, R., Elmaataoui, S., Alfeddy, M.N., and Jaiti, F. (2019). Assessment of genetic fidelity, biochemical and physiological characteristics of in vitro grown date palm cv. Al-Fayda. Vegetos *32* (*3*), 333–344 https://doi.org/10.1007/s42535-019-00034-3.

Meziani, R., Jaiti, F., Mazri, M.A., Anjarne, M., Ait Chitt, M., El Fadile, J., and Alem, C. (2015). Effects of plant growth regulators and light intensity on the micro-propagation of date palm (*Phoenix dactylifera* L.) cv. Mejhoul. J. Crop Sci. Biotechnol. *18* (*5*), 325–331 https://doi.org/10.1007/s12892-015-0062-4.

Meziani, R., Jaiti, F., Mazri, M.A., Hassani, A., Ben Salem, S., Anjarne, M., Ait Chitt, M., and Alem, C. (2016). Organogenesis of *Phoenix dactylifera* L. cv. Mejhoul: influences of natural and synthetic compounds on tissue browning, and analysis of protein concentrations and peroxidase activity in explants. Sci. Hortic. (Amsterdam) *204*, 145–152 https://doi.org/10.1016/j.scienta.2016.04.009.

Meziani, R., Mazri, M.A., Essarioui, A., Alem, C., Diria, G., Gaboun, F., El Idrissy, H., Laaguidi, M., and Jaiti, F. (2019a). Towards a new approach of controlling endophytic bacteria associated with date palm explants using essential oils, aqueous and methanolic extracts from medicinal and aromatic plants. Plant Cell Tissue Organ Cult. *137* (*2*), 285–295 https://doi.org/10.1007/s11240-019-01570-1.

Meziani, R., Mazri, M.A., Arhazzal, M., Belkoura, I., Alem, C., and Jaiti, F. (2019b). Evaluation of in vitro shoot elongation and rooting of date palm, and determination of physiological characteristics of regenerated plantlets. Not. Sci. Biol. *11* (*1*), 77–85 https://doi.org/10.15835/nsb11110402.

Quiroz-Figueroa, F.R., Rojas-Herrera, R., Galaz-Avalos, R.M., and Loyola-Vargas, V.M. (2006). Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. Plant Cell Tissue Organ Cult. *86* (*3*), 285–301 https://doi.org/10.1007/s11240-006-9139-6.

Rhiss, A., Poulain, C., and Beauchesne, G. (1979). La culture in vitro appliquée à la multiplication du palmier dattier (*Phoenix dactylifera* L.). Fruits *34*, 551–554.

Sedra, M.H. (2013). Genetic diversity analysis of Moroccan cultivar genotyping and rapid screening for bayoud disease resistance in date palm using molecular techniques. Acta Hortic. *994*, 271–285 https://doi.org/10.17660/ActaHortic.2013.994.27.

Sedra, M.H. (2015). Date palm status and perspective in Morocco. In Date Palm Genetic Resources and Utilization, J.M. Al-Khayri, S.M. Jain, and D.V. Johnson, eds. (Dordrecht: Springer), p.257–323.

Zaid, A., and Tisserat, B. (1983). In vitro shoot tip differentiation in *Phoenix dactylifera* L. Date Palm J. *2*, 163–182.

Zouine, J., and El Hadrami, I. (2007). Effect of 2,4-D, glutamine and BAP on embryogenic suspension culture of date palm (*Phoenix dactylifera* L.). Sci. Hortic. (Amsterdam) *112* (*2*), 221–226 https://doi.org/10.1016/j.scienta.2006.12.041.

Easy-PCR-XY kits for date palm and dioecious plants sex-determination using universal PCR-primers

M. El-Din Solliman[1,2,a] and H.A.A. Mohasseb[1,2,b]

[1]Plant Biotechnology Department, College of Agricultural and Food Sciences, King Faisal University, PO Box 400, Al-Ahsa 31982, Kingdom of Saudi Arabia; [2]Plant Biotechnology Department, National Research Centre, Dokki-Egypt, Cairo, PO Box. 12622, Egypt.

Abstract

Commercial production of date palm, one of the most important crops in Middle-Eastern countries, is hampered by difficulties in differentiating between male and female plants during early development. To address this issue, in our kits, we identified a sex determination region on the Y chromosome (date-SRY) in the date palm. To accomplish this, we developed a new date-sex determination kit by using the nested PCR method to amplify partial sequences of the data-SRY gene to identify a region of the gene that could be confidently used to identify male date palms. The purpose of the kit is to carry a PCR reaction with the plant DNA templates to determine male or female sex using PCR. The procedure is based on the amplification of a plant's Sry gene fragment. This gene is shorter by 550 bp on chromosome Y as compared with chromosome X, which allows for the determination of plants' sex by means of PCR. The expected PCR products are 550 bp long for males or 0 bp for females. The kit is designed to perform experiments during two laboratory applications (I – PCR set-up - 1.5 h; II – electrophoresis - 1.5-2 h). This invention provides novel universal primers that can amplify the fragment of SRY gene of the male human and dioecious plants using polymerase chain reaction (PCR). These primers can generate the molecular signature from any biological material of unknown human origin and dioecious plants male or female, which actually is the characteristic of its sex identity. The present invention relates to the sex determination kits for the dioecious plants, and particularly to genetic sex determination of the dioecious plants based on the first identification of the SRY gene in the dioecious plants.

Keywords: dioecy sex determination, SRY, sex determination, plant sex

INTRODUCTION

Dioecious plants have separate male and female plants. The male plants may be useful as pollinators, dioecious plants are entirely pollinated manually in both traditional oasis horticulture and in modern commercial orchards. The method by which distinction between male and female in an organism is under genetic control. The sex chromosomes are responsible for the determination of separate sexes. It is a biological system that determines the development of sexual characteristics in an organism. Traditionally, the symbol ♂ designates male and the symbol ♀ designates female. Uni-sexual diploid individuals have two sex chromosomes, and the rest are auto-somes (El-Din Solliman et al., 2019). The polymerase chain reaction was used to isolate the SRY gene with homologs of the conserved motif of the SRY gene from humans and most other mammals. Several plant 'sex chromosomes' have the sex-determining genes located within a small region (blue; only 10% of chromosome 1 of papaya) where recombination does not occur (Liu et al., 2004). Some fish sex chromosomes may be similar and small size means young, or primitive. Other plants have heteromorphic sex chromosomes like those of humans and *Drosophila*, or neo-sex chromosomes (Liu et al., 2004).

In most plants, male and female reproductive organs are found in the same flower and

[a]E-mail: msolliman@kfu.edu.sa; solliman@yahoo.com

[b]E-mail: hmohasseb@kfu.edu.sa

this is called a bisexual or hermaphrodite. In nematodes, earthworms both male and female reproductive organs are found in the same individual. Male and female reproductive organs being found in the different flowers of the same plant is called monoecious (e.g., maize, castor, coconut). In some plants, male and female flowers are produced on different plants called dioecious (e.g., papaya, date palm, jojoba, etc.). The physical separation of male and female plants from each other is usually referred to as sex determination. The mechanisms used for sex determination in different crop species have been documented in many reviews (Geber et al., 1999; Matsunaga and Kawano, 2001; Negrutiu et al., 2001; Barrett, 2002; Charlesworth, 2002a, b).

The date palm is a dioecious, perennial, monocotyledonous fruit tree belonging to the family *Arecaceae*. It is a staple food in western Asia and Africa. The date palm plant is also considered to be an ornamental plant in these regions. Hence, it plays a major role in the socio-economic conditions of these countries (Morton, 1987). For the desired production of date palms, the number of female plants must be higher than the number of male plants. However, it is currently impossible to know the sex of the plant in the early stages of plant development. The sex of the plant can only be identified when it blooms (at approximately five years of age). Many attempts have been made in the past to identify the sex of the plant, but most were unsuccessful and resulted in an unpredictable number of female plants in the field and hence an uncertain production potential of the crop. Breeding date palm plants is also challenging because of the long phases of juvenility and dioecism. These characteristics necessitate determining the sex of the date palm plant in the early stages of development so that the correct plantation can be planned (El-Din Solliman et al., 2019).

MATERIALS AND METHODS

Isolation of plant DNA

All DNA manipulations and purifications were performed according to the protocols by El-Din Solliman et al. (2019).

Polymerase chain reaction

The reaction conditions for PCR were performed according to the protocols by El-Din Solliman et al. (2019) and Domínguez and López-Larrea (1994).

Method to early distinguish the gender of dioecious plants

At first, we tested DNA from females and males, different samples were randomly selected from dioecious plants growing in King Faisal University Research and Training Station. DNA from these samples was isolated following the protocols described by El-Din Solliman et al. (2019). Human DNA samples for males and females were obtained from the College of Medicine, KFU. The amplification of a male-specific SRY marker situated in the Y chromosome sex determination region was amplified using a newly designed forward (F) and reverse (R) primer.

Easy PCR XY –lab., kit for plants sex determination by PCR reaction

The purpose of the kit is to carry a PCR reaction with the plant DNA templates to determine male or female sex using PCR. The kit is designed to perform experiments during two laboratory applications (I –PCR set-up - 1.5 h; II – electrophoresis - 1.5-2 h). Information about the kits is given here: Cat#: PCCSKU15257 PhoenixDx® Date Mix. Description: 2X Date PCR Mix: a reagent for research use, non-toxic, non-infectious, contains no material of human origin. Manufacturer: KFU Biotech GmbH.

RESULTS AND DISCUSSION

The diagnosis of sex in dioecious plants, humans, and most other mammals by SRY gene technique was used to amplify DNA segments, with the objective of finding markers linked to sex determination in the dioecious species, plants like date palm, jojoba, papaya, *Silene latifolia*, and pistachios. The sex of the plant has been identified as male after additional PCR reactions with primers for different markers that are specific to the Y chromosome (e.g., SRY and universal primer). The SRY marker has been previously shown to yield a 360 bp PCR product that overlaps in size with some human and dioecious plants sequences published before. Thus, additional PCR reactions must be performed to determine the gender of the plant. Amplification of the male sample resulted in only one 300 to 360 bp band, which was putatively identified as the 360-bp SRY amplification product. A primer pair according to our invention consists of a universal forward primer SRY_uF and reverse PCR primer SRY_uR. The sequence of the SRY-uF forward primer is given: SRY_F 5'-AGAAGTGAGTTTTGGATAG TAAAATAAGTTTCGA-3'. The SRY-uR reverse primer is given: 5'-CTCACCGCAGCAACGGGACC GCTACAGCCACTGG-3'.

A sex-determination method for a dioecious plant may include obtaining a sample of a dioecious plant and determining the presence or absence of the dioecious plant SRY gene (Figure 1) in the sample. The presence of the dioecious plant SRY gene (Figure 1) in the sample is indicative that the sample is from a male dioecious plant. Using the sex determination method for a dioecious plant, the sex of the dioecious plant may be determined when the dioecious plant is still young, i.e., prior to the flowering of the plants. Also provided are kits for the sex determination of a dioecious plant. These and other features of the present invention will become readily apparent upon further review of the following specification and drawings.

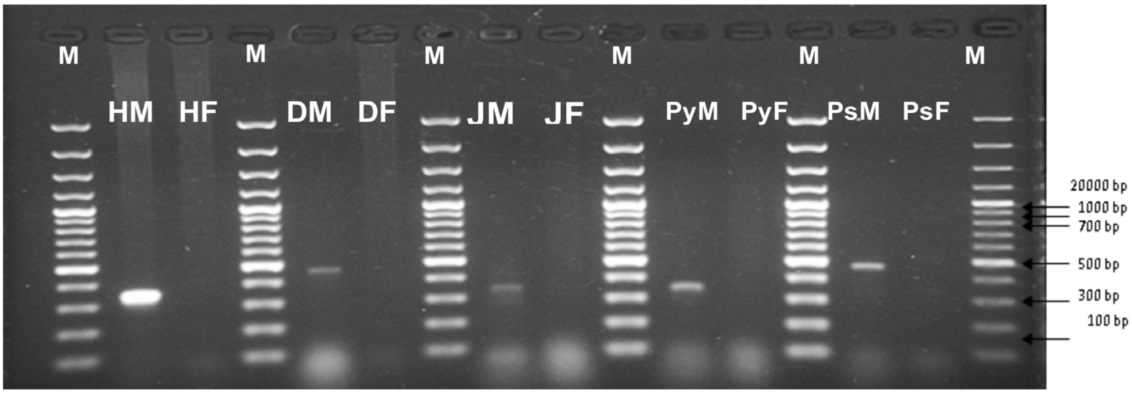


Figure 1. PCR amplification for screening of the presence of SRY gene with human male (HM and HF) and female compere with different variety of dioecious plant (dates (DM and DF), jojoba (JM and JF), papaya (PyM and PyF) and pistachios (PsM and PsF)) using specific primers universal SryF+ universal SryR lane M DNA 100 bp marker.

Easy-PCR XY –lab., kit for plants sex determination by PCR reaction

The purpose of the kit is to carry a PCR reaction with the plant DNA templates to determine male or female sex using PCR. The procedure is based on the amplification of a dioecious plant SRY gene fragment. This gene is shorter by 550 bp on chromosome Y as compared with chromosome X, which allows for the determination of dioecious plant sex by means of PCR. The expected PCR products are 550 bp long for males or 0 bp for females.

Due to the lack of morphological methods to identify sex at a very early stage for dioecious crops, sex-determination for dioecious crops during the long juvenile stage is an economically desirable objective for all scientists dealing with dioecious crops. A primer pair according to our invention consists of two universal PCR primers to identify SRY gene related to the human sex-determining region (SRY) at this stage is expected to facilitate breeding programs. A new invention and approach were adopted to isolate SRY gene which can be identified between males and females of dioecious crops. The SRY gene sequence of some dioecious crops was deposited in Gene Bank (date palm: BankIt1598036 DPSRY1 KC577225, and for Jojoba BankIt MK991776, 360pb). In this invention a major breakthrough in the methodology for sex-determining of dioecious crops such as jojoba, date palm, papaya, and pistachios. The correct sex gene was detected in all tested dioecious plants and humans. Our newly developed strategies for unambiguously selecting the female jojoba and date palm at a young age are very sample and very important to breeders, and therefore to limit the plantation costs associated with the cultivation of the non-productive male plants. Fragments of about 360 bp were isolated from dioecious plants, humans, and most other mammals by genomic walking. A 360-bp DNA fragment was amplified from all the male samples and none of the female samples. Therefore, this method can differentiate the sex of individual dioecious plants for the first time. Our novel method represents a breakthrough for sex determination of dioecious plants, humans, and most other mammals as it can identify sex at early developmental stages. A primer pair according to our invention consists of a Universal forward primer SRY-uF and reverse PCR primer SRY-uR. The sequence of the SRY-uF forward primer is given: SRY_F 5'-AGAAGTGAGTTTTGGATAGTAAAATAAGTTTCGA-3'. The SRY-uR reverse primer is given: 5'-CTCACCGCAGCAACGGGACCGCTACAGCCACTGG-3'.

Based on the discovery of the present kits inventors, the sex of dioecious plants may be genetically determined by determining the presence or absence of the dioecious plant SRY gene (Figures 1 and 2) in a dioecious plant sample. Determining the presence or absence of the dioecious plant SRY gene (Figure 2) in the dioecious plant sample may be carried out by any method known in the art. For example, determining the presence or absence of the dioecious plant SRY gene (Figure 1) in the dioecious plant sample may include extracting nucleic acids from the sample, contacting under amplification conditions the nucleic acid from the sample with a male-specific dioecious plant SRY primer pair, and detecting the presence or absence of amplification products or amplicons. The presence of amplification products may indicate the presence of the male-specific SRY gene in the dioecious plants, i.e., that the sample is from a male plant. The absence of amplification products may indicate that the sample is from a female plant.

Figure 2. Picture of PhoenixDx® Date Mix kits. Product Name: Sex-Determining Region Y (SRY), PCR Kit. Full Product Name: Plant Sex-Determining Region Y Protein (SRY) PCR Kit. Product Gene Name: SRY PCR kit.

Once the sex of the dioecious plants is determined, many dioecious plants breeders and growers proceed to maintain only the female dioecious plants. The earliest point, however, at which male and female trees can be distinguished by external morphology is when the plant flowers, usually 2-6 years after planting. A method of determining the sex of the dioecious plants at an earlier stage would avoid the need to invest time and expense in growing and maintaining unwanted male dioecious plants. Thus, a sex-determination method for dioecious plants solving the aforementioned problem is desired. Several plant species in which female flowering plants are more important than male have been identified. These species include *Actinidia deliciosa* (Shirkot et al., 2002), *Phoenix dactylifera* (El-Din Solliman et al., 2019), *Carica papaya* (Parasnis et al., 2000), *Borassus flabellifer* (George et al., 2007), *Hippophae rhamnoides* (Persson and Nybom, 1998; Sharma et al., 2010), *Myristica fragrans* (Shibu et al., 2000), *Piper longum* (Manoj et al., 2005-2008), *Pistacia vera* (Hormaza et al., 1994), and *Simmondsia chinensis* (Agrawal et al., 2007; Sharma et al., 2008).

CONCLUSIONS

In this report, a breakthrough in the methodology for determining the sex of dioecious plants such as date palm, jojoba, and pistachios has been developed. We concluded that PCR as a simple, rapid, and reliable technique can complement and also confirm sex-determination in both human and dioecious plants by using our specific universal primers pair according to our invention.

ACKNOWLEDGEMENTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors would like to thank King Faisal University, Saudi Arabia, for funding and supporting this research.

Literature cited

Agrawal, V., Sharma, K., Gupta, S., Kumar, R., and Prasad, M. (2007). Identification of sex in *Simmondsia chinensis* (jojoba) using RAPD markers. Plant Biotechnol. Rep. *1* (*4*), 207–210 https://doi.org/10.1007/s11816-007-0031-6.

Barrett, S.C. (2002). The evolution of plant sexual diversity. Nat Rev Genet *3* (*4*), 274–284 https://doi.org/10.1038/nrg776. PubMed

Charlesworth, D. (2002a). Plant sex determination and sex chromosomes. Heredity *88* (*2*), 94–101 https://doi.org/10.1038/sj.hdy.6800016. PubMed

Charlesworth, B. (2002b). The evolution of chromosomal sex determination. In The Genetics and Biology of Sex Determination, R. Short, ed. (Chichester, UK: John Wiley), p.207–219.

Domínguez, O., and López-Larrea, C. (1994). Gene walking by unpredictably primed PCR. Nucleic Acids Res *22* (*15*), 3247–3248 https://doi.org/10.1093/nar/22.15.3247. PubMed

El-Din Solliman, M., Mohasseb, H.A.A., Al-Khateeb, A.A., Al-Khateeb, S.A., Chowdhury, K., El-Shemy, H.A., and Aldaej, M.I. (2019). Identification and sequencing of Date-SRY Gene: a novel tool for sex determination of date palm (*Phoenix dactylifera* L.). Saudi J Biol Sci *26* (*3*), 514–523 https://doi.org/10.1016/j.sjbs.2017.08.002. PubMed

Geber, M.A., Dawson, T.E., and Delph, L.F., eds. (1999). Gender and Sexual Dimorphism in Flowering Plants (Berlin: Springer).

George, J., Karun, A., Manimekalai, R., Rajesh, M.K., and Remya, P. (2007). Identification of RAPD markers linked to sex determination in palmyrah (*Borassus flabellifer* L.). Curr. Sci. *93*, 1075–1077.

Hormaza, J.I., Dollo, L., and Polito, V.S. (1994). Identification of a RAPD marker linked to sex determination in *Pistacia vera* using bulked segregant analysis. Theor Appl Genet *89* (*1*), 9–13 https://doi.org/10.1007/BF00226975. PubMed

Liu, Z., Moore, P.H., Ma, H., Ackerman, C.M., Ragiba, M., Yu, Q., Pearl, H.M., Kim, M.S., Charlton, J.W., Stiles, J.I., et al. (2004). A primitive Y chromosome in papaya marks incipient sex chromosome evolution. Nature *427* (*6972*), 348–352 https://doi.org/10.1038/nature02228. PubMed

Manoj, P., Banerjee, N.S., and Ravichandran, P. (2005-2008). Development of sex specific molecular markers in dioecious *Piper longum* L. plants by differential display. J. Theor. Appl. Inf. Technol. *4* (*5*), 459–465.

Matsunaga, S., and Kawano, S. (2001). Sex determination by sex chromosome in dioecious plants. Plant Biol. *3* (*5*), 481–488 https://doi.org/10.1055/s-2001-17735.

Morton, J. (1987). Dates. In Morton Fruits of Warm Climates, J.F. Morton, ed. (Miami, FL: J.F. Morton), p.5–11.

Negrutiu, I., Vyskot, B., Barbacar, N., Georgiev, S., and Moneger, F. (2001). Dioecious plants. A key to the early events of sex chromosome evolution. Plant Physiol *127* (*4*), 1418–1424 https://doi.org/10.1104/pp.010711. PubMed

Parasnis, A.S., Gupta, V.S., Tamhankar, S.A., and Ranjekar, P.K. (2000). A highly reliable sex diagnostic PCR assay for mass screening of papaya seedlings. Mol. Breed. *6* (*3*), 337–344 https://doi.org/10.1023/A:1009678807507.

Persson, H.A., and Nybom, H. (1998). Genetic sex determination and RAPD marker segregation in the dioecious species sea buckthorn (*Hippophae rhamnoides* L.). Hereditas *129* (*1*), 45–51 https://doi.org/10.1111/j.1601-5223.1998.00045.x.

Sharma, K., Agrawal, V., Gupta, S., Kumar, R., and Prasad, M. (2008). ISSR marker-assisted selection of male and female plants in a promising dioecious crop: jojoba (*Simmondsia chinensis*). Plant Biotechnol. Rep. *2* (*4*), 239–243 https://doi.org/10.1007/s11816-008-0070-7.

Sharma, A., Zinta, G., Rana, S., and Shirko, P. (2010). Molecular identification of sex in *Hippophae rhamnoides* L. using isozyme and RAPD markers. For. Stud. China *12* (*2*), 62–66 https://doi.org/10.1007/s11632-010-0012-7.

Shibu, M.P., Ravishankar, K.V., Anand, L., Ganeshaiah, K.N., and Shaanker, U. (2000). Identification of sex-specific DNA markers in the dioecious tree, nutmeg (*Myristica fragrans* Houtt.). PGR Newsletter *121*, 59–61.

Shirkot, P., Sharma, D.R., and Mohapatra, T. (2002). Molecular identification of sex in *Actinidia deliciosa* var. *deliciosa* by RAPD markers. Sci. Hortic. (Amsterdam) *94* (*1-2*), 33–39 https://doi.org/10.1016/S0304-4238(01)00357-0.

Isolation and molecular detection of *Arthrobacter* species grown on the surface of date palm tissue culture media

A. Abdulrazzaq[1,a], H.S. Khierallah[2], S.M.H. Al-Rubaye[1] and S.M. Bader[2]

[1]Dept. Microbiology, Coll. Veterinary Medicine, University of Baghdad, Iraq; [2]College of Agriculture, University of Baghdad, Iraq.

Abstract

This study was aimed to found out the type of bacterial species grown on the surface of date palm tissue culture media. Shoot tips of date palm ('Barhi') were cultured on MS media supplemented with various combinations of hormones. During growth an infection appeared in all cultures which were gray in color. Primary identification proved a bacterial infection due to its color and appearance. One type of bacterial species was isolated from agar surface which exhibited many characteristics for the genus *Arthrobacter*. The main characteristics of these isolates were aerobic, Gram-positive, catalase-positive, spore forming non-motile. Antibiotic sensitivity test showed that the isolate was sensitive to cipro floxacin, Trimethoprim and Amikacin and resistant to Clarithromycin, Ceftriaxone and Gentamycin. Sequence analysis of 16 rRNA indicated new isolates were closely related to the *Glutamicibacter aitensis* strain ebst40 and arthro bacterial *aitensis* strain L11 with the highest sequence similarity (100%).

Keywords: shoot tips, *Glutamicibacter aitensis*, polymerase chain reaction, hormons

INTRODUCTION

In date palm tissue culture, shoot tip cultures exhibit two kinds of contaminants. External contaminants which can be removed by surface sterilization, while internal ones (existed in the explants) is hard to eliminate and appear during the course of growth in vitro.

The genus *Arthrobacter*, was defined by Conn and Dimmick (1947) and emended by Koch et al. (1995), belongs to the class *Actinobacteria*. The genus *Arthrobacter* comprises of 70 species with validly published names, species of the genus *Arthrobacter* have been isolated from a variety of environmental sources including soil, air, water, oil brine, plants, bio-films, cyanobacterial mats, sediment, poultry litter, cheese, human clinical specimens and animal specimens (Ganzert et al., 2011; Zhang et al., 2012).

Soil systems contain the greatest diversity of microorganisms on earth, with 5,000-10,000 species of microorganism per gram of soil. *Arthrobacter* spp. strains have a primitive life cycle and are among the most frequently isolated, indigenous soil bacteria, found in common and deep subsurface soils, arctic ice (TC1, 2006). All species in this genus are Gram-positive, obligate aerobes, rod shape during exponential growth and members of the genus *Arthrobacter* are catalase-positive, a spore forming bacteria that display a coryneform morphology and cocci in their stationary phase (Keddie et al., 1986).

They have a distinctive cell division called "snapping division" or reversion in which the outer bacterial cell wall ruptures at a joint and can be grown on mineral salts pyridone broth, where colonies have a greenish metallic center when incubated at 20°C (Camargo et al., 2004).

The Immigration of bacteria from soil represents one of the mechanisms to explain the presence of *Arthrobacter* on leaf and stem surfaces of plants (Mongodin et al., 2006). Soil particles are common on foliage of plants that are grown outdoors, wind and rain splatter may deliver soil particles to leaf surfaces, especially if leaves are close to the soil surface (Monier and Lindow, 2004). In a study that compared bacterial diversity of the lettuce phyllosphere to soil in which these plants were grown, it was revealed that many bacterial species were

[a]E-mail: aaldouri96@yahoo.com

common between the two compartments (Zwielehner et al., 2008). This was taken as indirect evidence for the movement of soil bacteria to the lettuce canopy, the transport of bacteria by soil particles across larger spatial scales has also been documented (Hua et al., 2007).

MATERIALS AND METHODS

Tissue culture work

Offshoots of date palm ('Barhi') were detached from the mother tree and brought to the laboratory for dissecting. Each offshoot was dissected and leaves were removed acropetally. Shoot tips (explants) were removed from the shoot terminals after all mature leaves were peeled away. All shoot tips then were stored in cold anti-oxidant solution (100 mg L^{-1} ascorbic acid and 150 mg L^{-1} citric acid) in a refrigerator until surface sterilization. Under a laminar air flow cabinet. Tips were surface sterilized with mercury chlorid 0.1% ($HgCl_2$) and few drops of Tween 20 for 15 min. Then rinsed three times in double steriled distilled water.

Explants were removed and transferred aseptically to sterile Petri dish. After that explants were cultured in jars containing MS medium, except hormone which were modified. Cultures were incubated under specific conditions for each stage. In the multiplication and rooting stages, gray color appeared (Figure 1), causing explants' death (Figure 2).

Figure 1. Appearance of bacterial infection with gray color.

Figure 2. Bacterial infection led to the death of the plantlet.

Isolation and identification

Twenty samples of palm tree tissue culture were brought to the department of microbiology in the Veterinary Medicine College, University of Baghdad to investigate the bacterial infection which caused the death of cultured explants.

Bacterial species were isolated from the agar surface after incubation at 37°C for 48 h using trypticase soy agar. Identification was done by biochemical tests such as catalase, gelatin hydrolysis, citrate utilization, motility, starch hemolysis test, indole production and urease test.

Antibiotic sensitivity test

Antibiotic sensitivity test was performed using a disc diffusion method on Mueller-Hinton agar according to Bauer et al. (1966). The discs of antibiotic which were used Amikacin 30 mg, amoxacillin 20 mg, clavulanic acid 10 mg, Ciprofloxacin 5 mg, trimethoprim-sulfamethoxazole 10 mg, clarithromycin 15 mg, ceftriaxone 30 mg and gentamycin 10 mg.

Colonies of suspected bacterial isolate were transfer from pure culture, to Muller-Hinton agar. The whole surface of the agar was covered by streaking. The antibiotic discs were placed on the surface of the medium by using sterilized forceps pressed firmly to ensure contact with the agar, then the plates were inverted and incubated aerobically at 37°C for 24 h. Recorded as inhibition zones were measured by mm using callipers, and the isolates were interpreted either susceptible, intermediate or resistant to a particular antibiotic (Benson, 2001).

DNA extraction

Genomic DNA was extracted using a wizard genomic DNA extraction kit supplied by Promega used as follows: strains were grown at 37°C for 24 h in a flask containing 500 mL BHI broth. Cultures were harvested at 8000 g for 10 min and washed twice with 50 mM Tris/HCl (pH 8). 15-50 mL (depending on the pellet size) of a solution containing Tris/HCl (50 mM), pH 8, sucrose (100 mM) and 0.5% (v/v) Triton X-100 was added, mixed and incubated overnight at 37°C. Subsequently, 250 mg of lysozyme and 375 µL mutanolysin (6 µL mL^{-1}) were added, mixed and incubated for 1 h at 37°C. Thereafter, 3.75 mL proteinase K (20 mg mL^{-1}) and 3.7 mL 10% Sarkosyl were added, mixed and incubated for 24 h at 37°C. Finally, 3.75 µL 25% SDS was added, mixed and incubated for 1 h at 55°C. DNA was purified according to Brenner et al. (1982).

Amplification of 16S rRNA gene

Amplification of 16S rRNA gene was performed using GoTaq® Green Master Mix (Promega, USA) according to the manufacturer's recommendations. The synthesized primers were used for amplification. Temperature-time profile of PCR was as follows: amplification was performed using chromosomal DNA as a template and oligonucleotides Ribo-For (5'-AGTTTGATCCTGGCTCAG-3'; and Ribo-Rev (5'-CCTACGTATTACCGCGGC-3'). Those two oligonucleotides were designed to amplify a 540 bp DNA fragment (Keddie et al., 1986). The nucleotide sequences were used for the analysis of sequence similarity through Blast (https://blast.ncbi. nlm.nih. gov/Blast.cgi). The percentage differences of the resultant partial 16S rRNA gene sequences among different species in the same group/genus of species were described previously (Zwielehner et al., 2008). Thirty cycles at 95°C for 0.5 min, 65°C for 2 min and 72°C for 2 min, all steps at maximal ramp rate (approximately 3°C s^{-1} in heating mode and 1.5°C s^{-1} in cooling mode). Analysis of PCR products was performed using electrophoresis in 2% gel, containing ethidium bromide. A marker containing DNA fragments of known size was used to know the expected product size. Then gel was visualized under ultraviolet (UV) light.

RESULTS AND DISUSSION

Isolation and identification of *Arthrobacter*

Tissues were cultured in nutrient agar in septic jar and incubated for seven months at 25°C (re-cultures were included). During the incubation period it was noticed that the color of the medium turned from greenish to yellowish and then all plantlets died.

Twenty samples from the surface of infected media were cultured and incubated overnight. The results gave one isolate, it was positive to Gram-stain, aerobic, none motile, rod-shaped, spore forming. The bacterial isolated from agar was found to exhibit many characteristics of the genus *Arthrobacter*.

Biochemical tests were done to complete the diagnosis of the *Arthrobacter* isolate, the results are shown in Table 1.

Table 1. Biochemical test for the *Arthrobacter* isolate.

Biochemical tests	Results
Catalase	+ve
Gelatinase	+ve
Citrate utilization	+ve
Motility	+ve
Spore formation	+ve
Starch hydrolysis	+ve
Oxidase	Variable
Indole	-ve
Urase	-ve
Pigment	-ve
Rod	+ve

Antibiotic sensitivity test

The pattern of antibiotic sensitivity to *Arthrobacter* species were determined using the disc diffusion method according to the guidelines recommended by the National Committee for Clinical Laboratory Standards as shown in Table 2.

Table 2. Antibiotic sensitivity test of the *Arthrobacter* isolate.

Antibiotic (µg)	Susceptibility results
Amoxacillin 20 + clavulanic acid 10 mcg (AMC30)	Sensitive (++)
Amikacin (AK30)	Sensitive (+++)
Trimethoprim + sulfamethoxazole (SXT25)	Sensitive (++++)
Ciprofloxacin (CIP5)	Sensitive(++++)
Clarithromycin (CLR 15)	Resistant
Ceftriaxone (CRO30)	Resistant
Gentamycin (CN10)	Resistant

The results shown in Table 2 exhibit the antimicrobial susceptibility patterns of the isolate studied. The markedly better sensitive were Ciprofloxacin Trimethoprim + sulfamethoxazole then Amikacin and Amoxacillin 20 + Clavulanic acid 10 µg. While the bacterial isolates were resistant to Clarithromycin, Ceftriaxone and Gentamycin.

Amplification of 16S rRNA gene and DNA sequences

Based on 16S rRNA gene sequence similarity for this isolate was related to species of the genus *Arthrobacter arilaitensis* strain L11 (100% similarity), *Glutamicibacter arilaitensis* ebst40 (100%), *Arthrobacter nicotianae* strain (100%), and *Glutamicibacter nicotianae* (100%) as shown in Table 3) according to the description sequences producing significant alignments from NCBI Blast: 9-16srRNA.

The genus *Arthrobacter* was defined by Conn and Dimmick (1947) and belongs to the class *Actinobacteria*. The bacterial genera that show up frequently in culture-independent surveys is *Arthrobacter* (high %GC Gram-positive, family *Micrococcaceae*, order *Actinomycetales*, phylum *Actinobacteria*) (Koch et al., 1995). Members of this genus *Arthrobacter* are Gram-positive, catalase-positive, aerobic and asporogenous bacteria that display a coryneform morphology (Keddie et al., 1986). This genus is phenotypically heterogeneous and over 35 species are currently recognized (Stackebrandt and Schumann, 2000).

Table 3. 16s rRNA gene sequences producing significant alignments (NCBI: 9_16sr RNA-R).

Description	Max	Total	Query	E	Ident	Accession
Arthrobacter arilaitensis strain L11 16S rRNAgene partial sequence	793	793	100%	0.0	100%	KT834847.1
Arthrobacter sp.RMR 28 16S rRNA gene partial sequences	793	793	100%	0.0	100%	KT387998.1
Arthrobacter sp.ARUP UnID 131 16S rRNA gene partial sequences	793	793	100%	0.0	100%	JQ259327.1
Arthrobacter sp. VTT E-073079 16S rRNA gene partial sequences	793	793	100%	0.0	100%	EU438937.1
Arthrobacter sp.EP_S_54 16S rRNA gene partial sequences	793	793	100%	0.0	100%	KJ642536.1
Arthrobacter sp.BF-2-2 16S rRNA gene partial sequences	793	793	100%	0.0	100%	EU668003.1
Arthrobacter nicotianae strain 0.184 B 16S rRNA gene partial sequences	787	787	100%	0.0	99%	KF254746.1

The phenotypic identification of *Arthrobacter* spp. is made particularly difficult by the fact that the description of almost every defined *Arthrobacter* species is based on a single strain (Keddie et al., 1986), therefore, it might be necessary at present to supplement the identification of *Arthrobacter* spp. by peptidoglycan and/or molecular genetics methods, although the knowledge of these methods is reserved for the reference laboratories. It is emphasized that members of the genus *Brevibacterium* may be readily confused with *Arthrobacter* species. Strains of both genera show a marked rod-coccus cycle when grown on suitable medium (Jones and Keddie, 1986). However, Gram stains *Arthrobacter* strains may partially present as jointed rods which are not seen in true *Brevibacterium* strains. In our experience jointed rods are rather rarely seen in clinical *Arthrobacter* isolates cultured on SBA. *Arthrobacter* strains may be motile, whereas *Brevibacterium* strains are invariably non-motile. Many *Brevibacterium* strains display a typical cheese-like odour which is not noted for *Arthrobacter* strains (Funke and Carlotti, 1994).

Arthrobacter arilaitensis is one of the major microorganisms found in plant tissue cultures, soil and at the surface of cheeses, remarkably in smear-ripened cheeses, where it is assumed to be responsible for yellow pigmentation of the cheese's rind because of its characteristic overall color and its involvement at the different stages of cheese ripening (Feurer et al., 2004; Larpin-Laborde et al., 2011).

The *Arthrobacter* strains that were isolated from agar of plant tissue culture were spores originally recovered from soil ground and when there found a suitable environment. It is evident from the phenotypic and molecular genetic data that this strain is representative to the three new *Arthrobacter* species. Analysis of the 16S rRNA gene of this isolate sequence (1482 bp) indicated that it belongs to the genus *Arthrobacter* according to the EzTaxon-e server. The most closely related strains to the strain *Glutamicibacter arilaitensis* ebst40 (100% similarity) were *Arthrobacter arilaitensis* strain L11 (100% similarity to Jensen (1960)), *Arthrobacter nicotianae* strain 184B (99% similarity to Kotoučková et al. (2004)). The phylogenetic analysis demonstrated close relationships between this isolate and the members of rRNA cluster 2 (including *Arthrobacter arilaitensis*, *Arthrobacter nicotianae*, and *Glutamicibacter arilaitensis*) (Komagata and Suzuki, 1988).

Therefore, it might be necessary at present to supplement the identification of *Arthrobacter* spp. by peptidoglycan and/or molecular genetics methods, although we acknowledge that these methods are reserved for the reference laboratories.

It is concluded that the *Arthrobacter* strains isolated from date palm tissue culture were spores originally recovered from soil ground and thrived on the surface of the medium.

ACKNOWLEDGEMENTS

Part of this work was supported by Pollution Food Research Center, Ministry of Science and Technology, Baghdad. We thank Dr. Saad Alrawi and Farked Alrawi for his help in molecular detection.

Literature cited

Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol *45* (*4*), 493–496 https://doi.org/10.1093/ajcp/45.4_ts.493. PubMed

Benson, H.J. (2001). Microbiological Applications, 8th edn (USA: The McGraw-Hill Company), p.264–270.

Brenner, D.J., McWhorter, A.C., Knutson, J.K., and Steigerwalt, A.G. (1982). *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J Clin Microbiol *15* (*6*), 1133–1140 https://doi.org/10.1128/jcm.15.6.1133-1140.1982. PubMed

Camargo, F.A.O., Bento, F.M., Okeke, B.C., and Frankenberger, W.T. (2004). Hexavalent chromium reduction by an actinomycete, *arthrobacter crystallopoietes* ES 32. Biol Trace Elem Res *97* (*2*), 183–194 https://doi.org/10.1385/BTER:97:2:183. PubMed

Conn, H.J., and Dimmick, I. (1947). Soil bacteria similar in morphology to mycobacterium and corynebacterium. J Bacteriol *54* (*3*), 291–303 https://doi.org/10.1128/jb.54.3.291-303.1947. PubMed

Feurer, C., Vallaeys, T., Corrieu, G., and Irlinger, F. (2004). Does smearing inoculum reflect the bacterial composition of the smear at the end of the ripening of a French soft, red-smear cheese? J Dairy Sci *87* (*10*), 3189–3197 https://doi.org/10.3168/jds.S0022-0302(04)73454-2. PubMed

Funke, G., and Carlotti, A. (1994). Differentiation of *Brevibacterium* spp. encountered in clinical specimens. J Clin Microbiol *32* (*7*), 1729–1732 https://doi.org/10.1128/jcm.32.7.1729-1732.1994. PubMed

Ganzert, L., Bajerski, F., Mangelsdorf, K., Lipski, A., and Wagner, D. (2011). *Arthrobacter livingstonensis* sp. nov. and *Arthrobacter cryotolerans* sp. nov., salt-tolerant and psychrotolerant species from Antarctic soil. Int J Syst Evol Microbiol *61* (*4*), 979–984 https://doi.org/10.1099/ijs.0.021022-0. PubMed

Hua, N.P., Kobayashi, F., Iwasaka, Y., Shi, G.Y., and Naganuma, T. (2007). Detailed identification of desert-originated bacteria carried by Asian dust storms to Japan. Aerobiologia *23* (*4*), 291–298 https://doi.org/10.1007/s10453-007-9076-9.

Jensen, V. (1960). *Arthrobacter ramosus* spec. nov. A new *Arthrobacter* species isolated from forest soils. Arsskr. K. Vet. Landbohojsk. *196*, 123–132.

Jones, D., and Keddie, R.M. (1986). Bergey's Manual of Systematic Bacteriology, Vol. 2 (Baltimore: The Williams & Wilkins Co.).

Jones, D., and Keddie, R.M. (2006). The genus *Arthrobacter*. In The Prokaryotes, M. Dworkin, S. Falkow, E. Rosenberg, K.H. Schleifer, and E. Stackebrandt, eds. (New York, NY: Springer), https://doi.org/10.1007/0-387-30743-5_36.

Keddie, R.M., Collins, M.D., and Jones, D. (1986). Genus *Arthrobacter* Conn and Dimmick 1947, 300AL. In Bergey's Manual of Systematic Bacteriology (Baltimore: The Williams & Wilkins Co.), p.1288–1301.

Koch, C., Schumann, P., and Stackebrandt, E. (1995). Reclassification of *Micrococcus agilis* (Ali-Cohen 1889) to the genus *Arthrobacter* as *Arthrobacter agilis* comb. nov. and emendation of the genus *Arthrobacter.* Int J Syst Bacteriol *45* (*4*), 837–839 https://doi.org/10.1099/00207713-45-4-837. PubMed

Komagata, K., and Suzuki, K. (1988). Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol. *19*, 161–207 https://doi.org/10.1016/S0580-9517(08)70410-0.

Kotoučková, L., Schumann, P., Durnová, E., Spröer, C., Sedláček, I., Neča, J., Zdráhal, Z., and Němec, M. (2004). *Arthrobacter nitroguajacolicus* sp. nov., a novel 4-nitroguaiacol-degrading *actinobacterium.* Int J Syst Evol Microbiol *54* (*3*), 773–777 https://doi.org/10.1099/ijs.0.02923-0. PubMed

Larpin-Laborde, S., Imran, M., Bonaïti, C., Bora, N., Gelsomino, R., Goerges, S., Irlinger, F., Goodfellow, M., Ward, A.C., Vancanneyt, M., et al. (2011). Surface microbial consortia from Livarot, a French smear-ripened cheese. Can J Microbiol *57* (*8*), 651–660 https://doi.org/10.1139/w11-050. PubMed

Mongodin, E.F., Shapir, N., Daugherty, S.C., DeBoy, R.T., Emerson, J.B., Shvartzbeyn, A., Radune, D., Vamathevan, J., Riggs, F., Grinberg, V., et al. (2006). Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. PLoS Genet *2* (*12*), e214 https://doi.org/10.1371/journal.pgen.0020214. PubMed

Monier, J.M., and Lindow, S.E. (2004). Frequency, size, and localization of bacterial aggregates on bean leaf surfaces. Appl Environ Microbiol *70* (*1*), 346–355 https://doi.org/10.1128/AEM.70.1.346-355.2004. PubMed

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. *15* (*3*), 473–497 https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Stackebrandt, E., and Schumann, P. (2000). Introduction to the taxonomy of actinobacteria. In The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community, 3rd edn (New York: Springer).

TC1. (2006). www.plosgenetics.org (accessed December 2006).

Zhang, J., Ma, Y., and Yu, H. (2012). *Arthrobacter cupressi* sp. nov., an actinomycete isolated from the rhizosphere soil of *Cupressus sempervirens.* Int J Syst Evol Microbiol *62* (*Pt 11*), 2731–2736 https://doi.org/10.1099/ijs.0.036889-0. PubMed

Zwielehner, J., Handschur, M., Michaelsen, A., Irez, S., Demel, M., Denner, E.B.M., and Haslberger, A.G. (2008). DGGE and real-time PCR analysis of lactic acid bacteria in bacterial communities of the phyllosphere of lettuce. Mol Nutr Food Res *52* (*5*), 614–623 https://doi.org/10.1002/mnfr.200700158. PubMed

Fingerprinting of Omani date palm cultivars

M. Al-Hinai[1], A. Al-Lawati[1], A. Al-Mamari[1], A. Al-Jabri[1], I. Al-Harrasi[1], A. Al-Amri[1], Y. Al-Raisi[1,a] and M. Ben Salah[2]

[1]Directorate General of Agriculture and Livestock Research, Ministry of Agriculture and Fisheries, PO Box 50 Seeb121, Sultanate of Oman; [2]International Center for Agricultural Research in the Dry Areas (ICARDA), Oman Office, PO Box 111 Barka-328, Sultanate of Oman.

Abstract

The numbers of known date palm cultivars that are distributed all over the world are approximately 5,000, out of which about 250 are found only in Oman. It is a dioecious, perennial, monocot plant, and its heterozygous form makes its progeny strongly heterogeneous. Most of these cultivars were described using morphological markers such as fruit and vegetative traits, but these are greatly affected by the environment and are also complex. In general, the identification and evaluation of genetic diversity between the cultivars on the basis of morphological markers is difficult. Recently DNA markers have been used to provide the information on the relatedness of date palm cultivars that are difficult to distinguish morphologically. Micro-satellite simple sequence repeat (SSR) have been used widely in date palm genetic diversity among GCC countries. In this study, the genetic diversity of 12 of Omani date palm cultivars was studied by using 10 micro-satellite markers which are the same markers used in GCC countries' date palm cultivars. Mature leaves samples of all cultivars, five replicates per cultivar, were collected from five governorates of Oman from north and south Al-Batinah (Sohar and Barka), Al-Dhahirah (Ibri), Al-Dakhilia (Wadi Quriate, Nizwa), Al-Sharqiya (Samad Al-Shan, Al-Kamel and Al-Alwafi) and Al-Burymi (Mahadah, Al-Buraymi). A total of 113 alleles were scored with average of 11.3 alleles per locus. It ranged from 5 alleles/locus for SSR (PDCAT 17) to 17 alleles/locus SSR (mPdCIR 10). The polymorphic information content (PIC) average was 0.668. There was genetic diversity within and among the selected cultivars and it were assessed by using micro-satellite markers.

Keywords: molecular marker, SSR, date palm

INTRODUCTION

Micro-satellite or simple sequence repeat (SSR) molecular markers have been proven to be very powerful in plant diversity analysis because they are locus specific, co-dominant, highly polymorphic and highly reproducible. Genetic variation in the date palm germplasm has been traditionally characterized using morphological descriptors. However, such morphological markers are often unreliable and ambiguous because of the influence of environmental factors and confounding effects of developmental stage of the plant (Barrow, 1998). The aim of this investigation was to study the genetic variation (polymorphisms) among different Omani date palm cultivars using SSR markers. Date palm (*Phoenix dactylifera* L.) is a dioecious perennial monocotyledon plant with long generation times (a period of 4 to 5 years is necessary to reach the first flowering) that belongs to the *Arecaceae* family (Elhoumaizi et al., 2002). It is important to study the genetic diversity of the Omani date palm cultivars, because it helps to find the identity of DNA that will help in documenting the Omani cultivars in order to preserve them. Also, to determine the strains within species, make sure and check conventional seedlings before being placed in the tissue propagation, definition promising new cultivars for farmers, to make sure the genetic stability of the output of agriculture and textile version genetic compatibility with the original certification and to ensure product quality.

[a]E-mail: yousufm68@yahoo.com

The most common characteristics that are used to identify different cultivars of date palm are the morphology of leaves, spines, and fruit, which are mainly based on the characterization of introduced date palm cultivars in California (Nixon, 1950). Over the years, many date palm cultivars have been transplanted to areas other than the area of their origin, and they may have been given different names. As a result, a cultivar may have different names in different areas, or two genetically different cultivars may have the same name. This may reduce the genetic diversity of the cultivars, making them vulnerable to biotic and abiotic stresses. In general, the identification and evaluation of genetic diversity between cultivars on the basis of morphological markers is difficult. The identification of trees is usually not possible until the onset of fruiting, which takes 3 to 5 years. Further, characterizing cultivars requires a large set of phenotypic data that are difficult to access statistically and are variable because of environmental effects (Sedra et al., 1993, 1996, 1998). Biochemical markers (isozymes and proteins) are effective in varietal identification (Bennaceur et al., 1991; Bendiab et al., 1992). However, they provide limited information and are an indirect approach for detecting genomic variation (Elmeer and Mattat, 2015).

In this study, we aimed to investigate the genetic diversity of date palms in Oman to improve production and reveal the genetic relationships among 10 date palm cultivars using 12 nuclear micro-satellite markers. These cultivars have names that were given by farmers after continuous selection.

MATERIAL AND METHODS

Collection of material

Date palm material was collected as 5 replicates of 12 cultivars from the different regions in Oman (North and South Al-Batinah, Al-Dhahirah, Al-Dakhliah, Al-Sharqiah, Al-Buraimi governorates) as shown in Table 1 and Figure 1. These cultivars represent the diversity of date palm genotypes in the Omani date palm plantations. Young leaves from mature, randomly sampled trees, were collected and stored at -80°C, until DNA extraction.

Table 1. Samples used in fingerprint study.

No.	Sample name	Code	Location	No. replicate
1	Selani	SL	North and South Al-Batinah, Al-Dhahirah, Al-Dakhliah, Al-Sharqiah, Al-Buraimi	5 each
2	Hasas	HSS		
3	Merzaban	MR		
4	Shahel	SHL		
5	Menaz	MNZ		
6	Manhi	MNH		
7	Lolo	LO		
8	Hilali Makran	HM		
9	Hilali Al-Hasa	HH		
10	Hilali Asfer	HA		
11	Nasho Al-Khashba	NK		
12	Kash Qantrah	KK		

Molecular analysis

NA was extracted by using (Maxi: kit Qiagen Cat # 68163 DNeasy plant). DNA concentration was measured using 1% agarose gel and then detected using UV light and the device Nanodrope was used to ensure the purity of DNA by absorption measurement at a wavelength between 260 and 280 nm to enter it in the next stage (Figure 2).The micro-satellite amplification reaction was performed by using Applied Bio-systems (2720 thermo cycler, Singapore) with 10 primer combinations. Table 2 shows the micro-satellite combination and its allelic ranges (Peakall and Smouse, 2012). The PCR program had initial denaturation at 95°C for 5 min, then 35 cycles of 95°C for 30 min, 52/55°C for 1 min, and 72°C for 1 min and final elongation step at 72°C for 7 min. Amplification products were separated using 2% agarose gel electrophoresis as shown in Figure 3. The micro-satellite alleles were detected using Beckman coulter CEQTM 8000 automated DNA sequencer machine. Control sample in replication was used in this experiment along with the samples to be analyzed, to ensure the repeatability and accuracy of results.

Table 2. List of microsatellite primers designed for date palm by Billotte el al. (2004) and Akkak et al. (2009), marker name, annealing temperature Tm (0), motif repeat, observed allelic size range (bp) and status of amplification.

No.	Locus name	Sequences (5 '-3')	Annealing T_m (°C)	Motif repeat	Expected allelic range (bp)	Observed allelic range (bp)
1	PDCAT10	F: CACTGCTCCTGTTGCCCTGT R: TGTAGAAGGGCAGAGGACGG	55	$(TC)_{16}$	107-127	114-128
2	PDCAT14	F: TGCTGCAAATCTAGGTCACGA R: GTTTACCCCTCGGCCAAATGTAA	55	$(TC)_{19}(TC)_{16}$	101-155	135-168
3	PDCAT17	F: CAGCGGAGGGTGGGCCTC R: GTTTCTCCATCTCCCTTTTTCTGCTACTC	55	$(GA)_{21}$	116-145	143-165
4	PDCAT20	F: TTTCAGACACATCAAGTAACGATGA R: GTTTACGTCCACCCCAAGTTACGA	55	$(GA)_{29}$	294-353	343-361
5	PDCAT21	F: GTGTTTGAAGATTGATTTTGTGTTATGAG R: GTTTCGAACTATGCACAATAGTATATTG	55	$(GA)_5T(GA)_2TA(GA)_2$ $GC(GA)_5(GT)_7$	144-150	143-163
6	mPdCIR 10	F: ACC CCG GAC GTG AGG TG R: CGT CGA TCT CCT CCT TTG TCT C	52	$(GA)_{22}$	118-161	130-152
7	mPdCIR 15	F: AGC TGG CTC CTC CCT TCT TA R: GCT CGG TTG GAC TTG TTC T	52	$(GA)_{15}$	120-156	140-157
8	mPdCIR 16	F: AGC GGG AAA TGA AAA GGT AT R: ATG AAA ACG TGC CAA ATG TC	52	$(GA)_{14}$	130-138	143-157
9	mPdCIR 85	F: GAG AGA GGG TGG TGT TAT T R: TTC ATC CAG AAC CAC AGT A	52	$(GA)_{29}$	152-183	174-200
10	mPdCIR 93	F: CCA TTT ATC ATT CCC TCT CTT G R: CTT GGT AGC TGC GTT TCT TG	52	$(GA)_{16}$	153-184	160-181

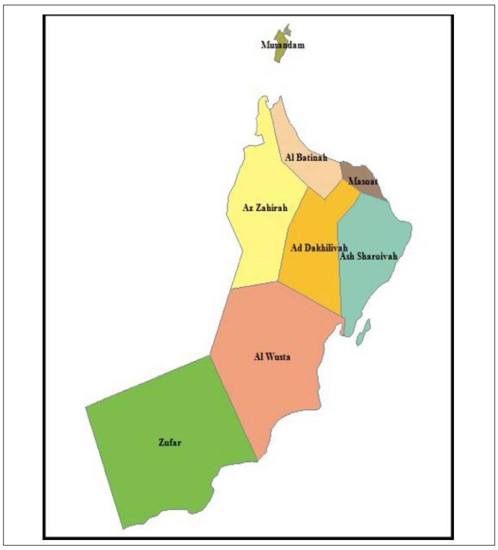


Figure 1. Locations of date palms collected.

Figure 2. Measuring the purity of the DNA using a device (Nanodrop).

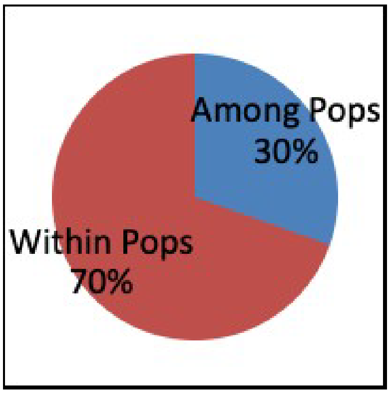


Figure 3. Analysis of molecular variance between Oman cultivars.

RESULTS AND DISCUSSION

The targeted fragments and allele scoring were performed by fragment analysis. For each marker, the average number of alleles per locus, the expected heterozygosity (He) and the observed heterozygosity (Ho) were calculated by Gene Alex 6.3 software .The genetic similarity and the analysis of molecular variance (AMOVA) and principal coordinates analysis (PCoA) were also calculated using Gene Alex 6.3 software. DARwin 6.0 software was used to make dendrogram which showed the distribution of different individuals.

A total of 113 alleles were scored with average of 11.3 alleles per locus. It ranged from 5 alleles/locus for SSR (mPdCIR 57) to 20 for locus SSR (mPdCIR 10). The polymorphic information content (PIC) average was 0.6650 and it ranged between 0.4818 (PDCAT21) and 0.9125 (mPdCIR 10) (Table 3). The average of expected heterozygosity (He) ranged between 0.227 (mPdCIR 85) and 0.718 (mPdCIR 10) and the average of observed heterozygosity (Ho) ranged between 0.150 (mPdCIR 85) and 0.700 (mPdCIR 16) (Table 3). For most of the markers, the observed heterozygosity value was higher than the expected one. The percentage of polymorphic loci per cultivar varied between 60 and 100% with an average of 85% (Table 4). Molecular variance analysis showed that 70% of the variation was due to differences within populations, while 30% was due to differences between populations in Omani cultivars (Figure 4).

The dendrogram shown in Figure 5 illustrates the divergence between the studied Omani date palm cultivars and suggests their tree brunching. The dendrogram is divided into four main groups, Group A has the following cultivars ('Lolo', 'Menaz', 'Kash Qantrah', 'Shahel', 'Hilali Makran'), while Group B has the following cultivars ('Selani', 'Hasas', 'Manhi') and group C has the following cultivars ('Nasho Al-khashba', 'Hilali Asfer', 'Merzaban', 'Hilali A-Hasa') and Group D has a combination with different following cultivars: 'Hilali Alhasa', 'Shahel', ‘Merzaban’ (Figure 6). The principal coordinates analysis (PCoA) of the 12 Omani cultivars showed that the majority of cultivars were grouped in cluster and also dispersed among different sub-clusters. DNA SSR markers are a powerful tool to provide information on the relatedness of culivars that are difficult to distinguish morphologically, thus helping in the management of plant accessions and in breeding programs. In this study, SSR markers have been used to assess the molecular characterization and the phylogenic relationships of Omani date palm cultivars. Present results provide evidence of a genetic diversity.

Table 3. The PIC values and alleles/loci of 17 microsatellite primer combination and heterozygoty of Omani cultivars calculated with GenAlex 6.3 software. He: average of expected heterozygosity; Ho: average of observed heterozygosity.

Locus name	Alleles/loci	PIC value	He	Ho
PDCAT10	10	0.639	0.627	0.683
PDCAT14	10	0.705	0.262	0.167
PDCAT17	5	0.573	0.375	0.483
PDCAT20	9	0.555	0.427	0.483
PDCAT21	7	0.548	0.420	0.517
mPdCIR 10	17	0.916	0.718	0.450
mPdCIR 15	15	0.879	0.672	0.683
mPdCIR 16	13	0.779	0.610	0.700
mPdCIR 85	11	0.570	0.227	0.150
mPdCIR 93	16	0.798	0.682	0.650
Total	113	-		
Average	11.3	0.668		

Table 4. Percentage of polymorphic loci of the studied Omani cultivars.

Population	Percentage (%)
Selani	80
Hasas	70
Merzaban	90
Shahel	100
Menaz	90
Manhi	100
Lolo	60
Hilali Makran	90
Hilali Al-Hasa	80
Hilali Asfer	90
Nasho Al-khashba	80
Kash Qantrah	90
Mean	85

CONCLUSIONS

This study showed the distribution of Omani date palm cultivars from different regions in Oman and analysis based on SSR markers. In the future this technique will help our study and provide a useful tool for research on genetic diversity, gene mapping, and marker-assisted selection in date palm. Therefore, while allowing studies on genetic variation, SSR markers also provide information on gene function related to possible phenotypic differences between the date palm cultivars.

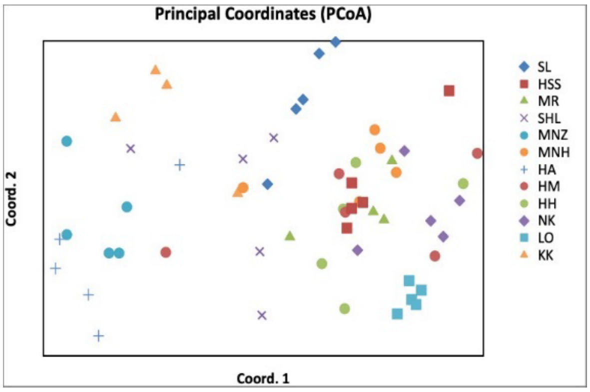


Figure 4. Principal coordinates analysis of the studied Omani date palm cultivars.

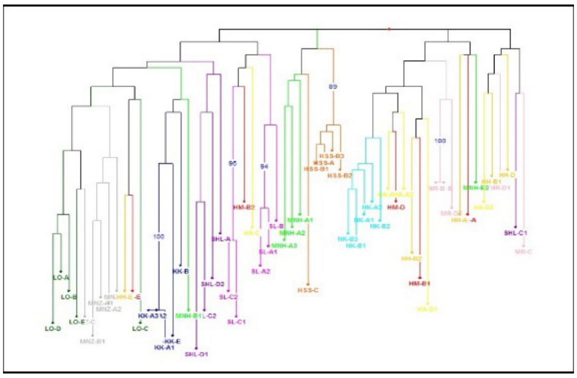


Figure 5. Dendrogram of similarity coefficients based on UPGMA cluster analysis of 12 genotypes using 10 microsatellite (SSR) primer pairs.

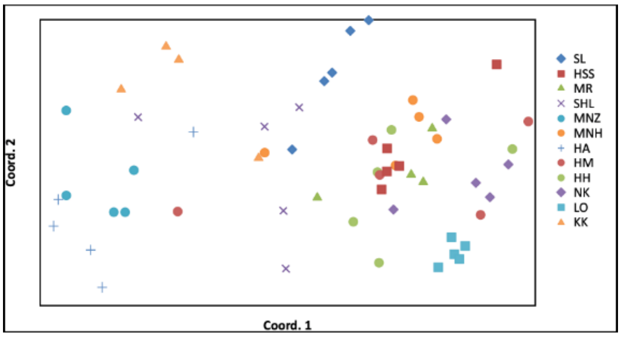


Figure 6. Principal coordinates analysis (PCoA) of 12 Omani date palm cultivars.

Literature cited

Akkak, A., Scariot, V., Torello Marinoni, D., Boccacci, P., Beltramo, C., and Botta, R. (2009). Development and evaluation of microsatellite markers in *Phoenix dactylifera* L. and their transferability to other *Phoenix* species. Biol. Plant. *53* (*1*), 164–166 https://doi.org/10.1007/s10535-009-0026-y.

Barrow, S. (1998). A monograph of *Phoenix* L. (*Palmae: coryphoideae*). Kew Bull. *53* (*3*), 513–575 https://doi.org/10.2307/4110478.

Bendiab, K., Baaziz, M., Brakez, Z., and Sedra, M.H. (1992). Correlation of isoenzyme polymorphism and Bayoud disease resistance in date palm cultivars and progeny. Euphytica *65* (*1*), 23–32 https://doi.org/10.1007/BF00022196.

Bennaceur, M., Lanaud, C., Chevallier, M.H., and Bounaga, N. (1991). Genetic diversity of date palm (*Phoenix dactylifera* L.) from Algeria revealed by enzyme markers. Plant Breed. *107* (*1*), 56–69 https://doi.org/10.1111/

j.1439-0523.1991.tb00528.x.

Billotte, N., Marseillac, N., Brottier, P., Noyer, J.-L., Jacquemoud-Collet, J.-P., Moreau, C., Couvreur, T., Chevallier, M.-H., Pintaud, J.-C., and Risterucci, A.-M. (2004). Nuclear microsatellite markers for the date palm (*Phoenix dactylifera* L.): characterization and utility across the genus *Phoenix* and in other palm genera. Mol. Ecol. Notes *4* (*2*), 256–258 https://doi.org/10.1111/j.1471-8286.2004.00634.x.

Elhoumaizi, M.A., Saaidi, M., Oihabi, A., and Cilas, C. (2002). Phenotypic diversity of date-palm cultivars (*Phoenix dactylifera* L.) from Morocco. Genet. Resour. Crop Evol. *49* (*5*), 483–490 https://doi.org/10.1023/A:1020968513494.

Elmeer, K., and Mattat, I. (2015). Genetic diversity of Qatari date palm using SSR markers. Genet Mol Res *14* (*1*), 1624–1635 https://doi.org/10.4238/2015.March.6.9. PubMed

Nixon, R.W. (1950). Imported Cultivars of Dates in the United States. Circular No. 834 (Washington, D.C.: United States Department of Agriculture).

Peakall, R., and Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics *28* (*19*), 2537–2539 https://doi.org/10.1093/bioinformatics/bts460. PubMed

Sedra, M.H., Filali, H., and Frira, D. (1993). Observations sur quelques caracteristiques phenotypiques et agronomiques du fruit des varieties et clones du palmier dattier selectionnes. Al Awamia *82*, 105–120.

Sedra, M.H., Fila, H., Benzine, A., and Allaoui, M. (1996). La palmeraie dattière marocaine: évaluation du patrimoine phenicicole. Fruits *1*, 247–259.

Sedra, M.H., Lashermes, P., Trouslot, P., Combes, M.C., and Hamon, S. (1998). Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. Euphytica *103* (*1*), 75–82 https://doi.org/10.1023/A:1018377827903.

Endophytic bacteria are an immense constraint in date palm micropropagation but their abundance is a good plant health indicator

A. Nasri[1], O. Chkir[1], E. Baklouti[1], A. Chabaane[2], M.A. Triki[3], A. Mliki[2], S. Symanczik[4], H.M. Shumacher[5], N. Drira[1] and L. Fki[1,a]

[1]Laboratory of Plant Biotechnology, Faculty of Sciences of Sfax, University of Sfax, Road Sokra BP 1171, 3000, Sfax, Tunisia; [2]Laboratory of Plant Molecular Physiology, Biotechnology Centre of Borj-Cédria, BP 901, Hammam-Lif, Tunisia; [3]Olive Tree institute, Route Elmatar Sfax, Tunisia; [4]Department of Soil Sciences, Forschungsinstitut für Biologischen Landbau (FiBL) Research Institute of Organic Agriculture, Ackerstrasse 113, Postfach 219, CH-5070 Frick, Switzerland; [5]Leibniz Institute DSMZ German Collection of Micro-organisms and Cell Cultures GmbH, Inhoffenstr. 7b, 38124 Braunschweig, Germany.

Abstract

Two strains of endophytic bacteria hampering date palm micropropagation were isolated from two cultivars ('Deglet Nour' and 'Barhee'). Significant differences in bacterial population densities were observed in the cultures of the two genotypes and in cultures established from healthy and brittle leaf disease-affected plants. Indeed, the percentage of contaminated recipients in 3-year-old in vitro tissue cultures established from 'Deglet Nour' and 'Barhee' were 15 and 30%, respectively. Moreover, we found that percentage of contaminated recipients in 3-year-old in vitro tissue cultures established from healthy and BLD-affected 'Deglet Nour' palms were 15 and 2%, respectively. Embryogenic and organogenic cultures were seriously damaged by these fastidious bacteria. They slowly turned yellow and brown and then died within three months. On the other hand, the two bacteria were not toxic for vitro-plants as growth rates were similar in vitro-plants growing with and without bacteria.

Keywords: date palm, micro-propagation, endophytic bacteria, brittle leaf disease

INTRODUCTION

In the last three decades, in vitro technology has been widely applied for the large-scale propagation of plants since benefits arising from this technology are numerous. In vitro plants are generally true-to-type and pathogen-free. Moreover, in vitro highly regenerable tissues can be considered as being the most suitable plant material to create cryo banks (Fki et al., 2011b) and to carry out gene transfer experimentations (Fki et al., 2011a). However, several constraints may hamper the regeneration of in vitro plants such as the recalcitrance and/or the genetic instability of some species or cultivars; and in some cases, the difficulty to establish clean in vitro tissue cultures.

Concerning this latter constraint, the fungal contaminations are easily detected and contaminated cultures can so be discarded early (Leifert et al., 1991). In contrast, the presence of latent bacteria is a serious problem in plant tissue cultures (Leifert and Waites, 1992). While endophytes are generally beneficial to plants in situ (Leifert et al., 1994; Benhamou et al., 1996; Berg et al., 2005), they may affect culture growth under the modified conditions in vitro.

In previous studies, we showed that date palm can be propagated through somatic embryogenesis and organogenesis (Fki et al., 2003, 2011c; Drira and Benbadis, 1985). Few are the authors who had accorded importance to the constraint of endophytic bacterial contaminations in date palm tissue culture although it is well known that endophytic contaminants with somaclonal variations constitute the two major constraints hampering large-scale propagation program (Fki et al., 2011c).

[a]E-mail: lotfifki@yahoo.fr

Today a new lethal threat, brittle leaf disease (BLD), is spreading at a fast pace, as more than 36,000 trees are infected in Tunisia (Triki et al., 2003). The causal agent of this disease is not yet known; it seems to be this physiological disease may be because plants find difficulties to assimilate manganese (Saidi et al., 2012; Marqués et al., 2011). Tissue culture technology has never been used to uncover the secrets of this disease.

This study aimed: 1) to identify the most fastidious endophytic bacterial strains affecting date palm in vitro tissue culture, 2) to determine if populations of indigenous bacterial endophytes in tissue cultures are influenced by host genotype and disease such as the brittle leaf disease, 3) to determine their toxicity against plantlets and in vitro cultures which are in proliferation phase, and 4) to find the factors that must be taken into consideration to establish a relatively clean tissue culture.

MATERIAL AND METHODS

Plant material

Leaves, less than 2 cm in size, were excised from healthy and BLD affected date palm offshoots for the cultivar 'Deglet Nour'. To determine if populations of endophytic bacteria in date palm could be influenced by host genotype or not, in vitro tissue cultures were also established from leaves taken from 'Barhee' offshoots.

Tissue culture

The leaves were disinfected with 0.01% $HgCl_2$ for 1 h, washed three times in sterile distilled water, fragmented to 3-mm pieces and then cultured for 2 years on MS medium to which 50 g L^{-1} sucrose, and 8 g L^{-1} agar were added. Media were supplemented with 0.1 mg L^{-1} 2,4-D to induce callogenesis and 1 mg L^{-1} 2,4-D to induce embryogenesis. The cultures were maintained in the dark at 28°C during the first 2 years and then transferred on free-PGRs MS medium in a growth chamber where photo period was 18 h. The temperature of incubation was 28°C. Subcultures were performed once every 2 months. All media were adjusted to pH 5.8 prior to autoclaving. The antibiotic cefotaxime (Duchefa, Haarlem, The Netherlands) was dissolved in water, the pH adjusted to 5.7, and sterile filtered. Antibiotic was added to the medium after autoclaving and cooling.

Endophytic bacterial identification using API galleries

Our study was focused on bacteria hampering the large-scale micro-propagation of date palm; bacteria which can be detected after the second and third subcultures were not the subject of our study. Endophytic bacteria were isolated from 3-year-old in vitro tissue culture and then purified by successive subcultures on BSM-Sigma.

Estimation of endophytic bacteria abundance

The percentages of contaminated cultures were determined in 3-year-old in vitro tissue culture after a visual examination of 100 tubes. We considered that tissue in one vessel is a culture. Measurements were repeated 3 times using different cultures.

Bacterial indexing of tissue cultures

For bacterial indexing of tissue cultures, we used 50 explants and each explant was divided into three parts and then tested for endophytic bacteria using bacteria screening medium (BSM-Sigma). Explants were cultivated on BSM-Sigma which contains 8 g L^{-1} agar, 8 g L^{-1} casein hydrolysate, 35.8 mg L^{-1} magnesium sulfate anhydrous, 2 g L^{-1} potassium phosphate monobasic, 10 g L^{-1} sucrose and 4 g L^{-1} yeast extract. Cultures were incubated at 27°C in the dark.

Leaf anatomy

Transverse sections were carried out at the base of leaves using a razor blade. Sections were first treated with 2% sodium hypochlorite for 20 min and then washed three times in water. After that, they were treated with acetic acid for 5 min. Next, they were stained with carmino green and then washed in water. Finally, sections were examined under a stereo microscope.

Statistical analysis

Results are expressed as mean percentages±standard error. The statistical analysis was computed using SPSS 13 software. Data were analyzed by one-way ANOVA and Duncan's test. Values of $P<0.05$ were considered to be statistically significant. Experiments were replicated three times. Data expressed in percentage were transformed by arcsin transformation and then analyzed. Arcsin transformation ($y' = \arcsin y^{1/2}$, y = original percentage/100) was done to stabilize the variance of data.

RESULTS

Isolation, identification, abundance estimation of the most fastidious endophytic bacteria affecting date palm tissue culture and eventual relationship with the brittle leaf disease

Diverse endophytic contaminants were detected in date palm cv. Deglet Nour tissue cultures (Figure 1). Fungal contaminations and some bacterial strains were detected within 2-4 weeks since they were unable to maintain a latent state in in vitro conditions.

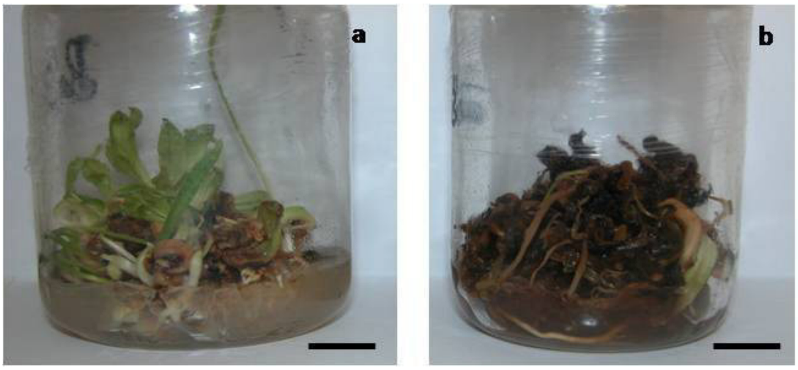


Figure 1. Evaluation of endophytic bacteria phytotoxicity (a) before endophytic bacteria proliferation and (b) after (2 months) endophytic bacteria proliferation. Scale bars: 10 mm.

On the other hand, two bacteria started their proliferation after 2-3 subcultures, especially when leaves are in advanced developmental stages (>1 cm). However, in case of juvenile leaves (<1 cm) we have to wait for more than two years (12 subcultures) to see this kind of bacteria. The two most fastidious endophytic bacteria affecting date palm tissue culture were orange-yellow and white of colors. Microbiological testing using API galleries showed that they are *Photobacterium damsela* and *Yersinia ruckeri*, respectively. Furthermore, we showed that more than 85% of contaminations were caused by white bacteria.

Several arguments were in favor of internal origin of these contaminants: 1) the observed phenomenon was limited to date palm tissue cultures as it had never shown on the cultures of other plants studied in the laboratory including potatoes and olives; 2) the existence of clean date palm cell lines that had never expressed, up to now, an endophytic contamination, yet maintained in vitro for many years; and 3) the bacterial colonies always appeared on the tissues and never outside of their contact.

To find out if populations of endophytic bacteria in date palm could be influenced by host genotype or not, we determined the percentage of contaminated cultures in 3-year-old in vitro tissue cultures of two cultivars. These percentages for 'Barhee' and 'Deglet Nour' were 30±5 and 15±3%, respectively, indicating that endophytic bacteria were more abundant in 'Barhee'. On the other hand, there was a similarity between latent bacteria strains in the two cultivars.

Photobacterium damsela and *Yersinia ruckeri* remained the most common bacteria that can be detected in in vitro tissue cultures established from explants taken from BLD affected plant.

However, these bacteria were more abundant in in vitro tissue cultures established from explants taken from healthy plant. Indeed, using 1-2 cm leaves, we found that more than 15±5% of cultures established from healthy explants were contaminated, although this percentage was only 2±1% for cultures established from BLD affected explants.

Phyto-toxicity of the two endophytic bacteria

Embryogenic culture and bud clusters proliferation were seriously affected by the presence of endophytic bacteria. They slowly turned yellow and brown (Figure 1). Although some part of the tissue continued to form new shoot buds and somatic embryos, within 2 weeks all the tissues died after a severe necrosis (Figure 1). It became impossible to continue cultures any longer.

On the other hand, the two kinds of bacteria were without any effect on rooted vitroplants. In vitro plants, cultivated on media containing or no these two endophytic bacteria, showed a similar growth rate in in vitro conditions (0.4±0.1 cm in length per month) and had the same leaf color and morphology.

Early detection of endophytic bacteria and problem remediation

Bacterial indexing based on the cultivation of tissues that may harbour microorganisms on bacterial growth media (BSM-sigma) did not systematically lead to the detection of endophytic bacteria which growth requires particular physico-chemical conditions (Figure 2). Transfer of tissue to liquid medium stimulated endophytic bacteria proliferation which became accessible to observation after two to three weeks. Indeed 20% of cultures that were apparently clean expressed their endophytic bacteria when they were transferred to agitated MS liquid medium.

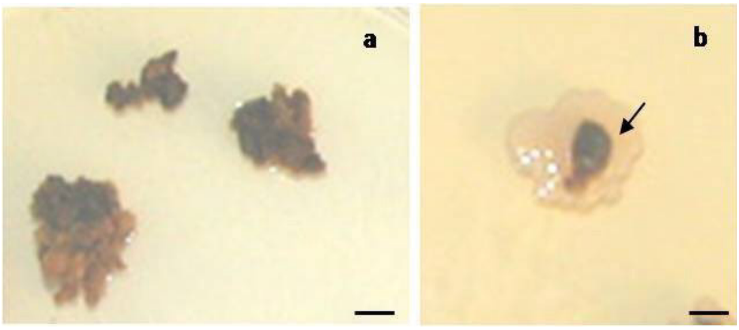


Figure 2. Bacterial indexing of date palm tissue cultures using BSM (a) day 1 and (b) day 15. Scale bars: 3 mm.

Cefotaxime was not toxic for date palm in vitro tissue cultures even if added at high concentration (500 mg L^{-1}). Control and tissue cultures cultivated on MS medium supplemented with 100, 250 and 500 mg L^{-1} had the same growth and were of the same appearance. For pure bacteria cultures cefotaxime was effective as it has bactericidal effect. On the other hand, this antibacterial compound could not be used to disinfect tissue culture since growth of bacteria resumed upon its removal. When apparently clean tissue was cultivated on MS medium enriched by 500 mg L^{-1} cefotaxime the percentage of contamination decreased enormously to be less then 2%.

Only juvenile leaves (0.5 cm) could be used to establish clean in vitro tissue culture. Immaturity of vascular tissue in these explants may explain the absence of endophytic contaminants (Figure 3).

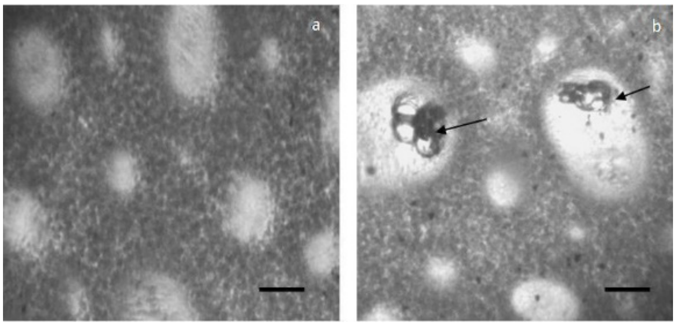


Figure 3. Portions of cross sections performed at the basal part of 7 mm leaf (a) and 25 mm leaf (b). Scale bars: 60 cm.

DISCUSSION

In this study we found that apparently clean date palm in vitro tissue cultures harbour latent bacteria. *Yersinia ruckeri* and *Photobacterium damsela* are the two most fastidious bacteria hampering large scale in vitro propagation. The first one was also purified by Irum et al. (2010) from endophyllosphere of *Convolvulus arvensis*. Concerning the second one, it was isolated from pomes in the eastern Anatolia region of Turkey (Kotan et al., 2006).

Factors that stimulate endophytic bacteria proliferation are not very known up to now. Nevertheless, it seems that stress situation was the key factor leading to endophytic bacteria expression. In a normal situation plant cells have the capacity to control endophytic bacteria. But stressed cells often loose this control and as consequence endophytic bacteria can start their high proliferation. Endophytic bacteria penetration mechanism is not well understood; this can explain the differential bacterial abundance between cultivars and species.

The endophytic bacteria detection is not easy to be accomplished as was already reported by several authors because of the difficulties occurring at several levels. This kind of bacteria requires very specific culture media (Leifert and Waites, 1992) and on the other hand, they require special activation and presence in large numbers to start their proliferation (Leifert and Waites, 1993). Moreover, the tissues of many plants release antimicrobial substances when transferred on bacterial indexing media (Leifert et al., 1991). It was also demonstrated that plant hormones may affect the growth of certain microorganisms by promoting their entry into a phase of latency (Lovrekovich and Farkas, 1963). Finally, residues of the disinfectant, used to sterilize primary explants, can block bacteria growth during the initiation phase (Leifert et al., 1994).

Moreover, our study which was started nine years ago showed that endophytic bacteria abundance in date palm tissues could reflect a correct plant health. Our findings, showing that endophytic bacteria are less abundant in BLD affected plant can help scientists working on the identification of the causal agent of BLD. We also showed that endophytic bacteria abundance in 'Barhee' was higher than in 'Deglet Nour'; this can explain the dissimilarity of plan vigor which is clearly higher for 'Barhee' when compared with 'Deglet Nour'. In this regard, several authors have shown the beneficial effect of some endophytic bacterial strains on the growth and the development of plant tissue culture (Leifert et al., 1994). Specific endophytic bacteria could be inoculated to the acclimatized in vitro plant to promote their growth rates. These bacteria are also widely implicated in plant protection against pathogens since they can induce ultra-structural changes in plant tissue and can neutralize the phytotoxicity of some aggressors (Leifert et al., 1994; Benhamou et al., 1996; Newman and Reynolds, 2004; Berg et al., 2005). In the near future, such bacteria could be used to prepare efficient bio-fertilizers.

In conifers, antibiotics have been used to disinfect some embryogenic cell lines (Ewald et al., 2000). However, many authors confirmed the inefficiency of antibiotics when added to tissue culture media. This may be because antibiotics cannot enter upon internal tissues (Reed and Tanprasert, 1995). We know, a long time ago that meristems, which are clusters of undifferentiated cells, do not harbour microorganisms, so they are used to generate pathogen-free plants. The establishment of clean in vitro tissue culture from juvenile explants can be explained by 1) the facile access of the disinfectant into internal tissues, and 2) the fact that in juvenile explants, conducting tissues have not yet reached the ultimate stage of differentiation. Similar results were obtained in banana by Van den Houwe and Swennen (2000) who had succeeded the establishment of healthy in vitro tissue culture from juvenile explants. If these bacteria are only present in the conducting tissue, the differentiation of in vitro tissue culture from epidermal and sub-epidermal tissues followed by a precocious separation of the neo-formations can facilitate the production of clean in vitro tissue culture. Defining physico-chemical conditions that mitigate bacterial growth, without affecting plant cell proliferation was efficient to resolve the problem of endophytic bacteria. High sucrose concentrations could be used to inhibit endophytic bacteria proliferation.

The observations in this study showed the complexity of the problem of endophytic bacteria in in vitro tissue culture since these fastidious microorganisms may impair multiplication, regeneration, in vitro conservation and safe exchange of cultures. It also suggests the need for taking into account such organisms while undertaking genetic transformation, physiological and other in vitro studies. Endophytic bacteria could affect molecular profiling and gene expression studies wherein DNA from the endophytes could get co-purified unaware of their presence in the tissue (Thomas et al., 2008). Leaky expression of some enzymes in cell and callus cultures, spurious GUS activity in transformation experiments and aberrant patterns during molecular profiling are some documented instances of interference by cryptic organisms.

Few are the tissue culturists who mentioned the proliferation of endophytic bacteria in their study because this problem started to be a serious constraint after only 2 to three years from primary explants cultivation.

CONCLUSIONS

In conclusion, this study showed the ubiquitous presence of endophytic bacteria in date palm in vitro tissue cultures. Such bacteria have negative impact on the applicability of the micro-propagation procedures and all other tissue culture related applications. However, we have to notify that abundance of endophytes is a good plant health indicator. Our findings showed that only juvenile explants could be used to establish relatively clean in vitro tissue cultures, since antibiotics such as cefotaxime have only a bacteriostatic effect. Immaturity of vascular tissue in these explants may explain the absence of endophytic contaminants.

ACKNOWLEDGEMENTS

This work was supported financially by the Ministry of Higher Education and Scientific Research in Tunisia, the International Atomic Energy Agency, the Arab League Educational, Cultural and Scientific Organization, the COST office, the Swiss National Science Foundation and the Technical Centre of Dates in Tunisia.

Literature cited

Benhamou, N., Kloepper, J.W., Quadt-Hallman, A., and Tuzun, S. (1996). Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol *112* (*3*), 919–929 https://doi.org/10.1104/pp.112.3.919. PubMed

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A., and Hallmann, J. (2005). Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol Ecol *51* (*2*), 215–229 https://doi.org/10.1016/j.femsec.2004.08.006. PubMed

Drira, N., and Benbadis, A. (1985). Multiplication végétative du palmier dattier (*Phoenix dactylifera* L.) par réversion, en culture in vitro, d'ébauches florales de pieds femelles. J. Plant Physiol. *119* (*3*), 227–235 https://doi.org/10.1016/S0176-1617(85)80182-6.

Ewald, D., Zaspel, I., Naujoks, G., and Behrendt, U. (2000). Endogenous bacteria in tissue cultures of conifers - appearance and action. Acta Hortic. *530*, 137–144 https://doi.org/10.17660/ActaHortic.2000.530.14.

Fki, L., Masmoudi, R., Drira, N., and Rival, A. (2003). An optimised protocol for plant regeneration from embryogenic suspension cultures of date palm, *Phoenix dactylifera* L., cv. Deglet Nour. Plant Cell Rep *21* (*6*), 517–524 https://doi.org/10.1007/s00299-002-0558-5. PubMed

Fki, L., Bouaziz, N., Kriaa, W., Benjemaa-Masmoudi, R., Gargouri-Bouzid, R., Rival, A., and Drira, N. (2011a). Multiple bud cultures of 'Barhee' date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. J Plant Physiol *168* (*14*), 1694–1700 https://doi.org/10.1016/j.jplph.2011.03.013. PubMed

Fki, L., Bouaziz, N., Sahnoun, N., Swennen, R., Drir, N., and Panis, B. (2011b). Palm cryobanking. Cryo Letters *32* (*6*), 451–462. PubMed

Fki, L., Masmoudi, R., Kriaa, W., Mahjoub, A., Sghaier, B., Mzid, R., Mliki, A., Rival, A., and Drira, N. (2011c). Date palm micro-propagation via somatic embryopgenesis. In Date Palm Biotechnology, S.M. Jain, J.M. Al-Khayri, and D.V. Johnson, eds. (The Netherlands: Springer), p.47–68.

Irum, M., Ibatsam, K., Mushtaq, S., and Amna, A. (2010). Diversity of epiphytic and endophytic micro-organisms in some dominant weeds. Pak. J. Weed Sci. Res. *16* (*3*), 287–297.

Kotan, R., Sahin, F., and Ala, A. (2006). Identification and pathogenicity of bacteria isolated from pome fruit trees in the Eastern Anatolia region of Turkey. J. Plant Dis. Prot. *113* (*1*), 8–13.

Leifert, C., and Waites, W.M. (1992). Bacterial growth in plant tissue culture. J. Appl. Bacteriol. *72* (*6*), 460–466 https://doi.org/10.1111/j.1365-2672.1992.tb01859.x.

Leifert, C., and Waites, W.M. (1993). Dealing with microbial contaminants in plant tissue and cell culture: hazard analysis and critical control points. In Physiology, Growth and Development of Plants in Culture, P.J. Lumsden, J.R. Nicholas, and B.J. Davies, eds. (The Netherlands: Springer), p.363–378.

Leifert, C., Ritchie, J.Y., and Waites, W.M. (1991). Contaminants of plant-tissue and cell cultures. World J Microbiol Biotechnol *7* (*4*), 452–469 https://doi.org/10.1007/BF00303371. PubMed

Leifert, C., Morris, E.M., and Waites, W.M. (1994). Ecology of microbial saprophytes and pathogens in tissue culture and field-grown plants: reasons for contamination problems in vitro. Crit. Rev. Plant Sci. *13* (*2*), 139–183 https://doi.org/10.1080/07352689409701912.

Lovrekovich, L., and Farkas, G.L. (1963). Kinitin as an antagonist of toxic effect of *Pseudomonas tabaci.* Nature *198* (*4881*), 710 https://doi.org/10.1038/198710a0.

Marqués, J., Duran-Vila, N., and Daròs, J.A. (2011). The Mn-binding proteins of the photosystem II oxygen-evolving complex are decreased in date palms affected by brittle leaf disease. Plant Physiol Biochem *49* (*4*), 388–394 https://doi.org/10.1016/j.plaphy.2011.02.008. PubMed

Newman, L.A., and Reynolds, C.M. (2004). Bacteria and phytoremediation: new uses for endophytic bacteria in plants. Trends Biotechnol. *15* (*3*), 215–224. PubMed

Reed, B.M., and Tanprasert, P. (1995). Detection and control of bacterial contaminants of plant tissue culture. A review of recent literature. Plant Tissue Cult. Biotechnol. *1* (*3*), 137–142.

Saidi, M.N., Jbir, R., Ghorbel, I., Namsi, A., Drira, N., and Gargouri-Bouzid, R. (2012). Brittle leaf disease induces an oxidative stress and decreases the expression of manganese-related genes in date palm (*Phoenix dactylifera* L.). Plant Physiol Biochem *50* (*1*), 1–7 https://doi.org/10.1016/j.plaphy.2011.09.016. PubMed

Thomas, P., Swarna, G.K., Patil, P., and Rawal, R.D. (2008). Ubiquitous presence of normally non-cultivable endophytic bacteria in field shoot-tips of banana and their gradual activation to quiescent cultivable form in tissue cultures. Plant Cell Tissue Organ Cult. *93* (*1*), 39–54 https://doi.org/10.1007/s11240-008-9340-x.

Triki, M.A., Zouba, A., Khouldia, O., Ben Mahmoud, O., Takrouni, M.I., Garnier, M., Bové, J.M., Montarone, M., Poupet, A., Flores, R., et al. (2003). "Maladie des feuilles cassantes" or brittle leaf disease of date palms in Tunisia: biotic or abiotic disease? J. Plant Pathol. *85* (*2*), 71–79.

Van den Houwe, I., and Swennen, R. (2000). Characterization and control of bacterial contaminants in in vitro cultures of banana (*Musa* spp.). Acta Hortic. *530*, 69–79 https://doi.org/10.17660/ActaHortic.2000.530.6.

Semiochemicals and date palm IPM: an overview

J.R. Faleiro[a]

225 Mariella, Arlem-Raia, Salcette, Goa, India.

Abstract

Chemical signals play an important role in the survival of insects, and is being increasingly exploited as an IPM tool. Semiochemicals are organic compounds used by insects to convey specific chemical messages that modify insect behavior or physiology and are species specific and harmless to the environment. These chemicals are essential for pest survival including location of habitat, food and mates. Semiochemicals which mediate interactions among individuals of the same species (intra-specific reactions) are called pheromones, while those that mediate interactions among individuals of different species (inter-specific interactions) are known as allelo chemicals. It has been possible to identify chemical signals/cues for thousands of insect species. Most identified insect pheromones are from the orders *Lepidoptera* and *Coleoptera*. Sex pheromones are used to successfully control lepidopteran insects while aggregation pheromones have been developed to control coleopteran pests. Effective attractants, pheromones, repellents and/or host volatiles are known only for some of the major date palm pests viz., lesser date moth *Batrachedraa mydraula*, greater date moth *Aphomia* (= *Arenipses*) *sabella*, Indian meal moth *Plodia interpunctela*, raisin moth *Cadra* (= *Ephestia*) *figulilella*, carob moth *Spectrobates* (= *Ectomyelois*) *ceratoniae*, red palm weevil *Rhynchophorus ferrugineus*, rhinoceros beetle *Orytes elegans*, dried fruit beetle *Carpophilus hemipterus*, etc. This paper gives an overview on the use of semiochemicals in date palm IPM with emphasis on red palm weevil.

Keywords: attractants, repellents, insect control

INTRODUCTION

Date palm, *Phoenix dactylifera* L. (*Arecaceae* or *Palmae*) is cultivated mostly in the arid regions of the world. The crop has played a significant role in the economy of these countries, provides nutritional security, besides helping to mitigate the adverse effects of desertification and climate change over centuries. It is estimated that there are 150 million date palms worldwide, and 75% of these in the Near East and North Africa (NENA) region. Currently the crop is cultivated over 1.40 million ha (FAOSTAT, 2019) with ~150 million date palms worldwide. Nearly 90% (8.14 million t) of the global date production comes from the top ten date producing countries (Figure 1). Date palm is important to the agrarian economy of several countries with the ability to withstand severe abiotic stresses prevalent in the arid regions of the world including hot and dry climatic conditions, water stress and salinity. Date palm thrives in the NENA region between 24 to 34°N but is distributed between 10 and 39°N latitude (Johnson et al., 2013). In the last three to four decades the crop is also gaining importance in Australia, India, Indonesia, Mexico, Namibia, southern Africa, South America, Pakistan, and the United States (Chao and Krueger, 2007).

Buxton (1920) documented the insect pests of date palm in Mesopotamia (present day Iraq) and elsewhere. 112 species of insects and mites associated with date palm worldwide including 22 species attacking stored dates are enlisted in a recent report on the arthropod fauna of date palm(El-Shafie, 2012). Wakil et al. (2015) reported that increased monoculture of date palm, coupled with global warming; unrestrained use of chemical insecticides and extensive international trade is likely to adversely impact the pest complex and the related natural enemies in the date agro-ecosystems. Developing sustainable IPM techniques to control insect pests in date palm is essential. In this context the use of semiochemicals in date

[a]E-mail: jrfaleiro@yahoo.co.in

palm IPM could help develop and deploy ecologically sound, economically feasible and socially acceptable IPM techniques.

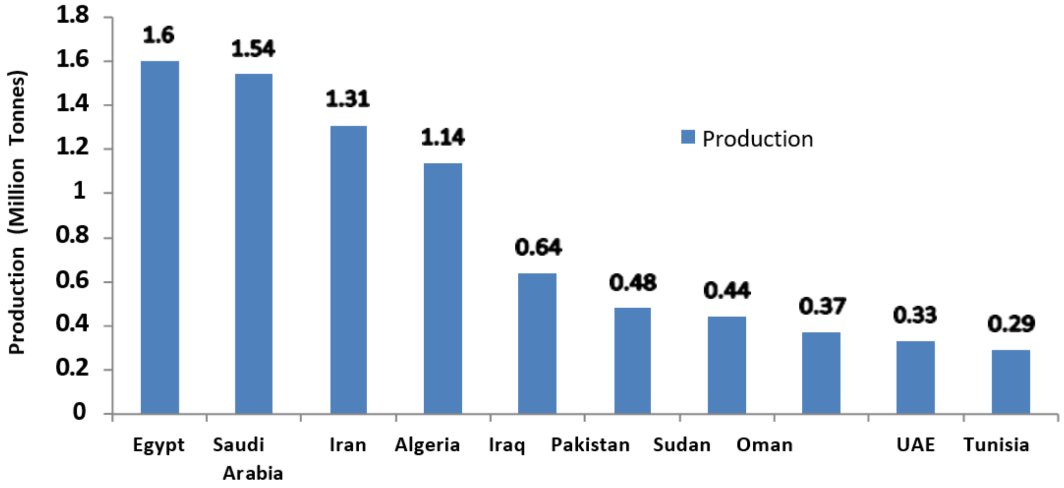


Figure 1. Top ten date producing countries (FAOSTAT, 2019).

SEMIOCHEMICALS AND IPM

Semiochemicals are organic compounds used by insects to convey specific chemical messages that modify insect behavior or physiology and are species specific and harmless to the environment. These chemicals are essential for pest survival including location of habitat, food and mates. Semiochemicals which mediate interactions among individuals of the same species (intra-specific reactions) are called pheromones, while those that mediate interactions among individuals of different species (inter-specific interactions) are known as allelo chemicals. It has been possible to identify chemical signals/cues for thousands of insect species. Semiochemicals are characterized and synthesized using sophisticated equipment and chemical methods involving nuclear magnetic resonance (NMR), solid-phase micro extraction (SPME), gas chromatography-electroantennography (GC-EAG), gas chromatography-mass spectrometry (GC-MS) etc. (Soroker et al., 2015). Most identified insect pheromones are from the orders *Lepidoptera* and *Coleoptera*. Sex pheromones are used to successfully control lepidopteran insects while aggregation pheromones have been developed to control coleopteran pests (Witzgall et al., 2010). Often the attraction of aggregation pheromones is synergized by plant volatiles. Synthetic pheromone lures are powerful tools that indicate early stages of pest or invasion, thereby enabling proper timing of selected control measures.

Several serious agricultural pests including the carob moth *Ectomyelois ceratoniae*, the army worm *Spodoptera frugiperda*, tomato leaf miner *Tuta absoluta*, fruit flies *Bactrocera* sp., mountain pine beetle *Dendroctonus ponderosae*, Asian citrus psyllid *Diaphorinacitri* and the red palm weevil *Rhynchophorus ferrugineus* have been successfully managed by using semiochemicals (El-Shafie and Faleiro, 2017a). Appropriate formulation technology for effective delivery of the semiochemical in the field is essential. A controlled-release system of pheromone dispenser that mimic the natural pheromone release by insect pest is required (Baker, 2011). Limitations such as inconsistency of semiochemical product to maintain release rate and short field longevity, together with high dispensing cost and physical limitations of the active ingredient of the semiochemicals (instability, volatility, and sensitivity to environmental factors like temperature and light) are the major challenges encountered in the formulation and dispensation of semiochemicals (Mafra-Neto et al., 2014). Following are the classical IPM approaches using semiochemicals;

- Mass elimination of the pest population may be achieved by mass trapping, attract and kill, attract and infect or push-pull methods;

- Mating disruption aims at interfering with the mating process of insect pests with the expected result of reduced oviposition. Classical mating disruption is usually achieved by permeation of the air with the volatile sex pheromone.

DATE PALM IPM AND SEMIOCHEMICALS

Effective attractants, pheromones, repellents and/or host volatiles are known only for some of the major date palm pests viz., lesser date moth *Batrachedra amydraula*, greater date moth *Aphomia* (= *Arenipses*) *sabella*, Indian meal moth *Plodia interpunctela*, raisin moth *Cadra* (= *Ephestia*) *figulilella*, carob moth *Spectrobates* (= *Ectomyelois*) *ceratoniae*, red palm weevil *Rhynchophorus ferrugineus*, rhinoceros beetle *Orytes elegans*, dried fruit beetle *Carpophilus hemipterus*, etc. Soroker et al. (2015) enlisted the pheromones of date palm pests (Table 1).

Table1. Pheromones of date palm pests.

Lepidoptera
Lesser date moth *Batrachedra amydraula*: *Batrachedridae* Z4, Z7-10Ac Z4-10Ac Z5-10OH Z5-10Ac Levi-Zada et al. (2011)
Greater date moth *Aphomia* (= *Arenipses*) *sabella*: *Pyralidae* Benzaldehyde sulcatol phenyl acetaldehyde 2-phenylpropenal geranyl acetone fuscamol Levi-Zada et al. (2014)
Indian meal moth *Plodia interpunctela*: *Pyralidae* Z9,E12-14Ac Z9,E12-14AldZ9,E12-14OH Z9-14Ac Zhu et al. (1999)
Raisin moth *Cadra* (= *Ephestia*) *figulilella*: *Pyralidae* Z9E12-14Ald Brady & Daley (1972)
Carob moth *Spectrobates* (= *Ectomyelois*) *ceratoniae*: *Pyralidae* Z9E11,13-14Ald Z9E11-14AldZ9-14Ald Baker et al. (1991)
Coleoptera
Red palm weevil, *Rhynchophorus ferrugineus*: *Curculionidae* 4S, 5S-nonanol 4S, 5S nonanone Hallett et al. (1993)
Rhinoceros beetle, *Orytes elegans*: *Scarabaeidae* 4-methyloctanoic acid Rochat et al., (2004)
Dried fruit beetle, *Carpophilus hemipterus*: *Nitidulididae* (Sap beetles) (2E, 4E, 6E, 8E)-3,5,7-trimethyl -2,4,6,8-decatetraene 2E, 4E, 6E, 8E)-3,5,7-trimethyl -2,4,6,8-undecatetraene (2E, 4E, 6E, 8E)- 7-ethyl-3,5dimethyl-2,4,6,8 decatetraene (2E, 4E, 6E, 8E)- 7-ethyl-3,5dimethyl-2,4,6,8 undecatetraene Bartelt et al. (1992)
Corn sap beetle, *C. dimidiatus*: *Nitidulididae* 3E,5E,7E,9E-6,8-diethyl-4meyhyl- dodecatetraene 3E,5E,7E,9E-5,7-diethyl-9methyl- tridecatetraene Bartelt et al. (1995)
Flower beetle *C. mutilatus*: *Nitidulididae* 3E,5E,7E-5-ethyl- 7methyl- undecatriene 3E,5E,7E-6-ethyl-4methyl-decatriene Bartelt et al. (1993)

By far, the most widely used pheromone in date palm is the red palm weevil aggregation pheromone synthesized by Hallett et al. (1993), used for both monitoring and mass trapping the pest (El-Shafie and Faleiro, 2020). Over the years trapping protocols with regard to trap design, lure-bait synergy, trap placement, lure longevity, trap servicing (periodic change of food bait and water) have been widely studied and reported from several countries. (Hallett et al., 1993; Oehlschlager, 2016; Soroker et al., 2015; Faleiro and Al-Shawaf, 2018; Faleiro, 2020). Pheromone trapping for RPW control cannot be relied as the sole technique but has to be combined with other IPM tactics for the effective control of the pest (El-Shafie and Faleiro, 2017b). Dry trapping systems involving attract and kill (El-Shafie et al., 2011) and the bait free Electrap™ (Al-Saroj et al., 2017) have also been reported. Although dry trapping is advancement in RPW trapping, periodic validation of the RPW control program based on weevil captures in traps calls for recording data on weevil captures. This is another bottle neck especially in area-wide operations (El-Shafie and Faleiro, 2020). In this context Spotta UK, has recently developed a prototype of a smart RPW pheromone trap that not only eliminates servicing, but also records and automatically transmits weevil capture data on a 24×7 basis (https://blog-spotta.co/2021-red-palm-weevils-detection-trials). Reports of other semiochemical mediated control technologies against RPW involving the use of repellents

(Guarino et al., 2013) and the spread of biological control agents (EPFs) using pheromone traps (Hajjar et al., 2015) need to be studied and developed further. Antony et al. (2016) identified genes involved in odorant reception and detection in the palm weevil *Rhynchophorus ferrugineus* that could potentially lead to the development of a bio-sensor enabling early detection of RPW.

In general, semiochemical technology in date palm is in the early stages of development offering a vast potential for future innovations and interventions.

Literature cited

Al-Saroj, S., Al-Abdallah, E., Al-Shawaf, A.M., Al-Dandan, A.M., Al-Abdullah, I., Al-Shagag, A., Al-Fehaid, Y., Ben Abdallah, A., and Faleiro, J.R. (2017). Efficacy of bait free pheromone trap (Electrap™) for managementof red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera*: *Curculionidae*). Pest Manage. Hortic. Ecosyst. *23* (*1*), 55–59.

Antony, B., Soffan, A., Jakše, J., Abdelazim, M.M., Aldosari, S.A., Aldawood, A.S., and Pain, A. (2016). Identification of the genes involved in odorant reception and detection in the palm weevil *Rhynchophorus ferrugineus*, an important quarantine pest, by antennal transcriptome analysis. BMC Genomics *17* (*1*), 69 https://doi.org/10.1186/s12864-016-2362-6. PubMed

Baker, T.C. 2011. Insect pheromones: useful lessons for Crustacean pheromone programs? In Chemical Communication in Crustacean, T. Breithaupt, and M. Thiel, eds. (Springer Science+Business Media LLC), https://doi.org/10.1007/978-0-387-77101-4-27.

Baker, T.C., Francke, W., Millar, J.G., Löfstedt, C., Hansson, B., Du, J.W., Phelan, P.L., Vetter, R.S., Youngman, R., and Todd, J.L. (1991). Identification and bioassay of sex pheromone components of carob moth,*Ectomyelois ceratoniae* (Zeller). J Chem Ecol *17* (*10*), 1973–1988 https://doi.org/10.1007/BF00992582. PubMed

Bartelt, R.J., Dowd, P.F., Vetter, R.S., Shorey, H.H., and Baker, T.C. (1992). Responses of *Carpophilus hemipterus* (*Coleoptera*: *Nitidulidae*) and other sap beetles to the pheromone of *C. hemipterus* and host-related coattractants in California field tests. Environ. Entomol. *21* (*5*), 1143–1153 https://doi.org/10.1093/ee/21.5.1143.

Bartelt, R.J., Carlson, D.G., Vetter, R.S., and Baker, T.C. (1993). Male-produced aggregation pheromone of *Carpophilus mutilatus* (*Coleoptera*: *nitidulidae*). J Chem Ecol *19* (*1*), 107–118 https://doi.org/10.1007/BF00987476. PubMed

Bartelt, R.J., Weaver, D.K., and Arbogast, R.T. (1995). Aggregation pheromone of *Carpophilus dimidiatus* (F.) (*Coleoptera*: *Nitidulidae*) and responses to *Carpophilus* pheromones in South Carolina. J Chem Ecol *21* (*11*), 1763–1779 https://doi.org/10.1007/BF02033675. PubMed

Brady, U.E., and Daley, R.C. (1972). Identification of a sex pheromone from the female raisin moth, *Cadra figulilella.* Ann. Entomol. Soc. Am. *65* (*6*), 1356–1358 https://doi.org/10.1093/aesa/65.6.1356.

Buxton, B.A. (1920). Insect pests of date and date palm in Mesopotamia and elsewhere. Bull. Entomol. Res. *11* (*3*), 287–304 https://doi.org/10.1017/S0007485300044709.

Chao, T.C., and Krueger, R.R. (2007). The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. Hortic. Sci. (Prague) *42* (*5*), 1077–1082.

El-Shafie, H.A.F. (2012). Review: list of arthropod pests and their natural enemies identified worldwide on date palm, *Phoenix dactylifera* L. Agric. Biol. J. N. Am. *3* (*13*), 516–524 https://doi.org/10.5251/abjna.2012.3.12.516.524.

El-Shafie, H.A.F., and Faleiro, J.R. (2017a). Semiochemicals and their potential use in pest management. In Biological Control of Pest and Vector Insects, V.D.C. Shields, ed. (Rijeka, Croaia: INTEC).

El-Shafie, H.A.F., and Faleiro, J.R. (2017b). Optimizing components of pheromone-baited trap for the management of red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in date palm agroecosystem. J. Plant Dis. Prot. *124* (*3*), 279–287 https://doi.org/10.1007/s41348-017-0097-5.

El-Shafie, H.A.F., and Faleiro, J.R. (2020). Red palm weevil *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*): global invasion, current management options, challenges and future prospects. In Invasive Species – Introduction Pathways, Economic Impact, and Possible Management Options, H.A.F. El-Shafie, ed. (IntechOpen), p.1–29.

El-Shafie, H.A.F., Faleiro, J.R., Al-Abbad, A.H., Stoltman, L., and Mafra-Neto, A. (2011). Bait-free attract and kill technology (Hook™ RPW) to suppress red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera*: *Curculionidae*) in date palm. Fla. Entomol. *94* (*4*), 774–778 https://doi.org/10.1653/024.094.0407.

Faleiro, J.R. (2020). Guidelines on RPW pheromone trapping with respect to trap design, trap density and servicing. In Red Palm Weevil: Guidelines on Management Practices, M. Elkakhy, and J.R. Faleiro, eds. (Rome, Italy: FAO), https://doi.org/10.4060/ca7703en.

Faleiro, J.R., and Al-Shawaf, A.M. (2018). IPM of red palm weevil. In Date Palm Pests and Diseases: Integrated Management Guide 2018, M. El Bouhssini, and J.R. Faleiro, eds. (International Centre for Agricultural Research in the Dry Areas (ICARDA)), pp.179.

FAOSTAT. (2019). Food and Agriculture Organization of the United Nations - Statistics Division (Crop Production). www. faostat.fao.org (accessed on 25 July, 2019).

Guarino, S., Peri, E., Lo Bue, P., Germanà, M.P., Colazza, S., Anshelevich, L., Ravid, U., and Soroker, V. (2013). Assessment of synthetic chemicals for disruption of *Rhynchophorus ferrugineus* response to attractant-baited traps in an urban environment. Phytoparasitica *41* (*1*), 79–88 https://doi.org/10.1007/s12600-012-0266-9.

Hajjar, M.J., Ajlan, A.M., and Al-Ahmad, M.H. (2015). New approach of *Beauveria bassiana* to control the red palm weevil (*Coleoptera*: *Curculionidae*) by trapping technique. J Econ Entomol *108* (*2*), 425–432 https://doi.org/10.1093/jee/tou055. PubMed

Hallett, R.H., Gries, G., Gries, R., Borden, J.H., Czyzewska, E., Oehlschlager, A.C., Pierce, H.D., Jr., Angerilli, N.P.D., and Rauf, A. (1993). Aggregation pheromones of two Asian palm weevils, *Rhynchophorus ferrugineus* and *R. vulneratus.* Naturwissenschaften *80* (*7*), 328–331 https://doi.org/10.1007/BF01141908.

Johnson, D.V., Al-Khayri, J.M., and Jain, S.M. (2013). Seedling date palms (*Phoenix dactylifera* L.) as genetic resources. Emir. J. Food Agric. *25* (*11*), 809–830 https://doi.org/10.9755/ejfa.v25i11.16497.

Levi-Zada, A., Fefer, D., Anshelevitch, L., Litovsky, A., Bengtsson, M., Gindin, G., and Soroker, V. (2011). Identification of the sex pheromone of the lesser date moth, *Batrachedra amydraula*, using SPME auto-sampling. Tetrahedron Lett. *52* (*35*), 4550–4553 https://doi.org/10.1016/j.tetlet.2011.06.091.

Levi-Zada, A., David, M., Fefer, D., Seplyarsky, V., Sadowsky, A., Dobrinin, S., Ticuchinski, T., Harari, D., Blumberg, D., and Dunkelblum, E. (2014). Circadian release of male-specific components of the greater date moth, *Aphomia* (*Arenipses*) *sabella*, using sequential SPME/GC/MS analysis. J Chem Ecol *40* (*3*), 236–243 https://doi.org/10.1007/s10886-014-0391-7. PubMed

Mafra-Neto, A., Fettig, C.J., Munson, A.S., Rodriguez-Saona, C., Holdcraft, R., Faleiro, J.R., El-Shafie, H., Reinke, M., Bernardi, C., and Villagran, K.M. (2014). Development of specialized pheromone and lure application technologies (SPLAT®) for management of coleopteran pests in agricultural and forest systems. In Biopesticides: State of the Art and Future Opportunities, ACS Symposium Series, A.D. Gross, J.R. Coats, S.O. Duke, and J.N. Seiber, eds., (Washington, DC: American Chemical Society), https://doi.org/10.1021/bk-2014-1172.ch015.

Oehlschlager, A.C. (2016). Palm weevil pheromones – discovery and use. J Chem Ecol *42* (*7*), 617–630 https://doi.org/10.1007/s10886-016-0720-0. PubMed

Rochat, D., Mohammadpoor, K., Malosse, C., Avand-Faghih, A., Lettere, M., Beauhaire, J., Morin, J.P., Pezier, A., Renou, M., and Abdollahi, G.A. (2004). Male aggregation pheromone of date palm fruit stalk borer *Oryctes elegans.* J Chem Ecol *30* (*2*), 387–407 https://doi.org/10.1023/B:JOEC.0000017984.26917.52. PubMed

Soroker, V., Harari, A., and Faleiro, J.R. 2015. The role of semiochemicals in date pest management. In Sustainable Pest Management. In Date Palm: Current Status and Emerging Challenges, W. Wakil, J.R. Faleiro, and T. Miller, eds. (Switzerland: Springer International Publishing), pp.445.

Wakil, W., Faleiro, J.R., Miller, T., Geoffery, O., Bedford, G.O., and Krueger, R.R. (2015). Date palm production and pest management challenges In Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges, W. Wakil, J.R. Faleiro, and T. Miller, eds. (Switzerland: Springer International Publishing), pp.445.

Witzgall, P., Kirsch, P., and Cork, A. (2010). Sex pheromones and their impact on pest management. J Chem Ecol *36* (*1*), 80–100 https://doi.org/10.1007/s10886-009-9737-y. PubMed

Zhu, J.W., Ryne, C., Unelius, C.R., Valeur, P.G., and Löfstedt, C. (1999). Re-identification of the female sex pheromone of the Indian meal moth, *Plodia interpunctella*: evidence for a four-component pheromone blend. Entomol. Exp. Appl. *92* (*2*), 137–146 https://doi.org/10.1046/j.1570-7458.1999.00533.x.

Management of damping-off and wilt diseases of cucumber in Oman: challenges and opportunities

N.A. Al-Saadi[1], R. Velazhahan[1], R. Abdel-Jalil[2] and A.M. Al-Sadi[1,a]

[1]Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, PO Box 34, Al-Khod 123, Oman; [2]Department of Chemistry, College of Science, Sultan Qaboos University, Al-Khod, Oman.

Abstract

Oman is an agricultural country in the southeastern part of the Arabian Peninsula. Cucumber is among the five top crops in terms of production. Despite its importance, soil borne diseases of cucumbers, especially damping-off and wilt, have been a major challenge to cucumber production, resulting in losses that were reported to reach as high as 100%. Damping-off and wilt are caused by different fungal and oomycete species, including *Pythium*, *Fusarium*, *Rhizoctonia* and *Monosporascus*. Management of soil borne diseases has relied mainly on the use of fungicides. However, the frequent application of fungicides has brought with it concerns from environmental and health hazards. In addition, it resulted in the development of fungicide resistance among populations of fungal and oomycete pathogens. Cultural practices such as soil solarization and soil change in greenhouses have been reported to reduce pathogen inoculum. The shift toward organic agriculture over the last decade necessitates the development of native agents that can be used to control plant diseases. Biological control of plant diseases has received attention from researchers due to its safety to humans and the environment. Several bio-control agents that are effective against plant diseases and tolerant to environmental stress conditions have been investigated. An overview is presented about the management of damping-off and wilt diseases of cucumber.

Keywords: *Oomycetes*, *Pythium*, control, vine decline

INTRODUCTION

Oman has been known for long as an agricultural country in the Arabian Peninsula. Dates are the most important agricultural product in Oman, with an annual production of about 369,000 t. Tomatoes (340,000 t), cucumbers (103,000 t), chili and peppers (93,000 t) and melons (51,000 t) are among the top crops in terms of production in the country (Figure 1) (FAO, 2021).

The increase in cultivation and production has been associated with an increase in plant diseases, mainly soil borne diseases. Surveys over the last two decades have shown that soil borne diseases of cucurbits result in up to 100% yield losses to farmers (Al-Sadi et al., 2011). The most common soil-borne disease are damping-off and wilt, which are primarily caused by *Fusarium*, *Pythium*, *Rhizoctonia* and *Monosporascus* species(Al-Sadi et al., 2011; Al-Mawaali et al., 2012; Al-Balushi et al., 2018).

In Oman, cucumber seedlings are vulnerable to damping-off and wilt diseases, which are predominant in more than 70% of greenhouses (Al-Sadi et al., 2011; Al-Balushi et al., 2018). Wilt disease, caused by *P. aphanidermatum*, *Rhizoctonia*, *Fusarium* and *Monosporascus* species, also prevails in many greenhouses grown crops (Al-Sadi et al., 2011). Damping-off incidence is usually highest during the first 10 days of transplanting seedlings into greenhouses, while wilt symptoms start appearing during fruit setting (Al-Sadi et al., 2011).

Management of soil borne diseases in Oman relies mainly on the use of fungicides (Al-Sadi, 2012; Al-Sadi et al., 2015; Al-Balushi et al., 2018). Many growers also practice soil solarization in summer and soil replacement supplementary to chemical control (Deadman

[a]E-mail: alsadi@squ.edu.om

et al., 2006). Biological control is limited to farms practicing organic agriculture(Al-Ghafri et al., 2020; Halo et al., 2021).

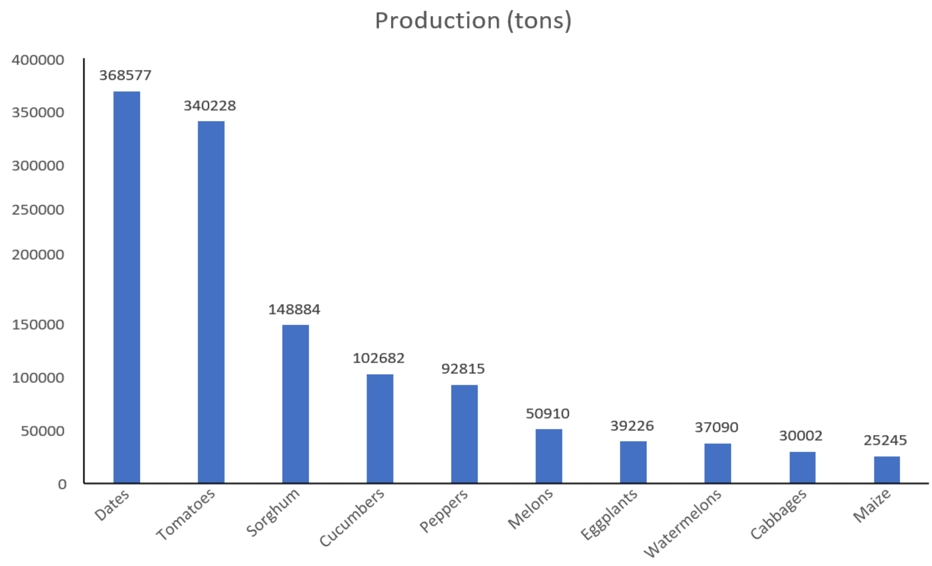


Figure 1. Top crops in terms of production in Oman in 2020 (FAO, 2021).

MANAGEMENT OF DAMPING-OFF AND WILT DISEASES

Several strategies have been developed and implemented all over the world to manage damping-off and wilt of cucumber (Al-Mawaali et al., 2012; Fuchs et al., 2017; Abbasi et al., 2019; Al-Taie et al., 2020; Halo et al., 2021). The choice for these strategies depends on several criteria including disease threshold, availability of resources and applicability to the areas in which the strategies are to be implemented. Strategies which are developed and implemented to manage damping-off and wilt diseases can be divided into different types, the most common of which are cultural, chemical, and biological.

Cultural practices

Cultural practices are important in the management of soil borne diseases of cucumber and other horticultural crops. They include soil solarization, crop rotation, replacement of old soil with new soil and irrigation management (Farrag and Fotouh, 2010; Shlevin et al., 2018; Flower et al., 2019). Soil solarization has been reported to reduce populations of *Pythium*, *Fusarium* and *Rhizoctonia* species (Deadman et al., 2006; Farrag and Fotouh, 2010; Yao et al., 2016; Kokalis-Burelle et al., 2017; Shlevin et al., 2018). Soil solarization also proved to be effective when combined with organic amendments (Bidima et al., 2022). However, it is known to reduce bacterial and fungal communities, so subsequent application of bio-control agents to the soil is recommended (Funahashi et al., 2022). Crop rotation is also effective in managing root diseases caused by soil borne pathogens (He et al., 2021). Rotating cucumber with cress has been found to decrease pathogens and increase cucumber yield (Gong et al., 2021). In addition, controlling the amount of water applied to seedlings and plants helps reduce disease levels (Al-Sadi et al., 2010).

Soil solarization and soil replacement are commonly used to manage damping-off and wilt of cucumber in Oman. Soil solarization was reported to reduce pathogen inoculum in greenhouses (Deadman et al., 2006). In addition, combining solarization with bio-fumigation using cabbage (*Brassica oleracea* L.) leaves gave promising results (Deadman et al., 2006). Soil replacement is another cultural practice employed by growers in Oman to manage *Pythium* inoculum in greenhouse systems (Al-Sadi et al., 2008b). Soil replacement involves the removal of the top layer of cultivated soil (~30-60 cm) and introducing new uncultivated soil.

Chemical control

Control of damping-off and wilt of cucumber is mainly achieved through the use of chemical fungicides. Several fungicides are used for the control of these diseases, including metalaxyl (or mefenoxam) (Al-Sadi, 2012; Liu et al., 2018; Naz et al., 2018; Matić et al., 2019), propamocarb (Rekanovic et al., 2007) and hymexazol (Al-Balushi et al., 2018). These fungicides however, vary in their characteristics and efficacy in the management of damping-off and wilt diseases.

Metalaxyl (mefenoxam) continues to be a leading fungicide for the control of *Oomycete* diseases and it is registered for use in over 100 agricultural crops as seed, soil and foliar treatments (Meena et al., 2019; White et al., 2019; Subharathinam et al., 2020; Hassanisaadi et al., 2021). It is a phenylamide fungicide that was introduced into agriculture in the late 1970s. It comes under several trade names such as Ridomil®, Apron®, Delta-Coat AD® and Subdue 2E®. Metalaxyl is usually applied as a soil treatment before or after transplanting seedlings into greenhouses. In addition, several growers were reported to have used multiple applications of metalaxyl per growing season in order to manage subsequent infections from *Pythium*.

The frequent use of fungicides, including metalaxyl, has been accompanied with problems, the most important of which are the development of fungicide resistance and rapid bio-degradation in soil. Experiments showed that phenylamide resistance is due to a change at the target binding site, resulting in a lack of inhibition of the process of RNA synthesis. Isolates exhibiting resistance to one fungicide of the group phenylamides can become resistant to the others; a phenomenon called cross resistance. In Oman, resistance was reported among *Pythium* species to hymexazol (Al-Balushi et al., 2018), but no report of resistance to metalaxyl has been reported so far.

Rapid bio-degradation of fungicides is another challenge to the use of these chemicals (Al-Sadi et al., 2008a). In Australia, fluctuations in the efficacy of metalaxyl fungicides in the control of cavity spot of carrots caused by some *Pythium* species between sites revealed that the continuous use of metalaxyl fungicide in some sites resulted in decreased persistence of the fungicide in soil, with the half-life in previously untreated soil of 82 days, being reduced to less than 10 days where the fungicide was used previously (Davison and McKay, 1999). In Oman, rapid bio-degradation of metalaxyl is common in some greenhouse soils (Al-Sadi et al., 2008a), which led to failure of control of damping-off disease.

Biological control

Biological control of damping-off and wilt diseases gained increased popularity. Suppression of these diseases by fungal bio-controller bacterial agents has been attained using several fungal and bacterial strains (Bělonožníková et al., 2022; Chowdhury et al., 2022; Marian et al., 2022; Praveena et al., 2022; Siddiqui et al., 2022). Mycorrhiza are also known to improve resistance of cucumber to root diseases (Ravnskov et al., 2020a). Several *Trichoderma* species are used as bio-control agents for cucumber root pathogens (Chen et al., 2018; De Corato et al., 2018; Abed et al., 2021; Mahmoud and Abdalla, 2021). *Trichoderma brevicrassum* showed promising results in the management of cucumber root diseases caused by *Rhizoctonia* species (Zhang and Zhuang, 2020). *T. harzianum* is a commonly used in the management of root diseases of cucumber (Javanshir Javid et al., 2016; Chen et al., 2017, 2019; Abed et al., 2021). *Aspergillus*, *Cladosporium*, *Talaromyces* and *Funnelliformis* species have also been reported to have antagonistic activities against *Pythium* species infecting cucumber (Table 1).

In Oman, *Pseudomonas aeruginosa* showed antagonistic activities against *Pythium* and *Fusarium* (Al-Hinai et al., 2010). Tomato rhizosphere bacteria, *Exiguobacterium indicum* and *Bacillus cereus* (Al-Hussini et al., 2019) and the endophytic fungus *Aspergillus terreus* isolated from native plants *Rhazyastricta* and *Tephrosia apollinea* (Halo et al., 2018) were found effective against *P. aphanidermatum*. A novel fungus, *Cladosporium omanense*, and an endophytic bacterium *Enterobacter cloacae* showed antagonistic activities against cucumber damping-off (Kazerooni et al., 2020; Halo et al., 2021). Several other bio-control agents were reported from Oman during the last 3 years (Al-Badri et al., 2020; Al-Daghari et al., 2020; Gaya

Karunasinghe et al., 2020; Al-Nadabi et al., 2021).

Table 1. Examples of fungal bio-control agents with antagonistic activities against *Pythium* species.

Biocontrol agents	Oomycete pathogens	References
Aspergillus insulicola	*P. aphanidermatum*	Karunasinghe et al., 2020
A. melleus	*P. aphanidermatum*	Karunasinghe et al., 2020
A. terreus	*P. aphanidermatum*	Halo et al., 2018; Al-Shibli et al., 2019
Cladosporium omanense	*P. aphanidermatum*	Halo et al., 2021
Funnelliformis mosseae	*P. ultimum*	Ravnskov et al., 2020b
Talaromyces pinophilus	*P. aphanidermatum*	Kazerooni et al., 2019
Trichoderma hamatum	*P. aphanidermatum*	Al-Taie et al., 2020
T. harzianum	*P. aphanidermatum*	Al-Taie et al., 2020; Abed et al., 2021
T. viride	*P. aphanidermatum*	Abed et al., 2021

CONCLUSIONS

Soil borne diseases represent a challenge to the cultivation and production of cucumber in soil-based systems. Although they are widely distributed in greenhouses in Oman, their successful management depends on the combined use of cultural and chemical methods. Proper solarisation of greenhouse soil in summer is recommended to reduce pathogen inoculum. Alternation of fungicides is important to prevent the development of fungicide resistance. In addition, fungicides should be applied before transplanting to reduce the chance of appearance of pre-emergence or post-emergence damping-off. Also, a follow up treatment is usually recommended before fruit setting to reduce the chance of development of wilt symptoms. The use of bio-control agents, especially *Trichoderma* species, can help reduce disease incidence in farms practicing organic agriculture.

Literature cited

Abbasi, P.A., Renderos, W., and Fillmore, S. (2019). Soil incorporation of buckwheat as a pre-plant amendment provides control of *Rhizoctonia* damping-off and root rot of radish and Pythium damping-off and root rot of cucumber. Can. J. Plant Pathol. *41* (*1*), 24–34 https://doi.org/10.1080/07060661.2018.1559224.

Abed, J.M., Farhan, T.A., and Khadhum, A.A. (2021). Inducing of systemic resistance in cucumber against *Pythium aphanidermatum*, which causes damping off and root rot disease. Int. J. Agric. Stat. Sci. *16*, 1953–1957.

Al-Badri, B.A.S., Al-Maawali, S.S., Al-Balushi, Z.M., Al-Mahmooli, I.H., Al-Sadi, A.M., and Velazhahan, R. (2020). Cyanide degradation and antagonistic potential of endophytic *Bacillus subtilis* strain BEB1 from *Bougainvillea spectabilis* Willd. All Life *13* (*1*), 92–98 https://doi.org/10.1080/26895293.2020.1728393.

Al-Balushi, Z.M., Agrama, H., Al-Mahmooli, I.H., Maharachchikumbura, S.S.N., and Al-Sadi, A.M. (2018). Development of resistance to hymexazol among *Pythium* species in cucumber greenhouses in Oman. Plant Dis *102* (*1*), 202–208 https://doi.org/10.1094/PDIS-11-16-1680-RE. PubMed

Al-Daghari, D.S.S., Al-Sadi, A.M., Janke, R., Al-Mahmooli, I.H., and Velazhahan, R. (2020). Potential of indigenous antagonistic rhizobacteria in the biological control of Monosporascus root rot and vine decline disease of muskmelon. Acta Agric. Scand. B Soil Plant Sci. *70* (*5*), 371–380 https://doi.org/10.1080/09064710.2020.1748703.

Al-Ghafri, H.M., Velazhahan, R., Shahid, M.S., and Al-Sadi, A.M. (2020). Antagonistic activity of *Pseudomonas aeruginosa* from compost against *Pythium aphanidermatum* and *Fusarium solani.* Bio-control Sci. Techn. *30* (*7*), 642–658 https://doi.org/10.1080/09583157.2020.1750562.

Al-Hinai, A.H., Al-Sadi, A.M., Al-Bahry, S.N., Mothershaw, A.S., Al-Said, F.A., Al-Harthi, S.A., and Deadman, M.L. (2010). Isolation and characterization of *Pseudomonas aeruginosa* with antagonistic activity against *Pythium aphanidermatum*. J. Plant Pathol. *92*, 653–660.

Al-Hussini, H.S., Al-Rawahi, A.Y., Al-Marhoon, A.A., Al-Abri, S.A., Al-Mahmooli, I.H., Al-Sadi, A.M., and Velazhahan, R. (2019). Biological control of damping-off of tomato caused by *Pythium aphanidermatum* by using native antagonistic rhizobacteria isolated from Omani soil. J. Plant Pathol. *101* (*2*), 315–322 https://doi.org/10.1007/s42161-018-0184-x.

Al-Mawaali, Q.S., Al-Sadi, A.M., Khan, A.J., Al-Hasani, H.D., and Deadman, M.L. (2012). Response of cucurbit rootstocks to *Pythium aphanidermatum.* Crop Prot. *42*, 64–68 https://doi.org/10.1016/j.cropro.2012.07.017.

Al-Nadabi, H.H., Al-Buraiki, N.S., Al-Nabhani, A.A., Maharachchikumbura, S.N., Velazhahan, R., and Al-Sadi, A.M. (2021). In vitro anti-fungal activity of endophytic bacteria isolated from date palm (*Phoenix dactylifera* L.) against fungal pathogens causing leaf spot of date palm. Egypt. J. Biol. Co. *31*, 65.

Al-Sadi, A.M. (2012). Efficacy of mefenoxam is affected by a lag period between application and inactivation of *Pythium* species. Phytopathol. Mediterr. *51*, 292–297.

Al-Sadi, A.M., Drenth, A., Deadman, M.L., Al-Said, F.A., Khan, I., and Aitken, E.A.B. (2008a). Association of a second phase of mortality in cucumber seedlings with a rapid rate of metalaxyl bio-degradation in greenhouse soils. Crop Prot. *27* (*7*), 1110–1117 https://doi.org/10.1016/j.cropro.2008.01.007.

Al-Sadi, A.M., Drenth, A., Deadman, M.L., Al-Said, F.A., Khan, I., and Aitken, E.A.B. (2008b). Potential sources of *Pythium* inoculum into greenhouse soils with no previous history of cultivation. J. Phytopathol. *156* (*7-8*), 502–505 https://doi.org/10.1111/j.1439-0434.2008.01396.x.

Al-Sadi, A.M., Al-Masoudi, R.S., Al-Habsi, N., Al-Said, F.A., Al-Rawahy, S.A., Ahmed, M., and Deadman, M.L. (2010). Effect of salinity on *Pythium* damping-off of cucumber and on the tolerance of *Pythium aphanidermatum.* Plant Pathol. *59* (*1*), 112–120 https://doi.org/10.1111/j.1365-3059.2009.02176.x.

Al-Sadi, A.M., Al-Said, F.A., Al-Kiyumi, K.S., Al-Mahrouqi, R.S., Al-Mahmooli, I.H., and Deadman, M.L. (2011). Etiology and characterization of cucumber vine decline in Oman. Crop Prot. *30* (*2*), 192–197 https://doi.org/10.1016/j.cropro.2010.10.013.

Al-Sadi, A.M., Al-Masoodi, R.S., Al-Ismaili, M., and Al-Mahmooli, I.H. (2015). Population structure and development of resistance to hymexazol among *Fusarium solani* populations from date palm, citrus and cucumber. J. Phytopathol. *163* (*11-12*), 947–955 https://doi.org/10.1111/jph.12397.

Al-Shibli, H., Dobretsov, S., Al-Nabhani, A., Maharachchikumbura, S.S.N., Rethinasamy, V., and Al-Sadi, A.M. (2019). *Aspergillus terreus* obtained from mangrove exhibits antagonistic activities against *Pythium aphanidermatum*-induced damping-off of cucumber. PeerJ *7*, e7884 https://doi.org/10.7717/peerj.7884. PubMed

Al-Taie, A.H., Al-Zubaidi, N.K., and Al-Shammary, M.K. (2020). Allelopathy effect of *Trichoderma* spp. and some plant extracts against *Pythium aphanidermatum* (in vitro). Indian J. Agric. Res. *54*, 757–762.

Bělonožníková, K., Hýsková, V., Chmelík, J., Kavan, D., Čeřovská, N., and Ryšlavá, H. (2022). *Pythium oligandrum* in plant protection and growth promotion: secretion of hydrolytic enzymes, elicitors and tryptamine as auxin precursor. Microbiol Res *258*, 126976 https://doi.org/10.1016/j.micres.2022.126976. PubMed

Bidima, M.G.S., Chtaina, N., Ezzahiri, B., El Guilli, M., and Barakat, I. (2022). Effect of soil solarization and organic amendments on *Sclerotium rolfsii* Sacc sclerotia. Arch. Phytopathol. Pflanzenschutz *55* (*8*), 1014–1030 https://doi.org/10.1080/03235408.2022.2081526.

Chen, S.C., Zhao, H.J., Wang, Z.H., Zheng, C.X., Zhao, P.Y., Guan, Z.H., Qin, H.Y., Liu, A.R., Lin, X.M., and Ahammed, G.J. (2017). *Trichoderma harzianum*-induced resistance against *Fusarium oxysporum* involves regulation of nuclear DNA content, cell viability and cell cycle-related genes expression in cucumber roots. Eur. J. Plant Pathol. *147* (*1*), 43–53 https://doi.org/10.1007/s10658-016-0978-7.

Chen, S., Yu, H., Zhou, X., and Wu, F. (2018). Cucumber (*Cucumis sativus* L.) seedling rhizosphere *Trichoderma* and *Fusarium* spp. communities altered by vanillic acid. Front Microbiol *9*, 2195 https://doi.org/10.3389/fmicb.2018.02195. PubMed

Chen, S.C., Ren, J.J., Zhao, H.J., Wang, X.L., Wang, T.H., Jin, S.D., Wang, Z.H., Li, C.Y., Liu, A.R., Lin, X.M., and Ahammed, G.J. (2019). *Trichoderma harzianum* improves defense against *Fusarium oxysporum* by regulating ROS and RNS metabolism, redox balance, and energy flow in cucumber roots. Phytopathology *109* (*6*), 972–982 https://doi.org/10.1094/PHYTO-09-18-0342-R. PubMed

Chowdhury, N., Hazarika, D.J., Goswami, G., Sarmah, U., Borah, S., Boro, R.C., and Barooah, M. (2022). Acid tolerant bacterium *Bacillus amyloliquefaciens* MBNC retains biocontrol efficiency against fungal phytopathogens in low pH. Arch Microbiol *204* (*2*), 124 https://doi.org/10.1007/s00203-021-02741-5. PubMed

Davison, E.M., and McKay, A.G. (1999). Reduced persistence of metalaxyl in soil associated with its failure to control cavity spot of carrots. Plant Pathol. *48* (*6*), 830–835 https://doi.org/10.1046/j.1365-3059.1999.00401.x.

De Corato, U., Salimbeni, R., and De Pretis, A. (2018). Suppression of soil-borne pathogens in container media amended with on-farm composted agro-bioenergy wastes and residues under glasshouse condition. J. Plant Dis. Prot. *125*, 213–226 https://doi.org/10.1007/s41348-017-0133-5.

Deadman, M., Al-Hasani, H., and Al-Sa'di, A.M. (2006). Solarization and bio-fumigation reduce *Pythium aphanidermatum* induced damping- off and enhance vegetative growth of greenhouse cucumber in Oman. J. Plant Pathol. *88*, 333–335.

FAO. (2021). FAOSTAT. http://www.fao.org/faostat/en/#data/QC/visualize (accessed January 5, 2021).

Farrag, E.S.H., and Fotouh, Y.O. (2010). Solarization as a method for producing fungal-free container soil and controlling wilt and root-rot diseases on cucumber plants under greenhouse conditions. Arch. Phytopathol. Pflanzenschutz *43* (*6*), 519–526 https://doi.org/10.1080/03235400701875679.

Flower, K.C., Hüberli, D., Collins, S.J., Thomas, G., Ward, P.R., and Cordingley, N. (2019). Progression of plant-parasitic nematodes and foliar and root diseases under no-tillage with different crop rotations. Soil Tillage Res. *191*, 18–28 https://doi.org/10.1016/j.still.2019.03.010.

Fuchs, J.G., Hedrich, T., Hofer, V., Koller, M., Oberhaensli, T., Ribera Regal, J., Tamm, L., Thuerig, B., Schwarze, F.W.M.R., and Herforth-Rahmé, J. (2017). Development of disease-suppressive organic growing media. Acta Hortic. *1164*, 181–188 https://doi.org/10.17660/ActaHortic.2017.1164.23.

Funahashi, F., Myrold, D.D., and Parke, J.L. (2022). The effects of soil solarization and application of a *Trichoderma* bio-control agent on soil fungal and prokaryotic communities. Soil Sci. Soc. Am. J. *86* (*2*), 369–383 https://doi.org/10.1002/saj2.20361.

Gaya Karunasinghe, T., Hashil Al-Mahmooli, I., Al-Sadi, A.M., and Velazhahan, R. (2020). The effect of salt-tolerant antagonistic bacteria from tomato rhizosphere on plant growth promotion and damping-off disease suppression under salt-stress conditions. Acta Agric. Scand. B Soil Plant Sci. *70* (*1*), 69–75 https://doi.org/10.1080/09064710.2019.1668956.

Gong, X., Shi, J., Zhou, X., Yuan, T., Gao, D., and Wu, F. (2021). Crop rotation with cress increases cucumber yields by regulating the composition of the rhizosphere soil microbial community. Front Microbiol *12*, 631882 https://doi.org/10.3389/fmicb.2021.631882. PubMed

Halo, B.A., Al-Yahyai, R.A., and Al-Sadi, A.M. (2018). *Aspergillus terreus* inhibits growth and induces morphological abnormalities in *Pythium aphanidermatum* and suppresses *Pythium*-induced damping-off of cucumber. Front Microbiol *9*, 95 https://doi.org/10.3389/fmicb.2018.00095. PubMed

Halo, B.A., Al-Yahyai, R.A., and Al-Sadi, A.M. (2021). Biological control of *Pythium aphanidermatum*-induced cucumber and radish damping-off by an endophytic fungus, *Cladosporium omanense* isolate 31R. Bio-Control Sci. Techn. *31* (*3*), 235–251 https://doi.org/10.1080/09583157.2020.1844148.

Hassanisaadi, M., Shahidi Bonjar, G.H., Hosseinipour, A., Abdolshahi, R., Barka, E.A., and Saadoun, I. (2021). Biological control of *Pythium aphanidermatum*, the causal agent of tomato root rot by two streptomyces root symbionts. Agronomy (Basel) *11* (*5*), 846 https://doi.org/10.3390/agronomy11050846.

He, X.J., Khashi u Rahman, M., Zhu, W.W., and Wu, F.Z. (2021). Effects of crop rotations on microbial community in rhizosphere soil of cucumber seedlings and its feedback. Allelopathy J. *52* (*2*), 225–238 https://doi.org/10.26651/allelo.j/2021-52-2-1318.

Javanshir Javid, K., Mahdian, S., Behboudi, K., and Alizadeh, H. (2016). Biological control of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* by some *Trichoderma harzianum* isolates. Arch. Phytopathol. Pflanzenschutz *49* (*17-18*), 471–484 https://doi.org/10.1080/03235408.2016.1242195.

Karunasinghe, T.G., Maharachchikumbura, S.S.N., Velazhahan, R., and Al-Sadi, A.M. (2020). Antagonistic activity of endophytic and rhizosphere fungi isolated from sea purslane (*Sesuvium portulacastrum*) against *Pythium* damping off of cucumber. Plant Dis *104* (*8*), 2158–2167 https://doi.org/10.1094/PDIS-01-20-0003-RE. PubMed

Kazerooni, E.A., Velazhahan, R., and Al-Sadi, A.M. (2019). *Talaromyces pinophilus* inhibits *Pythium* and *Rhizoctonia*-induced damping-off of cucumber. J. Plant Pathol. *101* (*2*), 377–383 https://doi.org/10.1007/s42161-018-0186-8.

Kazerooni, E.A., Al-Shibli, H., Nasehi, A., and Al-Sadi, A.M. (2020). Endophytic *Enterobacter cloacae* exhibits antagonistic activity against *Pythium* damping-off of cucumber. Cienc. Rural *50* (*8*), e20191035 https://doi.org/10.1590/0103-8478cr20191035.

Kokalis-Burelle, N., McSorley, R., Wang, K.H., Saha, S.K., and McGovern, R.J. (2017). Rhizosphere micro-organisms affected by soil solarization and cover cropping in *Capsicum annuum* and *Phaseolus lunatus* agroecosystems. Appl. Soil Ecol. *119*, 64–71 https://doi.org/10.1016/j.apsoil.2017.06.001.

Liu, B., Feng, C., Matheron, M.E., and Correll, J.C. (2018). Characterization of foliar web blight of spinach, caused by *Pythium aphanidermatum*, in the desert southwest of the United States. Plant Dis *102* (*3*), 608–612 https://doi.org/10.1094/PDIS-06-17-0859-RE. PubMed

Mahmoud, A.F., and Abdalla, O.A. (2021). Biological control of fungi associated with damping-off and root rot disease of cucumber (*Cucumis sativus* L.). Arch. Phytopathol. Pflanzenschutz *54* (*13-14*), 870–885 https://doi.org/10.1080/03235408.2020.1860412.

Marian, M., Takashima, Y., Harsonowati, W., Murota, H., and Narisawa, K. (2022). Bio-control of Pythium root rot on lisianthus using a new dark septate endophytic fungus *Hyaloscypha variabilis* J1PC1. Eur. J. Plant Pathol. *163* (*1*), 97–112 https://doi.org/10.1007/s10658-022-02459-0.

Matić, S., Gilardi, G., Gisi, U., Gullino, M.L., and Garibaldi, A. (2019). Differentiation of *Pythium* spp. from vegetable crops with molecular markers and sensitivity to azoxystrobin and mefenoxam. Pest Manag Sci *75* (*2*), 356–365 https://doi.org/10.1002/ps.5119. PubMed

Meena, R.P., Kalariya, K.A., Saran, P.L., and Roy, S. (2019). Efficacy of fungicides and bio-control agents against Pythium aphanidermatum causes damping off disease in ashwagandha (*Withania somnifera* L. Dunal). Med. Plant *11* (*4*), 404–409 https://doi.org/10.5958/0975-6892.2019.00052.2.

Naz, R., Nosheen, A., Yasmin, H., Bano, A., and Keyani, R. (2018). Botanical-chemical formulations enhanced yield and protection against *Bipolaris sorokiniana* in wheat by inducing the expression of pathogenesis-related proteins. PLoS One *13* (*4*), e0196194 https://doi.org/10.1371/journal.pone.0196194. PubMed

Praveena, R., Srekha, K., Revathy, R., Srinivasan, V., Sarathambal, C., George, P., Subila, K.P., and Dinesh, R. (2022). New rhizobacteria strains with effective antimycotic compounds against rhizome rot pathogens and identification of genes encoding antimicrobial peptides. Rhizosphere *22*, 100515 https://doi.org/10.1016/j.rhisph.2022.100515.

Ravnskov, S., Cabral, C., and Larsen, J. (2020a). Mycorrhiza induced tolerance in *Cucumis sativus* against root rot caused by *Pythium ultimum* depends on fungal species in the arbuscular mycorrhizal symbiosis. Biol. Control *141*, 104133 https://doi.org/10.1016/j.biocontrol.2019.104133.

Ravnskov, S., Cabral, C., and Larsen, J. (2020b). Mycorrhiza induced tolerance in *Cucumis sativus* against root rot caused by *Pythium ultimum* depends on fungal species in the arbuscular mycorrhizal symbiosis. Biol. Control *141*, 104133 https://doi.org/10.1016/j.biocontrol.2019.104133.

Rekanovic, E., Milijasevic, S., Todorovic, B., and Potocnik, I. (2007). Possibilities of biological and chemical control of Verticillium wilt in pepper. Phytoparasitica *35* (*5*), 436–441 https://doi.org/10.1007/BF03020601.

Shlevin, E., Gamliel, A., Katan, J., and Shtienberg, D. (2018). Multi-study analysis of the added benefits of combining soil solarization with fumigants or non-chemical measures. Crop Prot. *111*, 58–65 https://doi.org/10.1016/j.cropro.2018.05.001.

Siddiqui, Z.S., Habib, A., Umar, M., and Shams, Z.I. (2022). Effect of *Pythium aphanidermatum* (root rot pathogen) on the physiology of *Luffa cylindrica* (sponge gourd) and its management by microbial antagonists. S. Afr. J. Bot. *146*, 614–623 https://doi.org/10.1016/j.sajb.2021.11.038.

Subharathinam, M., Sanjeevkumar, K., Balabaskar, P., and Kumar, S. (2020). Effect of seed and soil application with different doses of *Serratia marcescens* on plant growth and incidence of damping-off (*Pythium aphanidermatum* (Edson) Fitz.) of brinjal under pot culture. Plant Arch. *20*, 1889–1894.

White, D.J., Chen, W., and Schroeder, K.L. (2019). Assessing the contribution of ethaboxam in seed treatment cocktails for the management of metalaxyl-resistant *Pythium ultimum* var. *ultimum* in Pacific Northwest spring wheat production. Crop Prot. *115*, 7–12 https://doi.org/10.1016/j.cropro.2018.08.026.

Yao, Y., Xue, Z., Hong, C., Zhu, F., Chen, X., Wang, W., Cai, Z., Huang, N., and Yang, X. (2016). Efficiency of different solarization-based ecological soil treatments on the control of Fusarium wilt and their impacts on the soil microbial community. Appl. Soil Ecol. *108*, 341–351 https://doi.org/10.1016/j.apsoil.2016.09.015.

Zhang, Y., and Zhuang, W.Y. (2020). *Trichoderma brevicrassum* strain TC967 with capacities of diminishing cucumber disease caused by *Rhizoctonia solani* and promoting plant growth. Biol. Control *142*, 104151 https://doi.org/10.1016/j.biocontrol.2019.104151.

Oryctes Nudi virus against coconut rhinoceros beetle and possibility of its use against date palm dynastid beetles

G.O. Bedford[a]

School of Natural Sciences, Macquarie University, NSW 2109, Australia.

Abstract

The culture, release, establishment, and effect of *Oryctes Nudi virus* (OrNV) against the coconut palm rhinoceros beetle *Oryctes rhinoceros* L. is summarised. The occurrence of possible tolerance to OrNV in certain beetle populations in recent years is discussed. It is suggested that field-collected strains or variants of OrNV may be tested against the larvae and adults of date palm dynastid pests *Oryctes elegans* and *Oryctes agamemnon* for possible biological control.

Keywords: *elegans*, *agamemnon*, *Elais*, *Cocos nucifera*, *Phoenix, dactylifera*

INTRODUCTION

Certain species of dynastid beetles are important pests of palms. *Oryctes rhinoceros* L. attacks the coconut palm (*Cocos nucifera* L.) by boring into the heart at the crown causing emerging fronds to show typical V-shaped cuts reducing photosynthetic area (Figure 1). Heavy prolonged damage to the crown meristem causes death of the palm (Figure 2) (Bedford, 1980, 2013a, b). It causes similar damage or death to young oil palms (*Elais guineensis* Jacq.) (Bedford, 2014). *Oryctes elegans* Prelland, *Oryctes agamemnon* Burmeister cause damage to date palms (*Phoenix dactylifera* L.) (Bedford et al., 2015). The current paper provides commentary rather than giving new data.

RESULTS AND DISCUSSION

Oryctes Nudi virus (OrNV) against the coconut palm rhinoceros beetle (CRB) *Oryctes rhinoceros*

Since the 1970s OrNV has been released and established in many coconut palm locations, initially by release into the pest's breeding sites, later by infecting and releasing adult beetles. OrNV at that time was produced by having larvae feed on OrNV-contaminated food, and when they died the dead larvae contained much fresh OrNV material. Since the 1980s OrNV has been produced by infecting cells in tissue culture of the African black beetle *Heterony chusarator* F. (Crawford and Sheehan, 1984, 1985). After establishment, OrNV has lowered *O. rhinoceros* populations and damage by killing larvae and reducing life span of adults (for results obtained by workers at various locations; see Bedford, 1976, 1980, 1981, 2013 a). Fall in damage as an index of population fall is often particularly noted in localities where there had been high beetle populations and damage at the start which may facilitate OrNV establishment and transmission. Rhinoceros beetle populations persist but often at a lower level than before OrNV establishment, as every patch of coconut palms is a unique ecosystem with many different parameters and variables. At three locations in Fiji where there had been high initial damage (as an index of beetle population), damage fell considerably after establishment of OrNV and was found to be still at a lower level 35 years later (Bedford, 2013b) (Figure 3).

[a]E-mail: geoffrey.bedford@mq.edu.au

Figure 1. Coconut palm damaged by *Oryctes rhinoceros* near Togo were, Viti Levu, Fiji. It would be scored as damaged in a rapid damage survey, and the number of fronds damaged would be counted for a detailed damage survey (Reproduced with permission from the Annual Review of Entomology, Volume 58 ©2013 by Annual Reviews, http://www.annualreviews.org).

Figure 2. Coconut palms killed and reduced to poles due to repeated heavy attacks by *Oryctes rhinoceros*, Drauniivi, Fiji. Breeding is now taking place in the tops. (Reproduced with permission from the Annual Review of Entomology, Volume 58 ©2013 by Annual Reviews, http://www.annualreviews.org).

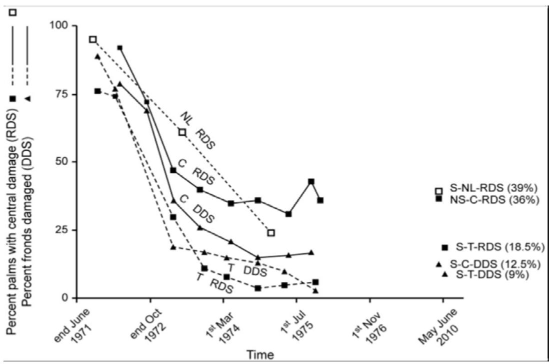


Figure 3. Change in palm damage at the 3 sites studied. C = Caboni, NL = Nadi - Lautoka Road, T = Tamavua, DDS = detailed damage survey (percentage of fronds damaged in a palm sample), RDS = rapid damage survey (percentage of palms in a sample with recent damage i.e., to central 3-4 fronds), S = significant difference $p<0.05$, NS = no significant difference $p>0.05$, and 2010 results are shown in brackets. (Reproduced with permission from the African Journal of Agriculture *8* (*49*), 6422–6425).

Recent developments with OrNV and CRB

Recent CRB invasions in the Pacific e.g., Guam showed apparent resistance to samples of OrNV strains applied. Specimens showed a base change at a particular nucleotide position in the partial CO1 gene sequence of mitochondria and were named haplotype CRB-G (Marshall et al., 2017). It is not known at present if this mitochondrial genome change has any causal relation to OrNV tolerance or resistance. Another mitochondrial haplotype, CRB-S, long established in South Pacific countries, remained susceptible to OrNV. But, as viruses may mutate, the possibility was raised that OrNV, produced by multiplication in *H. arator* tissue culture and given the brief generation time there of 12-16 h, might mutate and evolve to adapt to replicate in *H. arator* cells and undergo concomitant reduction or loss of ability to attack cells of the target, *O. rhinoceros* (Bedford, 2018). There is no genomic data available on this risk at present.

In the Solomon Islands OrNV has been found recently in CRB-G specimens. As CRB-S has also been found there, so there is possibility of interbreeding of the two haplotypes (Etebari et al., 2021) OrNV has also been found in CRB-G in Palau (Tanaka et al., 2021). So with OrNV now found in CRB-G, the classification of CRB according to mitochondrial haplotype may no longer be useful regarding susceptibility or non-susceptibility to OrNV. It is understandable that CRB of whatever haplotype may evolve tolerance or resistance to OrNV, and this could well be due to changes in the nuclear genome, in which case the CO1 mitochondrial genome may no longer be relevant. It is not known if there is any interaction between mitochondrial and nuclear genomes, regarding OrNV, in CRB.

It would be helpful to collect further strains or variants of OrNV from field populations of CRB over its range. And might it be possible to develop tissue culture of *O. rhinoceros*, the target, in which to multiply them, and thus move on from *H. arator* culture?

Application of OrNV work to date palm dynastids

The possibility of testing OrNV against date palm dynastids *O. elegans* and *O. agamemnon* has been suggested (Bedford et al., 2015). Field-collected variants of OrNV could be mixed with food material of date palm dynastid larvae to see if mortality occurs, and would indicate whether natural transmission is likely. OrNV variants could be applied to mouth parts of adults to see if infection occurs, with shortening of lifespan. Midgut and other tissues can be examined histologically and by OrNV genome detection techniques to look for evidence of OrNV multiplication. If there is evidence of adults being able to be infected, they could be confined with larvae in larval breeding site/food material to see if adults defaecate OrNV virions into the material and thus infect larvae, which would be a good indication of the likelihood of transmission in the field. Infected adults could be confined with non-infected adults to see if body contact leads to transmission via mouth parts. If OrNV is found to infect species of date palm dynastids, it could be multiplied in bulk by infecting larvae, so it is thus being obtained from larvae it had killed. This would be an alternative to multiplying it in tissue culture of a non-target organism e.g., *H. arator*, and so avoid the possibility of it evolving and adapting to attack tissue culture cells while undergoing reduction or loss of its ability to attack the intended target, the species of date palm pest *Oryctes*.

CONCLUSIONS

OrNV has lowered populations hence damage of the CRB at many locations in the South Pacific. As viruses may mutate, there is a possibility it may evolve during production in tissue culture of cells which are not from the target pest and hence undergo reduced virulence against the target, CRB. Also, genotypes of CRB may appear which show tolerance or resistance to strains of OrNV.

Field-collected strains of OrNV could be tested for possible infectivity or virulence against larvae and adults of the two species of dynastid pests attacking date palms.

Literature cited

Bedford, G.O. (1976). Use of a virus against the coconut palm rhinoceros beetle in Fiji. PANS *22* (*1*), 11–25 https://doi.org/10.1080/09670877609411451.

Bedford, G.O. (1980). Biology, ecology, and control of palm rhinoceros beetles. Annu. Rev. Entomol. *25* (*1*), 309–339 https://doi.org/10.1146/annurev.en.25.010180.001521.

Bedford, G.O. (1981). Control of the rhinoceros beetle by baculovirus. In Microbial Control of Insects, Mites, and Plant Diseases, Chapter 20, H.D. Burges, ed. (London: Academic), p.409–426.

Bedford, G.O. (2013a). Biology and management of palm dynastid beetles: recent advances. Annu Rev Entomol *58* (*1*), 353–372 https://doi.org/10.1146/annurev-ento-120710-100547. PubMed

Bedford, G.O. (2013b). Long-term reduction in damage by rhinoceros beetle *Oryctes rhinoceros* (L.) (*Coleoptera*: *Scarabaeidae*: *Dynastinae*) to coconut palms at *Oryctes Nudi virus* release sites on Viti Levu, Fiji. Afr. J. Agric. Res. *8* (*49*), 6422–6425 https://doi.org/10.5897/AJAR2013.7013.

Bedford, G.O. (2014). Advances in the control of rhinoceros beetle, *Oryctes rhinoceros*, in oil palm. J. Oil Palm Res. *26* (*3*), 183–194.

Bedford, G.O. (2018). Possibility of evolution in culture of the *Oryctes Nudi virus* of the coconut rhinoceros beetle *Oryctes rhinoceros* (*Coleoptera*: *Scarabaeidae*: *Dynastinae*). Adv. Entomol. *6* (*1*), 27–33 https://doi.org/10.4236/ae.2018.61004.

Bedford, G.O., Al-Deeb, M.A., Khalaf, M.Z., Mohammadpour, K., and Soltani, R. (2015). Dynastid beetle pests. In Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges, Chapter 5, W. Wakil, J.R. Faleiro, and T.A. Miller, eds. (Heidelberg: Springer), p.73–108 https://doi.org/10.1007/978-3-319-24397-9_5.

Crawford, A.M., and Sheehan, C. (1984). An *Oryctes rhinoceros Coleoptera Scarabaeidae baculovirus* inoculum derived from tissue culture. J. Econ. Entomol. *77* (*6*), 1610–1611 https://doi.org/10.1093/jee/77.6.1610.

Crawford, A.M., and Sheehan, C. (1985). Replication of *Oryctes baculovirus* in cell culture: viral morphogenesis, infectivity and protein synthesis. J. Gen. Virol. *66* (*3*), 529–539 https://doi.org/10.1099/0022-1317-66-3-529.

Etebari, K., Hereward, J., Sailo, A., Ahoafi, E.M., Tautua, R., Tsatsia, H., Jackson, G.V., and Furlong, M.J. (2021). Examination of population genetics of the Coconut Rhinoceros Beetle (*Oryctes rhinoceros*) and the incidence of its biocontrol agent (*Oryctes rhinoceros nudivirus*) in the South Pacific Islands. Curr Res Insect Sci *1* (*100015*), 100015 https://doi.org/10.1016/j.cris.2021.100015. PubMed

Marshall, S.D.G., Moore, A., Vaqalo, M., Noble, A., and Jackson, T.A. (2017). A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros nudivirus* and is invading Pacific Islands. J Invertebr Pathol *149*, 127–134 https://doi.org/10.1016/j.jip.2017.07.006. PubMed

Tanaka, S., Harrison, R.L., Arai, H., Katayama, Y., Mizutani, T., Inoue, M.N., Miles, J., Marshall, S.D.G., Kitalong, C., and Nakai, M. (2021). Confirmation of *Oryctes rhinoceros nudivirus* infections in G-haplotype coconut rhinoceros beetles (*Oryctes rhinoceros*) from Palauan PCR-positive populations. Sci Rep *11* (*1*), 18820 https://doi.org/10.1038/s41598-021-97426-w. PubMed

Biology, ecology, and management of the longhorn date palm borer *Jebusaea hammerschmidtii* (*Coleoptera*: *Cerambycidae*)

H.A.F. El-Shafiea[1,a], M.E. Mohammed[1,2] and A.A. Sallam[2,3]

[1]Date Palm Research Center of Excellence, King Faisal University, Saudi Arabia; [2]Agricultural Engineering Department, Faculty of Agriculture, Menoufia University, Egypt; [3]College of Technology and Development, Zagazig University, Egypt.

Abstract

The longhorn beetle (LHB) *Jebusaea hammerschmidtii* (Reiche) (*Coleoptera: Cerambycidae*) is a major pest of date palm *Phoenix dactlylifera* L. in the Middle East. The larvae tunnel and feed inside the palm trunk and thus difficult to control with insecticides. The flight characteristics of the beetle were studied using a computerized flight mill under controlled environment. For the management, two trials were carried out to evaluate the efficacy of phosphine gas and ECO2FUME (EF) in the management of this beetle. In the first trial, artificially infested date palms, under field conditions, were used to test the efficacy of phosphine, while the second trial dealt with the efficacy of EF on naturally infested date palm offshoots in airtight fumigation chamber. The maximum cumulative distance flown by the beetle was found 11.5 km at a temperature of 35°C, while a minimum distance of 2.4 km was recorded at 45°C. EF in combination with phosphine concentration of 1500 ppm and exposure period of 72 h at 25°C was effective against *J. hammerschmidtii*.

Keywords: cerambycid beetle, phosphine, fumigation, management

INTRODUCTION

The longhorn beetle *Jebusaea hammerschmidtii* (Reiche 1878) (*Coleoptera: Cerambycidae*) is a highly destructive pest of date palm *Phoenix dactylifera* L. (El-Shafie, 2015; Al-Saeedi, 2019; Dias et al., 2021; El-Shafie et al., 2022) in almost all date palm-growing countries where it inflicts serious economic damage. The larvae of *J. hammerschmidtii* are the main feeding stage that consumes the palm internal tissues leading to considerable economic damage (Khalaf et al., 2010; Ali and Hama, 2016; El-Shafie and Mohammed, 2016; El-Shafie, 2019, 2021). Recently, Dias et al. (2021) announced the mitochondrial genome of *J. hammerschmidtii*, which consists of 15619 bp and contains 13 protein coding, 22 tRNAs, and two rRNAs genes. Due to the cryptic nature of the beetle, chemical control is difficult, however, treatment of date palms with insecticides as spray, injection into the trunk, and soil application proved to be effective in controlling this pest (Al-Deeb and Khalaf, 2015; Al-Saeedi, 2019). Phosphine gas could be used as curative treatment for severely infested palms (El-Shafie, 2019). This article aims at evaluating the efficacy of phosphine and ECO2FUME in the management of the longhorn date palm borer in the field and for quarantine purposes.

MATERIALS AND METHODS

Flight characteristics

Forty un-sexed adult beetles of unknown age and mating status were flown on the flight mill (Mohammed et al., 2021) to obtain flight metrics such as distance flown, flight velocity, and frequency and length of flight bouts. To elucidate the effect of temperature on LHB flight characteristics, six different treatment temperatures of 20, 25, 30, 35, 40, and 45°C were applied. Three replicates of each temperature were used under a controlled relative humidity

[a]E-mail: elshafie62@yahoo.com

of 35%. The statistical analysis was conducted using one-way analysis of variance (ANOVA) by the statistical software package IBM SPSS (SPSS Inc., Version 24, Chicago, USA). In addition, Tukey multiple range tests were used to find means significantly different between the different treatment temperatures of 20, 25, 30, 35, 40, and 45°C with three replicates at $P<0.05$. The regression models and the correlation coefficients were determined using Microsoft Excel (Microsoft 365).

Management

To evaluate the performance of phosphine in the management of *J. hammerschmidtii*, two trials were carried out which are described hereunder:

1. Experiment I.

This experiment was done in the field on May 25, 2019. Twenty-four 10-year old date palms of the popular cultivar 'Khalas' were selected for this experiment. Each date palm was artificially infested with 5 medium size larvae of *J. hammerschmidtii* by making holes in the trunk using an electric drill. The holes were then carefully sealed with the sawdust that had oozed out during drilling to keep away predators, particularly ants. The larvae were allowed for a week to acclimatize before the commencement of the trial. Treatments used were 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 g of aluminum phosphide m^{-3} of the date palm trunk (3 g aluminum phosphide generate about 1 g of phosphine). Two controls wre used, namely a palm with plastic cover and another palm without cover. After application of the phosphine, each treatment was perfectly covered with a gas-proof plastic sheet. After five days, the larvae were dug carefully out of the trunk and mortality was then calculated. Collected data were statistically analyzed using one-way analysis of variance (ANOVA) by the statistical software package IBM SPSS (SPSS Inc., Version 24, Chicago, USA).

2. Experiment II.

In this experiment, date palm offshoots naturally infested with *J. hammerschmidtii* were fumigated in an airtight fumigation chamber using ECO2FUME (EF). EF is a mixture of liquefied gas consisting of 2% phosphine and 98% carbon dioxide (CO_2) by weight in high-pressure cylinder of ~50 bar (31 kg of CO_2 containing 620 g of phosphine).

Twelve offshoots were selected from 30-year-old plantations and were then detached from their mother and kept in a humid shaded place until used. The infested offshoots were then placed inside the fumigation chamber where temperature and relative humidity were measured using digital data loggers. Temperatures ranged between 20 and 25°C and relative humidity was 58-60% during the trial. The EF was released in the chamber at a concentration of phosphine equivalent to 1500 ppm for exposure times of 24, 48 and 72 h. After the respected exposure periods, the offshoots were dissected using sickle and electric chain saw to look for larvae of the longhorn beetle. The insects retrieved from the dissected offshoots were put on a white cork board in an aerated place for a few hours before dead larvae were counted. This is a recovery period for the paralyzed larvae to be noticed if there is any. Collected data were then used to calculate mortality percentage and efficacy of the EF gas. The median lethal time (LT_{50}) and 95% lethal time (LT_{95}) with 95%, confidence limits for the longhorn insect were determined using the probit analysis in IPM SPSS statistics version 22.The regression models and the correlation coefficients were determined using Microsoft Excel (Microsoft 365).

RESULTS AND DISCUSSION

The LHB was found to stop flying at 20°C, while a normal flight was noted from 25 to 40°C (Figure 1). Based on these results, the temperature of 35°C is considered optimal for flying the LHB with a high cumulative flight distance of 11.20±2.4 km. However, the maximum flight speed of 107.05±7.1 m min^{-1} was achieved at 40°C. Under semi-field conditions, the optimum temperature for adult emergence of *J. hammerschmidtii* in Iraq ranged between 33 and 34°C (Al-Saeedi, 2019) which conforms with our study.

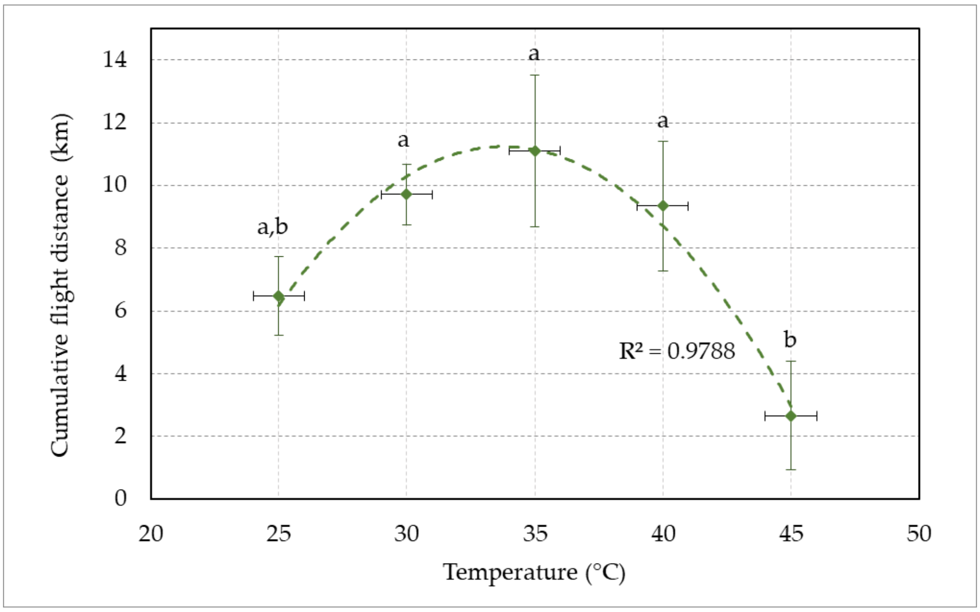


Figure 1. Effect of atmosphere temperature treatments on the cumulative flight distance of *J. hammerschmidtii* using the computerized flight mill at RH of 35%. The different superscript letters show a significant difference between the means values ($P<0.05$).

The mortality percent of the different treatments ranged between 30 and 100% and there seems to be a dose-dependent mortality (Figure 2). There was no significant difference among the concentrations of 6, 7.5, and 9 g.

The LHB exhibited slow response to the gas during the 24-48 h. exposure period. However, at 72-h exposure period, all larvae of LHB were dead. The estimated lethal time that kills 95% of the larvae of LHB found at the surface of the offshoot and deeper inside the trunk were 50 and 66 h, respectively (Table 1). The hiding behavior of the LHB larvae in sealed feeding tunnels may account for the reason behind the delayed response to the gas treatment. The nature of damage by the LHB is characterized by making separate narrow feeding tunnels in the internal tissue sealed with remains of consumed palm tissues and frass of the larvae (El-Shafie, 2015, 2019).

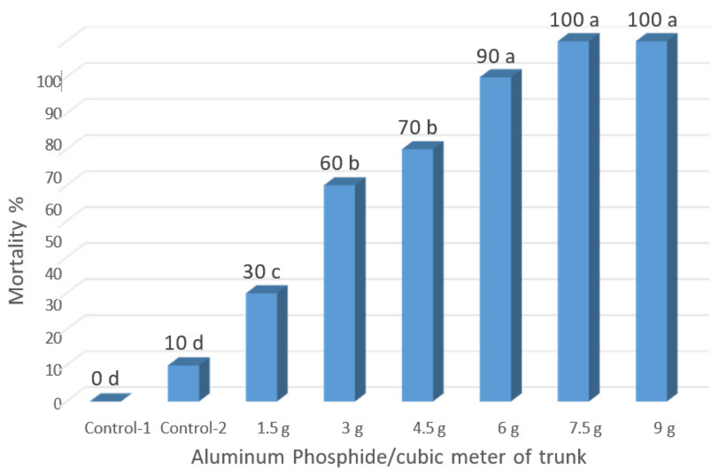


Figure 2. Mortality percentage of *J. hammerschmidtii* larvae in date palm trunk treated with different concentration of phosphine gas.

Table 1. Median lethal time (LT_{50}) and lethal time for 95% mortality (LT95) in hours of the longhorn beetle *J. hammerschmidtii* larvae exposed to ECO2FUME gas at phosphine concentration of 1500 ppm.

Infestation depth[a]	LT_{50} (%)		LT_{95} (%)		Intercept	SE	Chi-square
	Estimate	Bound	Estimate	Bound			
1&2 FW	31.07	27.4-34.7	49.68	45.3-55.1	-2.746	0.189	12.89
3&4 FW	38.93	35.3-42.6	57.54	53.2-62.9	-3.441	0.223	
5&6 FW	47.07	43.5-50.7	65.68	61.3-71.2	-4.160	0.254	

[a]FW = frond (leaves) whorl that constitute the offshoot trunk, 1&2 FW represent the outer most leaves while 5&6 FW are the inner most leaves LT_{50} and LT_{95}, calculated at 95% confidence limits.

The compact layers of sheaths may hinder the infiltration of gas and may provide explanation of survival of older larvae after 24 h exposure to EF gas (El-Shafie et al., 2020). Al Ballaa and Faleiro (2019) recommended the use of solid phosphine formulation as fumigant for quarantine treatment of date palm offshoots against the red palm weevil, *Rhynchophorus ferrugineus*. Unlike dipping in insecticides, fumigation with EF gas allows cost-effective handling of large number of offshoots in relatively shorter time without adversely affecting the environment. Recently, Wakil et al. (2021) reported the development of phosphine resistance in four key stored products pests, including one that infest dates. The application of phosphine against date palm borers including *Jebusaea hammerschmidtii* should be cautiously taken for the safety of operators and avoidance of resistance development. The use of light traps for mass capturing of adult beetles, pruning of old fronds, proper watering and fertilization of date palms help to a great extent in the management of this beetle.

CONCLUSIONS

J. hammerschmidtii has become a serious pest of date palm in recent years. It has a relatively long life cycle and the larval stage lasts for almost a year. The beetle is strong flyer and has the capacity to disperse in date palm plantations. For the management, phosphine gas and ECO2FUME at phosphine concentration of 1500 ppm for 72 h exposure time proved to be effective for controlling *J. hammerschmidtii* in the field for quarantine purposes, respectively. However, because phosphine and ECO2FUME have no residual effect after treatment, precautions have to be taken to prevent post-treatment infestation.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the financial support provided by the Date Palm Research Center of Excellence, King Faisal University through the research project No. DPRC-9-(2018).

Literature cited

Al Ballaa, S.R., and Faleiro, J.R. (2019). Studies on curative treatment of red palm weevil, *Rhynchophorus ferrugineus* Olivier infested date palm palms based on an innovative fumigation technique. Arab J. Plant Protect. *37* (*2*), 119–123 https://doi.org/10.22268/AJPP-037.2.119123.

Al-Deeb, M.A., and Khalaf, M.Z. (2015). Longhorn stem borer and frond borer. In Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges, 1st edn, W. Wakil, J.R. Faleiro, and T.A. Miller, eds. (Springer International Publishing), p.63–72 https://doi.org/10.1007/978-3-319-24397-9_4.

Al-Saeedi, H.M.L. (2019). Ecology, biology, and evaluation of some pest management methods of longhorn date palm stem borer, *Jebusaea hammerschmidtii* (*Coleoptera*: *Cerambycidae*). Ph.D. dissertation (Iraq: University of Baghdad).

Ali, A.-S.A., and Hama, N.N. (2016). Integrated management for major date palm pests in Iraq. Emir. J. Food Agric. *28* (*1*), 24–33 https://doi.org/10.9755/ejfa.2016-01-032.

Dias, G.B., Aldossary, A.M., El-Shafie, H.A.F., Alhoshani, F.M., Al-Fageeh, M.B., Bergman, C.M., and Manee, M.M. (2021). Complete mitochondrial genome of the longhorn date palm stem borer *Jebusaea hammerschmidtii* (Reiche, 1878). Mitochondrial DNA B Resour *6* (*11*), 3214–3216 https://doi.org/10.1080/23802359.2021.1989334. PubMed

El-Shafie, H.A.F. (2015). Biology, ecology, and management of the longhorn date palm stem borer *Jebusaea* *hammerschmidti* (*Coleoptera*: *cerambycidae*). Outlooks Pest Manag. *26* (*1*), 20–23 https://doi.org/10.1564/v26_feb_06.

El-Shafie, H.A.F. (2019). The use of phosphine as curative treatment against date palm borers. Outlooks Pest Manag. *30* (*5*), 204–207 https://doi.org/10.1564/v30_oct_04.

El-Shafie, H.A.F. (2021). The longhorn beetle *Jebusaea hammerschmidtii* Reiche (*Coleoptera*: *Cerambycidae*): an old serious pest undermining date palm plantations. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. *2021*, PAVSNNR202116033 https://doi.org/10.1079/PAVSNNR202116033.

El-Shafie, H.A.F., and Mohammed, M.E.A. (2016). Description and quantification of damage incurred by the longhorn date palm stem borer *Jebusaea hammerschmidti* Reiche, 1877 (*Coleoptera*: *Cerambycidae*) on date palm (*Phoenix dactylifera* Linnaeus, 1753). Int. J. Entomol. Res. *4*, 55–65.

El-Shafie, H.A., Mohammed, M.E., and Sallam, A.A. (2020). Quarantine protocol against coleopteran borers in date palm offshoots using Eco2fume gas. Outlooks Pest Manag. *31* (*4*), 190–192 https://doi.org/10.1564/v31_aug_10.

El-Shafie, H., Mohammed, M., and Alqahtani, N. (2022). A preliminary study on flight charcteristics of the longhorn date palm stem borer *Jebusaea hammerschmidtii* (Reiche 1878) (*Coeloptera*: *Cerambycidae*) using a computerized flight mill. Agriculture *12* (*1*), 120 https://doi.org/10.3390/agriculture12010120.

Khalaf, M.Z., Naher, F.A., and Ali, A.A. (2010). Population density of *Oryctes elegans* Prell. (*Coleoptera*: *Scarabaeidae*) on some date palm varieties in south Baghdad orchards. Agric. Biol. J. N. Am. *1* (*3*), 238–242 https://doi.org/10.5251/abjna.2010.1.3.238.242.

Mohammed, M., El-Shafie, H., and Alqahtani, N. (2021). Design and validation of computerized flight-testing systems with controlled atmosphere for studying flight behaviour of red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Sensors (Basel) *21* (*6*), 2112 https://doi.org/10.3390/s21062112. PubMed

Wakil, W., Kavallieratos, N.G., Usman, M., Gulzar, S., and El-Shafie, H.A.F. (2021). Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. Insects *12* (*4*), 288 https://doi.org/10.3390/insects12040288. PubMed

Efficacy of *Beauveria bassiana* and chlorantraniliprole against date palm dubas bug

M.U. Ghazanfar[1,a] and W. Wakil[2,b]

[1]Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan; [2]Department of Entomology, University of Agriculture, Faisalabad, Pakistan.

Abstract

Dubas bug (*Ommatissus lybicus* de Bergevin; *Homoptera: Tropiduchidae*) is a serious sucking insect of date palm all over the world. Its attack destroys the tree by feeding on the nutrient sap and reduces the fruit production. Various strategies have been used to control this bug like trunk injection (dichlorvos, aldicarb, butocarboxim) and aerial sprays of chemicals. However, the application of chemicals is harmful for human, environment and other species. Moreover, pesticide residues have also been reported from date palm fruits. To address these concern in date palm production, we evaluated two different isolates of *Beauveria bassiana* and one dose rate of chlorantraniliprole each individually and in combination against dubas bug. *Beauveria bassiana* was applied at 1×10^7 conidia mL^{-1} while chlorantraniliprole was used at 0.05 ppm, respectively. Mortality data were recorded at 3, 5 and 7 days post-application. The results revealed that the combination of *B. bassiana* with chlorantraniliprole gave significantly higher mortality than when used alone. The combination of chlorantraniliprole at 1×10^7 conidia mL^{-1} with Bb2 isolate of *B. bassiana* resulted in the highest mortality of dubas bug. The mortality of dubas bug with *B. bassiana* was increased with exposure interval. The results showed that *B. bassiana* with insecticide can be a promising strategy to be considered as part of IPM program of dubas bug in date palm.

Keywords: entomopathogenic fungi, anthranilic diamide, biological control, IPM, *Ommatissus lybicus*

INTRODUCTION

Pakistan ranked 4th place in terms of area and production of date palm and its cultivation is confined to hot areas of Pakistan (Shah et al., 2013). A variety of insect pests and pathogen attacks on date palm result in substantial yield losses all over the world. Among insect pests, dubas bug *Ommatissus lybicus* de Bergevin (*Hemiptera*: *Tropiduchidae*) infestation in the Middle East, Africa and in Pakistan is an emerging threat to its production (Al-Kindi et al., 2018). Its first infestation was reported in 1999 and ever since, it is spreading with alarming rate and causes tree decline (Zamani et al., 2013). The adults and nymphs suck the cell sap and damage trees by excretion of honeydew. Its nymph passes five instars with two generations per year. Chemicals are extensively used to control this insect but the continuous application resulted in the development of resistance in this insect (Dehghan et al., 2021). Entomopathogenic fungi may serve as alternative as part of IPM being eco-friendly in nature (Zamani et al., 2013).

The current study was conducted with the objective to control dubas bug with *Beauveria bassiana* in combination with chlorantraniliprole in order to develop a successful integrated management program of this insect pest.

[a]E-mail: usmanghazanfar1972@gmail.com
[b]E-mail: waqaswakeel@hotmail.com

MATERIALS AND METHODS

Rearing of insect

The colony of dubas bug was collected from date palm growing area and reared on date palm seedlings at 27°C, 65% RH and 14:10 (L:D) photo period. Newly emerged adults were collected from the colony and used for bioassays.

Entomopathogenic fungi

Two *Beauveria bassiana* isolates were taken from the cultural collection of Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan. These isolates were cultured on PDA and stored at 25°C. The plates were dried under the aluminum foil pans. The conidia were harvested with sterile scalper. The desired concentration (1×10^7 conidia mL^{-1}) were achieved using hemocytometer.

Insecticide

The suspension concentrate which contains 200 g L^{-1} chlorantraniliprole (a.i.), anthranilic diamide (Coragen 20 SC), FMC United Pvt. Limited (Lahore, Pakistan) was used in bioassays.

For bioassays the adults (alive) were surface sterilized with 70% ethanol, then later on washed with sterilized water in order to remove the excessive ethanol. They were treated with desired concentration of fungal isolates by spraying the spore suspension with an airbrush (Master Multi-purpose Airbrush, USA). Different airbrushes were used for chlorantraniliprole and conidia suspension. For combined treatments, the chlorantraniliprole was sprayed first and followed by conidial suspension. The treated insects were kept at 27°C, 65% RH and 14:10 (L:D) photo period. Treatments were arranged in complete randomized blocks and consisted of 25 dubas bugs (mixed sex) per treatment. Each treatment was replicated thrice while the experiment was repeated three times independently in order to avoid pseudo replications. For control treatments the adults were treated with sterilized water only. The mortality was recorded after 3, 5 and 7 days post-application.

Statistical analysis

The control mortality was corrected using Abbott's formula (Abbott, 1925). The analysis was done with Mini-tab 17 statistical software (Mini- tab, 2010), and the means were compared with Tukey-Kramer (HSD) test at 5% significance level (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

When the control agents were applied alone, *B. bassiana* provided lower mortality than the tested dose rate of chlorantraniliprole at all exposure intervals (Table 1). Among *B. bassiana* isolates, Bb2 was more effective compared to Bb1 with maximum mortality of 34.06 and 26.13%, respectively, at 7 d of exposure. The combined treatments of fungi and insecticide gave higher mortality than alone treatments and the trend was same at each exposure interval. The mortality was more than 94% when Bb2 was combined with chlorantraniliprole, while it was more than 86% when Bb1 plus chlorantraniliprole was used against test insect species at 7 d post treatment. The mortality was increased with extended exposure intervals for each treatment and highest mortalities were observed at 7 days of treatment in each case (Table 1).

Dubas bug is considered as notorious pest of date palm inflicting heavy damage to overall yield of dates in different countries of the world (Shah et al., 2013; Dehghan et al., 2021). Our results clearly exhibited that entomopathogenic fungi has great potential to control this insect. Our results are in supportive to Diwan and Wehed (2017), they reported that infection of adult female *O. lybicus* by *Metarhizium anisopliae* significantly reduced their fecundity. Similarly, endophytic *B. bassaiana* gave successful control up to 100% of this insect under laboratory and filed conditions (Khudhair et al., 2016). The commercial formulation Mycotol® (*Verticillium lecanii*) also proved affective against dubas bug nymphs compared to adults (Livingston and Al Mafargi, 2005). The efficacy of chlorantraniliprole against dubas bug was also reported by Arbabtafti et al. (2014) with 100% mortality. Sub-lethal dose of chlorantraniliprole and entomopathogenic fungi *B. bassiana* and *M. anisopliae* found compatible against different insect pests (Wakil et al., 2013; Jia et al., 2016) were also in line with our findings.

Table 1. Mean mortality (%±SE) of *Ommatissus lybicus* adults exposed for 3, 5 and 7 days treated with *Beauveria bassiana* (Bb1: 1×10^7 conidia mL^{-1} and Bb2: 1×10^7 conidia mL^{-1}), one dose of chlorantraniliprole (0.05 ppm), and their combinations (Bb1 + chlorantraniliprole, Bb2 + chlorantraniliprole) in laboratory trials.

Treatment	Interval			$F_{2,17}$	P
	3 d	5 d	7 d		
Bb1	11.12±1.40Be	15.23±1.39Bd	26.13±1.08Ae	35.5	<0.01
Bb2	18.33±2.54Bd	23.12±1.72Bc	34.06±1.84Ad	15.2	<0.01
Chlorantraniliprole	31.67±0.74Cc	44.04±2.01Bb	62.49±1.27Ac	116	<0.01
BB1+chlorantraniliprole	42.21±1.11Cb	67.24±1.63Ba	86.40±1.16Ab	282	<0.01
Bb2+chlorantraniliprole	56.67±1.21Ca	73.41±1.20Ba	94.31±1.15Aa	252	<0.01
$F_{4,29}$	142	256	523	-	-
P	<0.01	<0.01	<0.01	-	-

Within each treatment, means followed by the same upper-case letter are not significantly different; Tukey-Kramer (HSD) test at P=0.05. Within each exposure interval followed by the same lower-case letter are not significantly different; Tukey-Kramer (HSD) test at P=0.05.

CONCLUSIONS

The findings indicate that *B. bassiana* and chlorantraniliprole may provide good control under IPM scenario of dubas bug in date palm.

Literature cited

Al-Kindi, K.M., Al-Wahaibi, A.K., Kwan, P., Andrew, N.R., Welch, M., Al-Oufi, M., and Al-Hinai, Z. (2018). Predicting the potential geographical distribution of parasitic natural enemies of the Dubas bug (*Ommatissus lybicus* de Bergevin) using geographic information systems. Ecol Evol *8* (*16*), 8297–8310 https://doi.org/10.1002/ece3.4286. PubMed

Arbabtafti, R., Sheikhigarjan, A., Gharalari, A.H., Damghani, R., Tajbakhsh, M.R., and Arab Jafari, K.M. (2014). Drenching efficacy of imidacloprid and thiamethoxam against Dubas bug, *Ommatissus lybicus* (*Hem.*: *tropiduchidae*). Egypt. Acad. J. Biol. Sci. E Toxicol. Pest Control *6* (*1*), 43–52 https://doi.org/10.21608/eajbsf.2014.17258.

Dehghan, A., Payandeh, A., and Imani, S. (2021). Lethal and sublethal effects of Eforia on the reproduction, development and feeding behaviour of *Ommatissus lybicus* (*Hemiptera*: *tropiduchidae*). Int. J. Trop. Insect Sci. *41* (*1*), 123–130 https://doi.org/10.1007/s42690-020-00183-y.

Diwan, H.M., and Wehed, A.Q. (2017). Effect of Interaction between temperatures and formulations of biopesticide Fytomaxn and some oils on viability of fungus *Metarhizium anisopliae* and its efficacy against Dubas bug *Ommatissus lybicus* Bergevin. Iraq J. Agricul. Res. *22* (*8*), 19–27.

Jia, M., Cao, G., Li, Y., Tu, X., Wang, G., Nong, X., Whitman, D.W., and Zhang, Z. (2016). Biochemical basis of synergism between pathogenic fungus *Metarhizium anisopliae* and insecticide chlorantraniliprole in *Locusta migratoria* (Meyen). Sci Rep *6* (*1*), 28424 https://doi.org/10.1038/srep28424. PubMed

Khudhair, M.W., Alrubeai, H.F., and Khalaf, M.Z. (2016). Innovative method to control dubas bug, *Ommatissus lybicus* (Deberg) (*Homoptera*: *Tropiduchidae*) in date palm orchards using endophytic *Beauveria bassiana* isolates. J. Agric. Sci. Technol. *6*, 394–402.

Livingston, S., and Al Mafargi, K. (2005). Effect of *Verticillium lecanii* on date palm Dubas bug (*Ommatisscus lybicus* Asche & Wilson) in vitro. Arab J. Pl. Prot. *23*, 58–60.

Mini-tab. (2010). Mini-tab 17 Statistical Computer Software (State College, PA, USA: Mini-tab Inc.).

Shah, A., Mohsin, A.U., Hafeez, Z., Naeem, M., and Haq, M.I.U. (2013). Eggs distribution behaviour of Dubas bug (*Ommatissus lybicus*: *Homoptera*: *Tropiduchidae*) in relation to seasons and some physico-morphic characters of date palm leaves. J. Insect Behav. *26* (*3*), 371–386 https://doi.org/10.1007/s10905-012-9354-4.

Sokal, R.R., and Rohlf, F.J. (1995). Biometry, 3rd edn (New York, USA: Freeman & Company).

Wakil, W., Ghazanfar, M.U., Riasat, T., Qayyum, M.A., Ahmed, S., and Yasin, M. (2013). Effects of interactions among *Metarhizium anisopliae*, *Bacillus thuringiensis* and chlorantraniliprole on the mortality and pupation of six geographically distinct *Helicoverpa armigera* field populations. Phytoparasitica *41* (*2*), 221–234 https://doi.org/10.1007/s12600-012-0282-9.

Zamani, Z., Aminaee, M.M., and Khaniki, G.B. (2013). Introduction of *Beauveria bassiana* as a biological control agent against the *Ommatissus lybicus* in Kerman province. Arch. Phytopathol. Pflanzenschutz *46* (*15*), 1821–1830 https://doi.org/10.1080/03235408.2013.778454.

F_1 sterility and its application to control *Ephestia* spp. in date warehouses in Iraq

A.A. Al-Taweel[1,a], A.A. Hameed[2], S.A. Khylawi[2] and I.J. Al-Jboory[3]

[1]Al-Esraa University College, Baghdad, Iraq; [2]Ministry of Science and Technology, Baghdad, Iraq; [3]University of Baghdad, College of Agriculture, Department of Plant Protection, Baghdad, Iraq.

Abstract

A modification of sterile insect technique (SIT) called partial sterility/inherited sterility or F_1 sterility is suggested for controlling stored product insect pest belonging to *Lepidoptera* order such as *Ephestia* spp. This modification showed longer population suppression with a low number of released insects. The percentage of egg hatch of *Ephestia cautella*, *Ephestia figulilella* and *Ephestia calidella* males and females irradiated as pupae at age 5-6 days with either 0.25 or with 0.35 kGy and the produced adults were mated either together or to the opposite sex were as follows: *E. cautella*/0.0, 13.9 and 19.9% for the dose 0.25kGy and 0.0,7.6 and 13.4% for the dose 0.35 kGy in comparison with the 96.9% for the control group; *E. figulilella*/2.7, 7.3 and 21.7% for the dose 0.25 kGy and 0.0, 10.4 and 12.3% for the dose 0.35 kGy in comparison with the 82.9% for the control group; *E. calidella*/3.0, 8.9 and 20.0% for the dose0.25 kGy and 0.0, 15.0 and 13.0% for the dose 0.35 kGy in comparison with the 94.9% for the control group. Presented results representing the following mating for the above three species: irradiated male × irradiated female; irradiated female × un-irradiated male and un-irradiated female × irradiated male in comparison with the control group (un-irradiated female × un-irradiated male). We observed very interesting results because it showed that the percentage of egg hatch of F_1 progeny of the above three species produced from the above mentioned crosses and mated either together or with un-irradiated opposite sex caused reduction in the egg hatch to an average ranged between 0.0 and 5.2% and most of the progeny produced were malformed. In conclusion these results are very important as we mentioned above because it may reduce the number of released insect in the warehouses through generation.

Keywords: F_1 sterility, partial sterility, *Ephestia* spp., gamma ray, date warehouses, Iraq

INTRODUCTION

Ephestia spp. are a serious pest of stored dry dates in Iraq and other date production countries, that causes damage during several steps of harvest, storage, processing and exportation (Hussain, 1985). A modification of sterile insect technique called partial sterility/inherited sterility was suggested for controlling *Ephestia* spp. (Lachance, 1985; Klassen, 1993; Al-Taweel et al., 1997, 2016; Al-Rubeai et al., 1995; Al-Taweel and Al-Jboory, 2007). This technique is species specific, which relies on the mass rearing, exposing pupae to specific dose of gamma rays causing partial/inherited sterility and the emerged adult released in either infested date orchards or warehouses to reduce insect population to zero. Therefore, the aim of this study is designed to investigate the effect of two doses of gamma rays that induce partial/inherited (F_1) sterility in three species of *Ephestia*, these are: *E. cautella*, *E. figulilella* and *E. calidella*.

MATERIALS AND METHODS

The stock cultures of the above three species were reared and irradiated according to the methods described by Ahmed et al. (1972) and Al-Taweel et al. (1990) and then crossed

[a]E-mail: ayadaltaweel90@gmail.com

as mentioned in Tables 1 and 3, the doses of gamma rays were 0.25 and 0.35 kGy, respectively. The produced F_1 generation were crossed as illustrated in Tables 2 and 4. Data were analyzed using ANOVA and mean differences were determined by Duncan's multiple range test at $P \leq 0.05$.

Table 1. Average number of eggs laid female^{-1} and its hatch percentage for three species of *Ephestia* exposed as pupae at age 5-6 days to 0.25 kGy dose of gamma rays.

Species	Type of mating female × male	No. of replicates	Average no. of eggs female^{-1}	% egg hatch
Ephestia cautella	N × N	6 (15)	104.8 a	96.9 a
	T × T	6 (15)	31.8 d	0.0 d
	T × N	6 (15)	54.6 b	13.3 b c
	N × T	6 (15)	67.5 b	19.9 b
Ephestia figulilella	N × N	4 (15)	102.6 a	88.9 a
	T × T	6 (15)	28.7 d	2.7 d
	T × N	8 (15)	47.3 bcd	21.7 b
	N × T	6 (15)	50.5 bc	7.3 c
Ephestia calidella	N × N	6 (15)	96.4 a	94.9 a
	T × T	6 (15)	42.1 d	3.0 c d
	T × N	6 (15)	51.8 bc	8.9 c
	N × T	5 (15)	47.6 bc	20.0

N means control un-irradiated either female or male and T means treated either female or male with a dose of gamma rays as mentioned in the title of the table.
Number between parentheses represent the number of pairs/replicate.
Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ (Duncan's test).

Table 2. Average number of eggs laid female^{-1} and its hatch percentage for the F_1 progeny of three *Ephestia* species produced from paternal/maternal exposed to 0.25 kGy and mated as shown in this table.

Species	Parental crosses F × M	F_1 crosses F × M	Average no. of eggs laid female^{-1}	% eggs hatch
Ephestia cautella	N × N	$F_1 \times F_1$	108.4 a	94.6 a
	T × T	/	/	/
	T × N	$F_1 \times F_1$	19.9 c	0.0 b
		$F_1 \times N$	16.6 c	1.4 b
		$N \times F_1$	41.7 b	0.0 b
	N × T	$F_1 \times F_1$	18.8 c	0.0 b
		$F_1 \times N$	17.6 c	0.8 b
		$N \times F_1$	13.9 c d	0.0 b
Ephestia figulilella	N × N	$F_1 \times F_1$	101.4 a	89.2 a
	T × T	/	/	/
	T × N	$F_1 \times F_1$	19.4 a	0.0 b
		$F_1 \times N$	14.8 c d	2.5 b
		$N \times F_1$	33.6 b	1.4 b
	N × T	$F_1 \times F_1$	14.4 c d	0.0 b
		$F_1 \times N$	16.2 c	3.2 b
		$N \times F_1$	24.4 b	1.2 b
Ephestia calidella	N × N	$F_1 \times F_1$	98.2 a	96.9 a
	T × T	/	/	/
	T × N	$F_1 \times F_1$	8.4 c d	0.0 b
		$F_1 \times N$	24.4 b	5.2 b
		$N \times F_1$	34.8 b	2.4 b
	N × T	$F_1 \times F_1$	34.4 b	0.8 b
		$F_1 \times N$	16.2 c	3.4 b
		$N \times F_1$	24.4 b	1.2 b

F, M means either female or male; N means normal un-irradiated either female or male ; T means irradiated either female or male; F_1 means F_1 progeny.
F_1 progeny from cross T×T are all malformed and we did not able to make any crosses.
Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ (Duncan's test).

Table 3. Average number of eggs laid female^{-1} and its hatch percentage for three species of *Ephestia* exposed as pupae at age 5-6 days to 0.35 kGy dose of gamma rays.

Species	Type of mating	No. of replicates	Average no. of eggs female^{-1}	% eggs hatch
Ephestia cautella	N × N	6 (15)	104.8 a	96.9 a
	T × T	6 (15)	30.8 b	0.0 c
	T × N	6 (15)	21.6 c	7.5 b c
	N × T	6 (15)	34.4 b	13.4 b
Ephestia figulilella	N × N	4 (15)	102.6 a	88.9 a
	T × T	6 (15)	28.3 b c	0.0 c
	T × N	6 (15)	23.2 c	10.4 b
	N × T	8 (15)	29.8 b c	12.3 b
Ephestia calidella	N × N	6 (15)	96.4 a	94.9 a
	T × T	6 (15)	31.4 b	0.0 c
	T × N	6 (15)	27.6 c	15.0 b
	N × T	5 (15)	29.2 b	13.0 b

N means control un-irradiated either female or male and T means treated either female or male with a dose of Gamma rays as mentioned in the title of the table.
Number between parentheses represent the number of pairs/replicate.
Means followed by the same letter in a column are not significantly different at P≤0.05 (Duncan's test).

Table 4. Average number of eggs laid female^{-1} and its hatch percentage for the F_1 progeny of three *Ephestia* species produced from paternal/maternal exposed to 0.35 kGy and mated as shown in this table.

Species	Parental crosses F × M	F_1 crosses F × M	Average no. of eggs laid female^{-1}	% eggs hatch
Ephestia cautella	N × N	F_1 × F_1	98.4 a	94.2 a
	T × T	/	/	/
	T × N	F_1 × F_1	/	/
		F_1 × N	/	/
		N × F_1	14.8	0.0 b
	N × T	F_1 × F_1	/	/
		F_1 × N	24.4 c	0.0 b
		N × F_1	68.6 b	0.8 b
Ephestia figulilella	N × N	F_1 × F_1	114.6 a	89.4 a
	T × T	/	/	/
	T × N	F_1 × F_1	/	/
		F_1 × N	/	/
		N × F_1	44.6 b c	0.2b
	N × T	F_1 × F_1	/	/
		F_1 × N	8.6 c	0.0 b
		N × F_1	78.6b	0.3b
Ephestia calidella	N × N	F_1 ×F_1	124.2 a	94.4 a
	T × T	/	/	/
	T × N	F_1 × F_1	/	/
		F_1 × N	/	/
		N × F_1	58.6 B	0.0 b
	N × T	F_1 × F_1	/	/
		F_1 × N	42.6 b c	4.1b
	N × F_1		56.2 b	0.6 b

F, M means female and male; N means normal un-irradiated either female or male; T means irradiated either female or male; F_1 means F_1 progeny.
Non F_1 progeny are produced from T×T for all species under investigation and also from same F_1 crosses of either T×N or N×T.
Means followed by the same letter in a column are not significantly different at P≤0.05 (Duncan's test).

RESULTS AND DISCUSSION

Results presented in Tables 1 and 2 represent the effect of 0.25 kGy on the three species of *Ephestia* under consideration (parental and F_1 progeny), while Tables 3 and 4 illustrate the effect of 0.35 kGy on the same three species of *Ephestia* (parental and F_1 progeny). The results of these four tables are very interesting and promising in using gamma radiation either for dates disinfestation with one of these two low doses of gamma rays or for inducing partial/F_1 sterility at the laboratory level in the reared insect (irradiated with gamma rays as pupae at 5-6 days old) and releasing the adults either in the date orchards or in the date warehouses to reduce the population of these three species to minimum because it showed that the percentage of eggs hatch of F_1 progeny mated as illustrated in Tables 3 and 4 ranged from zero to 5.2% for the dose 0.25 kGy and range from zero to 4.1% for the dose 0.35 kGy. Moreover, these results suggested building sterility within the population of these three *Ephestia* spp. In the warehouses which resulted in elimination of these pest from date warehouses during storing period.

CONCLUSIONS

The results of Tables 1-4 for using gamma rays to induce partial sterility/F_1 sterility in the three species of *Ephestia* under investigation are very encouraging and promising for controlling these species in the warehouses. However, further investigations are needed.

Literature cited

Ahmed, M.S.H., Al-Hakkak, Z.S., and Al-Saqur, A.M. (1972). Inherited sterility in the fig moth, *E. cautella*. Paper presented at: Inter. Conf. Peaceful Uses of Atom. Energy (Geneva, Vienna: UN).

Al-Rubeai, H.F., Al-Garbawi, Z.A., and Al-Taweel, A.A. (1995). Radiation induced sterility in *E. calidella*. Iraqi J. Biol. Sci. *14*, 46–55.

Al-Taweel, A.A., and Al-Jboory, I.J. (2007). Date moth insect and their control by using different IPM elements in rehabilitation of the date palm in Iraq. Paper presented at: IPM Inception Workshop (Amman, Jordan).

Al-Taweel, A.A., Ahmed, M.S.H., Kadhum, S.S., and Hameed, A.A. (1990). Effect of gamma radiation on the progeny of irradiated *Ephestia cautella* (Walker) (*Lepidoptera*: *Pyralidae*) males. J. Stored Prod. Res. *26*, 233–236 https://doi.org/10.1016/0022-474X(90)90028-Q.

Al-Taweel, A.A., Ahmed, M.S.H., Waheed, A.K., and Nasser, M.J. (1997). Inherited sterility in progeny of gamma irradiated adults of raison moth, *E. figulilella*. Dirasat J. *24*, 128–138.

Al-Taweel, A.A., Al-Jboory, I.J., and Hammed, A.A. (2016). Implementation of nuclear techniques, Inesfly paint and some integrated pest management elements for controlling *Ephestia* spp. in date palm orchards and date warehouses in Iraq. Entomol.and Zool. Studies J. *4* (*5*), 783–787.

Hussain, A.A. (1985). Date Palms and Dates with Their Pest in Iraq (Basrah University Press), pp.583.

Klassen, W. (1993). Use of inherited sterility for the suppression of pest *Lepidoptera*. Lecture presented at: Arab Meeting on Insect Control Using Nuclear Techniques (Cairo, Egypt).

Lachance, L.E. (1985). Genetic Methods for the Control of *Lepidoptera* Species: Status and Potential (USDA, ARS-28).

Integrated management for palm borers

M.Z. Khalaf[1,a], S.K. Mahmood[2] and M.H. Al-Seria[3]

[1]Agricultural Research Directorate, Ministry of Science & Technology, Iraq; [2]College of Biotechnology, Al-Nahrain University, Baghdad, Iraq; [3]Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Jadyria, PO Box 4732, Iraq.

Abstract

An integrated management program was conducted to control the palm borers, *Oryctes* sp. in date palm orchards of central Iraq during the years 2015-2020. The following control factors were used: cultural practices, such as sanitation and pruning frond bases, hand collection of larvae during regular annual service work, solar light traps with lamp of 320-420 nm wavelength and bio-control agents (entomopathogenic fungi). Results indicated that the population density of *Oryctes* spp. reduced in percentage to 91.6 and 53.0% for larvae and adults, respectively. The yield increased in percentage to 28 and 32% after one and two year of following the program. The results depicted the effectiveness of some integrated ecologically sound control methods for monitoring and population suppression of *Oryctes* spp. on date palm orchard environment.

Keywords: cultural practices, light traps, bio-control, systemic insecticides

INTRODUCTION

Date palm (*Phoenix dactylifera*) is one of the major crops in Iraq and most adapted to climatic conditions of the region (Hussain, 1974; Khalaf et al., 2011). It suffers damages from several pests, especially borers like palm frond borer, *Phonapate frontalis*, long horn palm stem (trunk) borer, *Jebusaea hammerschmidtii* and several *Oryctes* spp.: fruit stalk (bunch) borer, *Oryctes elegans*, *Oryctes agamemnon*, Arabian rhinoceros beetle, *Oryctes agamemnon arabicus* and *Oryctes gamemnon matthisseni* which are wide spread in date palm orchards of Iraq and other countries (Khalaf et al., 2010, 2013, 2014).

Oryctes spp. complex caused severe damages to the bases of fronds and bunches making long tunnels inside tissue, which act as weakening and breaking factors for these parts (Al-Khawaja, 1999; Abass, 2000; Khalaf and Al-Taweel, 2015). Boring and feeding on plant tissue thus leads to infestation with pathogens, and associated with weakness of plant and low productivity (Bedford, 1973; Kaaka, 2009; Khalaf et al., 2011). Ecological control measures play an important role as a solution responding to the economic, sanitary and environmental requirements. Such practices and as a major component of IPM strategy can conserve the biodiversity by natural balance and minimizing and rationalizing to pesticide use (Alrouechdi, 2000).

Al-Bahi et al. (2000), Steibauer (2003), and Khalaf et al. (2012) have pointed to the possibility of using light traps in a management strategy of insect and date palm borers. This study aims to suppress population density of Arabian rhinoceros beetle, *Oryctes agamemnon arabicus* in Iraqi date palm orchards, through application some of ecological sound control methods.

MATERIALS AND METHODS

Three integrated pest management techniques were field administered to evaluate its efficacies to suppress the population density of *Oryects* spp. in date palm orchards (1 ha each) located in the Almadain district according to the global positioning system: latitude 33.15 north, longitude 44.56 east, and altitude 15.40, 14 feet, 208 SW (30 km south of Baghdad) contains common date cultivars (‘Barhee’, ‘Brem’, ‘Umrani’, ‘Khastawi’ and ‘Zahdi’) during the

[a]E-mail: mkhalaf34@yahoo.co.uk

years 2015-2020. The control options were conducted as follows: first orchard experience: cultural practices (pruning frond bases), hand collection of larvae and light trap for adults capture for five years period. The second orchard experience: cultural practices for two years 2015 and 2017, collecting adults by light trap for one year 2015 only, while no hand collection of larvae was practiced in a five-year period. The third orchard was used as a control treatment, where no cultural practices, no hand collection of larvae and no light trap were practices in five years period. The *Oryctes* spp. larvae were collected and counted from the crown of palm tree during annual sanitation practices start in January till March of each orchard. The numbers of ARB adults were counted daily in each light trap during June and July to determine the population density of adults in each orchard. Meanwhile a Sony HD-R 50 camera was used to image the daily moon phases through the period from June 13/14 to July 12/13 in 2015, June 18/19 to July 17/18 in 2018 to measure the effect of moon light intensity on adults flight ability. In addition, two local entomopathogenic isolates were laboratory tested for their pathogen city against *Oryctes* larvae, which are MARD 34 and 46 (Table 1); selected from the Entomopathogenic Fungal Isolates Bank at the Agricultural Research Directorate, Iraqi Ministry of Science and Technology (Khudhair et al., 2014).

Table 1. Isolates of *Metarhizium anisopliae* and *Beauveria bassiana* used.

Isolate code	Species	Location	Longitude	Latitude	Isolation source
MARD 34	*Metarhizium anisopliae*	Basrah Province	47°77'E	30°68'N	Infected adult of *O. agamemnon arabicus* Date palm and citrus orchard
MARD 46	*Beauveria bassiana*	Dhi Qar Province Nasrea city	46°48'E	31°13'N	Date palm, orchard soil

The spore concentrations were determined using hemocyto meter and adjusted to 1×105, 1×107, 1×109 and 1×1011 conidia mL^{-1}. The four concentrations were applied separately by direct spraying of each on the larvae and on their food. Three replicates (5 larvae each) were used for each treatment. Borer larvae in each replicate were transferred into a new sterilized cage 30×20×22.5 cm. Cages were kept under rearing room conditions 25±2°C and 70% RH and they were checked every three days, counting the dead larvae along with monitoring their behavior and any noticeable morphological changes.

RESULTS AND DISCUSSION

Cultural practices and hand collection of larvae as a control methods

The results in Table 2 indicate the efficacy of cultural practices (sanitation and pruning fronds bases) and hand collection of larvae during annual regular service work in date palm orchards in reducing the population density of *Oryctes* spp. Number of collected larvae reduced from 9.5 to 0.8 and from 10.9 to 5.1 per tree crown in the 1st and 2nd orchards during 2010 and 2015, respectively, in comparison with 11.8 to 21.7 in the 3rd orchard (control). Obviously, the control practices applied decreased the population density of larvae year after year from 2015 to 2018. Such reduction represent in values of 91.6 and 53.0% for 1st and 2nd orchards, respectively, compared with the control orchard. The results of the control orchard showed an increase in larval number collected in 2015 (total 118 larvae) and that in 2018 (total 217 larvae), which is almost two times higher. Such results clearly demonstrated the efficacy of the administered management practices, which could be applied routinely during annual services of date palm orchards. These results are in agreement and confirm that found with *Oryctes elegans* in Iraq date palm orchards (ICARDA, 2011).

Table 2. Effect of integrated control measures on population density of *Oryctes* spp. larvae on date palm orchards in five years period 2015-2020.

No. of tree	Larva tree^{-1} (in crown only) at season 2015-2020					
	Orchard No.1		Orchard No.2		Orchard No.3	
	2015	2020	2015	2020	2015	2020
1	7	0	13	5	10	12
2	4	1	12	4	9	29
3	12	0	11	3	13	31
4	15	0	12	5	14	19
5	10	2	15	2	14	28
6	13	0	9	6	12	22
7	12	2	12	2	13	18
8	14	1	6	7	14	16
9	8	1	9	8	9	17
10	9	1	10	9	10	25
Total	**95**	**8**	**109**	**51**	**118**	**217**
Mean	9.5	0.8	10.9	5.1	11.8	21.7
Reduction%	91.6%		53.0%		---	

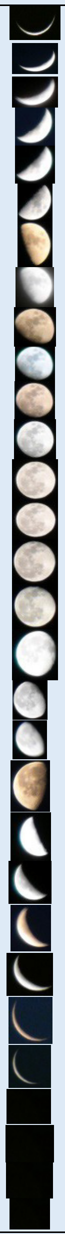
First orchard = annually (cultural practices, light traps, hand collection of larvae) during five years period.
Second orchard = cultural practices once each two years, 2015 and 2017, light trap for one year only 2015, without application of larval collection during five years period.
Third orchard (control) = no cultural practices, no light traps and no hand collection of larvae, during five years period.

Light traps as a control method of adults

The results in Table 3 show that the total adults of *Oryctes* spp. caught during season of 2015 were 134, 157 and 139 adults trap^{-1} month^{-1} in the 1st, 2nd and 3rd orchards, respectively. While, after five years of using light traps to monitor and control, the numbers of adults caught reached 32, 93 and 237 adult trap^{-1} month^{-1} during 2020. These values represent a reduction in adults population of 71.1% in the 1st orchard after five years of following annual cultural practices, hand collection of larvae and light trap during the whole period of experimentation and 41.1% in the 2nd orchard (cultural practices every two years, 2015 and 2017 and light trap for one year 2010, without application of larval collection during the whole period of experiment) compared with the 3rd orchard (as a control treatment). It is obvious that management practices decreased the population density of *Oryctes* spp. adults after five years. It is worth mentioning that the number of captured adults decreased gradually till it reached the lowest number in the mid of Islamic month (11-20) and increased gradually again from 21 to 30 in last final 10 days of Islamic month, which could be effect of moon light on flight ability of adults. These results are in agreement and confirm with which was found by ICARDA (2011) and Khalaf et al. (2011), when they used light traps to monitor and control *O. elegans* in Iraq date palm orchards.

The results presented in Figure 1 show the relation between moon phase light intensity and flight ability of *Oryctes* spp. adults. Results showed that the numbers of adults caught in trap were affected negatively by the light intensity (full moon). Adults number caught were 19, 32 and 30 adult in the 1st, 2nd and 3rd orchards, respectively, compared with 39:76, 48:77 and 42:71 during the first(moon age 1-10 day): last ten days (moon age 21-30 day) in the 1st, 2nd and 3rd orchards, respectively. These results indicated a relation between the flight ability of *Oryctes* spp. adults and moon light intensity. These result match with those reported by Khalaf et al. (2011), Steibauer (2003), and Morton et al. (1981) about impact of brightness of the moon light on *Oryctes elegans*, *Heliothis armigera* and *Mnesampela privata* caught in light traps.

Table 3. No. of *Oryctes* spp. adults caught in light traps in date palm orchards at seasons of 2015-2020.

Islamic month	Moon phase	2015 June/ July	2020 June/ July	Number of *Oryctes* spp. adults (June-July, 2015 and 2020)					
				Orchard No.1		Orchard No.2		Orchard No.3	
				2015	2020	2015	2020	2015	2020
1/2		13/14	18/19	6	2	7	5	6	13
2/3		15/15	19/20	5	1	6	4	5	13
3/4		15/16	20/21	4	2	6	3	6	12
4/5		16/17	21/22	4	1	5	4	6	12
5/6		17/18	22/23	5	1	4	3	2	10
6/7		18/19	23/24	2	0	4	3	2	6
7/8		19/20	24/25	4	1	6	3	5	7
8/9		20/21	25/26	3	0	4	2	5	4
9/10		21/22	26/27	3	1	3	2	2	3
10/11		22/23	27/28	3	1	3	2	3	3
11/12		23/24	28/29	3	0	2	0	2	1
12/13		24/25	29/30	0	1	2	1	2	3
13/14		25/26	30/1	2	0	4	3	3	5
14/15		26/26	1/2	2	0	5	3	3	3
15/16		27/28	2/3	1	1	3	2	2	0
16/17		28/29	3/4	0	0	2	0	2	3
17/18		29/30	4/5	2	0	3	1	2	3
18/19		30/1	5/6	2	1	3	0	2	4
19/20		1/2	6/7	3	1	3	2	3	0
20/21		2/3	7/8	4	1	5	1	5	3
21/22		3/4	8/9	3	0	5	2	4	6
22/23		4/5	9/10	3	0	7	3	6	8
23/24		5/6	10/11	6	1	7	4	7	12
24/25		6/7	11/12	6	1	8	5	6	14
25/26		7/8	12/13	7	2	6	6	5	14
26/27		8/9	13/14	8	2	8	4	8	13
27/28		9/10	14/15	10	3	8	5	9	17
28/29		10/11	15/16	11	2	9	5	8	14
29/30		11/12	16/17	12	3	9	7	8	16
30/1		12/13	17/18	10	3	10	8	10	15
Total				**134**	**32**	**157**	**93**	**139**	**237**
Mean				4.5	1.1	5.2	3.1	4.6	7.9
Reduction %				76.1		41.1%		-	

Survival percentages of *Oryctes* spp. larvae after treating them with entomopathogenic fungal isolate *B. bassiana* MARD 46 spore suspensions revealed that the concentration 1×10^{11} inflicted the highest mortality among larvae reaching 93.33% after 19 days, followed by the concentration 1×10^{9} that recorded mortality of 66.66% at the same time (Figure 2). In addition, mortality reached 53.33% after 19 days using concentration of 1×10^{7} and the lowest mortality was 40% at the concentration 1×10^5 after the same period. All concentrations used decreased larval survivals with time progression reaching 0.0% at the end of the experiment (29 days).

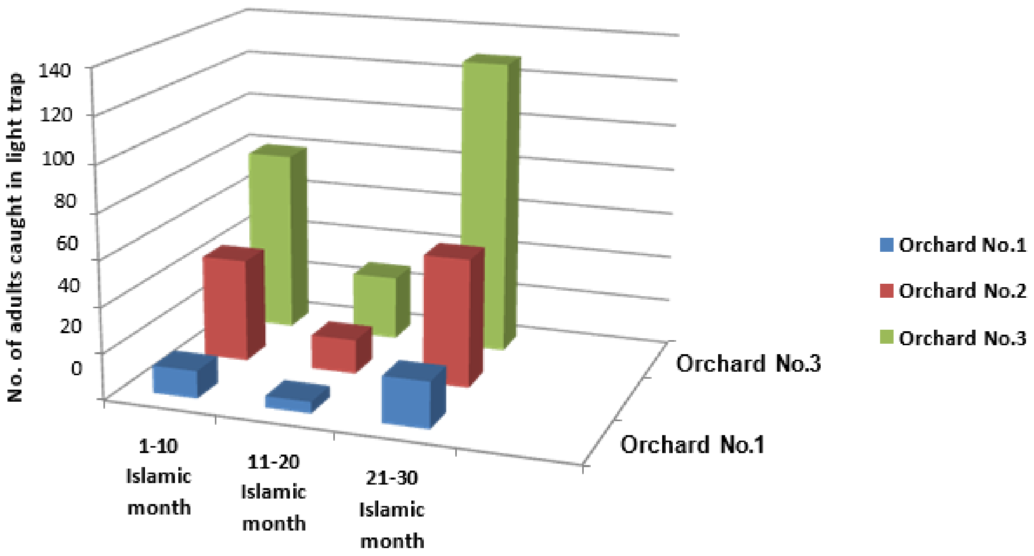


Figure 1. Effect of moon light on flight ability of *Oryctes* spp. in date palm orchards.

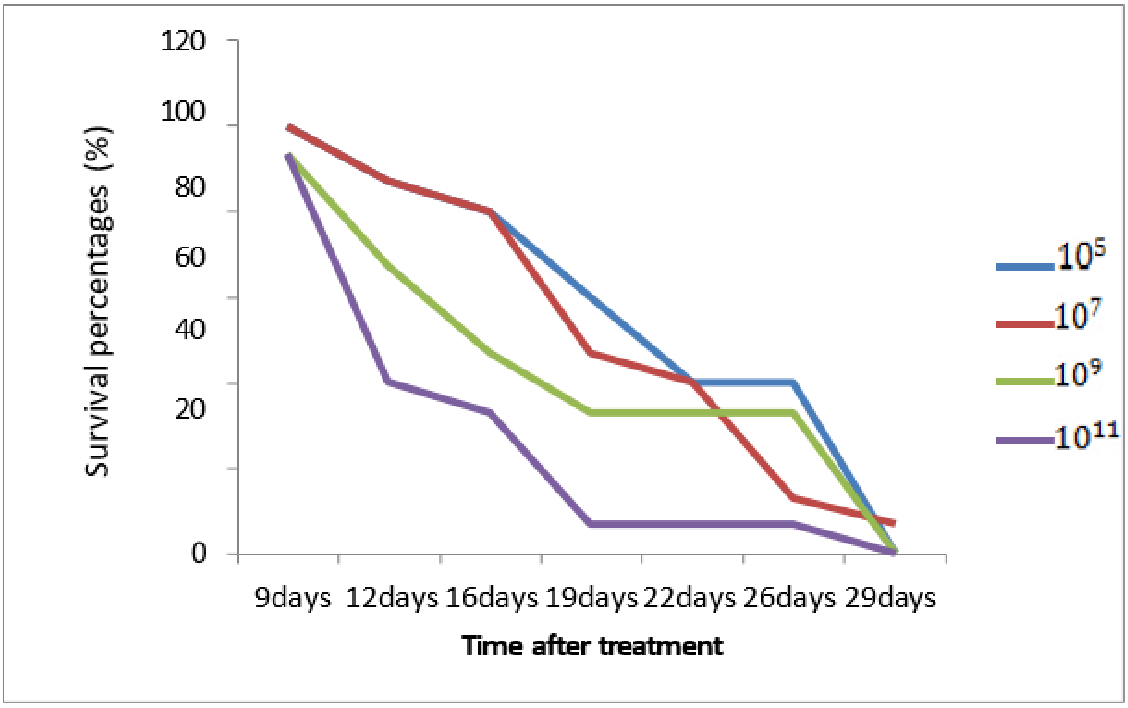


Figure 2. Survival percentages among date palm borer larvae *Oryctes* spp. treated with the *Beauveria bassiana* isolate (MARD46).

Figure 3 illustrates survival percentages of date palm borers' larvae during experimentation duration (29 days) following treated them with different spore suspension concentrations of *M. anisopliae* (MARD 34). The result revealed that the highest mortality after

19 days was 66.66% at concentration 1×10^{11} followed by the concentration 1×10^{9} with 53.33% at the same time. The lowest mortality scored at the concentrations 1×10^{5} and 1×10^{7} reaching 46.66% after 19 days of the treatment. All concentration recorded decrease of survival with time progression reaching 0.0% at the end of the experiment.

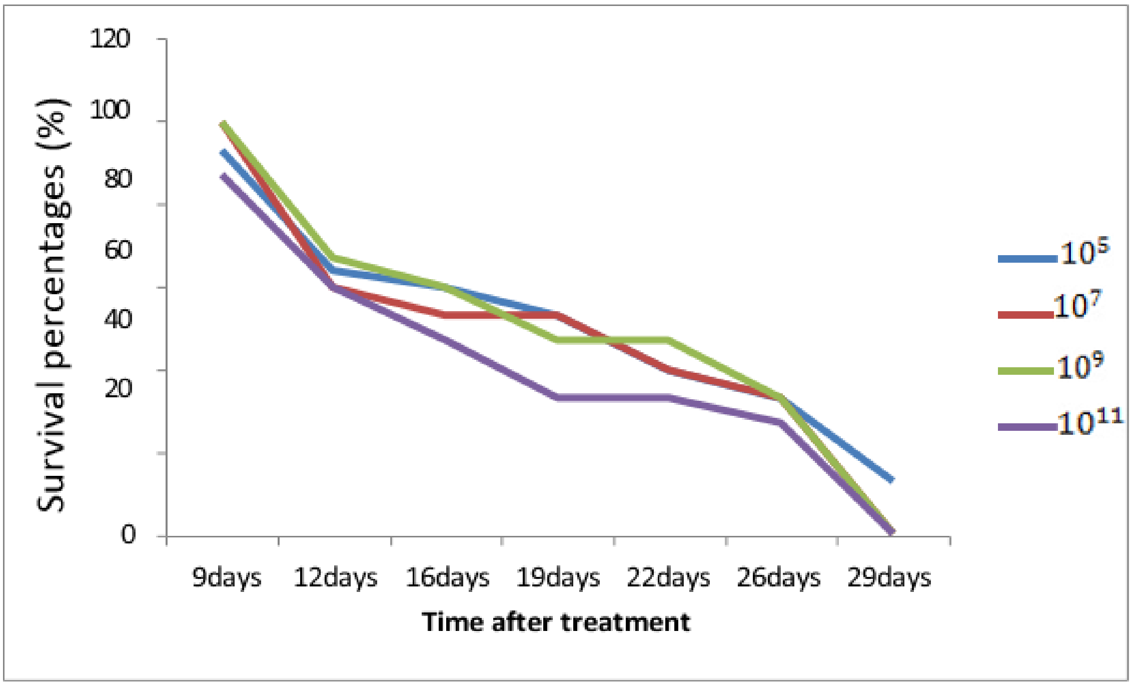


Figure 3. Survival percentages among date palm borer larvae *Oryctes* spp. treated with the *Metarhizium anisopliae* isolate (MARD34).

Ricano et al. (2013) found that using more than one formula of *B. bassiana* can remarkably reduce survival and increase mortality rate among red palm weevil larvae and adults. *B. bassiana* can increase mortality rate of *Helicoverpa armigera* larvae as well as mentioned by Agarwal (2012). Such practice, using entomopathogenic fungi infested of conventional insecticides which failed in achieving acceptable control level, could be effective alternative and sound method from ecological and health viewpoints.

CONCLUSIONS

The results demonstrated the high infestation rates of *Oryctes* spp. to date palm trees. Application of cultural practices, hand collection of larvae, light traps for adults and entomopathogenic fungi as ecological sound pest management's practices showed clearly the efficacies of such practices to control of *Oryctes* spp. (especially *Oryctes agamemnon arabicus* as a dominant species in Iraq) in date palm orchards.

Literature cited

Abass, A.H. (2000). Laboratory and field observation on palm stem borer *Oryctes elegans* (*Coleoptera*: *Scarabaeidae*). Paper presented at: 7th Arab Congress of Plant Protection (Amman, Jordan).

Agarwal, R. (2012). Biopesticidal formulation of *Beauveria bassiana* effective against larvae of *Helicoverpa armigera*. J. Biofertil. Biopestic. *3* (*3*), 1–3.

Al-Bahi, A., Abu-Qeeleh, I., and Al-Dungla, A. (2000). A survey of a carnie species associated with palm trees in Sabah, South Libya. Paper presented at: 7th Arab Congress of Plant Protection (Amman, Jordan).

Al-Khawaja, H.K. (1999). Control of fruit stalk borer *Oryctes elegans* (*Coleoptera*: *Scarabaeidae*) by using agricultural, chemical and biological methods. Nongxue Xuebao *4* (*1*), 20–31.

Alrouechdi, K. (2000). Pests control and biodiversity conservation. Paper presented at: 7th Arab Congress of Plant Protection (Amman, Jordan).

Bedford, O. (1973). Experiments with the virus *Rhabdion virus Oryctes* against the coconut palm rhinoceros beetles *Oryctes rhinoceros* and *Scapanesaustralis groosepunctatus* in New Guinea. J. Inver. Path. *25*, 309–339.

Hussain, A.A. (1974). Date Palm and Dates with Their Pest in Iraq (Iraq: Univ. of Baghdad, Ministry of High Education and Scientific Researches), pp.166.

ICARDA (2011). Integrated control measures for stem borer. Final report 2011. In Improved Livelihoods of Small Farmers in Iraq through Integrated Pest Management and Organic Fertilization. Part: IPM Date Palm – Palm Borers, M.Z. Khalaf, A.A. Ali, and M. El-Bouhssini, eds. (IRAQ-IFAD Project), p.37.

Kaaka, W.A. (2009). Fruit Stalk Borer *Oryctes elegans* (Section: Palm Pests). http://www.iraqi-datepalms.net (in Arabic).

Khalaf, M.Z., and Al-Taweel, A.A. (2015). Palm borers in Iraqi environment: species- damages- methods of control. J. of The Blessed Tree *07* (*01*), 54–64.

Khalaf, M.Z., Naher, F.H., and Ali, A.A. (2010). Population density of *Oryctes elegans* Prell (*Coleoptera*: *Scarabaeidae*) on some date palm varieties in South Baghdad orchards. Agric. Biol. J. N. Am. *1* (*3*), 238–242 https://doi.org/10.5251/abjna.2010.1.3.238.242.

Khalaf, M.Z., Shbar, A.K., Al-Seria, M.H., Sami, R.A., and Naher, F.H. (2011). Some aspects of biology and control methods of fruit stalk borer *Oryctes elegans* Prell (*Coleoptera*: *Scarabaeidae*). J. Agric. Sci. Technol. A *1*, 142–147.

Khalaf, M.Z., Shbar, A.K., Naher, F.H., Jabo, N.F., Abdul-Hamza, B.H., and Sami, R.A. (2012). Activity of insect fauna during the night in the palm orchards of central Iraq. J. Food Sci. Eng. *2*, 277–282.

Khalaf, M.Z., Al-Rubeae, H.F., Al-Taweel, A.A., and Naher, F.H. (2013). First record of Arabian rhinoceros beetle *Oryctes agamemnon arabicus* Fairmaire on date palm trees in Iraq. Agric. Biol. J. N. Am. *4* (*3*), 349–351 https://doi.org/10.5251/abjna.2013.4.3.349.351.

Khalaf, M.Z., Naher, F.H., Khudair, M.W., Hamood, J.B., and Khalaf, H.S. (2014). Some biological and behavioural aspects of Arabian rhinoceros beetle, *Oryctes agamemnon arabicus* Fairmaire (*Coleoptera*: *Scarabaedae*: *Dynastinae*) under Iraqi Conditions. Iraqi J. Agric. Res. *19* (*Special Issue 2*), 122–133.

Khudhair, M.W., Alrubeai, H.F., Khalaf, M.Z., Shbar, A.K., Hammad, B.S., and Khalaf, H.S. (2014). Occurrence and distribution of entomopathogenic fungi in Iraqi agro-ecosystem. Int. J. Entomol. Res. *2*, 117–124.

Morton, R., Taurt, L.D., and Wardhaugh, K.G. (1981). The analysis and standardization of light- trap catches of *Heliothis armigera* (Hubner) and *H. punctiger* wollengren (*Lepidoptera*: *noctuidae*). Bull. Entomol. Res. *71* (*2*), 207–225 https://doi.org/10.1017/S0007485300008245.

Ricano, J., Guerri-Agullo, B., Serna-Sarrias, M.J., Rubio-Llorca, G., Asensio, L., Barranco, P., and Lopez-Llorca, L.V. (2013). Evaluation of the pathogenicity of multiple isolates of *Beauveria bassiana* (*Hypocreales*: *Clavicipitaceae*) on *Rhynchophorus ferrugineus* (*Coleoptera*: *Dryophthoridae*) for the assessment of a solid formulation under simulated field conditions. Fla. Entomol. *96* (*4*), 1311–1324 https://doi.org/10.1653/024.096.0410.

Steibauer, M.J. (2003). Using ultra-violet light traps to monitor autumn gum moth, *Mnesampela privata*, (*Lepidoptera*: *Geometridae*), In south-eastern Austrila. Aust. For. *66* (*4*), 279–286 https://doi.org/10.1080/00049158.2003.10674922.

Role of manganese in curing of date palm leaf brittle disease in El-Kharga Oasis, Egypt

E.F.S. Ahmed[1], A. Talaat[2,a] and A.M.A. Hassan[3]

[1]Central Laboratory for Date Palm Research and Development, Egypt; [2]Soil, Water & Environment Research Institute, Egypt; [3]Central Laboratory for Agricultural Climate, Egypt.

Abstract

Several years ago, a new phenomenon began to appear on the leaves of the 'Saidy' date palm in El-Kharga Oasis in southwestern Egypt. This phenomenon was known as a disease of leaf brittle in the date palm. This disease begins with the leaves losing their elasticity and ending with their brittle completely in hand fist, the development of this disease leads to the death of the palm. Chemical analyses were conducted on adult leaflets and soil samples in an infected and non-infected area. Results showed that concentrations of all nutrients in the tissue were at the normal levels in leaves of unhealthy palms, except manganese, which was lower in the unhealthy palms compared with healthy palms. It could be concluded that adding manganese led to avoiding leaf brittle disease and restoration of the vitality of the palm trees.

Keywords: date palm, manganese, 'Saidy', leaf brittle

INTRODUCTION

The New Valley Governorate is Egypt's largest governorate and one of the biggest on the African continent (Ibrahim et al., 2014). The date palm (*Phoenix dactylifera*) distribution in Egypt covers a great area extending for the far south to north and the far east to west (Al-Wasfy and Mostafa, 2008).

The farmers in New Valley usually do not care about applying micro-nutrients to date palm and they are interested in applying macro-nutrients (NPK) only, which led to a decreasing in the concentrations of micro-nutrients in cultivated soil with the time. So, it was noticed that adult leaflets of date palm have a dull olive green color and lose their elasticity and become brittle and break when squeeze. Affected trees stop growing and eventually die. This phenomenon was known as brittle leaf disease (Figure 1). Brittle leaf disease was accompanied by Mn deficiency in leaflets of date palm (Namsi et al., 2007) without pathogens (Marqués et al., 2012). So, many attempts were conducted to treat this disease by adding manganese by spraying, soil application or trunk injection. In this context, many investigators reported that applying trunk injection with Mn salts was more effective in increasing foliar Mn concentrations than soil or foliar applications (Broschat and Doccola, 2010; Zaen El-Deen et al., 2018).

Figure 1. Symptoms associated with the brittle leaf disease of date palms.

[a]E-mail: ahmed.talaat@arc.sci.eg

Despite the harm of this phenomenon, no studies were conducted in Egypt. This research aims to investigate the effect of magnesium application on preventing brittle leaf disease of date palm.

MATERIALS AND METHODS

Survey study

Brittle leaf disease was diagnosed in five 'Saidy' cultivar date palm orchards in 2019. These orchards are located in El-Kharga oasis, New Valley, Egypt (Figure 2). The study area has a hyper-arid climate with a normal annual precipitation of 0.0 mm. The average annual temperature is 25°C with maximum values of up to 41°C in summer (July) and minimum values as low as 7°C in winter (January) according to the Egyptian Meteorological Authority (2021). 'Saidy' is the main cultivar in this region. Fruitful female palms were selected and they ranged from 10 to 40 years of age.

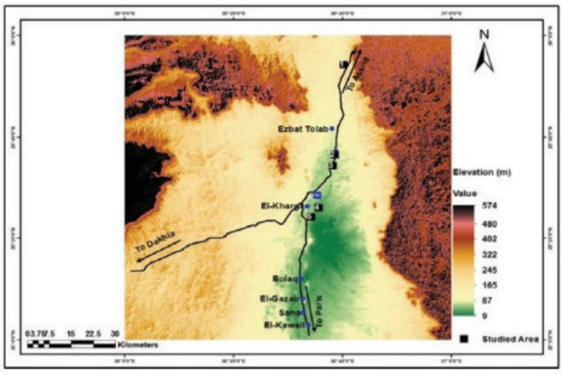


Figure 2. Map of study area.

On brittle leaf disease affected trees a composite sample, containing a minimum of 10 leaflets was collected from the central part of adult fronds at the maturity stage of dates (tamer stage). Leaflet samples were cleaned with distilled water, dried in a forced-air circulation oven at 70°C until constant weight, and ground to 0.5 mm. Leaflet samples were subjected to analysis using the wet digestion in H_2SO_4-H_2O_2 mixture as described by Parkinson and Allen (1975) for determination of N content and nitric digestion for the other nutrients (P, K, Ca, Mg, Fe, Mn and Zn); P content was determined using the molibdovanadate method (colorimetry) described by Miller (1998); N content was determined by distillation using the Kjeldahl method (Horneck and Miller, 1997); nutrients Ca, Mg, Fe, Mn and Zn contents were assessed using atomic absorption spectrometry following the method described by Hanlon (1997).

Two composite samples were collected one from the surface soil (0-30 cm) and the other from the subsurface soil (30-60 cm) under each selected tree. Soil pH in H_2O, total nitrogen, organic carbon, total $CaCO_3$, available P, K, Ca, Mg, Fe, Mn and Zn were done according to Baruah and Barthakur (1997).

Irrigation water samples were collected from each orchard. The pH, EC, Ca, Mg, Na, K, Cl and SO_4 were determined.

Effect of application of Mn on preventing brittle leaf disease of date palm

This study was carried out in one of the aforementioned areas during 2020 and 2021 on 'Saidy' date palms. Fifteen palms were selected under flooding irrigation system, planted at 6×6 m apart and were uniform, at similar age (25 years old). They were subjected to the same management and cultural practices, such as artificial pollination, pruning, irrigation, fertilization and manuring. Bunches were thinned to 10 per palm by removing excess, earliest, latest and smallest ones. Artificial pollination was uniformly performed with respect of source, date and method. Soil application of nitrogen fertilization was as urea (46.5 N) at 2.15 kg palm^{-1} was divided into three equal doses applied three times a year i.e., March, May and July in each season. Potassium sulfate (48% K_2O) at 1.5 kg palm^{-1} was added at two equal doses in middle of April and June. Calcium super phosphate (15.5% P_2O_5) was applied at 1.0 kg palm^{-1}. Farmyard manure (FYM) and calcium super phosphate were mixed and added once in a circle surrounding each palm in the middle of December.

Three replicate palms were randomly assigned to each of the following treatments: 1) control without Mn, 2) soil application 153 g of $MnSO_4.H_2O$ (50 g Mn) palm^{-1} broadcast to the soil surface under the canopy, 3) 10 mL of a 5% Mn solution from $MnSO_4.H_2O$ (=0.5 g Mn), 4) 20 mL of a 5% Mn solution from $MnSO_4.H_2O$ (= 1.0 g Mn), 5) 40 mL of a 5% Mn solution from $MnSO_4.H_2O$ (= 2.0 g Mn). The treatment number 3, 4 and 5 were injected into the trunk about 100 cm above the ground. The injection application was conducted by performing A hole (7-mm diameter) drilled to radius of the trunk, the injector hammered into the hole, the hole around the injector was filled with silicon, the opened end of the tube joined to the bottles of the nutrient solutions by suitable tubes each has a valve to control the flow of each one (Figure 3). All bottles were hanged above the injection hole.

Figure 3. Trunk injection treatment.

The study had five treatments in a simple experiment which were arranged in a complete randomized block design in three replicates and one palm for each.

Leaflet nutrient contents samples were collected at the maturity stage of dates (tamer stage). Twenty leaflets palm^{-1} were collected. Collected samples were prepared and analyzed for macro- and micro-nutrients as mentioned above.

The bunches were harvested when the fruits reached full maturity (last week of September). The yield of the experimental palms was measured. All data were statistically analyzed according to the technique of analysis of variance (ANOVA) as published by Gomez and Gomez (1984), using "MstatC 2.1" Computer software package. Least significant differences (LSD) at 5% was used to test the differences between treatment means.

RESULTS AND DISCUSSION

Survey study

1. Nutrient contents of date palm leaflets.

Data in Tables 1 and 2 show the nutrient contents of date palm leaflets. It is clear that the critical levels of N, P, K, Ca and Mg in date palm leaflets is 11.7, 0.7, 2.0, 7.1 and 1.4 g kg^{-1} dry weight (1.17, 0.07, 0.2, 0.71 and 0.14%). Nitrogen, phosphorus, potassium, calcium and magnesium concentrations in date palm leaflets ranged from 12.2 to 12.6, 0.76-0.83, 2.0-2.8, 7.6-8.8 and 1.76-2.08 g kg^{-1} dry weight (1.22 to 1.26%, 0.076 to 0.083%, 0.2 to 0.28%, 0.76 to 0.88% and 0.176 to 0.208%), respectively. This means that concentrations of these nutrients are in adequate levels under the studied orchards.

Table 1. Average macro-nutrient contents of adult leaflets of date palm from studied orchards.

Orchard	Contents (g kg^{-1} dw)				
	N	P	K	Ca	Mg
Orchard 1	12.4	0.77	2.4	8.4	2.08
Orchard 2	12.6	0.76	2.2	7.6	1.92
Orchard 3	12.2	0.78	2.8	8.8	1.76
Orchard 4	12.6	0.84	2.0	7.6	2.08
Orchard 5	12.2	0.83	2.6	8.8	1.80
Optimum range[a]	11.7-17.1	0.7-0.8	2.0-2.7	7.1-10.8	1.4-2.2

[a]Utilized from Labaied et al. (2020).

Table 2. Average micronutrient contents of adult leaflets of date palm from studied orchards.

Orchard	Contents (mg kg^{-1} dw)		
	Fe	Mn	Zn
Orchard 1	188	22	7.4
Orchard 2	185	22	7.2
Orchard 3	191	23	7.8
Orchard 4	185	22	7.0
Orchard 5	191	23	7.6
Optimum range	160-206	26-82	6.0-8.0

Manganese concentration in date palm leaflets ranged from 22 to 23 mg kg^{-1} dry weight. It was observed that 100% of date palm leaflets were deficient in Mn, where the critical level of Mn is 26 mg kg^{-1} dry weight. Meanwhile, Fe and Zn values ranged between 185 and 191 and 7.0-7.8 mg kg^{-1}. The results indicate that Fe and Zn in the sufficient ranges. This result is in agreement with similar findings Namsi et al. (2007).

2. Soil nutrient content.

Horneck et al. (2011) stated that the N, P and K availability in the soils can be classified as shown in Table 3. The results in Table 4 show that the surface soils (0-30 cm) have a moderate soil available N content in all studied orchard. The highest value (54.3 mg kg^{-1}) of available N was recorded by orchard 4, while the lowest value (42.3 mg kg^{-1}) was recorded by orchard 2. Also, the results indicated that the soil available P content varied from moderate to high. The lowest (12.0 mg kg^{-1}) and highest (25.1 mg kg^{-1}) availability of the soil P in orchard 5 and orchard 3, respectively. Additionally, the results revealed that the soil available K content varied from moderate to very high. Orchard 2 recorded the highest value (168 mg kg^{-1}) of available K, while orchard 4 recorded the lowest value (1050 mg kg^{-1}) of this nutrient.

The availability levels of N, P and K in the studied soil samples in the different sites may be related to the fertilization rates and to the soil parent materials richness in some sources of them such as phosphate rock and shale, as well as soil properties. These results are in agreement with similar findings of Abd El-Rahim et al. (2016) and Tantawy et al. (2017).

Data in Table 3 show the classification of DTPA-extractable Fe, Mn and Zn (Lindsay and Norvell, 1978). The available Fe content in surface soils ranged from 2.41 to 3.61 mg kg^{-1}, so available Fe varied from low to moderate as shown in Table 4. The lowest value is associated with the surface layer of orchard 2, while the highest value was recorded in orchard 1. Regarding the available Mn, its values were ranged from 0.02 to 0.72 mg kg^{-1}. This means that available Mn at the low level according to Lindsay and Norvell (1978). The lowest value was reported in orchard 4, while the highest value is found in the surface layer of orchard 1. Available Zn was more adequate compared with Fe and Mn. Soil content of available Zn varied from moderate to high. Available Zn values ranged from 1.07 to 1.57 mg kg^{-1}. Orchard 1 gave the lowest value, while orchard 4 recorded the highest value of available Zn. Similar observations were recorded by Abd El-Khalik (2004).

Table 3. Classification of some nutrients in studied soils.

Nutrient	Classification			
	Low	Moderate	High	Very high
N (mg kg^{-1})[a]	<40	40-80	>80	-
P (mg kg^{-1})[a]	<10	10-25	25-50	>50
K (mg kg^{-1})[a]	<150	150-250	250-800	>800
Fe (mg kg^{-1})[b]	<2.5	2.5-4.0	>4.0	-
Mn (mg kg^{-1})[b]	<2	2.0	>2	-
Zn (mg kg^{-1})[b]	<1.0	1.0-1.5	>1.5	-

[a]Horneck et al. (2011); [b]Lindsay and Norvell (1978).

Table 4. Available nutrients of soil at 30 and 60 cm in studied date palm orchards.

Orchard	Available nutrients (mg kg^{-1})											
	N		P		K		Fe		Mn		Zn	
	30 cm	60 cm	30 cm	60 cm	30 cm	60 cm	30 cm	60 cm	30 cm	60 cm	30 cm	60 cm
Orchard 1	47.6	13.5	18.6	7.6	359	319	3.61	3.92	0.72	0.57	1.07	2.5
Orchard 2	42.3	9.3	18.8	10.1	1050	560	2.41	2.63	0.52	0.16	1.14	0.62
Orchard 3	46.7	15.0	25.1	15.4	721	601	2.96	2.89	0.70	0.29	1.11	0.91
Orchard 4	54.3	28.6	24.1	12.1	168	187	2.85	2.56	0.02	0.12	1.57	2.32
Orchard 5	48.5	12.4	12.0	6.7	200	188	2.80	2.18	0.38	0.50	1.25	2.07

3. Chemical analysis of irrigation water.

Several groundwater wells were used as irrigation water sources, irrigation waters were analyzed. Their pH ranged from 6.05 to 8.84. EC ranged from 0.65 to 1.40 dS m^{-1}, Ca (0.50 to 4.00 meq L^{-1}), Mg (0.40 to 5.00 meq L^{-1}), Na (0.62 to 7.63 meq L^{-1}) and K (0.40 to 0.50 meq L^{-1}), in addition HCO_3 (0.2 to 5.05 meq L^{-1}), Cl (0.90 to 4.73 meq L^{-1}) and SO_4 (0.20 to 10.33 meq L^{-1}).

Effect of application of Mn on preventing brittle leaf disease of date palm

1. Leaflets nutrient content.

Leaf nutrient contents of 'Saidy' date palm were significantly affected by adding Mn treatments through either soil application or trunk injection as shown in Tables 5 and 6. Results revealed that trunk injection treatments of Mn were more effective compared with soil application. 'Saidy' date palm leaf nutrient contents increased gradually by increasing application levels of Mn trunk injection. In addition, 2.0 g Mn $tree^{-1}$ was the best level of soil injection. The highest values in leaf, N (13.30 and 14.00 g kg^{-1}), P (0.80 and 0.85 g kg^{-1}) and K (2.40 and 2.44 g kg^{-1}), Ca (8.85 and 8.90 g kg^{-1}), Mg (1.55 and 1.50 g kg^{-1}), Fe (221.63 and 22.58 mg kg^{-1}), Mn (62.00 and 63.33 mg kg^{-1}) and Zn (14.57 and 14.84 mg kg^{-1}) concentrations obtained from injected trunk with 2.0 g Mn $tree^{-1}$ compared with the other treatments in the 1st and 2nd seasons, respectively. Meanwhile, control treatment gave the lowest leaf nutrient concentrations in both studied seasons.

The role of direct transfer of nutrients by trunk injection method was studied by Abo-Rekab et al. (2010), Broschat and Doccola (2010) and El-Merghany et al. (2016). They reported that improving leaf mineral content of date palm by trunk injection method.

Table 5. Effect of Mn treatments on leaf macro-nutrients content of 'Saidy' date palm during 2020 and 2021 seasons.

Treatments	Contents (g kg^{-1})									
	N		P		K		Ca		Mg	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Control	12.2	13.0	0.65	0.70	2.04	2.06	7.65	7.75	1.35	1.35
Soil application (50 g Mn tree^{-1})	12.5	13.2	0.65	0.70	2.06	2.10	8.00	8.10	1.45	1.35
Trunk injection (0.5 g Mn tree^{-1})	12.5	13.2	0.75	0.80	2.36	2.38	8.55	8.65	1.45	1.45
Trunk injection (1.0 g Mn tree^{-1})	13.0	13.8	0.80	0.80	2.36	2.40	8.80	8.90	1.50	1.45
Trunk injection (2.0 g Mn tree^{-1})	13.3	14.0	0.80	0.85	2.40	2.44	8.85	8.90	1.55	1.50
LSD 0.05	0.1	0.1	0.07	0.06	0.07	0.07	0.11	0.80	0.04	0.04
Optimum range	11.7-17.1		0.7-0.8		2.0-2.7		7.1-10.8		1.4-2.2	

Table 6. Effect of Mn treatments on leaf micro-nutrients content of 'Saidy' date palm leaflets during 2020 and 2021 seasons.

Treatments	Contents (mg kg^{-1})					
	Fe		Mn		Zn	
	2020	2021	2020	2021	2020	2021
Control	171.0	172.5	25.3	24.3	6.3	6.6
Soil application (50 g Mn tree^{-1})	184.1	185.6	48.0	47.0	6.6	7.1
Trunk injection (0.5 g Mn tree^{-1})	205.4	206.9	56.5	55.5	10.6	10.9
Trunk injection (1.0 g Mn tree^{-1})	219.75	221.3	62.0	62.5	13.9	14.2
Trunk injection (2.0 g Mn tree^{-1})	221.63	222.6	62.0	63.3	14.6	14.8
LSD 0.05	2.7	2.7	1.4	1.6	1.9	1.9
Optimum range	160-206		26-82		6.0-8.0	

2. Fruit yield.

The effect of applying Mn on yield parameters is shown in Table 7. Data showed significant differences between all studied treatments in bunch weight and yield tree^{-1} of 'Saidy' date palm in both studied seasons. Soil application and trunk injection of Mn gave significant increase of these characteristics compared with control (without Mn). Injection of the trunk with 2.0 g Mn $^{-1}$tree showed the best effect on fruit yield characters, where it gave 10.20 and 10.11 kg of bunch weight and 101.95 and 101.10 kg of tree yield in 2020 and 2021 seasons, respectively.

Table 7. Effect of Mn treatments on bunch weight and yield of 'Saidy' date palm during 2020 and 2021 seasons.

Treatments	Bunch weight (kg)		Yield (kg tree^{-1})	
	2020	2021	2020	2021
Control	7.57	7.85	75.70	78.50
Soil application (50 g Mn tree^{-1})	8.40	8.56	83.95	85.55
Trunk injection (0.5 g Mn tree^{-1})	9.31	9.22	93.10	92.15
Trunk injection (1.0 g Mn tree^{-1})	9.74	9.71	97.35	97.10
Trunk injection (2.0 g Mn tree^{-1})	10.20	10.11	101.95	101.10
LSD 0.05	0.42	0.44	4.15	4.35

These results may be due to the role of sufficient Mn on improving vegetative growth and nutrients status of 'Saidy' date palm which led to increase all yield parameters. These results are in agreement with the findings by Abdi and Hedayat (2010) on 'Kabkab', El-Merghany et al. (2016) on 'Sewy', Saleh et al. (2016) on 'Piarom' and Zaen El-Daen (2019) on 'Sewy'.

CONCLUSIONS

It can be concluded that applying 2.0 g Mn tree^{-1} through trunk injection is the most efficient method compared to soil application to curing of date palm leaf brittle disease. It could be added that trunk injection is saving a great amount of micro-nutrients fertilizers and it is an environmentally friendly fertilization method to overcome micro-nutrients deficiency problems in the soil.

Literature cited

Abd El-Khalik, M.A. (2004). Application of GIS in evaluation of some soils In New Valley governorate. M.Sc. thesis (Egypt: Fac. Agric., Zagazig University).

Abd El-Rahim, M.G.M., El-Desoky, M.A., Roshdi, N.M., and Hamed, M.H. (2016). Available phosphorus assessment of Gharb El-Mawhoob soils, El-Dakhla Oasis, Egypt. Assiut J. Agric. Sci. *47* (*4*), 186–200.

Abdi, G.H., and Hedayat, M. (2010). Yield and fruit physiochemical characteristics of 'Kabkab' date palm as affected by methods of potassium fertilization. Adv. Environ. Biol. *4* (*3*), 437–442.

Abo-Rekab, Z.A., Darwesh, R.S.S., and Hassan, N. (2010). Effect of arbuscular mycorrhizal fungi, NPK complete fertilizers on growth and concentration nutrients of acclimatized date palm plantlets. Mesopotamia J. of Agric. *38* (*Supplement 1*).

Al-Wasfy, M.M., and Mostafa, R.A.A. (2008). Effect of different methods of fruit thinning on 'Zaghloul' date palm production and fruit quality. Assiut J. Agric. Sci. *39*, 97–106 https://doi.org/10.21608/ajas.2008.269515.

Baruah, T.C., and Barthakur, H.P. (1997). A Text Book of Soil Analysis (New Delhi: Vikas Publishing House Pvt Ltd.).

Broschat, T.K., and Doccola, J.J. (2010). Effects of soil-applied and trunk and petiole-injected manganese on manganese content of coconut palm (*Cocos nucifera*). Arboric. Urban For. *36* (*6*), 272–274 https://doi.org/10.48044/jauf.2010.035.

Egyptian Meteorological Authority. (2021).

El-Merghany, S.I., Abd El-Rahman, E., and Zaen El-Dean, E.M.A. (2016). Influence of potassium fertilization on Barhee date palms growth, yield and fruit quality under heat stress conditions. Journal of Plant Production *9* (*1*), 73–80.

Gomez, K.A., and Gomez, A.A. (1984). Statistical Procedure for Agricultural Research, 2nd edn (New York: Wiley).

Hanlon, E.A. (1997). Elemental determination by atomic absorption spectrophotometry. In Handbook of Reference Methods for Plant Analysis, K. Yash, ed. (London, UK: CRC Press), p.161–168.

Horneck, D.A., and Miller, R.O. (1997). Determination of total nitrogen in plant tissue. In Handbook of Reference Methods for Plant Analysis, K. Yash, ed. (Boca Raton, FL, USA: CRC Press), p.85–93.

Horneck, D.A., Sullivan, D.M., Owen, J.S., and Hart, J.M. (2011). Soil Test Interpretation Guide. EC 1478-E (Oregon State University), http:// extension.oregonstate.edu/catalog/.

Ibrahim, A.I., Hashem, M.H., Hemeida, A.A., Hassan, M.M., and Maksoud, A.I.A. (2014) Characterization of genetic diversity of date palm (*Phoenix dactylifera* L.) cultivars collected from New Valley governorate (El-Kharga and Dakhleh) based on morphological variability and molecular markers. https://www.researchgate.net/publication/278819162.

Labaied, M.B., Khiari, L., Gallichand, J., Kebede, F., Kadri, N., Ben Ammar, N., Ben Hmida, F., and Ben Mimoun, M. (2020). Nutrient diagnosis norms for date palm (*Phoenix dactylifera* L.) in Tunisian oases. Agronomy (Basel) *10* (*886*), 1–15.

Lindsay, W.L., and Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Am. J. *42* (*3*), 421–428 https://doi.org/10.2136/sssaj1978.03615995004200030009x.

Marqués, J., Duran-Vila, N., Namsi, A., Bové, J.M., and Daròs, J.A. (2012). Bacteria associated with the rhizospere of manganese-deficient date palms affected by brittle leaf disease. J. Plant Pathol. *94* (*1*), 157–169.

Miller, R.O. (1998). Extractable chloride, nitrate, orthophosphate, potassium, and sulfate-sulfur in plant tissue: 2% acetic acid extraction. In Handbook of Reference Methods for Plant Analysis, K. Yash, ed. (Boca Raton, FL, USA: CRC

Press), p.115–118.

Namsi, A., Montarone, M., Serra, P., Ben Mahamoud, O., Takrouni, M.L., Zouba, A., Khoualdia, O., Bové, J.M., and Duran-Vila, N. (2007). Manganese and brittle leaf disease of date palm trees. J. Plant Pathol. *89* (*1*), 125–136.

Parkinson, J.A., and Allen, S.E. (1975). A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological materials. Commun. Soil Sci. Plant Anal. *6* (*1*), 1–11 https://doi.org/10.1080/00103627509366539.

Saleh, J., Hosseini, Y., and Ghoreishi, M. (2016). Is trunk injection more efficient than other iron fertilization methods in date palms grown in calcareous soils? J. Advanced Agricultural Technologies *3* (*3*), 160–163 https://doi.org/10.18178/joaat.3.3.160-163.

Tantawy, M.A., El-Desoky, M.A., Awad, M.Y. and Roshdi, N.M. (2017). Available potassium evaluation of Gharb El-Mawhoob soils, El-Dakhla Oasis, Egypt. Assiut J. Agric. Sci. *48* (*1-1*), 214–228.

Zaen El-Daen, E.M.A. (2019). Effect of fertilization by injection of soil and trunk with NPK on productivity and fruits quality of Sewy date palm. Fayoum J. Agric. Res. Dev. *33* (*1B*), 146–1600.

Zaen El-Deen, E.M.A., Attia, M.F., and Abd El-Hamied, S.A. (2018). Response of pear (Le Conte cv.) trees grown in calcareous soil to trunk injection and foliar application of some micro-nutrients. Alexandria Science Exchange J. *39* (*4*), 747–761.

The use of smart farming techniques to face palm pets and diseases in the Arab region

A. Houtia[a] and F. Houtia

University of Adrar, Adrar, Algeria.

Abstract

The Arab region faces various environmental challenges that have led to a decline in agricultural production and food insecurity due to many factors, including the increasing prevalence of pests and diseases causing losses in crops. Among the crops affected by these pests is palm cultivation that is of great importance in the Arab region; this latter has more than 160 million palm trees producing more than 6.6 million t (78% of global production). The increasing losses caused by palm pests and diseases make combating them a priority within agricultural policies, leading some Arab countries to resort to the use of smart farming techniques to improve palm productivity and to protect their crops from the pests they face, particularly the red palm weevil. The study aims to highlight the pests and diseases suffered by palm cultivation and its effects on date production in the Arab region, highlight the importance of using smart farming techniques to combat palm pests and improve the productivity of this wealth that the region has, with reference to the experiences of some Arab countries.

Keywords: Arab experiences, palm cultivation, diseases, pests, smart farming techniques

INTRODUCTION

The Arab region faces various environmental challenges that have led to a decline in agricultural production and food insecurity due to many factors, including the increasing prevalence of pests and diseases causing losses in crops. Among the crops affected by these pests is palm cultivation that is of great importance in the Arab region; this latter has more than 160 million palm trees producing more than 6.6 million t (78% of global production).There is no doubt that scientific development and progress have given human being many methods, procedures and techniques that have enabled him to control many economic pests on palm trees, among which smart farming techniques, which have contributed to reducing the damage and dangers of these diseases in some countries.

RESEARCH PROBLEM

In the absence of the ability of traditional methods to cope with the pests and diseases of palms prevalent in the Arab region, it is necessary to rely on modern farming techniques and use them to preserve the palm wealth of the region. Therefore, we wonder: to what extent can smart farming techniques be used to combat palm pests and diseases.

IMPORTANCE AND OBJECTIVES OF THE RESEARCH

Agricultural pests and diseases are one of the obstacles to agricultural production in the Arab world, especially those affecting palm wealth and the increased losses caused by them. Hence, the importance of research is in enhancing the potential to benefit from smart farming techniques and their use in the fight against these diseases, after proving their effectiveness. Through this study, we seek to achieve the following objectives:

- Highlighting palm pests and diseases and their effects on date production in the Arab region;
- Highlighting the importance of using smart farming techniques to combat palm pests and improve palm productivity;

[a]E-mail: hotiamar@yahoo.fr

- Tracking the efforts of some countries in the region, especially the GCC countries and their attempts to benefit from the experiences of smart farming, and employ their techniques in the fight against palm diseases.

RESEARCH METHODOLOGY

The descriptive approach has been adopted to highlight the pests and diseases of palm cultivation. The analytical approach was also adopted in demonstrating the extent to which smart farming techniques are used to control these pests and reduce their spread in the Arab region. References and reports of regional and local agricultural organizations and statistical indicators were used to prepare this study.

PALM PESTS AND DISEASES IN THE ARAB REGION

Like any other fruit tree, palms are exposed to many pests and diseases that affect their production, and inevitably lead to a decrease in quantity and quality. Therefore, we are going to get to know about the most important ones in the Arab region.

Concept of agricultural pests and their risks

Agricultural pests are the group of organisms that can be around the economic plant and compete for food, water and space, causing poor growth, low productivity or death. Therefore, it causes a lack of sources of human life, including insects, spiders, snake worms, fungal, viral and bacterial pathogens, weeds, rodents, birds, snails, mollusks, etc. (https://www.researchgate.net/.../almhadrt-5-afat-almhasyl-alzrayt-w/). Pests are among the greatest threats to agricultural wealth in most countries; the risks resulting from them increase, often in relatively large numbers in the agricultural environment. The extent of losses varies from region to region depending on the severity of the damage and the usual transactions and means of resisting them. These risks can be reflected in the following:

1. Decrease in plant production;
2. Decrease in soil productivity and value;
3. Pests and diseases are transported to crops and fruits, and then transmitted to humans, leading to a deterioration of public health. In addition, spending huge amounts to control diseases (Hasona, 2019);
4. Increase in agricultural crop production costs, as in most agricultural crops, including vegetables and fruits, pest control costs 25-35% of the total production cost. The report, released by "London-based Royal Botanic Gardens", suggests that the spread of agricultural pests may cost the world's agriculture sector $ 540 billion a year (https://www.skynewsarabia.com/business/949690-.);
5. The negative impact on the quality of crops themselves; in fact, pests even affect crops after harvesting and storing them, which causes corruption. Such risks and losses make pest control one of the key procedures in agricultural programs.

Pests and diseases facing palms in the Arab region

According to FAO data, global date palm cultivation covers an estimated area of more than 1.09 million has. Total production is over 8.5 million t year^{-1}, while there are about 5,000 species of date palms worldwide. Thus, palm cultivation is particularly concentrated globally in Asia, which accounts for 55.8% of global production, followed by Africa with 43.4% of production (https://www.aljazeera.net/ebusiness/2021/4/27/). Palm cultivation (Arafat, 2012) is one of the main pillars of agricultural activity in the region of 160 million palm trees producing more than 6.6 million, which represents about 78% of the world's total date production of about 8.5 million t (https:// www.mewa.gov.sa/ar/MediaCenter/News/). However, this palm wealth suffers from many diseases, the severity of which varies according to the stages of growth, pathogens, prevailing environmental, climatic conditions and the type of agricultural varieties and factors available. Moreover, these diseases can be classified according to the symptoms they cause, such as *Spetoria*, *Alternaria solani*, charreds, rot, wilting, etc. In addition, according to the plant member, that affects them, such as leaf diseases, stem diseases, root diseases, norovirus diseases and fruit diseases, and a third classification classifies date palm diseases according to the pathogen, called fungal, bacterial, viral, nematodic or physiological diseases (El Meliegi, 2015). According to the latest classification, Table 1 lists some diseases facing palm cultivation in the Arab region.

Table 1. Classification of date palm diseases by pathogen (Arafat, 2012, p.20-30).

Type of diseases/ pests	**Name of diseases**
Fungal diseases	Fusarium wilt disease or sydden decline syndrome; Al Khamedj or inflorescense rot of date palm; Black scorch (majnoon); Leaf spots disease; Balaat disease; Graphiola leaf spot or false smut disease; Fruit rot disease; Diplodia rot disease or diplodia leaf base rot; Alternaria dieback of date palm leaves, *Alternaria* spp.; Thielaviopsis bud rot (stem bleeding, bitten leaf, black scorch, dry basal rot, heart rot); Palm rachis blight; Leaf blight disease or anthrancnose disease; Fruit rot disease; Calyx-end rot; Bayoud disease
Phytoplasmas diseases	Lethal yellowing disease; Al-Wijam disease; Brittle leaves disease
Physiological diseases	White end; F failure of fertilization; Cross cuts disease, crosscuts or transverse notches
Symptoms/phenomena of unknown cause	Bending head; External browning of dates; Barhee disorder; Bastard offshoot; Dry bone disease
Other phenomena	Simple swelling and desquamation of date; Sun blight or sun burn; Yellowing of the inner leaves of date palm (false Bayoud or albinism); Fruit shrivel; Fruit drop; Constriction of date fruits; Pesticide damage on date fruits; Effects of drought; Effects of salinity; Wind injury; Cold injury
Nematode disease	Parasitic nematodes
Bacterial disease	Pink rot of florescence
Insect pests	Termites (Order: *Isoptera*); Longhorn date palm stem borer; Red palm weevil (RPW), Indian palm weevil; Mole cricke; Young date palm borer; Dubas bug or date palm leafhopper; Bark beetle; Giant palm bore; Fruit stalk borers; Date moth; Fronds borer; Date palm giant mealybug; Inflorescence weevil; Date palm leafhopper; Desert locust; Lesser date moth (LDM); Greater date moth; Mediterranean flour moth; Fronds beetle; Date palm inflorescense beetle; Oriental wasp, date red wasp
Harmful weed	*Lactuca serrola* L.; Prickly lettuce (*Compositae*); Green foxtail; Knotgrass; *Polygonum aviculare* L. (*Polygonaceae*); *Zygophyllum simplex* L. (= *Z. portulacoides* Forssk.); Egyptian crowfoot grass, *Dactyloctenium aegyptium* (L). Beauve
Other animal palm pests	Birds; Commensal rodents; Bats; Palm snails; Predatory spider

From Table 1 we see the multiplicity and diversity of diseases to which palms are exposed to at all stages of their growth. There is no doubt that the increasing numbers or density of the pest means entering the stage of damage and economic loss because of the low quantity and quality of production. Therefore, this requires combating them and not wasting time to ensure sufficient effectiveness to achieve the required results. One way to help is the use of smart farming techniques.

Modern methods to control palm diseases

Due to the importance of palm cultivation in many Arab countries, these countries, especially the GCC countries, have made significant efforts in the field of controlling palm diseases. In order to reduce their damage, they have developed many techniques in the field of controlling palm pests and dates, especially red palm weevil (*Rhynchophorus ferrugineus*), including:

1. Tissue palm culture, to produce high quality cultivars, especially rare palm cultivars in large numbers or to produce resistant to certain diseases such as eggs in the Maghreb countries. The cultivation of palm seedlings from tissue culture laboratories means that they are pest-free. Furthermore, tissue transplant laboratories in the Arab countries such as Saudi;
2. Organic agriculture, using the remnants of forest fires, which gives palms strong growth, has evolved and become a successful alternative to the use of agrochemicals such as pesticides and fertilizers. In addition, agricultural areas depended on has expanded in some countries such as UAE, Saudi Arabia, Egypt and Morocco;
3. Using smart technology in agricultural pest control, by adapting modern technology and digital technologies to develop the agricultural sector. We will focus in our study on the potential of their use to control palm diseases;
4. Modern techniques for injecting palm trees with pesticides, including the use of implants Encapsulated to inject palm trunk, and modern devices have been developed to do so (Al Tarihi, 2009, p.42-46).

SMART FARMING TECHNIQUES AND IMPORTANCE OF THEIR USE IN ARAB COUNTRIES

The Arab region is one of the regions facing major environmental crises such as lack of arable water, climate change, drought and desertification, which will lead to decrease in agricultural production and food insecurity. This will make Arab region one of the region's most in need of smart farming techniques.

Concept of smart farming

The Global Economic Forum predicts that the world's population will increase to about 9.7 billion by 2050, and food demand will increase by up to 60%. Therefore, specialists believe smart farming, also known as the Third Green Revolution after the plant breeding and genetics revolution, should be used (Mohamed, 2021; Figure 1) in order to meet the needs.

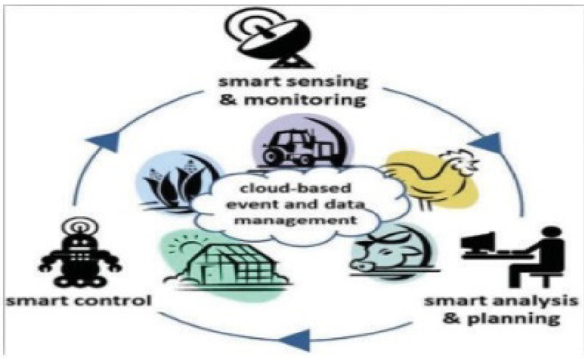


Figure 1. Objectives of smart farming (Mohamed, 2021).

Smart farming objectives

The shift to smart farming will enable the achievement of the following objectives (Hedada, 2018):

1. Promoting agricultural innovation;
2. Creating green jobs;
3. Implementation of sustainable management of natural resources;
4. Protection of the environment through better management of natural resources.

Technology used in smart farming

- Internet stuff technology (IoT): its essence is to extract data from objects and devices that are connected to the internet, enabling farmers to respond quickly to emerging issues and circumstances. Its most important applications include: in greenhouses, by

- monitoring temperature, light levels, humidity and atmospheric pressure, and water consumption within the glass house. So are drones to facilitate oversight tasks;
- Artificial intelligence: in front of the huge amount of daily farm data (temperature, humidity weather, etc.) AI techniques are used to obtain an accurate picture of agricultural land conditions by analyzing and processing data and extracting valuable predictions. For example, knowing the exact time to seeding, irrigation, harvesting, fertilizer, pests, etc. Robots in developed countries are making their way into agriculture, with 'Ox' of the United States opening its first farm in late 2019, where robots act as farmers rather instead of humans (2021);
- Geographic information system (GIS): a system designed to capture, store, manipulate, analyze, manage, and present all types of geographical data. The keyword to this technology is Geography – this means that some portion of the data are spatial, i.e., words, data that is in some way referenced to locations on the earth;
- Global position systems (GPS) is a technology that uses satellites to obtain data that determines our location on Earth very accurately;
- Remote sensing, for example, with a view to obtaining accurate data; for example, remote sensors placed in the fields allow farmers to obtain detailed maps of both terrain and resources in the region. As well as, they measure variables such as acidity, soil temperature and humidity, and can also predict weather patterns for days and weeks to come.

Smart farming techniques used to control palm diseases

Palm diseases have caused significant losses to this sector, for example, the disease of Bayoud has destroyed more than 60% of palms in Morocco, and more than 30% in Algeria. There is no doubt that the use of smart farming techniques will enable the control of many pests of palm trees, contribute to the success of many different control programs, and reduce the damage and dangers of these pests. Among the techniques that can be utilized in this area are (http://meleigi.com/2017/02/14): drones, remote sensors, artificial intelligence, and the use of GIS.

Arab efforts in controlling palm diseases using smart techniques

We will try to focus on some efforts from some Gulf countries that used some of these techniques, among which:

1. United Arab Emirates.

The UAE is the first country in the Arab region to adopt smart farming with the aim of achieving sustainability in agricultural production. Among the research centers distributed across the country, we find the Hamriya Center, which specializes in palms, includes the largest palm complex in the country, and contains integrated pest control laboratories (https://www.alkhaleej.ae/).

2. Saudi Arabia.

Palm cultivation in the Kingdom is exposed to about 225 types of pests, the most dangerous of which is the red weevil. Among the techniques, that Saudi Arabia has benefited from in the fight against palm diseases are GIS. From 2008 to 2014, FAO selected researcher Valerio to lead the international research team between King Faisal University and the Palm Research Center in Al-Ahsa, Saudi Arabia, and FAO. GIS was presented as an effective technology in the world's palm disease control programs. With his research team, he was able to work on an integrated program to combat palm pests, notably the red palm weevil. Based on controlling agriculture at the level of large areas including thousands of hectares, and adopts several levels:

- The human resource that is keen to train him well, and to keep him in the agriculture area consistently;
- The use of traps containing insect repellents or catch them, developed within different laboratories in Al-Ahsa, that take a certain numbering related to their geographical

location on the map;
- Monitoring via GIS technology.(https://researchguides.library.wisc.edu/GIS).

3. Bahrain.

The National Agricultural Sector Development Initiative and the National Space Science Authority and agriculture and marine resources agency at the Ministry of Works, Municipal Affairs and Planning and Arabian Gulf University have signed a contract to implement the project of the early detection system for red palm weevil. In addition, to implement the experience of under-irrigation with the Dutch company 'Smart Farm Sensing' with technical expertise in this field. As the company has implemented similar projects in the region in Saudi Arabia, UAE and Jordan Morocco and Sudan to increase crop production and quality and reduce losses in date production due to pests and irrigation inefficiency. This national project aims to take advantage of the internet of things technology through its use to early detect the presence of red palm weevil in palm trees before its spread, which contributes to the speed of response to this insect. In addition, the second part of the project contributes to the study of determining the tolerance of palms for incomplete irrigation and the impact on the quality of dates and nutritional value. Reduce the waste of irrigation water, and assess the use of hydro rock (water rock) to provide irrigation water for palm trees and reduce loss through what is known as deep leakage, especially under the conditions of the light soil. This latter characterizes the date production areas in the Gulf Arab states and most of its production areas in the Arab world. The study also evaluates the experience of using soil moisture data and weather factors to manage irrigation water in integration with artificial intelligence systems and smart farm management (https://www.bna.bh/).

Despite efforts, the introduction of smart palm-growing technologies faces difficulties, including:

- Poor communications and Internet infrastructure in some Arab countries;
- These techniques needs some skills that many agricultural workers do not have;
- The material cost that is an obstacle to many (Khaldi, 2020);
- How to activate the role of scientific research centers in finding solutions to diseases that have not been completely eliminated.

CONCLUSIONS

This study shows the several benefits that smart farming will bring to the agricultural sector; including supporting the efforts of some, Arab countries, especially the GCC countries, in developing an important sector, namely palm cultivation. In addition, taking advantage of its techniques in controlling palm diseases and pests, and we have reached the following results:

- The implementation of smart agriculture in the Arab countries aims to develop and improve the agricultural system, which is dominated by the use of traditional methods. Also, to try to take advantage of smart farming techniques in the fight against agricultural pests;
- Palm pests are among the greatest threats to agricultural wealth in the Arab region, and have significant damage to date production and quality;
- The use of smart farming techniques, which have many means, will enable the control of many palm pests and reduce their damage and dangers;
- The GCC countries are making great efforts to control palm diseases to adopt smart technologies by taking advantage of international experiences and making agreements with international companies in this field;
- Some palm diseases, including red palm weevil, can be eliminated by taking advantage of international experiments, such as the Canary Islands, which eliminated the disease in May 2016.

Based on the findings of this study, the following recommendations can be made:

- Create an enabling policy environment to overcome barriers to the adoption of intelligent palm-growing techniques;
- Encourage private investment, particularly in the areas of soil conservation, disease

control, infrastructure such as secondary roads and energy, electrical supplies and wireless communication networks in palm plantation areas;
- Encourage innovation, participatory research, knowledge and education on the use of smart palm farming techniques;
- Support joint Arab action and share knowledge and ideas about smart farming technology and its uses;
- Strengthening cooperation and coordination between Arab countries in the field of early warning and sharing of information and knowledge, in order to effectively control widespread diseases such as Bayoud and red palm weevil;
- Develop human capabilities in the use of smart technologies as (GIS) to take advantage of them in the fight against palm pests.

Literature cited

Al Tarihi, I.H. (2009). Modern Techniques in the Fight against Palm Pests and Dates, the Blessed Tree (United Arab Emirates: Khalifa International Date Palm Award).

Arafat, K.H. (2012). Date palm diseases. Paper presented at: The First Regional Conference on Date Palm Pest Management (Al Ain, United Arab Emirates: Arab Development Organization Agriculture, League of Arab States).

El Meliegi, M.A. (2015). Dates palm diseases in Saudi Arabia and ways to combat them.

Hasona, M. (2019) How crop pests negatively affect the agricultural area. https://www.ts3a.com/

Hedada, A. (2018). 'Smart Farming' and Its Areas of Application in the Arab World (Arab Chambers).

Khaldi, A. Al. (2020). Smart Farming Green Revolution to Achieve Food Security UAE as a Model. https://omran.org/ar/ 04/02/2022.

Mohamed, H.R. (2021). The future of smart farming technologies and food security globally and in the Arab world: the experience of the United Arab Emirates. https://omran.org/ar 06/02/2022.

Stenotrophomonas sp. associated with leaf spot and leaf blight diseases of date palm in Iran

M. Azadvar[1,a], M. Rezaee[2] and Z. Azami Sardoee[2]

[1]Plant Protection Research Department, Kerman Agricultural and Natural Resources Research and Education Center, AREEO, Kerman, Iran; [2]Plant Protection Department, College of Agriculture, University of Jiroft, Jiroft, Iran.

Abstract

Leaf spot and leaf blight are the most important and new diseases of date palm in the world as well as Iran. This research was aimed to study the association of the bacteria with leaf spot and leaf blight diseases of date palm in Kerman province, Iran. For this purpose, during 2018-2019, date palm leaves showing leaf spot and blight symptoms were sampled from different areas of the province. Isolation of bacteria from the collected samples was performed according to the standard plant bacteriological method. Pathogenicity of the isolates was investigated by inoculation on midrib and leaflets of date palm 'Mazafati' cultivar using attached and detached leaves for several times. Selected bacteria isolates were identified by performing the key phenotypic bacteriological tests and nucleotide sequence analysis of a part of 16S rRNA gene after PCR assay. In this study, a total of 15 bacterial strains were isolated from the collected samples. Artificial inoculation tests showed that four isolates were able to cause mild leaf spot symptoms on date palm leaves. The results of phenotypic and molecular analysis revealed that the selected isolates were related to *Stenotrophomonas* sp. This is the first report on association of a bacterium with date palm leaf spot and leaf blight diseases in Iran and introduction of *Stenotrophomonas* sp. as an opportunistic pathogen on date palm in the world.

Keywords: bacteria, date palm, opportunistic pathogen, 16S rRNA, PCR

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) cultivation is one of the main horticultural activities in south of Iran. Plant pathogens, mainly fungi and prokaryotes, cause considerable losses on date production in the world and Iran as well. Leaf spot and leaf blight have been the two main newly emerged, unknown etiology, and widespread diseases on all date palm cultivars of date-producing provinces of southern Iran during the last decade. A few studies have been conducted on leaf blight and leaf spot diseases of date palm in Iran. Symptoms include spots in different sizes, shapes and colors on all parts of the leaves, and yellowing and early drying of the lower leaves. Leaf spot and blight are caused by a wide range of the pathogens worldwide. Several fungi including *Nigrosporas phaerica*, *Graphiola phoenici*, *Alternaria* spp., pseudo pest *Alotiopsis theae*, *Helmintosporium* sp., *Derschlera spicifera*, *Fusarium torulosum*, *Sordaria fimicola*, *Serenomyces phoenicisi*, *Pestalotiopsis* sp., *Chaetomium* sp. and *Nattrassia mangifera* (Al-Rokibah, 1991; Fröhlich et al., 1997; Livingston et al., 2002; Fayad and Mania, 2006; El-Deeb et al., 2007; El-Gariani et al., 2007; Najafinia and Azadvar, 2008; Ammar and El-Naggar, 2011; Abass et al., 2013; Alam et al., 2020; Namsi et al., 2019; Al-Nadabi et al., 2020) have been reported in association with leaf spot and leaf blight diseases of date palm in the different countries. So far, no bacteria have been reported in association with date palm leaf spot and blight disease in the world. *Serratia marcescens* and *Pseudomonas alboprecipitans* have been reported as causal agent of inflorescence pink rot and leaf blight bacterial diseases of palm, respectively (Knauss et al., 1979; Riaz et al., 2009).

Little information is available on etiology of leaf spot and leaf blight diseases of date palm in Iran. The aim of this study was to isolate and identify the bacteria associated with leaf

[a]E-mail: mehdiazadvar@gmail.com

blight and leaf spot diseases of date palm trees in Kerman province, Iran.

MATERIALS AND METHODS

Disease survey and sampling

Surveys were conducted during February 2017 to June 2019 in order to diagnose leaf spot and leaf blight diseases of date palm and isolate the associated bacteria in Kerman province of Iran. A total of 53 leaf samples showing leaf spot and leaf blight symptoms were collected from 12 different regions on eight commercial date palm cultivars.

Isolation and purification of bacteria

The collected leaf samples showing leaf spot and leaf blight symptoms were washed with tap water, surface sterilized with 0.5% sodium hypochlorite, then washed thoroughly with sterilized distilled water and subjected for bacteria isolation according to the standard plant bacteriological methods (Schaad et al., 2001). The isolated bacteria were purified by streak method and the selected single colonies were stored on distilled water and culture media for further use.

Pathogenicity tests

Pathogenicity tests were conducted for all of the 15 bacteria isolates on leaf midribs and leaflets of date palm 'Mazafati' cultivar using attached and detached leaves for several times. The leaves were first surface disinfected with 1% sodium hypochlorite, washed with sterilized distilled water, injured by a sterilized needle (on leaflet) or scalpel (on midrib), and inoculated with each of the bacteria isolates by suspension spray method. Injured leaves were sprayed with sterilized distilled water as control. The inoculated leaves were covered with transparent plastic bags for a week. After pathogenicity confirmation, bacteria were isolated on nutrient agar medium from the symptomatic leaves (Schaad et al., 2001).

In vitro antagonistic activity test

The selected bacteria isolates were used in direct confrontation method (Abdennabi et al., 2017) in order to investigate their putative antagonistic activity against three fungal leaf spot associated fungi i.e., two *Alternaria* spp. and a *Colletotricum* sp. isolate (kindly provided by Dr. Mousa Najafinya, Iranian Research Institute of Plant Protection, Tehran, Iran). The antagonistic activity of the bacterial isolates was evaluated by presence or absence of the inhibition zone, a week after treatment.

Identification of the bacteria isolates

The four bacteria isolates, namely N2, N4, N6 and N7, that induced mild leaf spot and blight symptoms in pathogenicity assays, were tested for some key phenotypic bacteriological tests as Gram staining, catalase, oxidase, lecitinase, levan formation, starch and gelatin hydrolysis, and potato soft rot by the standard protocols (Schaad et al., 2001). Fine identification of the bacteria isolates was done through PCR amplification and sequencing of a part of the 16S rRNA gene.

DNA extraction and polymerase chain reaction

Total genomic DNA was extracted from the selected bacteria isolates using a CTAB based protocol as described by Wilson (1987). Quality and quantity of the extracted DNA were assessed and adjusted with spectrophotometer (Nano DroponeC, USA) according to the manufacturer's instruction.

Polymerase chain reaction for amplification a part of 16 rDNA was performed using universal prokaryote primers fD1 andrP1 (Weisburg et al., 1991) in a total volume of 50 µL reaction mixture containing 50 ng of DNA template, 2 µL of each the oligonucleotides (10 pm) and 25 µL of Taq 2× PCR master mix Red, 1.5 mm $MgCl_2$ (Ampliqon, Denmark). The thermo-cycling conditions consisted of an initial denaturation at 94°C for 5 min., followed by 35 cycles by denaturation at 94°C for 50 s, annealing at 52°C for 50 s, extension at 72°C for 1 min and

45 s, and the final extension at 72°C for 10 min using Master Cycler (Eppendorf, Germany) thermocycler.

PCR product and nucleotide sequence analysis

PCR products were electrophoresed in 1% agarose gel containing ethidium bromide. A part of the PCR products were directly subjected for bi-directionally nucleotide sequencing (Bioneer, South Korea). The obtained sequences were edited and assembled with the BioEdit v 7.1.9 program (Hall, 1999), and were aligned and compared with databases in the Gene Bank, using the NCBI Basic Local Alignment Search Tools (BLASTn) program (http://www.ncbi.nlm.nih.gov/BLAST). The phylogenetic tree was constructed by maximum likelihood analysis using MEGAX software (Kumar et al., 2018) using reference sequences from GenBank.

RESULTS AND DISCUSION

The collected leaf samples showed high variation of leaf spot symptoms varied from 1 mm to 2 cm in size, irregular to rectangle in shape, yellow to brown color, with or without water soaking or yellowing margins, and with or without greyish center. The spots were most prevalent on the leaf midribs, than the leaflets and spins. Results showed that although all the commercial date palm cultivars were susceptible to leaf spot, but leaf blight symptoms were mostly observed on 'Mazafati' and 'Kalote' cultivars. The symptoms were mostly apparent in April and October and the upper young leaves were never infected by the diseases. Young palms and palms inter-cropped with alfalfa showed more symptom severity than the old ones, and non-intercropped orchards.

In this study, a total of 15 bacterial isolates were isolated from the collected samples. Among these isolates, only four isolates namely N2, N4, N6 and N7 were able to cause mild symptoms on the detached date palm midrib of 'Mazafati' cultivar. The same isolates could produce leaf spot on the leaflets in detached method and extensive blotch in the attached method during summer in the pathogenicity test.

Selected isolates were identical in phenotypic features as Gram positive, catalase and oxidase positive, growth in presence of 3% NaCl but not in 5%, Negative in levan formation and starch hydrolysis but positive in gelatin hydrolysis, lecinitase, and potato soft rot tests (Schaad et al., 2001). The expected 1.5 kb amplicons corresponding to sequences of the bacterial 16S rRNA gene were amplified from the N2, N4, N6 and N7 isolates (Figure 1).

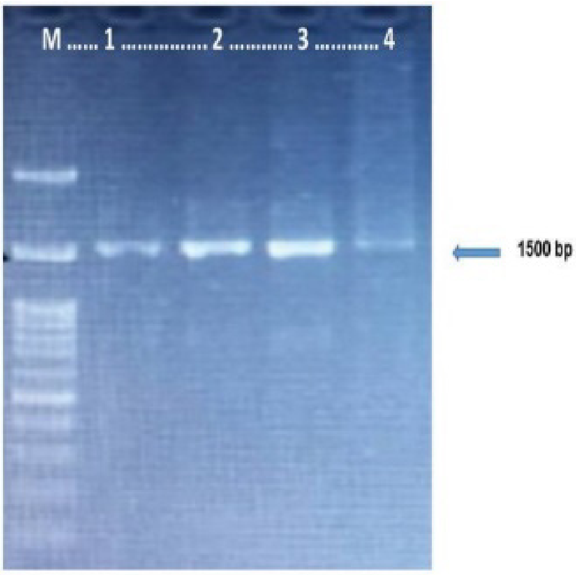


Figure 1. Agarose gel photograph of PCR products from the 16S rRNA gene using fD1/rP1 primer pairs. 1-4: date palm isolates, M: DNA size marker.

BLAST analyses of the sequences indicated that the bacteria isolated from date palm leaf spot and leaf blight diseases in Iran were identical, and also showed the highest similarity (99.9%) with the 16S rDNA sequence of different species of *Stenotrophomonas* sp. including *S. maltophilia*. Based on this, bacterial isolates N2, N4, N6 and N7 isolated from leaf spot and leaf blight of palm trees in Iran belonged to the genus *Stenotrphomonas* sp. The phylogenetic tree constructed based on the 16S rDNA sequences confirmed that the *Stenotrophomonas* sp. isolates N2, N4, N6 and N7, obtained from date palm leaf spot and leaf blight diseases in southern Iran, were more closely related and clustered with *Stenotrophomonas maltophilia* (GenBank acc. No. LT222224) and *Stenotrophomonas cyclobalanopsidis* strain TPQG1-4 (GenBank acc. No. MNO36524) (Figure 2). Species identification of these isolates requires sequence analysis of the less conserved genes other than 16S rRNA such as the gyrase gene.

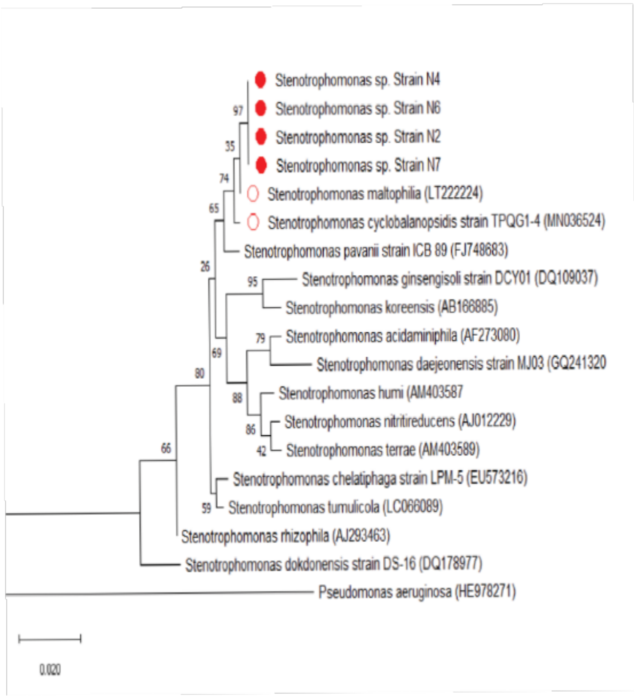


Figure 2. Phylogenetic tree constructed by maximum likelihood analysis of 16S rRNA sequences of the *Stenotrophomonas* isolates associated with date palm leaf spot and leaf blight diseases from Iran and some of the *Stenotrophomonas* species from GenBank. *Pseudomonas aeroginosa* was used as out group.

Now, the genus *Stenotrophomonas* includes 18 different species with different ecological characteristics and includes clinical and environmental isolates. Among them, several species such as *S. maltophilia*, *S. rhizophila*, *S. chelatiphaga*, *S. acidaminiphilia* and *S. pavane* showed the ability to enhance plant growth and are considered as plant growth promoting bacteria. In addition, some species of *Stenotrophomonas* show antagonistic activity against plant and human pathogenic fungi and bacteria and have been introduced as biological control agents (Dunne et al., 1997; Wolf et al., 2002; Ryan et al., 2009; Nayomi and Thangavel, 2017). The four isolated bacteria in the current study had no antagonistic effect on the date palm leaf spot associated fungi by in vitro test (data not shown).

On the other hand, some of the species such as *Stenotrophomonas cyclobalanopsidis*, has recently been introduced and described as the causative agent of leaf spot disease in *Cyclobalanopsis patelliformis* (Bian et al., 2020). To the best our knowledge, this is the first report of the association of a *Stenotrophomons* sp. with leaf spot and leaf blight diseases of palm and introduction of *Stenotrophomonas* sp. as an opportunistic or a weak pathogen on palm trees in the world.

CONCLUSIONS

The current study provided evidence for association of *Stenotrophomonas* sp. with leaf spot and leaf blotch diseases of date palm in Iran. Further in depth studies are needed to molecular interaction of *Stenotrophomonas* sp. with date palm, species identification of the bacterium, and pathogenicity of the bacterium on different date palm cultivars under various weather conditions.

ACKNOWLEDGEMENTS

Financial support of the Agricultural Research, Education and Extension Organization, AREEO (project number 24-70-16-130-981197) is gratefully acknowledged. Thanks to Mobina Azadehvar for editing the manuscript.

Literature cited

Abass, M.H., Hameed, M.A., and Ahmed, A.N. (2013). First report of *Nigrosporasphaerica* (Sacc.) Mason as a potential pathogen on date palm (*Phoenix dactylifera* L.). Can. J. Plant Pathol. *35* (*1*), 75–80 https://doi.org/10.1080/07060661.2012.732612.

Abdennabi, R., Triki, M.A., Ben Salah, R., and Gharsallah, N. (2017). Anti-fungal activity of endophytic fungi isolated from date palm sap (*Phoenix dactylifera* L.). EC Microbiol. *13*, 123–131.

Al-Nadabi, H., Maharachchikumbura, S.S.N., Al-Gahaffi, Z.S., Al-Hasani, A.S., Velazhahan, R., and Al-Sadi, A.M. (2020). Molecular identification of fungal pathogens associated with leaf spot disease of date palms (*Phoenix dactylifera*). All Life *13* (*1*), 587–597 https://doi.org/10.1080/26895293.2020.1835740.

Al-Rokibah, A. (1991). Leaf blight of date palm caused by *Glomerella cingulate* in Al-Qassim region. J. King. Saud. Univ. *3*, 109–115.

Alam, M.W., Rehman, A., Ahmad, S., Sarwar, M., Nawaz, A., Khan, S.M., Ali, S., Aslam, S., and Mannan, A. (2020). First report of *Nigrosporasphaerica* causing leaf spot of date palm in Pakistan. J. Plant Pathol. *102* (*1*), 223 https://doi.org/10.1007/s42161-019-00360-0.

Ammar, M.I., and El-Naggar, M.A. (2011). Date palm (*Phoenix dactylifera* L.) fungal diseases in Najran, Saudi Arabia. Int. J. Plant Pathol. *2* (*3*), 126–135 https://doi.org/10.3923/ijpp.2011.126.135.

Bian, D.R., Xue, H., Piao, C.G., and Li, Y. (2020). *Stenotrophomonas cyclobalanopsidis* sp. nov., isolated from the leaf spot disease of *Cyclobalanopsis patelliformis.* Antonie van Leeuwenhoek *113* (*10*), 1447–1454 https://doi.org/10.1007/s10482-020-01453-y. PubMed

Dunne, C., Crowley, J.J., Moënne-Loccoz, Y., Dowling, D.N., Bruijn, S., and O'Gara, F. (1997). Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. Microbiology (Reading) *143* (*12*), 3921–3931 https://doi.org/10.1099/00221287-143-12-3921. PubMed

El-Deeb, H.M., Lashin, S.M., and Arab, Y.A. (2007). Distribution and pathogenesis of date palm fungi in Egypt. Acta Hortic. *736*, 421–429 https://doi.org/10.17660/ActaHortic.2007.736.39.

El-Gariani, N.K., El Rayani, A.M., and Edongali, E.A. (2007). Distribution of phytopathogenic fungi on the coastal region of Libya and their relationships with date cultivars. Acta Hortic. *736*, 449–455 https://doi.org/10.17660/ActaHortic.2007.736.42.

Fayad, M.A., and Mania, A.O. (2006). Study of date palm leaf spot disease in Basrah, Iraq and the relation of age of palm and wax content with Infection. Abstract presented at: Ninth Arab Congress of Plant Protection (Damascus, Syria).

Fröhlich, J., Hyde, K.D., and Guest, D.I. (1997). Fungi associated with leaf spots of palms in north Queensland, Australia. Mycol. Res. *101* (*6*), 721–732 https://doi.org/10.1017/S095375629600322X.

Hall, T.A. (1999). BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. *41*, 95–98.

Knauss, J.F., Miller, J.W., and Virgona, R.J. (1979). Bacterial blight of fishtail palm, a new disease. Proc. Fla. State Hon. Soc. *91*, 245–247.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol *35* (*6*), 1547–1549 https://doi.org/10.1093/molbev/msy096. PubMed

Livingston, S., Al Mufargi, K., and Al Sunkeli, M. (2002). Chemical control of leaf spot of date palm (*Phoenix dactylifera*) in Sultanate of Oman. Plant Pathol. J. *18* (*3*), 165–167 https://doi.org/10.5423/PPJ.2002.18.3.165.

Najafinia, M., and Azadvar, M. (2008). Leaf die-back of date palm as caused by *Nattrassiae mangiferae* in Kerman

Province. Iranian J. Plant Prot. Sci. *39*, 25–30.

Namsi, A., Gargouri, S., Rabaoui, A., Mokhtar, N., Takrouni, M.L., Moretti, A., Masiello, M., Touil, S., Dieb, L., and Werbrouck, S.P.O. (2019). First report of leaf blight caused by *Alternaria mali* and *A. arborescens* on date palm (*Phoenix dactylifera*) in Tunisia. Plant Dis. *103* (*11*), 2962 https://doi.org/10.1094/PDIS-05-19-1121-PDN.

Nayomi, J., and Thangavel, M. (2017). *Stenotrophomonas maltophilia*: a novel plant growth promoter and bio-control agent from marine environment. Int. J. Adv. Res. (Indore) *5* (*4*), 207–214 https://doi.org/10.21474/IJAR01/3797.

Riaz, M., Kumar, V., Mansoury, E., Al-Kandari, F., Al-Attar, E., and Al-Ameer, F. (2009). Pink rot of inflorescence: a new disease of date palm in Kuwait. Mycopath *7*, 1–4.

Ryan, R.P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M.B., Berg, G., van der Lelie, D., and Dow, J.M. (2009). The versatility and adaptation of bacteria from the genus *Stenotrophomonas.* Nat Rev Microbiol *7* (*7*), 514–525 https://doi.org/10.1038/nrmicro2163. PubMed

Schaad, N.W., Jones, J.B., and Chun, W. (2001). Laboratory Guide for the Identification of Plant Pathogenic Bacteria, 3rd edn. (American Phytopathological Society (APS Press)).

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol *173* (*2*), 697–703 https://doi.org/10.1128/jb.173.2.697-703.1991. PubMed

Wilson, K. (1987) Preparation of genomic DNA from bacteria. In Current Protocols in Molecular Biology, F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, eds. (New York: Wiley & Sons), p.2.4.1–2.4.5.

Wolf, A., Fritze, A., Hagemann, M., and Berg, G. (2002). *Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal properties. Int J Syst Evol Microbiol *52* (*Pt 6*), 1937–1944. PubMed

Operationalized multi-stakeholders research, technology and innovation platform to enhance date palm integrated production system in the NENA region

R. Shibli[1,2,a], N. Thaher[3], M. Malkawi[4], A. Swelam[5] and S. Ramasamy[5]

[1]Association of Agricultural Research Institutions in the Near East and North Africa, Baqaa, Jordan; [2]Faculty of Agricultural Technology, Al-Ahliyya Amman University, Balqaa, Jordan; [3]National Agricultural Research Center, Baqaa, Jordan; [4]Jordan University of Science and Technology, Irbid, Jordan; [5]Food and Agriculture Organization of the United Nations, Innovation, Research and Extension Unit, Rome, Italy.

Abstract

Date palm (*Phoenix dactylifera* L.) is one of high economical value crops in the NENA region in general and the Arab Peninsula and North Africa Sub Regions in particular where date palm represents a large cultivated area with an estimation of 1.30 million ha. However, constraints to production, improved genetic resources, integrated pest management, postharvest handling and processing, marketing and trade, limit the competitiveness of the date palm sector in local, regional and international markets. On the other hand, the region is experiencing a huge knowledge gap, as the knowledge produced locally is not easily and widely accessible. This platform aims at establishing an interactive and collaborative multi-stakeholder regional platform to promote the date palm. The main objectives of this platform are to interconnect relevant stakeholders effectively, to share, exchange and consolidate available knowledge, technologies and innovations related to date palm value chain, and to actively contribute to addressing major problems in the date palm production. The platform was designed to facilitate a full cycle model from idea to innovation and vice versa including back end server for storing data, processing information, providing artificial intelligence support and providing web services to communicate with other parts of the platform. Front end application provides interface to various stakeholders and displays reports and statistics via dashboard. The platform is characterized by active dashboard for discussion, forum, projects, news, activities, reports and statistics and performed as a knowledge hub that effectively utilize any new opportunities in an economic or social process. The operationalized platform demonstrates a model of collaboration among relevant stakeholders on research, development, innovations, regulations and funding. The platform also identifies a set of research priorities, gaps and practices, promising technologies and technical innovations. Therefore, this generated knowledge with better integration among stakeholders, enhanced learning processes with multiple benefits, and created business opportunities for stakeholders.

Keywords: knowledge, interconnect, collaborative, back end, front end, server, model

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the main crops grown in the Near East and North Africa (NENA) region and especially at the Arab Peninsula and North Africa Sub-Regions where the date palm represents a large cultivated area with an estimation of 1.30 million ha (Cheng and Krueger, 2007). It is an important part of the religious, cultural, and economic heritage of the Arabian Peninsula and it is one of the most important income-generating crops, providing a major source of export earnings and livelihood for millions of farmers (Aregawi et al., 2018). The production and distribution systems of the date palm crop dominate value chains through modernized sustainable systems and expanding exports

[a]E-mail: r.shibli@cgiar.org

which contribute to the achievements of a number of sustainable development goals (SDGs) (Xia, 2020). However, constraints to production, improved genetic resources, integrated pest management, postharvest handling and processing, marketing and trade, limit the competitiveness of the date palm sector in local, regional and international markets. On the other hand, the region is experiencing a huge knowledge gap as the knowledge produced locally is not accessible widely with necessary improvement. The economic, social, environmental and nutritional importance of the date palm sector in the NENA region, and its important role in improving the livelihood of rural people and food security in rural areas, are contributing to achieve sustainable agricultural development (Arias et al., 2016). However, challenges in the production, marketing and trade, improved genetic resources, pests and diseases, postharvest handling and processing limit the competitiveness of the date palm sector in local, regional and international markets (Arias et al., 2016). Nevertheless, opportunities exist for the expansion of date palm cultivation areas through the application of sustainable crop, soil and water management techniques, as well as undertake efforts to improve germ plasm, develop income-generating activities, and promote sustainable technologies that support the development of resilient farming systems. The sustainability and growth of this significant crop can only be maintained by continued innovation aiming at containing rising problems and improving the quality and increasing the quantity of the product. Such endeavor requires effective collaboration of stakeholders in the region at large, therefore, this paper presents an innovation platform designed to provide a means of collaboration between researchers, producers, investors, and government agencies in a manner to promote the cause of date palm in the NENA region to maintain high competitiveness in a dynamic global market.

Due to high potential and increasing importance of date palm in the region and to close the knowledge gap, Food and Agriculture Organization (FAO) and Association of Agricultural Research Institutions in the Near East and North Africa (AARINENA) joined efforts to explore opportunities to sustainably improve the quality and quantity of date palm production and support the small-scale producers. This joint effort resulted in establishing a research, technology and innovation date palm virtual platform. The main goal of this platform is to promote the date palm integrated production system in NENA region through the establishment of a multi-stakeholder regional platform. The specific objectives of this project are to: create and design interactive and collaborative innovation platform, interconnect relevant stakeholders effectively to share and consolidate the available knowledge, information, and innovations related to date palm value chain and production, to address the problems and promote knowledge exchange and experiences between stakeholders of the platform at regional and international levels. The platform was designed to facilitate a full cycle model from idea to innovation and vice versa and this included back end server and software for storing data, processing information, providing artificial intelligence support and providing web services to communicate with other parts of the platform. Front end application provides interface to various stakeholders and displays reports and statistics via dashboard. The platform was characterized by active dashboard for discussion, forum, projects, news, activities, reports and statistics and performed as a knowledge hub that effectively utilize any new opportunities in an economic or social process. The operationalized research, technology and innovation platform demonstrates a model of collaboration among relevant stakeholders and partners on research, development, innovations, regulations and funding. The platform also identifies a set of research priorities, gaps and practices, promising technologies and technical innovations related to the date palm value chain. Therefore, this generated knowledge with better integration among stakeholders, enhanced learning processes with multiple benefits, created business opportunities by deploying new technologies, markets, learning and networking, and leveraged change in resource mobilization. The platform can also actively enhance the capacity of producers, stakeholders and young professionals in the region. To that end, the operationalized research, technology and innovation date palm platform provides, for the first time, a digital means for collaboration between researchers, producers, funders, policy makers, private industries, government agencies and other stakeholders to promote the cause of specific innovation in the region and to maintain high competitiveness of date palm products in a dynamic global market. The importance of the established platform lays in promoting the accessibility of information, knowledge and technologies available. Private industries, specifically, provide new agricultural inputs and output markets, identify, pilot and mainstream new market opportunities. While farmers have always sought, out and adopted new technologies in an effort to increase yield and efficiency and reduce the cost of production. Therefore, farmers are the users of knowledge, and apply innovative products and practices to increase agricultural productivity and market accesses. In turn, farmers' associations represent farmers' interest, needs and opportunities in value chains and the community. They facilitate access to agricultural inputs, credits and market and promote specific innovation through collaborative research and organizing logistical support.

MATERIALS AND METHODS

Sub-section 1 related to methods

The research, technology and innovation date palm platform was designed and implemented based on a general view of innovation platforms, which can be translated into a specific purpose platform such as the current one under investigation. The development process facilitates the implementation of a full cycle innovation model as shown in Figure 1. This model describes only the innovation side of the platform.

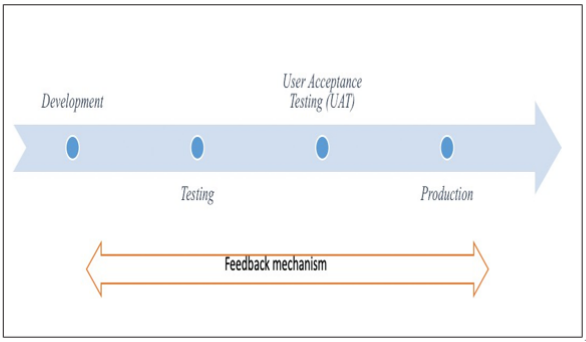


Figure 1. The development process of research, technology and innovation date palm platform.

The development of the main architecture of the platform carried out with operational back end server and software which was established for storing and processing information, providing artificial intelligence support, and providing web services for communicating among the innovation platform partners. Furthermore, another main component was carried out with operational front end application for providing interface to various members and partners and displaying reports via the dashboard, and providing instant real-time statistics on various parameters on the date palm platform. News feedback related to the date palm industry was provided and updated on a timely basis; this included alerts, warning, seasonal procedures and alerts, crops collection, and so on. Smart phone Apps (Android and iPhone iOS) were implemented which meant to provide stakeholders, more specifically farmers with immediate notifications and alerts. The platform also allowed stakeholders to interact on a real-time basis to keep confidentiality of discussions, suggestions, solutions and others. The overall structure and flow of events, activities, and interactions within the platform are shown in Figure 2.

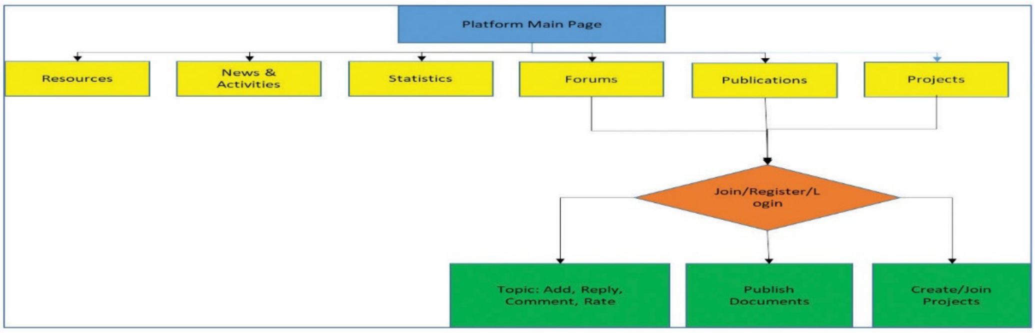


Figure 2. The layout structure of the research, technology and innovation date palm platform.

Sub-section 2 related to methods

The physical platform was developed using a state-of-the-art technology for web platform design: Word Press (PHP and My Sql).The pilot testing, designing and structuring of the platform were accomplished in two testing phases: alpha and beta versions testing and then the platform was ready for launch as shown in Figure 3.

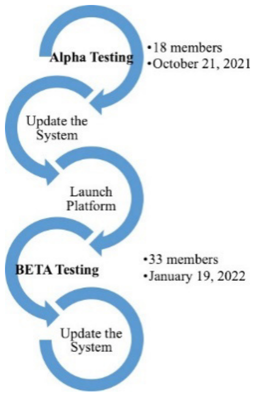


Figure 3. Testing and validation process of the research, technology and innovation date palm platform.

The first phase was alpha pilot testing which focused on the state-of-the-art for development of the date palm platform and the pilot operation of the whole system in the real world. This included evaluation of processes and protocols that were developed along with the usage of the e-platform to support research, technology and innovation related to the date palm. Alpha testing is a type of software testing performed to identify bugs before releasing the product to real users or the public. This is referred to as an alpha testing only because it is done early on, near the end of development of the software. This was the last testing stage before the software was released into the real world through a beta version. The main characteristics of alpha testing approach were the following: minimizing the failure risks performed by users in various fields such as research, management, productions, institutions, administrations involved in the date palm crop industry. The purpose of alpha version verification was to ascertain that the software requirements were met and the system was completed and to determine that the function of the software was proper and consistent with the environment for which it was intended. This testing and verification process helped the designers to improve the usability of the platform before beta testing, and checking the quality of the product before forwarding to beta testing. The testing process covered important components such as: functional testing, usability testing, interface testing, and compatibility testing. There were 18 stakeholders' participants from diverse background and organizations representing the date palm industry (alpha testing often performed with 10-15 participants) who signed up on the platform http://aarinena.org/palm/, filled in the registration form online, signed in into their account, and then testing procedures started step by step with participants. At the end, there were corrections and proper operations of the e-tools and the overall of the e-platform in accordance with the system specification requirements.

The second phase was beta version testing which focused on the strategy of launching, full scale testing with stakeholders, review comments and feedback, review ease of use, issues and needs, etc. Participants were asked ahead of time and prior to the testing to sign up and create account on the date palm platform to have an interactive session, discussion and feedback with stakeholders. Beta testing is performed by real users of the software application in a real environment. Beta testing is one of the types of user acceptance testing and this case feedback needed and released to a limited number of end-users of the product to obtain feedback on the product quality. The full-scale testing was verified by 33 stakeholders' participants from diverse background and organizations related to the date palm industry who signed in into the platform "http://aarinena.org/palm/", and then testing procedures started systematically with participants. This testing helped in minimization of product failure risks, provided increased quality of the product through customer validation, and helped unravel many unnoticed defects. Usually, it is the last testing stage before the software is released into the real world. The main characteristics of beta testing approach were to further minimize the failure risks, to perform by end users, to be done before the large-scale pilot testing, and to ensure the quality of the product before issuing the final version of the platform. There were corrections and proper operations of the e-tools and the overall e-platform in accordance with the systems' specification requirements, which elaborated based on the actual experience of the end-users. During beta version testing, the evaluation criteria were covered issues such as functional requirements fulfilment, and more specifically, performance and usability tests. The procedures of these two tests were the same as the ones described under alpha testing. The feedback from pilot application of beta version was taken into consideration and adjustments were made as necessary to the platform. The platform was ready for use by stakeholders.

RESULTS AND DISCUSSION

The scope of this research project aimed to provide an online "meeting place" (forum) that is used to engage with others, to debate and share knowledge and to communicate with others about a wide range of date palm topics that participants are interested in discussing. A central place for organizing, planning and tracking projects that are developed in a distributed environment. It therefore is a web application that should be compatible with majority of browsers/devices to provide user friendly interface for project administrators, project managers and project members working far apart all around the world. The platform as it stands is in its beta version. It was tested and used by selected number of stakeholders and revised based on the feedback from key stakeholders. The platform was tested in order to perform final adjustments and modifications. The platform was validated and now ready for interaction and use by stakeholders. Currently, the platform consists of the following major components: Forums, Resources, Stakeholders, News & Activities, Projects, Profile, and Registration. These components were designed to be dynamically modified, renamed, edited, and replaced. The design and architecture of the platform were accomplished to allow complete collaboration within stakeholders' groups or across stakeholders. In the current status, the platform provides the following services, which are aligned with the main objectives of the platform: interactivity and collaboration between participants, interconnection of stakeholders, enabling collaboration on issues, research, innovation, regulations and funding, providing forums for various groups, where members of a group can add new topics, enables the creation and management of projects, enables the creation and management of events, and enables the dissemination of information through publications, news, newsletters and other means.

The main difference between this operationalized date palm platform and other platforms lies in the ability of this platform to promote, manage, and support collaboration between various stakeholders. However, other innovation platforms serve the agriculture industry including palm trees such as the TAPipedia platform which is managed by the Food and Agriculture Organization (Food and Agriculture Organization (FAO), 2021a). TAPipedia is mainly a platform for disseminating information, sharing knowledge and promoting the agriculture industry at a global scale. TAPipedia platform is designed to provide vital information on tropical agriculture and publishes important information and data in this field, as well as important resources on tropical agriculture (Food and Agriculture Organization (FAO), 2021b). In contrast, the date palm innovation platform is a collaborative platform that enables the collaboration of researchers, producers, investors, marketers, funding agencies, and government agencies to collaborate in real time on various issues of mutual concern. This platform is also designed to enable real time interaction between all stakeholders. The development of the date palm innovation platform has benefited from the advancement in the software and information technology where collaboration and interaction between stakeholders has become more available with various tools and architectures.

Some of the main components of the platform such as Resources, Statistics, News and Activities were open for any person to browse and view. However, other main components such as Forums, Publications, and Projects were dedicated to stakeholders of the platform who can join, participate, comment, interact, rate, and carry all activities designated to any of these items. For a user to become an active member of the platform, a registration is required, which is in turn monitored and controlled by AARINENA administration. For example, a new member can register and create a profile to indicate which member group he/she belongs to: research, producer, farmer, marketer, government official, or investor. Each member group has its specific Forum. Members within the same group are automatically registered to the group Forum. For example, a researcher will be able to view all topics published within the Research Group. He/she can add his/her topic, interact with other topics within the group, rate the topic, and so on. In order to facilitate interaction among all groups (researchers, producers, farmers, etc.), a general Forum is created, where all members of the platform can view and interact by making comments, suggestions, replies, and rating. Within each group, members can create projects. Once a new project idea is created, the owner of the project may open the project item for all members from all categories, restrict it to one category, or share it only with specific members. This item within the platform was designed to allow stakeholders to collaborate on project development, project execution, and project funding.

General features of the platform were developed and these are: self-base registration which was enabled for the portal platform by the system administrator. This was to enable a user to create his/her own account. Then the data captured on the registration page was the standard information required for account creation. An extra authorization to approve the registration's request by administrator. A modern and informative profile system was established for profile system, with member statistics, bio, setting, activity and subscription pages. User profile, editing profile and joining groups were all established through the platform. Bilingual: English and Arabic. Mobile friendly responsive on all kind of devices. Key features of the platform were developed and these are: downloadable file list of publications. Add news, activities, and events in the announcement. Reports and parameter estimates. Bulk import and export users. Forums for small and extremely large communities with multi layout: extended forum layout, simplified forum layout, question and answer forum layout, threaded forum layout. Subscription and RSS feed. User groups and permissions were built-in, a powerful user group system to allow creating a new, managing and deleting. All user groups have permissions with separate managing options. Member rating and badges. Project manager is a simple project management tool to manage work and deliver projects timely that: ensure the best use of resources and planning. Properly organize and track all tasks and projects. Meet your deadline more efficiently. Advanced time management tool for efficient time tracking. Make team collaboration more effective than ever. Get advanced reports for full insights about everything. Project management permissions.

Standard features of the date palm platform were developed and these are: add as many to-do lists as you want with title and description. Add tasks, assign users, assign a due date. See progress bar on the list. Add comments on individual to-do lists. Mark to-do as complete or incomplete. The results show that there were three types of milestones and these are: a) upcoming, b) completed and c) late milestone. Assign messages and to-do list on milestone. Messages were used for discussing the project with co-workers of that project. You can add attachments on messages. Comments can be made for discussion. There were files sharing feature to ensure better understanding and work flow between co-workers. Uploading all files on messages and comments and navigate to individual attached threads. Uploading and sharing any file anytime in one place (Pro). The Pro features for managing projects from front end and allowing users the ability to view your projects from the front end. Managing projects without going to back end interface. Gantt chart for observing the duration of the tasks through horizontal bar graphs. Changing the start and end dates of tasks or projects. Assessing dependencies among different tasks and subtasks. Subtasks for more simple task management system such as breaking long tasks into as many portion as you need. Assigning several workers for several tasks. Reports for tasks and user activities to make project management efficient. This will also help to make the right decisions for your projects and team. The reports are very much self-explanatory and depend on the user activities. In addition, there were overdue tasks, completed task, user activities, task by milestone, unassigned tasks, and summary. Time tracker with start, stop or pause feature for every task and calculation and tracking of time spent on a task.

CONCLUSIONS

An innovative, interactive and collaborative date palm platform has been created to serve a large community of stakeholders within the date palm industry in the NENA regions. An inventory of relevant stakeholders related to date palm production system were mapped and identified. Research priorities, technologies and technical innovations related to the date palm value chain were identified. There were interactive protocols and functioning for operationalized a distance coaching protocol and capacity building activities throughout the research project. The research, technology and innovation date palm platform was developed and operationalized in a dynamic manner to minimize the maintenance cost and to allow maximum flexibility for the management of the platform. Finally, we conclude that the outcome of the research project was a physical and governance structure model of innovation platform for date palm industry and adoption of this platform by member countries, and regional model for innovation platform monitoring and evaluation system ready for use. In general, this would contribute to developing innovation capacities in all levels and improving knowledge and information exchange to enhance linkages between researcher, academics, producers, investors, and policy and decision makers.

ACKNOWLEDGEMENTS

The authors would like to thank the Food and Agriculture Organization of the United Nations for their support and funding of the project. The authors would also like to thank the technical and research team for their assistance. Finally, sincere appreciation and thanks to Khalifa International Award for Date Palm and Agricultural Innovation for their generous support and launching the research, technology and innovation date palm platform at the 7th International Date Palm Conference on March 14, 2022 in Abu Dhabi.

Literature cited

Aregawi, L., Alemayehu, M., and Endris, M. (2018). Date palm production practices and constraints in the value chain in afar regional state, Ethiopia. Adv. Agric. *2018*, 6469104 https://doi.org/10.1155/2018/6469104.

Arias, E., Hodder, A.J., and Oihabi, A. (2016). FAO support to date palm development around the world: 70 years of activity. Emir. J. Food Agric. *28* (*1*), 1–11 https://doi.org/10.9755/ejfa.2015-10-840.

Cheng, T.C.C., and Krueger, R.R. (2007). The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. HortScience *42* (*5*), 1077–1082 https://doi.org/10.21273/HORTSCI.42.5.1077.

Food and Agriculture Organization (FAO). (2021a). Tropical Agriculture Platform (TAP). Enhancing Knowledge Exchange for Agricultural Innovation. https://www.fao.org/in-action/tropical-agriculture-platform/tapipedia/en/.

Food and Agriculture Organization (FAO). (2021b). Tropical Agriculture Platform: TA Pipedia. Exchange

Knowledge and Supporting Capacity Development for Agricultural Innovation Systems. https://www.tapipedia.org/.

Xia, J. (2020). Food and Agriculture Organization (FAO), Committee on Agriculture. 27th session. Proposal for an international year of date palm. COAG/2020/21 https://www.fao.org/3/nd767en/nd767en.pdf.

Comparison between nitrogen biofertilizers source (blue green algae and bacteria) on growth of date palm plantlets (*Phoenix dactylifera* 'Malacabe') during acclimatization stage

L.M. Abdel-Galeil[1,a], E.M. Aref[2] and M.A. Mona[2]

[1]Central Laboratory of Date Palm and Research and Development, ARC, Giza, Egypt; [2]Department of Agricultural Microbiology, Soils, Water and Environment Research Institute; ARC, Giza, Egypt.

Abstract

The acclimatization phase is the most important stage in the protocol of date palm micro-propagation because if not optimized, the whole process will be in efficient. Factors affecting the successful production of free-living date palm, including length of plantlets, number of leaves, strength of root system, humidity of the growing environment, and composition of the soil have been studied. Bio-fertilizers such as cyanobacteria and bacteria considered which have efficient potential to enhance the vegetative growth, chemical composition in leaves and improved nutrients uptake were evaluated. The purpose of this study was to investigate the influence of either mixture of bacteria (*Azospirillum lipoferum*, *Azotobacter chroococcum* and *Paenibacillus polymyxa*) or mixture of cyano bacteria (*Nostoc muscorum* and *Anabaena oryzae*) and the combination between the mixture of bacteria and cyano bacteria in presence of two levels of ammonium nitrate (33.5% (as nitrogen source), 50 and 75% of recommended dose) on growth, Soil biological activity and chemical composition of date palm plants during acclimatization stage. Experimental results indicated that all bio-fertilizer (blue green algea and bacteria) significantly increased plant height (cm), number of leaves, leaf width (cm), indole (mg g^{-1} fw) and chlorophyll a, b and c (mg g^{-1} fw) contents in both seasons, with the superiority of mixture between (*Nostoc muscorum*+ *Anabaena oryzae* + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenibacillus polymyxa* + 75% N, which gave in general, the high means in plant height (cm), number of leaves plant^{-1} and leaf width (cm). A similar trend was also gained concerning the content of indoles, chlorophylla, activity of dehydrogenase, nitogenase and carbons content compared to the control and other treatment. So, It may be recommended to use a mixture of *Nostoc muscorum* + *Anabaena oryzae* + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenibacillus polymyxa* + 75% to enhance the vegetative growth, increase soil biological activity such as dehydrogenase, CO_2 and nitrogenase activity and chemical composition in leaves during acclimatization stage.

Keywords: bacteria, bio-fertilizers, indole acetic acid, chlorophyll, carotenoids, nitrogenase activity, dehydrogenase, ammonium nitrate

INTRODUCTION

Factors affecting the successful production of date palm by micro-propagation include length of plantlets, number of leaves, strength of root system, humidity conditions, and composition of the soil (Abul-Soad and Mushtaque, 2014). Efficacy of the protocol of date palm micro-propagation referred to the acclimatization phase deemed the most critical period till transfer to the open field. The most important obstacles confronting date palm plantlets through the first acclimatization stage is sluggishness in vegetative growth with low survival rate (Lobna and Eldawayati, 2017). Plant growth promoter rhizobacteria (PGPR) such as *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Paenibacillus polymyxa*, individually or

[a]E-mail: lobnamohamed822@yahoo.com

in combination maximized the counts and the activity of microorganisms in rhizosphere region and convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003). PGPR activated some mechanisms which could encourage the plant outgrowth through nitrogen fixation, phosphate solubilization, siderophore production, inhibition of bio-film formation, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses and interference with pathogen toxin production (Bhattacharyya and Jha, 2012). Fuentes-Ramirez and Caballero-Mellado (2005) reported high microbial counts and activity occurred by inoculation with the mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Paenibacillus polymyxa*. Cyano-bacteria acted as potent bio-fertilizer because of enhancing all the morphological and biochemical criteria of plants such as proteins, carbohydrates, total nitrogen contents, lowering the chemical nitrogen fertilizers up to 50%, boosting nutrient uptake and limiting the growth weeds (Jagannath et al., 2002; Ojaghloo et al., 2007). Cyano-bacteria and bacteria were recommended as bio-fertilizers substitution for chemical fertilizers to avert the high cost and environmental problems. Lobna et al. (2018) mention that using a mixture of plant growth promoting rhizobacteria (PGPR) such as *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus polymyxa* increase the highest significant values of plant height number of leaves and width in the early stages in greenhouse during acclimatization stage of ‘Malacabe’ date palm plantlets. The main purpose was to investigate the influence of either mixture of bacteria (*Azospirillum lipoferum*, *Azotobacter chroococcum* and *Paenibacillus polymyxa*) or a mixture of cyano-bacteria (*Nostoc muscorum* and *Anabaena oryzae*) and the combination between the mixture of bacteria and cyano-bacteria in the presence of two levels of ammonium nitrate (33.5%) as nitrogen source (50 and 75% of recommended dose) on growth, soil biological activity and chemical composition of date palm plants during acclimatization stage.

MATERIALS AND METHODS

Experimental layout

This experiment was carried out under greenhouse conditions at the Central Laboratory of Date Palm Research and Development, Giza, Egypt for two successive seasons to investigate the influence of either mixture of bacteria (*Azospirillum lipoferum*, *Azotobacter chroococcum* and *Paenibacillus polymyxa*) or mixture of cyano-bacteria (*Nostoc muscorum* and *Anabaena oryzae*) and the combination between the mixture of bacteria and cyano-bacteria in the presence of two levels of ammonium nitrate as nitrogen source (50 and 75% of recommended dose).

Plant material

Six-month-old healthy ‘Malacabe’ date palms were obtained from a tissue culture laboratory with average 20-25 cm height and 3-4 leaves plantlet^{-1}. The plantlets were cultured in 25 diameter plastic pots (one plantlet pot^{-1}) and filled with mixture of peat moss and sand (1:2 volume/volume). The additives were applied every month from March to November for two seasons. All treatments had 3 replicates and every replicate contains 3 plantlets.

Source of bacterial strains

All bacterial and cyanobacterial strains were obtained from the Department of Agricultural Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Centre.

Azospirillum lipoferum was grown on nitrogen deficient semi solid malate medium (Dobereiner et al., 1976). *Azotobacter chroococcum* was cultured on modified Ashby medium (Abd El Malek and Ishac, 1968). *Paenibacillus polymyxa* was grown according to Hino and Wilson (1958). Cyanobacterial strains (*Nostoc mucorum* and *Anabaena oryzae*) were propagated on BG11 liquid medium used by Rippka et al. (1979).

Treatments used

1. Control: 1 g of recommended dose of ammonium nitrate (33.5% mineral fertilizers) (T1);
2. Mixture of *Nostoc muscorum* + *Anabaena oryzae* + 50% of N (T2);
3. Mixture of *Nostoc muscorum* + *Anabaena oryzae* + 75% N (T3);
4. Mixture of *Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenebacillus polymyxa* + 50% N (T4);
5. Mixture of *Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenebacillus polymyxa* + 75% N (T5);
6. Mixture of *Nostoc muscorum* + *Anabaena oryzae* + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenibacillus polymyxa* + 50% N (T6);
7. Mixture of *Nostoc muscorum* + *Anabaena oryzae* + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenebacillus polymyxa* + 75% N (T7).

Data recorded

1. Vegetative growth: plant height (cm), number of leaves plant^{-1} and leaf width (cm);
2. Chemical composition: in fresh leaf samples chlorophyll a, b and carotenoids (mg g^{-1} fw) were determined according to the methods described by Saric et al. (1967) while total indole (ppm) was determined according to Larsen et al. (1962). Soil biological activity such as dehydrogenase, CO_2 evolution and nitrogenase activity were determined according to Skujiņš and Burns (1976) and Dilowarth (1970), respectively.

Statistical analyses

The experimental layout was complete randomized design and the data were subjected to analyses of variance at 5% significance according to Steal and Torrie (1983).

RESULTS AND DISCUSSION

Table 1 demonstrates that the mixture of cyan-bacteria and bacteria in combination with 75% N recorded the tallest height (66.00 and 81.70 cm), number of leaves plantlet^{-1} (7.66 and 8.67) and leaf width (2.05 and 2.5 cm) in the first and second season, respectively, compared with the same mixture of cyano-bacteria and bacteria in combination with 50% N, but there is no significant difference between them. On the other hand bacterial treatment alone was best compared with cyano-bacteria alone or control. Enhancing growth and morphological properties of date palm as a result of biological treatments was ascertained to excrete growth promoting regulators such as gibberellin and auxin, vitamins, amino acids, polypeptides, antibacterial and anti-fungal substances (Lugtenberg and Kamilova, 2009). Enhancing of growth and morphological properties of date palm as a result of biological treatments was ascertained to excrete growth promoting regulators such as gibberellin and auxin, vitamins, amino acids, polypeptides, antibacterial and anti-fungal substances (Lugtenberg and Kamilova, 2009). Farrag et al. (2011) that *Azospirillum brasilense* might play a significant role in improving the growth response of date by producing good quality planting stock. These plants may perform better growth, survival and more fruit production due to IAA production. Lobna et al. (2018) also recommended that using a mixture of plant growth promoting rhizobacteria (PGPR) such as *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus polymyxa* increase and showed significant values of plant height number of leaves and width in the early stages in greenhouse during acclimatization stage of 'Malacabe' date palm plantlets. Saadaoui et al. (2019) observed that supplementation of soil with 0.5 g of algal biomass led to higher plant growth rates, 100% survival rates, high ability of rooting, higher number of leaves, largest stem thickness and longer shoot.

Table 1. Effect of nitrogen bio-fertilizers source (blue green algea and bacteria) on vegetative growth of date palm plants (*Phoenix dactylifera* 'Malacabe') during acclimatization stage.

Treatment	After 8 months			After 16 months		
	Means of leaves numbers	Plant height (cm)	Leaf width (cm)	Means of leaves numbers	Plant height (cm)	Leaf width (cm)
T1	5.330	47.000	1.017	6.670	57.130	1.217
T2	4.360	48.000	0.983	5.000	30.608	1.250
T3	5.370	55.270	1.224	6.000	30.665	1.533
T4	70 6.0	56.330	1.483	7.370	74.230	1.831
T5	6.330	58.470	1.499	7.680	74.670	1.842
T6	7.330	64.370	2.033	8.330	80.700	2.446
T7	7.600	66.000	2.050	8.670	81.700	2.503
LSD at 5%	0.768	2.479	0.110	0.736	3.987	0.121

Indoles content (mg 100 g^{-1} FW)

The data in Figure 1 show that high significant values of indoles in leaves resulted from the mixture of cyano-bacteria and bacteria with 75% of nitrogen (6.91 and 6.97 mg g^{-1} FW) treatment in the first and second season, respectively. There was no significant difference between the same mixture treatment with 50% nitrogen, while the control treatment gave the lowest values (3.29 and 3.61 mg g^{-1} FW), respectively. On the other hand the mixture between cyano-bacteria and bacteria was the best treatment compared with the use cyano-bacteria alone or bacteria. Dilfuza (2011) reported that the auxin level is usually higher in the rhizosphere, where high percentage of rhizosphere bacteria is likely to synthesize auxin as secondary metabolites because of the rich supplies of root exudate. The author also stated that the production of auxin (IAA), has been recognized as an important factor in direct plant-growth-promoting abilities of rhizosphere bacteria and they stimulate proliferation of lateral roots that increase nutrient absorbing surfaces and results in better assimilation of water and nutrients from the soil.

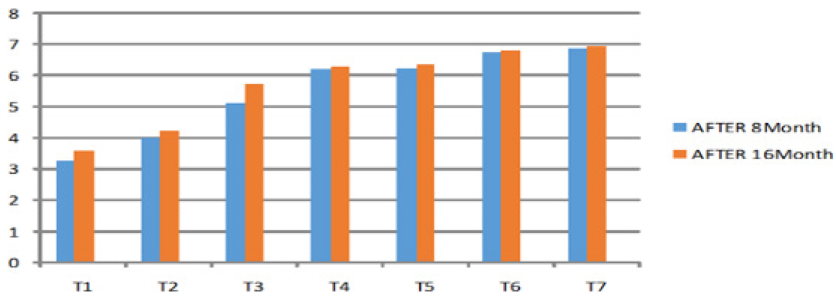
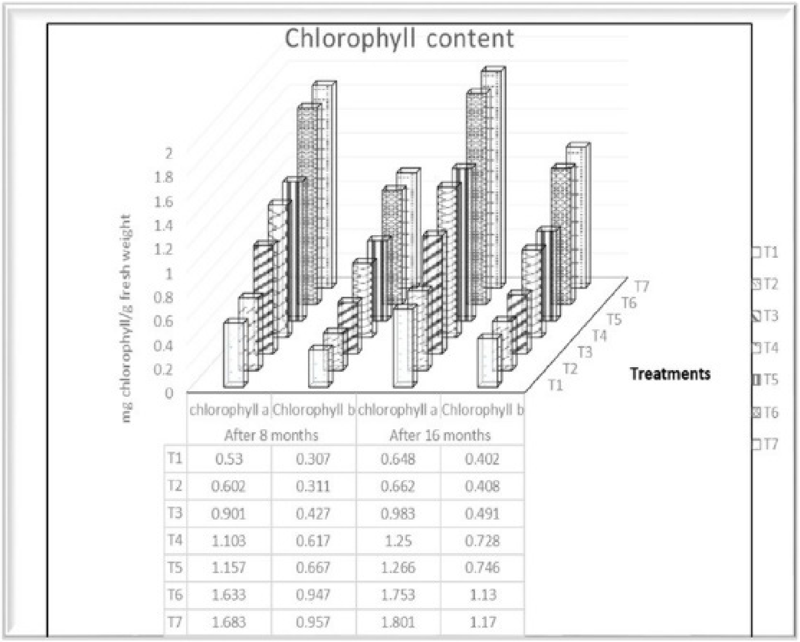


Figure 1. Effect of nitrogen bio-fertilizers source (blue green algea and bacteria) on indole content (mg 100 mg g^{-1} FW) of date palm plants (*Phoenix dactylifera* 'Malacabe') during acclimatization stage.

Chlorophyll contents (mg g FW)

The results presented in Figure 2 demonstrated that highest values in chlorophyll a and b were recorded in the leaves of plantlets treated with the mixture of cyano-bacteria and bacteria combined with 75% N increased chlorophyll a by 1.68 and 1.80 mg g^{-1} FW and chlorophyll b by 0.95 and 1.17 mg g^{-1} FW in the first and second season, respectively. However, there are no significant differences among the values of chlorophyll a and b noticed at cyano-bacteria and bacteria with 50 and 75% N, while the lowest values were recorded in the control treatment.



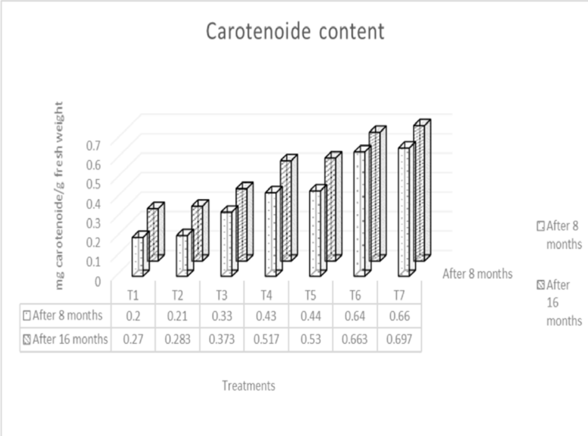
	After 8 months		After 16 months	
	chlorophyll a	Chlorophyll b	chlorophyll a	Chlorophyll b
T1	0.53	0.307	0.648	0.402
T2	0.602	0.311	0.662	0.408
T3	0.901	0.427	0.983	0.491
T4	1.103	0.617	1.25	0.728
T5	1.157	0.667	1.266	0.746
T6	1.633	0.947	1.753	1.13
T7	1.683	0.957	1.801	1.17

Figure 2. Effect of nitrogen bio-fertilizers source (blue green algea and bacteria) on chlorophyll contents a and b (mg g^{-1} FW) of date palm plants (*Phoenix dactylifera* 'Malacabe') during acclimatization stage.

Free-living nitrogen-fixing bacteria e.g., *Azotobacter chroococcum* and *Azospirillum lipoferum*, were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Abd El-Lattief, 2013). Covindjee and Shevela (2011) stated that most cyano-bacteria contain carotenoids, chlorophyll a, phycocyanin and allophycocyanin as pigments for photosynthesis. Lobna et al. (2018) reported that the highest values of chlorophyll a chlorophyll b were recorded in the leaves of plantlets treated with the mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus polymyxa* in the early stages in greenhouse during acclimatization stage of 'Malacabe' date palm plantlets. Saadaoui et al. (2019) reported that supplementation soil with 0.5 g of algal biomass led to higher plant growth rates, 100% survival rates, high ability of rooting, higher number of leaves, largest stem thickness, longer shoot and higher total chlorophyll as compared to conventional fertilizer.

Carotenoids content (mg g^{-1} FW)

The data in Figure 3 indicate that the highest values in carotenoids content were recorded in the leaves of plantlets treated with the mixture of cyano-bacteria and bacteria combined with 75% N (66.0 and 69.0 mg g^{-1} FW) in the first and second season, respectively. However, there are no significant differences noticed at cyano-bacteria and bacteria with 50 or 75% N, while the control treatment recorded the lowest value (0.2 and 0.3 mg g^{-1} FW) in the first and second season, respectively. Lobna et al. (2018) reported highest values of carotenoids content in the leaves of plant lets treated with the mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus polymyxa*) in the early stages in greenhouse during acclimatization stage. Saadaoui et al. (2019) on date palm (*Phoenix dactylifera* L.) reported that supplementation soil with 0.5 g of algal biomass led to higher plant growth rates, high ability of rooting, number of leaves, largest stem thickness, longer shoot, largest stem thickness and higher carotenoids as compared to conventional fertilizer.



	T1	T2	T3	T4	T5	T6	T7
After 8 months	0.2	0.21	0.33	0.43	0.44	0.64	0.66
After 16 months	0.27	0.283	0.373	0.517	0.53	0.663	0.697

Figure 3. Effect of nitrogen bio-fertilizers source (blue green algea and bacteria) on carotenoid contents (mg g^{-1} FW) of date palm plants (*Phoenix dactylifera* 'Malacabe') during acclimatization stage.

Nitrogenase activity

The data in Figure 4 indicate that the maximum activities of niterogenase were 164.63 and 167.1 mol C_2H_4 g^{-1} soil that were obtained by the mixture of cyano-bacteria and bacteria with 50% N in first and second season respectively. While bacterial treatment, the activities of nitrogenase were 149.37 and 153.8 mol C_2H_4 g^{-1} soil h^{-1} at the same level of nitrogen in first and second season, respectively. The mixture of all microbes was superior followed by bacterial treatment. Microorganisms like cyano-bacteria, *Azotobacter*, *Azospirillum*, etc. are the natural nitrogen fixers. The natural process of nitrogen fixation is accomplished by a complex but important enzyme system known as nitrogenase. This important biological fixation of nitrogen into ammonia makes it accessible for synthesis of nucleotide, DNA, RNA, amino acid and protein. Nitrogenase enzyme's primary biological function generates ammonia from dinitrogen. This activity is essential for all organisms because it sequesters atmospheric nitrogen gas in a biologically accessible form. Artificial agricultural fertilizer production is now the largest source of fixed nitrogen in the earth's ecosystem (Dighe et al., 2010).

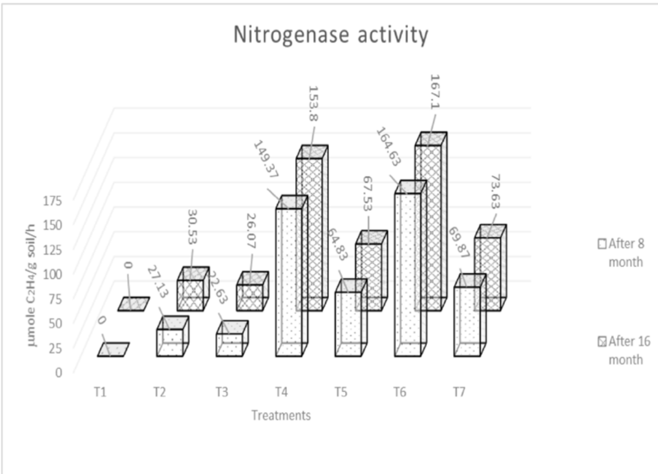


Figure 4. Influence of nitrogen bio-fertilizers source (blue green algea and bacteria) on nitrogenase activity of date palm plants (*Phoenix dactylifera* 'Malacabe') during acclimatization stage.

CO_2 evolution

The data in Figure 5 demonstrate the role of microorganisms which enhance the respiration rate throughout acclimatization of date palm stage. All biological treatments encouraged CO_2 evolution compared to the control. The mixture of all tested microorganisms or bacterial treatments were superior to the cyanobacterial treatment after 8 or 16 months of cultivation. The increase of CO_2 evolution reached to about two-folds at either 50 or 75% N compared to the control.

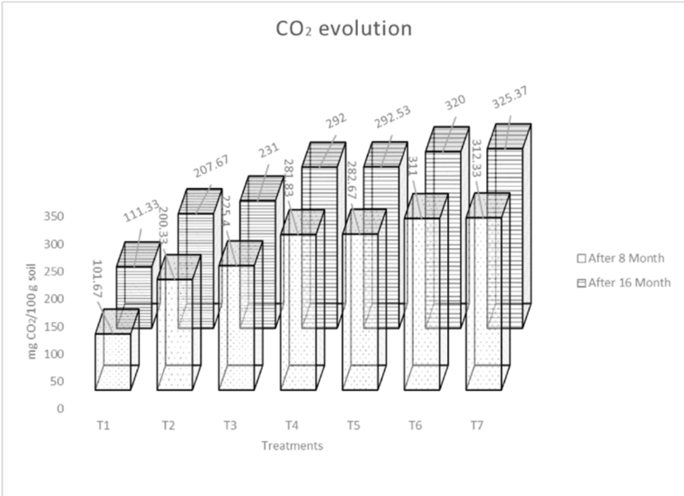


Figure 5. Influence of nitrogen bio-fertilizers source (blue green algea and bacteria) on CO_2 evolution of date palm plants (*Phoenix dactylifera* 'Malacabe') during acclimatization stage.

Dehydrogenase activity

The mixture of all tested microbes was the best treatment in dehydrogenase activity followed by bacterial treatment (Figure 6). The cyanobacterial treatment exerted the lowest effect on dehydrogenase activity. Dehydrogenase activity after 16 months was better than after 8 months and also at 75% N was more preferable than at 50% N for cyanobacterial treatment. Dehydrogenase activity reached to 189.03 g TPF/soil with mixture of all tested microbes at 75% N after 16 months versus 28.07 g TPF/soil for the control in the same condition.

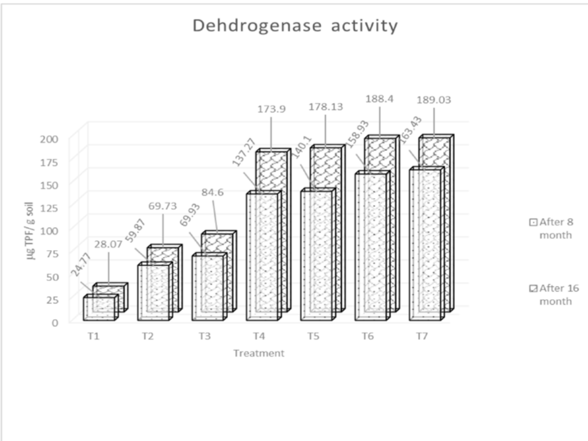


Figure 6. Influence of nitrogen bio-fertilizers source (blue green algea and bacteria) on dehydrogenase activity of date palm plants (*Phoenix dactylifera* 'Malacabe') during acclimatization stage.

El-Kassas (2002) reported that inoculation with the nitrogen fixing *Azospirillum* to wheat increased the soil *Azospirilla* and other microbial population including fungi, actinomycetes and *Azotobacter*, and consequently increased both the dehydrogenase activity and CO_2 evolution, which are considered as index for biological activity and soil fertility. de Caire et al. (2000) studied the identification changes in the activity of soil extracellular enzymes, b-glucosidase, phosphomonoesterase, arylsulphatase, protease and urease and the intracellular dehydrogenase following the addition of cyanobacterial exopolysaccharide and biomass to soil.

CONCLUSIONS

From the previous results, It could be recommended that at the early period of established date palm plantlets during acclimatization stage under greenhouse condition, it was preferred to treat plantlets with (fresh extract) mixture of cyanobacterial (*Nostoc muscorum* + *Anabaena oryzae*) + bacteria (*Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenibacillus polymyxa* + 75% N) (as a soil drench) every month to enhance the vegetative, increase soil biological activity such as dehydrogenase, CO_2 and nitrogenase activity and chemical composition in leaves, which accelerated the successful transfer to open filed (Figure 7).

Figure 7. Mixture of cyano bacteria (*Nostoc muscorum* + *Anabaena oryzae*) + bacteria (*Azotobacter chroococcum* + *Azospirillum lipoferum* + *Paenibacillus polymyxa*) + 75% N.

Literature cited

Abd El-Lattief, E. (2013). Impact of integrated use of bio and mineral nitrogen fertilizers on productivity and profitability of wheat (*Triticum aestivum* L.) under upper Egypt conditions. Int. J. Agron. Agric. Res. *3*, 67–73.

Abd-el-Malek, Y., and Ishac, Y.Z. (1968). Evaluation of methods used in counting azotobacters. J Appl Bacteriol *31* (*3*), 267–275 https://doi.org/10.1111/j.1365-2672.1968.tb00367.x. PubMed

Abul-Soad, A., and Mushtaque, A.J. (2014). Factors affecting in vitro rooting of date palm (*Phoenix dactylifera* L.). Pak. J. Agric. Sci. *51* (*2*), 477–48.

Bhattacharyya, P.N., and Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol *28* (*4*), 1327–1350 https://doi.org/10.1007/s11274-011-0979-9. PubMed

Covindjee, and Shevela, D. (2011). Adventures with cyano bacteria: a personal perspective: review article. Plant Sci. *2*, 28.

de Caire, G.Z., De Cano, M.S., Palma, R.M., and De Mule, C.Z. (2000). Changes in soil enzyme activities following additions of cyanobacterial biomass and exopolysaccharide. Soil Biol. Biochem. *32* (*13*), 1985–1987 https://doi.org/10.1016/S0038-0717(00)00174-7.

Dighe, N.S., Shukla, D., Kalkotwar, R.S., Laware, R.B., Bhawar, S.B., and Gaikwad, R.W. (2010). Nitrogenase Enzyme: a Review. Pelagia Research Library. Pharm. Sin. *1* (*2*), 77–84.

Dilfuza, E. (2011). Indole-acetic acid production by root associated bacteria and its role in plant growth and development. In Auxins: Structure, Biosynthesis and Functions (Botanical Research and Practices), A.H. Keller, and M.D. Fallon, eds. (Nova Science Pub Inc.), pp.135.

Dilowarth, M.J. (1970). The acetylene reduction method for measuring biological nitrogen fixation. Rhizobium Newsletter *15*, 155–163.

Dobereiner, J., Marriel, I.E., and Nery, M. (1976). Ecological distribution of *Azopirillum lipoferum* Beijerinck. Can J Microbiol *22* (*10*), 1464–1473 https://doi.org/10.1139/m76-217. PubMed

El-Kassas, A.R. (2002). Studies on cyano bacteria and their effect on some soil properties. M.Sc. thesis (Kalubia, Egypt: Soils Dept., Faculty of Agriculture, Benha University).

Farrag, H.M.A., Abd-El Kareim, A.H.E., and Darwesh, R.S.S. (2011). Growth promotion of date palm plantlets ex vitro by inoculation of rizosphere bacteria. J. Hortic. Sci. Ornam. Plants *3* (*2*), 130–136.

Fuentes-Ramirez, L.E., and Caballero-Mellado, J. (2005). Bacterial biofertilizers. In PGPR: Bio-Control and Biofertilization, Z.A. Siddiqui, ed. (Dordrecht, The Netherlands: Springer), p.143–172.

Hino, S., and Wilson, P.W. (1958). Nitrogen fixation by a facultative *Bacillus.* J Bacteriol *75* (*4*), 403–408 https://doi.org/10.1128/jb.75.4.403-408.1958. PubMed

Jagannath, S., Umapati-Dengi, B., and Damakar, E. (2002). Algalization studies on chickpea (*Cicer arietinum* L.). Biotechnology of Microbes and Sustainable Utilization *21*, 145–150.

Larsen, P., Harbo, A., Klungsoyr, S., and Aasheim, T. (1962). On the biogenesis of some indole compounds in *Azotobacterium*. Physiol. Plant. *15* (*3*), 552–565 https://doi.org/10.1111/j.1399-3054.1962.tb08058.x.

Lobna, M. (2016). Influence of soaking date palm plantlets (*Phoenix dactylifera* L.) in different cyanobacteria, *Azolla pinnate* and humic acid on adventitious roots induction and survival percentage during acclimatization stage. Arab J. Biotechnol. *19* (*1*), 51–62.

Lobna, M., and Eldawayati, M.M. (2017). The promotive effect of blue green algae (BGA) on date palm plantlets (*Phoenix dactylifera*) during acclimatization stage. J. Biol. Chem. Environ. Sci. *12* (*2*), 539–555.

Lobna, M.A.-G., Maiada, M.E.-D., and Mahamad, A.M.M. (2017). The promotive effect of blue green algae (BGA) on date palm plantlet (*Phoenix dactylifera*) during acclimatization stage. J. Biol. Chem. Environ. Sci. *12* (*2*), 539–555.

Lobna, M.A.-G., Farrag, H.M.A., and Zayed, E.M.M. (2018). Plant growth promoting rhizobacteria (PGPR) protect date palm (*Phoenix dactylifera* L.) plantlets from fungal attack during acclimatization stage. American-Eurasian J. Agric. & Environ. Sci. *18* (*2*), 89–95.

Lugtenberg, B., and Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. Annu Rev Microbiol *63* (*1*), 541–556 https://doi.org/10.1146/annurev.micro.62.081307.162918. PubMed

Ojaghloo, F., Farah Vash, F., Hassan Zadeh, A., and Pourysef, M. (2007). Effect of inculation with *Azotobacter* and barvar phosphate bio-fertilizerers on yield of sunflower (*Carthamus tinctorius* L.). Journal of Agricultural Science, Islamic Azad University (Tabriz Branch), p.25–30.

Rippka, R., Deruelles, J., Waterburg, J.B., Herdma, M., and Stanier, R.Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. *111*, 1–16.

Saadaoui, I., Sedky, R., Rasheed, R., Bounnit, T., Almahmoud, A., Elshekh, A., Dalgamouni, T., al Jmal, K., Das, P., and Al Jabri, H. (2019). Assessment of the algae-based bio-fertilizer influence on date palm (*Phoenix dactylifera* L.) cultivation. J. Appl. Phycol. *31* (*1*), 457–463 https://doi.org/10.1007/s10811-018-1539-6.

Saric, M., Kostrori, R., Cupina, T., and Geric, I. (1967). Chlorophyll Determination (Haucana, Anjiga: Univ. Noven Sadu Prakitikum is kiziologize Bilijaka Beogard).

Skujiņš, J., and Burns, R.G. (1976). Extracellular enzymes in soil. CRC Crit Rev Microbiol *4* (*4*), 383–421 https://doi.org/10.3109/10408417609102304. PubMed

Steal, R.G.D., and Torrie, J.H. (1983). Principles of Procedures of Statistics. A Biometrical Approach (New York, USA: McGrow Hill Book Inc.), p.663–669.

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as bio-fertilizers. Plant Soil *255* (*2*), 571–586 https://doi.org/10.1023/A:1026037216893.

Study on the vegetative growth characters of several Iraqi date palm cultivars

E.A. Mawloud[a]

Independent Consultant of Dates & Palms company, Zafarania, Baghdad, Iraq.

Abstract

To establish database for the Iraqi date palm cultivars, 15 cultivars were chosen to study their vegetative growth characteristics. Those cultivars were 'Khastawi', 'Khadrawi Albasra', 'Zahdi', 'Sayer', 'Chabch-ab', 'Ashgar', 'Asabi Alaroose', 'Tiberzal', 'Barban', 'Jawzi', 'Khsab', 'Sultani', 'Owaynat Ayoub', 'Lolwi', and 'Hadal'. The studied vegetative growth characteristics included season growth rate, trunk circumference, comprehensive study of the leaves as well study the floral parts. All data were collected from Zafarania farm in previous seasons and updated in 2018. The results showed the season's growth of four of the studied cultivars such as 'Khadrawi Albasra' and 'Khastawi'.

Keywords: Iraqi date palm, vegetative growth

INTRODUCTION

The date palm is one of the oldest cultivated crop trees; the earliest known records show that its culture was already established in Iraq as early as 3000 BC (Nixon and Carpenter, 1978). The date palm is a dioecious plant, meaning that male and female flowers are produced on separate trees. The date palm is one of the most important members of the family *Palmaceae*, also known as *Phoenix dactelifera* L. The genus *Phoenix* is distinguished from other genera of pinnate-leaved palms by the upward lengthwise folding of the pinnae (Zaid and de Wet, 2002). In Iraq, there are more than 500 registered date palm cultivars. Each cultivar has specific thermal needs, relative humidity, and suitable soil. When those conditions are available, the palm tree would be at its best production and vegetative growth. Some cultivars grow and thrive in a specific area compared to if they were planted in other areas. An example is the 'Ashrasi' cultivar, which grows well in the Badra and Jassan regions east of Iraq compared to if it was planted in other areas (Al-Bekr, 1972). The date palm trunk, which is also called a stem, is vertical, cylindrical, and columnar, with the same girth all the way up. The circumference does not increase once the canopy of fronds has fully developed. In general, the leaves (fronds) are composite pinnae and have a thick semi-cylindrical rachis.

It is important to mention that some date palm cultivars use pinnae and fronds instead of leaflets and leaves, respectively (Al-Jibouri et al., 1971). Some vegetative growth characteristics of date palms in Iraq were classified into classes: growth rate (cm): fast, medium, slow; circumference of trunk (cm): large, medium, small; leaf length (cm): long, medium, short. Also the length of the leaflets and spines were studied by Al-Jibouri et al. (1971). Because the average of each characteristic varied across its range, the maximum and minimum ranges in each class for a single trait were classified by Al-Bekr (1972) and depicted in Figures 1-4. The goal of this study is to determine the vegetative (phenotypic) characteristics of several Iraqi date palm cultivars, so that the traits of each cultivar can be distinguished, and a scientific botanical key can be created.

MATERIALS AND METHODS

To create a database and botanical key for the Iraqi date palm cultivars, the vegetative growth characteristics of several date palm cultivars were studied. Fifteen date palm cultivars were chosen for this study: 'Khastawi', 'Khadrawi Albasra', 'Zahdi', 'Sayer', 'Chabchab', 'Ashgar',

[a]E-mail: esamd52@yahoo.com

‘Asabi Alaroose’, ‘Tiberzal’, ‘Barban’, ‘Jawzi’, ‘Khsab’, ‘Sultani’, ‘Owaynat Ayoub’, ‘Lolwi’, and ‘Hadal’. All the cultivars were located at Zafarania farm. Three symmetrical replications of each cultivar were selected and subjected to the same agriculture practices. The data for this work were collected in previous seasons and updated in 2018. The following characteristics were measured as follows:

1. The season’s growth rates: the length of the trunk was measured from the base of the new leaf to the soil surface. The season’s growth rate was calculated by dividing the total length of the palm by the number of seasons;
2. The trunk circumference (cm) was measured directly from the upper adventitious roots.
3. Leaf characteristics: three leaves for each replicate of the studied cultivars at the age of 2-3 years were discussed, according to Zaid and de Wet (2002) as follows: A) leaf length (in cm): the leaf length was measured from the first upper leaflet to the lower spine; B) rachis areas were calculated from the first spine located under the leaflet (pinnae) to the last lower spine. In addition, the length and number of spines were counted for all replications.
4. The fruit stalks and strands length were measured (cm).

Statistical analysis

Each trait was put in a group and classified by the season’s growth rate into slow, medium, and fast. For leaf characteristics and fruit area, they were grouped as small, medium, and large. Also, the number of spines was classified as low, medium, and high. The standard deviation was calculated for each trait according to the method mentioned by Kozak and Piepho (2020).

RESULTS AND DISCUSSION

Season’s growth rate

The data in Table 1 and Figure 1 show that ‘Khadrawi Albasra’, ‘Tibrzal’, ‘Hadal’, and ‘Khastawi’ had the lowest season’s growth rate. While other date palm cultivars studied showed medium growth rate levels, such as ‘Zahdi’ and ‘Sayer’. The highest seasonal growth was found with the cultivars ‘Jabjab’, ‘Sultani’, and ‘Aount Ayob’.

Table 1. Season’s growth rate groups of several Iraqi date palm cultivars.

Growth rate	Cultivar
Slow	Khadrawi Albasra, khastawi, Hadal, Tibearzal
Medium	Sayer, Asab Alaroose, Khasab, Jawzi, Barban,Lolwi, Ashgar, Zahdi
Fast	Sultani, Owayanat Ayou, Chabcgab

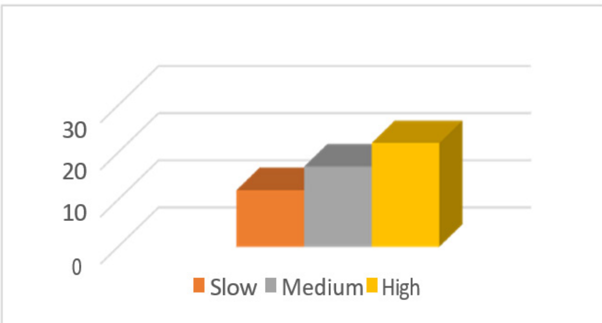


Figure 1. Season’s growth rates average (cm) of some Iraqi date palm cultivars.

Trunk circumference

The circumference of the cultivars under study was classified into three groups, as shown in Table 2. Small circumference trunk was shown by eight cultivars, such as 'Khadrawi', 'Albasra', and 'Zahdi'. On the other hand, the largest circumference was of 'Khasab', 'Barban', and 'Sultani'. The rest of the cultivars had medium circumferences.

Table 2. Circumference trunk groups of several Iraqi date cultivars.

Trunk circumference	Cultivar
Small	Khadrawi Albasra, Sayer, Zahdi, Lolwi, Tibearzal, Khastawi, Owayanat Ayou, Chabchab
Medium	Hadal, Ashkar, Asab Alaroose, Jawzi
Large	Khasab, Barban, Sultani

Leaf length

The palm fronds represent the most important vegetative parts that must be studied carefully (Zaid and de Wet, 2002). The leaves of eight cultivars under study, i.e., 'Khadrawi', 'Albasra', 'Sayer', and 'Zahdi', were in the short range, 331-398 cm. Among the date palm cultivars studied, only 'Sultani' had the longest leaves, measuring 465-532 cm. The rest of the studied cultivars had a medium-sized length of leaves (Table 3; Figure 2).

Table 3. Leaf length groups of several Iraqi date cultivars.

Leaf length	Cultivar
Short	Asab Alaroose, K. Albasra, Sayer, Zahdi, Hadal, Ashgar, Lolwi, Khasab
Medium	Chabchab, Tiberazal, Khastawi, Barban, Owaynat Ayoub, Jawazi
Long	Sultani

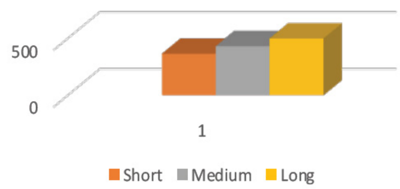


Figure 2. Leaves length average (cm) of some Iraqi date palm cultivars.

Rachis length

The data showed that six cultivars have a short range of rachis length, i.e., 'Sayer', 'Khastawi', and 'Chabchab'; 41-63 cm. While 'Zahdi', 'Ashkar', 'Khasab', 'Hadal', 'Owaynat Ayoub', and 'Khadrawi Albasra' are in the medium range of rachis length, 64-84 cm. The longest rachis was found for 'Tiberzal', 'Lolwi', and 'Sultani'; 85-105 cm (Table 4; Figure 3)

Table 4. Rachis groups of several Iraqi date cultivars.

Rachis length	Cultivar
Short	Sayer, Jwazi, Khastawi, Asab Alaroose, Chabchab, Barban
Medium	Zahdi, Ashgar, Khasab, Hadal, Khadrawi Albasra, Owaynat Ayoub
Long	Tibearzal, Lolwi, Sultani

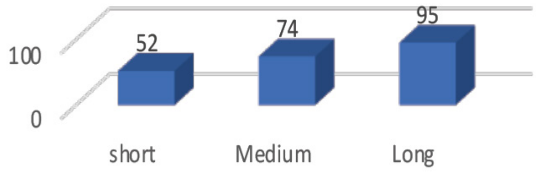


Figure 3. Rachis length average (cm) of some Iraqi date palm cultivars.

Spine number

'Jawzi', 'Kahdrawi Albasa', 'Sayer', 'Khastawi', 'Chabchab', and the other four cultivars were characterized by having low numbers of spines in the range of 12-16 leaf^{-1}. On the other hand, 'Barban' and 'Asab Alaroose' had a high number of spines, 26-34 leaf^{-1}. The medium number of spines was found with 'Zahdi', 'Khasab', 'Ashkar', and 'Sultani' (Table 5; Figure 4).

Table 5. Spine's number groups of several Iraqi date cultivars.

Spine number	Cultivar
Low	Jawzi, Chabchab, Khadrawi Albasra, Lolwi, Sayer, Khastawi, Owaynat Ayoub, Hadal, Tiberzal
Medium	Zahdi, Khasab, Ashgar, Sultani
High	Asab Alaroose, Barban

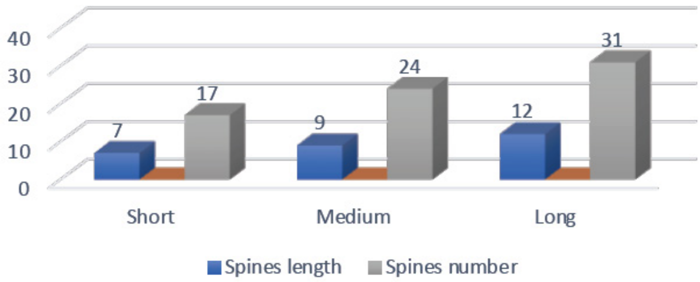


Figure 4. Number of spines and length average of some Iraqi date palm cultivars.

Spine length

The spine's length ranges from 5 to 12 cm. The spines of 'Khadrwi Albasra', 'Sayer', and 'Khastawi' were short (5-7). The spines of 'Zahdi', 'Hadal', 'Chabchab', 'Tiberzal', 'Sultani', and 'Jawzi' were in the medium group. Six of the cultivars had long spines (Table 6; Figure 4). In all the cultivars studied, the spine distribution system was single leaflet.

Table 6. Spine length groups of several Iraqi date cultivars.

Spine length	Cultivar
Short	Khadrawi Albasra, Khastawi, Sayer
Medium	Chabchab, Sultani, Teberzal, Jawzi, Hadal, Zahdi
Long	Khasab, Owaynat Ayoub, Asab Alaroose, Ashgar, Lolwi, Barban

Length of the fruit

Stalk and strands must be taken into consideration when selecting any date palm cultivar for planting (Nixon and Carpenter, 1978).

1. Fruit stalk.

Some of the cultivars under study have short fruit stalks, such as 'Khadrwi Albasra', ‘Zahdi’, ‘Khasab’, and ‘Chabchab’, whereas ‘Barban’, ‘Ashkar’, ‘Jawzi’, ‘Tiberzal’, and ‘Owaynat Ayoub’ fell in the range of medium stalk length. Only ‘Sultani’ has a long fruit stalk among the studied cultivars; 142.8 cm (Table 7; Figure 5).

Table 7. Fruit stalk’s length groups of several Iraqi date cultivars.

Fruit stalk length	Cultivar
Short	Chabchab, Khastawi. Khadrawi Albasra, Asab Alaroose, Sayer, Zahdi, Jawzi, Khasab
Medium	Berban, Ashgar, Lolwi, Teberzal, Owaynat Ayoub
Long	Sultani

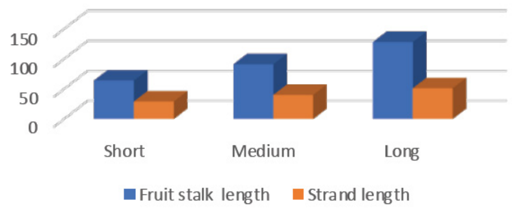


Figure 5. Length of fruit stalks and strands length average (cm) of some Iraqi date palm cultivars.

2. Strand length.

The longest strand length was found with ‘Barban’, ‘Sultani’, ‘Jawzi’, ‘Owaynat Ayoub’, ‘Khasab’, ‘Asaba Alarous’, and ‘Zahdi’ (44 to 55 cm), while ‘Khadrawi’, ‘Al Basra’, ‘Sayr’, ‘Khastawi’, ‘Hadal’, ‘Ashkar’, ‘Chabchab’, ‘Tiberzal’, and ‘Jawzi’ were in the range of medium strand length (Table 8; Figure 5).

Table 8. Strand length groups of several Iraqi date cultivars.

Strand length	Cultivar
Short	Null
Medium	Khastawi, Chatchab, Ashgar, K. Albasra, Sayer, Tiberzal, Lolwi, Hadal, Jawzi
Long	Sultani, Owaynat Ayoub, Barban, Khasab, Zahdi, Asab Alaroose

CONCLUSIONS

In reviewing the results of this study, it can be concluded that the commercial cultivar ‘Khadrawi Albasra’ showed the slowest season’s growth rate among the studied cultivars (Figure 6). This trait contributed crucially to carrying out agriculture practices with less effort and at a more convenient time for many seasons. While the other cultivars season’s growth rates are either medium, i.e., ‘Sayer’, ‘Khasab’, and ‘Zahdi’, or in the fast range, i.e., ‘Sultani’, and ‘Chabchab’.

Significant evidence suggests that all else being equal, the bearing capacity of a date palm is proportional to the number of green leaves carried by its carriers. The value of the green leaves is in producing date palm food for sizing and maturing the fruit. Therefore, it is desirable to retain all the green leaves (Dowson and Aten, 1962; Nixon and Carpenter, 1978). The low number of spines is desirable to carry out the agricultural practices easily and at a convenient time; some of the commercial cultivars, such as ‘Khadrawi Albasra’, ‘Lolwi’, ‘Sayer’, and ‘Khastawi’, had low numbers of spines. Moreover, the long fruit stalk facilitates the practices of pulling down the bunches and prevents tangling with fronds and spines. This practice could be done during the time when the fruit stalk is rather pliable and can easily be bent at the base. In this respect, most of the studied cultivars had short and/or medium fruit stalks. An interesting relationship was found between the leaves and fruit stalks; whenever the leaves were long, the fruit stalks were short and vice versa.

Figure 6. 'Khadrawi Al Basra', at the age of 40 years, has been cultivated with several date palm cultivars (Courtesy Huseein Samawi, 2022, Iraq).

ACKNOWLEDGEMENTS

Mr. Shalash, Mrs. Starr, and Mr. Hussein Samawi provided invaluable assistance. Thanks to Zaffarania Farm for providing all the samples of vegetative growth from various palm date cultivar. Also special thanks to Al-Hamza Dulaimy for editing this scientific paper.

Literature cited

Al-Bekr, A.J. (1972). The Date Palm: a Review of Its Past and Present Status; and Recent Advances in Its Culture, Industry, and Trade (Baghdad: Al-Ani Press), p.1085 (in Arabic).

Al-Jibouri, N.M., Hussain, Z.K., and Ibrahim, K.N. (1971). Description of Hundred and Ten Varieties of Iraqi Date Palm (Iraq: General Dir, Hort., MOA), p.1–44 (in Arabic).

Dowson, V.H.W., and Aten, A. (1962). Dates Handling, Processing, and Packing. No.72 (FAO).

Kozak, M., and Piepho, H.-P. (2020). Analyzing designed experiments: should we report standard deviations or standard errors of the mean or standard errors of the difference or what? Exp. Agric. *56* (*2*), 312–319 https://doi.org/10.1017/S0014479719000401.

Nixon, R.W., and Carpenter, J.B. (1978). Growing Date in the United States. Bulletin No. 207.

Zaid, A., and de Wet, P.F. (2002). Date Palm Cultivation, Chapter I, Plant Production and Protection, 156 (FAO).

Thinning treatments affect yield, fruit quality and skin separation of 'Medjool' date palm grown in semi-arid conditions in Jordan

R.L. Ahmad[1,a], N.T. Mazahreh, J.Y. Ayad[2], K.O. Al Sané[1] and O.A. Samra[1]

[1]National Agricultural Research Centre (NARC), Al-Balqa, Jordan; [2]Department of Horticulture and Crop Science, School of Agriculture, University of Jordan, Amman, Jordan.

Abstract

Date palm (*Phoenix dactylifera* L. 'Medjool') cultivation expanded very fast in the last few decades. Increased fruit size and high nutritive value were major quality characteristics that make it ranked first among other cultivars in Jordan. Skin separation is a major physiological disorder that lowers the commercial quality of 'Medjool' date palm. This study was undertaken for two growing seasons in two major areas of date palm production in Jordan to test for the effects of fruit thinning on enhancing fruit quality and reducing the incidence of skin separation. Six different fruit thinning methods were practiced by; removing 25% of strands number per bunch (T1), shortening strands by removing one third of strand length from terminal tips (T2), removing one third of central strands per bunch (T3), removing 50% of individual fruits per strand (T4), combining both shortening one third of total strands from terminal tips and removing one third of central strands (T5) and control non-thinned fruits (T6). Average fruit yield and bunch weight were significantly lower in response to all thinning treatments as compared to the control and the reduction increased by increasing the level of thinning. Fruit length, fruit weight, and average fruit diameter was the highest under fruit thinning (T5, T4). Fruit thinning resulted in significantly higher percentage of first grade fruits with lower levels of skin separation than control non-thinned fruits. Moreover, thinning treatments resulted in higher total soluble solids (TSS) of 'Medjool' fruits with values ranging from 60.0% (T6) to 71.0% (T4). We suggest fruit thinning at the beginning of kamri stage improved 'Medjool' fruit quality and lower skin separation percentage and, hence, help in enhancing the commercial value and marketability of this valuable date palm.

Keywords: fruit size, skin separation, thinning, TSS, yield

INTRODUCTION

Date palm is the second most important fruit tree after olives (*Olea europaea*) in Jordan. There is a significant expansion in the cultivation of date palm in Jordan and the area cultivated with palm trees has doubled from about 1.67 thousand ha in 2009 to 3.37 thousand ha in 2019, and the number of trees increased from 264,407 to 511,679 trees during the same period, respectively (Department of Statistics, 2009-2019). About 70% of these trees are 'Medjool' and 20% are 'Berhee', while 10% are from different cultivars, producing about 25,000 t $year^{-1}$. Furthermore, 'Medjool', also called 'Medjhool', 'Medjehuel', 'Mejhul' or 'Mejhoul', produces large soft fruits, with orange-yellowish flesh, and mildly rich and delicious taste. It has a unique appearance from other date palm fruits and it is highly priced in markets around the world (Elhoumaizi et al., 2006; Al-hajjaj and Ayad, 2018). In addition, 'Medjool' has a great economic importance and agricultural uses in Jordan. The middle Ghour region in Jordan has been considered the most suitable area for economic production of 'Medjool' dates (Altahat, 2015). In fact, dates play a significant rule in the economy, society, and environment of Jordan.

Although commercial value of 'Medjool' dates depends on fruit size, taste, and sugar

[a]E-mail: lmr_raed@yahoo.com

content, fruit appearance and the lack of disorders is also significant. Skin separation of date palm fruits is known in all cultivated varieties, but it is considered as a very serious problem in 'Medjool' because of its negative impact on commercial value (Gophen, 2021). During the final stages of 'Medjool' fruits ripening; known as "tamer" which is characterized by rapid water loss and shrinking of the mesocarp, the hard dry skin decreases in surface area and wrinkles which results in separating fruit skin from the flesh (Lustig et al., 2021). Fruit quality of dates is affected by various preharvest factors such as irrigation, pollination, fertilization, and fruit thinning as well as postharvest factors such as storage and processing (Al-Yahyai and Manickavasagan, 2013). Several assumptions have been reported regarding the possible reasons and solutions for this disorder: nutrient imbalances, temperatures and humidity fluctuations during fruit development and maturity stages (Lustig et al., 2021).

Date palm trees usually carry large number of fruits in their clusters known as bunches. The heavy bunches can cause some physiological disorders like reduced fruit size, increased percentage of skin separation, delayed ripening and alternate fruit bearing (Atawia et al., 2020). Fruit thinning is crucial for date palm growers (Soliman et al., 2010; El-Badawy et al., 2018; Moustafa et al., 2019) to control alternate bearing which is common in date palm (Soliman et al., 2010) and to alter source sink interaction that results in larger and more uniform size fruits (Soliman and Harhash, 2012).

Several methods have been reported for fruit thinning in date palm orchards. Researchers either recommend fruits thinning by reducing the number of fruits per strand or by reducing the number of strands or shorting strands per bunch or by reducing the number of bunches per tree and increasing the number of leaves per bunch (Panchal et al., 2021). Nevertheless, the method of thinning recommended was dependent on the date cultivars. Therefore, the objective of this current work is to test the proper thinning techniques that improve 'Medjool' date palm fruits yield and quality and reduces the incidence of skin separation.

MATERIAL AND METHODS

Planting material and experimental sites

Field studies were carried out for two growing seasons (2018 and 2019) at two different locations in Jordan Valley: Al-Karama and Wadi Araba. These two locations represent major date palm production areas in Jordan. Uniform 10-years-old of 'Medjool' date palm trees grown at 8×8 m spacing were selected for this study. Regular agricultural practices of irrigation and fertilizers management were followed according to farm recommendations. Furthermore, the number of bunches per tree were maintained to ten according to local recommendation.

Thinning treatments

Eighteen uniform date palm trees were randomly selected at both Al-Karama and Wadi Araba. Five weeks after pollination, the bunches were subjected to six different types of thinning practices in both seasons described as follows: T1 – strand thinning by removing 25% of strands per bunch; T2 –shortening strands by removing one third of strand length from terminal tips; T3 – strand thinning by removing one third of central strands per bunch; T4 – fruit thinning by removing 50% of individual fruits per strand; T5 – combining both shortening one third of total strands from terminal tips and removing one third of central strands; and T6 –control non-thinned fruits.

Fruit harvesting, yield and quality assessment

Trees were harvested at maturity ripening stage "tamer" in both locations. Average bunch weigh and fruit yield tree^{-1} were measured at harvest time. Fruit samples were randomly selected at mature tamer stage for fruit physical and quality assessment. 100 fruits per experiment unit were randomly selected to measure average fruit weight, fruit length, and fruit width. Total soluble solids were measured in fruit juice by refractometer.

Skin separation assessment

The percentage incidence of skin separation in 'Medjool' fruits at harvest time were classified into three levels by counting the number of fruits with skin separation to the total number of fruits; A-class with 0-35% skin separation, B-class with 35 to 75% skin separation, and C-class with skin separation that exceeds 75% (Gophen, 2021).

Experimental design

Experiment treatments of 6 different fruit thinning practices were arranged in a randomized complete block design (RCBD) with three replicates (according to Steel and Torrie, 1980). Analysis of variance type II and means of different treatments were analyzed using SAS software (version 9 for Windows). Duncan's multiple range test at p<0.05 was used to identify differences among thinning treatment means.

RESULTS AND DISCUSSION

Data given in Tables 1 and 2 summarize the effects of thinning treatments on average fruit physical properties; weight, length, diameter and %moisture. Average fruit weight significantly increased in response to different thinning treatments (Table 1). A similar trend was observed at the two experimental locations. The maximum average fruit weight was obtained at both T4 (23.04-24.51 g) and T5 (24.91-25.32 g) as compared to the control non-thinned fruits (17.94-20.48 g) at both Al-Karama and Wadi Araba, respectively. Maximum fruit weight in both locations was obtained by combining both T2 and T3 treatments which was accomplished by shortening one third of total strands from terminal tips and removing one third of central strands followed by removing 50% of individual fruits per strand.

Table 1. Effects of fruits/strands thinning on average fruit weight (g) and length (mm) of 'Medjool' date palm.

Treatment[a]	Average fruit weight (g)		Average fruit length (mm)	
	Al-Karama	Wadi Araba	Al-Karama	Wadi Araba
T1	17.94 d	20.48 c	46.0 de	44.0 f
T2	18.27 d	22.59 b	46.2 de	45.0 ef
T3	20.74 c	23.10 b	47.4 c	46.4 cd
T4	23.04 b	24.51 a	53.4 a	49.8 b
T5	24.91 a	25.32 a	54.4 a	50.2 b
T6	15.98 e	18.78 d	42.7 g	41.7 g
LSD value	0.96		1.3	

[a]T1 – strand thinning by removing 25% of strands per bunch; T2 – shortening strands by removing one third of strand length from terminal tips; T3 – strand thinning by removing one third of central strands per bunch; T4 – fruit thinning by removing 50% of individual fruits per strand; T5 – combining both shortening one third of total strands from terminal tips and removing one third of central strands; T6 – control non thinned fruits.

Means within each column followed with different letters indicate significant differences at p≤0.05.

The increased fruit weight primarily resulted from the increased average fruit size. Fruit length (Table 2) and diameter (Table 3) of 'Medjool' dates were positively increased in response to all thinning treatments in both studied locations. Thinning treatment T5 resulted in significantly increased average fruit length (54.4 mm in Al-Karama and 50.2 mm in Wadi Araba) followed by T4 thinning treatment which was carried out by removing 50% of individual fruits and resulted in 53.4 mm average fruit length in Al-Karama and 49.8 mm in Wadi Araba. The control non-thinned trees recorded the lowest fruit length in the two locations (42.7 and 41.7 mm), respectively.

Similarly, T5 and T4 thinning treatments resulted also in larger fruits diameter. The highest values of fruit diameter (29.9 and 29.8 mm) were obtained for T5 treatment in both Al-Karama and Wadi Araba, respectively, followed by T4, which gave 29.6 and 29.0 mm in both locations, respectively.

Fruit's moisture content at ripening was also affected by thinning treatment (Table 2).

Shortening strands by removing one third of strand length from terminal tips (T2) resulted in decreased fruit moisture content (27.8%) in Al-Karama location, while fruit thinning by removing 50% of individual fruits per strand (T4) resulted in the lowest value (27.5%) at Wadi Araba location as compared to the control (28.8 to 32.0%) in both locations.

Table 2. Effects of fruits/strand thinning on average fruit diameter (mm) and moisture content (%) of 'Medjool' date palm.

Treatment[a]	Average fruit diameter (mm)		Moisture %	
	Al-Karama	Wadi Araba	Al-Karama	Wadi Araba
T1	26.9 de	24.8 f	28.9 bcd	29.7 bc
T2	25.7 ef	26.0 def	27.8 cd	28.9 bcd
T3	27.9 bcd	27.6 cd	30.3 ab	28.4 bcd
T4	29.6 ab	29.0 abc	28.6 bcd	27.5 d
T5	29.9 a	29.8 a	30.2 ab	28.0 cd
T6	24.3 fg	22.7 g	28.8 bcd	32.0 a
LSD value	1.817		1.95	

[a]T1 – strand thinning by removing 25% of strands per bunch; T2 – shortening strands by removing one third of strand length from terminal tips; T3 – strand thinning by removing one third of central strands per bunch; T4 – fruit thinning by removing 50% of individual fruits per strand; T5 – combining both shortening one third of total strands from terminal tips and removing one third of central strands; T6 – control non thinned fruits.

Means within each column followed with different letters indicate significant differences at p≤0.05.

Table 3. Effects of thinning treatments on average bunch weight (kg), fruit yield (kg tree^{-1}) and TSS% of 'Medjool' date palm.

Treatment[a]	Average bunch weight (kg)		Average yield (kg tree^{-1})		TSS (%)	
	Al-Karama	Wadi Araba	Al-Karama	Wadi Araba	Al-Karama	Wadi Araba
T1	10.9 b	6.4 cd	108.6 b	63.9 cd	64.1 def	62.2 ef
T2	10.2 b	6.1 de	102.2 b	63.9 cd	65.3 cde	66.2 ef
T3	9.7 b	5.5 de	96.5 b	55.0 de	67.2 bcd	66.6 bcd
T4	9.9 b	4.9 e	98.7 b	48.8 e	71.0 a	68.6 abc
T5	10.0 b	5.6 de	99.7 b	55.5 de	70.1 ab	69.5 ab
T6	17.9 a	7.6 c	179.7 a	75.2 c	62.1 ef	60.3 f
LSD value	1.4		14.1		3.8	

[a]T1 – strand thinning by removing 25% of strands per bunch; T2 – shortening strands by removing one third of strand length from terminal tips; T3 – strand thinning by removing one third of central strands per bunch; T4 – fruit thinning by removing 50% of individual fruits per strand; T5 – combining both shortening one third of total strands from terminal tips and removing one third of central strands; T6 – control non thinned fruits.

Means within each column followed with different letters indicate significant differences at p≤0.05.

The positive effects of thinning practices on fruits physical properties might be attributed to the reduction in fruit competition for nutrients and photosynthetic products and to the reductions in compactness of fruits within bunches that improves their coloration and make maturity more uniform (Soliman et al., 2010).

In agreement with our findings, removing 15 to 30% of the central strands per bunch resulted in increased fruit weight of 'Khalas' dates as compared to the non-thinning treatments (Soliman et al., 2010). Moreover, fruit thinning by removing 30% of central strands per bunch of 'Khadrawi' date palm resulted in larger fruit size than those non-thinned or thinned by removing 15% of central strands (Moustafa et al., 2019). Moreover, fruits and bunch thinning practices are primarily used to manage alternate bearing problems associated with non-thinning and improve both fruit size and quality (Chao and Krueger, 2007).

Increased fruit size and decreased moisture content at maturity were also observed in response to strands thinning in date palm (Panchal et al., 2021; Soliman and Harhash, 2012). Furthermore, El-Badawy et al. (2018) have indicated increased fruit size of 'Sewi' date palm and decreased moisture content in response to increased percentage of thinning.

On the other hand, Marashi and Mousavi (2007) reported that 'Barhee' fruit size and quality was not affected by bunch and strand thinning treatments in an experiment conducted in Iran. Moreover, Moustafa et al. (2019) indicated no significant effect among all thinning treatments on fruits moisture content in one growing season, while strand thinning by removing 30% of total number of strands significantly decreased moisture content in the second season.

Effect on average bunch weight and yield

The results of various thinning treatments on average bunch weight and total yield tree^{-1} took a similar trend during the two growing seasons in both locations (Table 3). In general, all thinning treatments of 'Medjool' date palm significantly reduced bunch weight and yield tree^{-1} as compared to non-thinning treatments. Average bunch weight decreased ranged from 17.9 kg (T6) to 9.7 kg (T3) in Al-Karama area and from 7.6 kg (T6) to 4.9 kg (T4) in Wadi Araba area. Similarly, average fruit yield decreased in response to thinning from 179.7 to 96.5 kg tree^{-1} in Al-Karama and from 75.2 to 48.8 kg tree^{-1} at Wadi Araba.

Similar responses were reported about thinning practices by removing 15 and 30% of the total number of strands from the center of each bunch in 'Khalas' date palm (Soliman et al., 2010) and 'Succary' date palm (Soliman and Harhash, 2012) and 'Segae' dates (Al Saikhan, 2008). Furthermore, strand thinning to either 20 or 25 strands bunch^{-1} significantly decreased the bunch weight and yield tree^{-1} of 'Zaghloul' as compared to non-thinning.

On the other hand, Al-Saikhan (2008) reported that thinning practices by removal of 1/3 of central strands and shorting the tips of strands by 1/3 in 'Ruzeiz' date palm reduces significantly both bunch weight and fruit yield as compared to control and to either method of strand thinning techniques alone.

Effect on total soluble solids

Total soluble solids (TSS) significantly increased in response to different thinning treatments in both locations (Table 3). Thinning treatments of T4 and T5 resulted in the highest TSS in both Al-Karama and Wadi Araba with values ranging from 70.1 to 69.5% for T5 and from 71.0 to 68.6% for T4, respectively. On the contrary, non-thinning treatment resulted in the lowest value in both locations (62.1 to 60.3%), respectively. This might be because fruit thinning alters source sink relations through lowering the competition between fruits and thus increasing adequate allowable carbohydrates to the remaining (Moustafa et al., 2019) and hence increasing the total soluble solids and sugar content of fruits and hasten maturity and improve fruit quality. In agreement with our work, thinning treatments significantly increased the TSS in tamer stage of date palm and that might be due to the higher loading rate of photosynthetic products and other metabolites to the fruits (Al-Saikhan and Sallam, 2015). In this context, similar findings were reported by several investigators who have studied several date cultivars (Soliman et al., 2010; Soliman and Harhash, 2012).

Effect on the fruit grade and skin separation

Fruits thinning practices altered fruit physical properties as described previously. However, fruits were classified into 3 grades according to the percentage of skin separation disorder: Grade-A with 0 to 35% of skin separation, Grade-B with 35 to70% of skin separation and Grade-C with 70 to 100% of skin separation (Table 4).

All thinning treatments resulted in reduced fruits skin separation (0-35%) and therefore higher percentage of grade-A fruits compared to the non-thinning. Removing 50% of individual fruits per strand (T4) resulted in the highest percentage of grade-A fruits in Al-Karama (67.7%) and Wadi Araba (76.3%) and the lowest percentage of Grade-B and C. Whereas the control non-thinned trees in both locations produced the lowest percentage of Grade-A fruits (52.3 and 59.5%) in both locations, respectively.

Table 4. Effect of thinning treatments on fruit quality grades (% skin separation) of 'Medjool' date palm.

Treatment[a]	Grade-A[b]		Grade-B		Grade-C	
	Al-Karama	Wadi Araba	Al-Karama	Wadi Araba	Al-Karama	Wadi Araba
T1	58.5 f	65.9 cd	23.3 bcd	21.3 def	18.2 b	12.7 cd
T2	57.7 f	63.1 e	24.9 ab	24.8 abc	17.6 b	12.1 cd
T3	63.9 de	67.7 c	22.4 cde	20.2 ef	13.6 cd	12.1 cd
T4	67.7 c	76.3 a	19.3 f	16.8 g	13.0 cd	6.9 e
T5	65.9 cd	73.2 b	22.4 cde	19.2 fg	11.6 d	7.6 e
T6	52.3 g	59.5 f	21.8 de	25.9 a	25.8 a	14.6 c
LSD value	2.7		2.4		2.6	

[a]T1 – strand thinning by removing 25% of strands per bunch; T2 – shortening strands by removing one third of strand length from terminal tips; T3 – strand thinning by removing one third of central strands per bunch; T4 – fruit thinning by removing 50% of individual fruits per strand; T5 – combining both shortening one third of total strands from terminal tips and removing one third of central strands; T6 – control non thinned fruits.

[b]The levels of skin separation were as follows: Grade A (0-35% of skin separation), Grade B (35-70% of skin separation) and Grade C (70-100% of skin separation).

Means within each column followed with different letters indicate significant differences at $p \leq 0.05$.

Similar results were reported by Soliman et al. (2010) and Soliman and Harhash (2012) who have found that thinning treatments resulted in a higher percentage of first grade fruits than the non-thinned trees. On the other hand, Lustig et al. (2021) found that skin separation was affected by other factors such as the climatic condition during fruit maturity and ripening stages such as diurnal variations of the ambient relative humidity and temperature. They have suggested that skin separation can be reduced by reducing the difference of the vapor pressure between the fruit skin and the environment.

CONCLUSIONS

From the results of this study, it could be recommended that thinning treatments of 'Medjool' date palm have improved fruit chemical and physical characteristics compared to the control. In this regard, the most effective treatments wereT5 (combining both shortening one third of total strands from terminal tips and removing third of central strands) and T4 (fruit thinning by removing 50% of individual fruits per strand). These thinning practices increased average fruit weight, length, diameter, TSS% and reducing level of skin separation by 28-29% compared to control.

ACKNOWLEDGEMENTS

Authors would like to thank the Scientific Research Fund and Innovation for supporting this project.

Literature cited

Al-hajjaj, H.S., and Ayad, J.Y. (2018). Effect of foliar boron applications on yield and quality of Medjool date palm. J. Appl. Hortic. *20* (*3*), https://doi.org/10.37855/jah.2018.v20i03.32.

Al-Saikhan, M.S. (2008). Effect of thinning practises on fruit yield and quality of Ruzeizdate palm cultivar (*Phoenix dactylifera* L.) in Al-Ahsa Saudi Arabia. Asian J. Plant Sci. *7* (*1*), 105–108 https://doi.org/10.3923/ajps.2008.105.108.

Al Saikhan, M.S., and Sallam, A.A. (2015). Impact of chemical and non-chemical thinning treatments on yield and fruit quality of date palm. J. Food Res. *4* (*4*), 18–29 https://doi.org/10.5539/jfr.v4n4p18.

Al-Yahyai, R., and Manickavasagan, A. (2013). Quality of dates: influencing factors and assessment methods. Acta Hortic. *1012*, 1241–1246 https://doi.org/10.17660/ActaHortic.2013.1012.167.

Altahat, E.S. (2015). Analysis of agricultural policies affecting Medjool palm cultivation in Jordan. J. Agric. Sci. *7* (*4*), 129–134.

Atawia, A.A.R., El-Akkad, T.A.M., and Hassan, T.M. (2020). Effect of thinning treatments on some physical and chemical fruit characters of Barhy and Magdool date palm cultivars. Ann. Agric. Sci. *58* (*2*), 339–350.

Chao, C.C.T., and Krueger, R.R. (2007). The date palm (*Phoenix dactylifera* L.): overview of biology, uses and cultivation. HortScience *42* (*5*), 1077–1082 https://doi.org/10.21273/HORTSCI.42.5.1077.

Department of Statistics. (2009-2019). Agricultural Statistics (Amman, Jordan), www.dos.gov.jo.

El-Badawy, H.E.M., El-Gioushy, S.F., and Ahmed, I.A.M. (2018). Effect of some thinning practices on yield and fruit quality of Sewidate palm grown in Farafra Region. Asian Journal of Agricultural and Horticultural Research. *2* (*3*), 1–20 https://doi.org/10.9734/AJAHR/2018/45474.

Elhoumaizi, M.A., Devanand, P.S., Fang, J., and Chao, C.C.T. (2006). Confirmation of 'Medjool' date as a landrace variety through genetic analysis of 'Medjool' accessions in Morocco. J. Am. Soc. Hortic. Sci. *131* (*3*), 403–407 https://doi.org/10.21273/JASHS.131.3.403.

Gophen, M. (2021). Study on Skin separation in date fruits. Recent Progress in Plant and Soil Research. *3* (*27*), 42–52 https://doi.org/10.9734/bpi/rppsr/v3/13694D.

Lustig, I., Bernstein, Z., and Gophen, M. (2021). Study on skin separation in Majhul fruits. Recent Progress in Plant and Soil Research. *3* (*27*), 67–76 https://doi.org/10.9734/bpi/rppsr/v3/11232D.

Marashi, S., and Mousavi, A. (2007). Effects of Different methods and degrees of fruit thinning on yield and fruit characteristics of Barhee date cultivar. Acta Hortic. *736*, 187–192 https://doi.org/10.17660/ActaHortic.2007.736.15.

Moustafa, A.R., Abdel-Hamid, N., Abd El-Hamid, A., El-Sonbaty, R., and Abd El-Naby, S.K.M. (2019). Strand thinning of Khadrawi date palm cultivar in relation to yield and fruit quality. Bull. Natl. Res. Cent. *43* (*1*), 204 https://doi.org/10.1186/s42269-019-0234-3.

Panchal, C.N., Muralidharan, C.M., Baidiyavadra, D.A., and Mohan Sharma, K. (2021). Standardization of leaf: bunch ratio in date palm (*Phoenix dactylifera* L.). Plant Arch. *21* (*1*), 1757–1759 https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.279.

Soliman, S.S., Al-Obeed, R.S., and Harhash, M.M. (2010). Effects of bunch thinning on yield and fruit quality of 'Khalas' date palm. Acta Hortic. *882*, 725–732 https://doi.org/10.17660/ActaHortic.2010.882.79.

Soliman, S.S., and Harhash, M.M.Soliman S. S. (2012). Effects of strands thinning on yield and fruit quality of Succary date palm. Afr. J. Biotechnol. *11* (*11*), 2672–2676 https://doi.org/10.5897/AJB11.3277.

Steel, R.G.D., and Torrie, J.H. (1980). Principles and Procedures of Statistics (New York: McGraw-Hill).

Deficit irrigation: a viable option to improve irrigation efficiency of date palm in Saudi Arabia

A. Al-Omran[a], I. Louki and F. Alshammari

College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia.

Abstract

The continued expansion of agriculture and urban development in the GCC countries – which is located within the arid regions – is accompanied with growing demand for water supply in different sectors. All of this calls for the agricultural sector to look for practices that increase the irrigation efficiency and water conservation and increasing water productivity of crops. We determined the actual water requirements as an introduction to water conservation with deficit irrigation (DI) programmes. The study was conducted in eight different regions of Saudi Arabia to estimate monthly and annual crop water requirements (CWR).

Keywords: deficit irrigation (DI), water requirements, water balance method, Penman-Monteith method

INTRODUCTION

The agricultural expansion of date palm in Saudi Arabia faces huge challenges, which are typical of dry regions characterized with water scarcity, low rainfall and high evapotranspiration demand. Most of the irrigation water is obtained from groundwater storage. The agricultural sector consumed more than 85% of water consumption, which reached more than 23 billion m^3 in 2012 (Ministry of Water and Electricity, 2015). Due to the increases of water demand, an effective and accurate evaluation of crop water requirement (CWR) is essential for planning, designing, operating, managing farm irrigation systems. Accurate estimation of CWR can help to maintain efficient use of water resources for irrigation. Evapotranspiration (ET) plays a major role in irrigation water management (Allen et al., 1998). They reported that many factors may play a role in limiting crop development; these are water availability, soil salinity, poor land fertility, poor soil and water management, plant density, and soil water contents. In Saudi Arabia, the limiting factor in agricultural development is water availability to irrigate the increasing number of date palm trees. Based on the recent statistical reports (General Authority for Statistics, 2015), the total number of date palm trees under drip irrigation system is 54,000 ha and 53,200 ha under surface irrigation.

The objective of the present study was to determine the date palm water requirements of eight regions of Saudi Arabia taking in the consideration the shaded area of the tree and irrigation water quality, and compare date palm water requirements with the actual water added by farmers in the same regions.

MATERIAL AND METHODS

Experimental sites

This study was conducted on eight different regions of Saudi Arabia to estimate monthly and annual irrigation water requirements of date palm (*Phoenix dactyliferous* L.) of 'Klayas' cultivar. Fields measurements and determination of ETc were taken during one year starting October 2013-September 2014 on complete grown tree (more than 10 years old). Fields that have been selected are located in regions of the Medina (Al Ula), Tabuk (Teimaa), Makkah (Al Jumum), Al Jouf (Sakakah), Riyadh (Sodos), Qassim (Riyad Al Khabra, Hail (Al Kaedh), East

[a]E-mail: rasoul@ksu.edu.sa

Region (Al Ahsa) (Figure 1).

Figure 1. Location of date palm fields in eight different regions of Saudi Arabia.

Metrological data

Small weather stations were installed in each site of the study to monitor the changes of meteorological data during the study periods. The meteorological data recorded were: net radiation (MJ m^{-2} day^{-1}), wind speed (m h^{-1}), air temperature (°C), relative humidity (%) and rainfall (mm). The air water vapor pressure deficit (kPa) was calculated using daily and hourly average temperatures and relative humidity. Finally, the reference evapotranspiration (ETr, mm day^{-1}) was calculated according to the Penman-Monteith (PM) equation as specified by the FAO protocol (Allen et al., 1998).

Estimation method of ET

1. Penman-Monteith method.

The Penman-Monteith equation was used based on climate data on the farm as part of the national project of the rationalization of the irrigating water in agriculture (RIWA), Ministry of Environment, Water, Agriculture to estimate the water needs and then calculate the total irrigation water requirements based on the quality of irrigation water and soil salinity, taking into account the values of crop coefficient Kc for each month, irrigation efficiency and shaded area of date palm. The combined FAO Penman-Monteith method was used to calculate ETo through the following equation:

$$ET_o = \frac{0.408\,\Delta(Rn - G) + \gamma\left(\frac{900}{T+273}\right)U_2\,(e_s - e_a)}{\Delta + \gamma\,(1 + 0.34U_2)} \quad (1)$$

where ET_o = reference evapotranspiration (mm day^{-1}); Rn = net radiation at the crop surface (MJ m^{-2} day^{-1}); G = soil heat flux density (MJ m^{-2} day^{-1}); T = mean daily air temperature at 2 m height (°C); U_2 = wind speed at 2 m height (m s^{-1}); e_s = saturation vapor pressure (kPa); e_a = actual vapor pressure (kPa); e_s-e_a = saturation vapor pressure deficit (kPa); Δ = slope of saturation vapor pressure curve at temperature T (kPa °C^{-1}); γ = psychrometric constant (kPa °C^{-1}).

As crop evapotranspiration ET_c can be calculated as:

$$ET_c = K_c \times ET_r \quad (2)$$

where K_c = crop coefficient ranged from 0.8-1.0 depending on the month of year as noted in (Allen et al., 1998); ET_r = ET_o=reference crop evapotranspiration (mm day^{-1}); ET_c = crop evapotranspiration (mm day^{-1}).

The percentage of evapotranspiration area (S_e) was calculated from actual shaded are at noon in June to the actual area to each tree from the following equation as described by Hellman (2010) for grape:

$$S_e = \frac{Shaded\ area\ per\ tree}{Actual\ area}\ x\ 100 = \frac{\pi\ R^2}{10m\ x\ 10m} \quad (3)$$

where S_e = the percentage of evapotranspiration area; R = radius of tree (m); Shaded area = area of the shade of one tree measured at noon.

Leaching requirements were calculated using the following equation (Doorenbos and Pruitt, 1977):

$$LR = \frac{EC_{iw}}{2MaxEC_e} \times \frac{1}{Eff} \quad (4)$$

where LR = the fraction of the water to be applied that passes through the entire root zone depth and percolates below; EC_{iw} = electrical conductivity of irrigation water (dS m^{-1}); EC_e = electrical conductivity of the soil saturation extract for a given crop appropriate to the tolerable degree of yield reduction (dS m^{-1}); Max EC_e = maximum tolerable electrical conductivity of the soil saturation extract for a given crop (dS m^{-1}); Eff = leaching efficiency (90% for sandy and loamy sands).

The gross water requirements was calculated by (GWR):

$$GWR = \frac{ET_c \times S_e}{(1\text{-}LR) \times Effir} \quad (5)$$

where GWR = gross water requirement (m^3 ha^{-1}); ET_c = crop evapotranspiration (m^3 ha^{-1}); Effir = efficiency (%), 90%; LR = leaching requirements; S_e = the percentage of evapotranspiration area.

2. Water balance method.

Water balance method by difference of soil moisture content between two irrigations by measuring changes in moisture content after and before irrigation at the root zone using a device to measure moisture (Terra Sen Dacom) at depths of 10-120 cm all year, after verifying the accuracy of moisture sensitive, calibrated sensors with direct method (gravimetric laboratory method) with data from the sensors for a period of two months for three sites. The total amount of irrigation during one is calculated by the following equation:

$$ET = P + I - Dr \pm \Delta S \quad (6)$$

where ET = consumptive use (mm); P = precipitation (mm); I = irrigation added (mm); Dr = drainage (mm); ΔS = change in soil water content (mm).

3. Measurement.

1) 5TE: a total of 12 ECH2O-5TE sensors were used with 3 Em50 data loggers (METER Group, Inc., USA, formerly Decagon);
2) Em50 data logger: a 5-channel, self-contained data recorder designed for use with any ECH2O sensor. Two types of output data by this device can be obtained: raw count (unprocessed data) and processed data, which are converted to volumetric water content (m^3 m^{-3}) that is ready to use directly.

4. Volumetric water content (VWC) by gravimetric method and by sensors.

The volumetric water content in the soil sample was determined by a gravimetric method and measured by sensors. The sensor output of the processed data that were converted the raw data internally into engineering values of volumetric water content as (cm^3

cm^{-3}), electric conductivity (mS cm^{-1}), and temperature (°C) by factory calibration was used in this study to investigate the results and devise a correction equation. Sensors were inserted vertically into a 20-L plastic pot containing 17 kg of a soil sample at 15 cm in depth for 15 min. An ECH$_2$O-5TE sensor was connected to a continuous data logger (model EM50) and programmed to collect readings at 1-min intervals in order to determine the soil water content for a moisture experiment and EC test.

RESULTS AND DISCUSSION

Date palm water requirement in the experimental sites

The results of the study in Tables 1-3 show that the irrigation water requirements (m^3 ha^{-1}) after taking into account the proportion of cultivated area for each tree of the sites in, Medina, Tabuk, Makkah, Al Jouf, Riyadh, Qassim, Hail, East Region were 9495.24, 7340.18, 7298.93, 8913.59, 8614.96, 8568.68, 7996.99, and 8510.72 m^3 ha^{-1}, respectively, with 100 palm trees ha^{-1}. The total annual irrigation water requirements (m^3 tree^{-1}) in these sites were: 95, 73.4, 73, 89, 86, 85.7, 80, and 85 m^3, respectively, as the radius of shaded area tree^{-1} was 3.5 m with effective diameter of 90%, and the rate of leaching were: 12, 8, 13, 12, 14, 11, 13, and 13%, respectively. Irrigation efficiency was 90%, and it was found that the average overall irrigation water requirements in all sites was 8342.41 m^3 ha^{-1} year^{-1} with 100 (trees ha^{-1}). These values of ETc and CRW were attributed to the metrological conditions of each site. However, the reduction in the estimated CWR to an average of 8342 m^3 ha^{-1} compared to overall average of 20,000 m^3 ha^{-1} was reported by many researchers (Al-Amoud et al., 2012; Ismail et al., 2014; Mihoub et al., 2015; Dewidar et al., 2015) was mainly attributed to the percentage of vegetative cover or shaded area (S_e) of the tree, as we calculated the S_e values as (0.33) of the actual area of the tree. Therefore, the practice distance of 10×10 m between trees in the farms of Saudi Arabia was considered not adequate in all sites. This area of 100 m^2 for each tree was overestimating the crop water requirements and therefore, it must be changed to 7×7 m in order to have a higher vegetative cover in date palm farms.

Table 1. Mechanical analysis of three types of experimental soil.

Nº	Sand (%)	Silt (%)	Clay (%)	Texture	EC (dS m^{-1})	CEC (meq 100 g^{-1})
1	75.35	11.32	13.33	Sandy Loam	1.09	11.54
2	94.97	2.01	3.02	Sand	0.6	4.83
3	16.50	39.24	44.27	Clay	2.5	23.69

Table 2. Routine analysis of experimental sandy loam soil.

pH	SP (%)	$CaCO_3$ (%)	Na^+ (meq L^{-1})	K^+ (meq L^{-1})	Ca^{2+} (meq L^{-1})	Mg^{2+} (meq L^{-1})	HCO_3^- (meq L^{-1})	Cl^- (meq L^{-1})	SO_4^{2-} (meq L^{-1})
8.24	24	18.92	3.63	0.73	3	3.2	2.22	3.89	5.04

2. Water balance method.

Effect of soil moisture content on 5TE sensor measurement accuracy

Figure 2 shows that the effect of soil moisture on the sensor reading accuracy depends on moisture level, the EC level, and the temperature. There was no significant impact on the measurement accuracy when the EC and temperature of the soil were 2.42 dS m^{-1} and 25°C, respectively. However, the differences between the sensor readings and real moisture increased with increasing soil moisture levels as much as the differences in the soil EC and its temperature away from the indicated limits.

Table 3. The observed average values of the climatic variables eight sites.

Sites	Stat	T-mean (°C)	T-max (°C)	T-min (°C)	Rainfall (mm)	Radiation (MJ m^{-2})	RH-min (%)	Wind speed (m s^{-1})	ET_0 (mm)	Kc	ETc (mm)
Medina	Min.	9.30	13.50	2.80	0.00	2.05	6.00	0.40	2.23	0.80	1.78
	Max.	34.40	40.10	30.30	0.60	11.22	44.00	5.10	12.21	1.00	12.21
	Ave.	24.34	30.51	17.81	0.00	8.19	16.51	2.53	7.11	0.91	6.63
Tabuk	Min.	5.60	9.50	-3.30	0.00	0.79	6.00	0.20	1.18	0.80	0.94
	Max.	33.20	41.80	26.30	8.00	9.76	75.00	4.20	10.57	1.00	10.57
	Ave.	21.67	28.72	14.35	0.11	7.23	18.47	1.95	5.72	0.91	5.32
Makkah	Min.	20.20	23.90	13.10	0.00	1.81	6.00	0.50	2.43	0.80	1.94
	Max.	39.50	49.90	32.00	12.80	9.15	58.00	2.80	8.40	1.00	8.40
	Ave.	30.36	37.71	23.77	0.04	6.67	23.27	1.36	5.46	0.91	5.03
Al Jouf	Min.	4.10	7.00	-1.60	0.00	0.46	4.00	0.80	0.75	0.80	0.60
	Max.	37.50	43.60	32.30	42.00	10.89	92.00	6.50	15.65	1.00	15.65
	Ave.	22.47	28.62	15.93	0.38	7.45	22.05	2.72	6.59	0.91	6.19
Riyadh	Min.	7.40	10.70	-0.70	0.00	1.17	5.00	0.50	1.22	0.80	0.98
	Max.	37.10	44.10	30.70	16.60	10.00	87.00	4.30	12.72	1.00	12.72
	Ave.	24.90	31.81	16.95	0.26	7.52	17.73	1.91	6.29	0.91	5.86
Qassim	Min.	6.00	9.90	-1.90	0.00	1.41	5.00	0.70	1.51	0.80	1.21
	Max.	38.00	45.70	30.50	13.40	9.08	76.00	5.00	13.34	1.00	12.74
	Ave.	25.12	32.80	16.82	0.08	6.90	17.58	2.23	6.50	0.91	6.05
Hail	Min.	4.80	7.30	-2.40	0.00	0.76	7.00	0.40	0.76	0.80	0.61
	Max.	37.10	43.80	31.20	30.40	10.81	95.00	4.80	10.43	1.00	10.43
	Ave.	22.31	29.50	15.08	0.54	7.51	22.32	2.14	5.90	0.91	5.50
East Region	Min.	9.10	14.40	0.50	0.00	0.88	6.00	0.60	1.16	0.80	0.90
	Max.	39.20	47.50	32.70	26.80	10.28	89.00	4.80	12.69	1.00	12.70
	Ave.	25.60	34.28	18.81	0.40	6.98	20.96	2.08	6.30	0.91	5.88

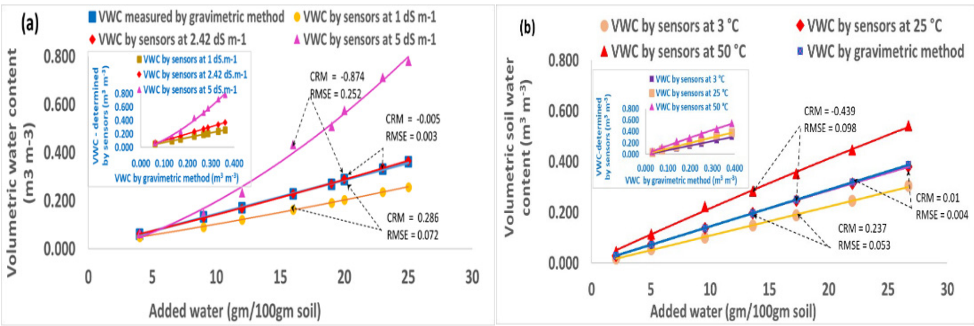


Figure 2. Correlation between actual added water level and sensor reading average at (a) different salinity levels and (b) temperature.

Effect of electrical conductivity (ECe) on sensor measurement accuracy.

The effect of gradually increased salinity levels expressed as the electrical conductivity on the sensor measurements of VWC at 25°C are shown in Figure 3. The sensor measurements were close to the real VWC values when the soil ECe was about 2.42 dS m^{-1}. However, the sensor underestimated or overestimated the real value when the ECe was under or over 2.42 dS m^{-1}. The difference between the sensor reading and the real moisture increased as much as the ECe differed from the indicated limit when the other factors were fixed. Rosenbaum et al. (2011) also reported similar results.

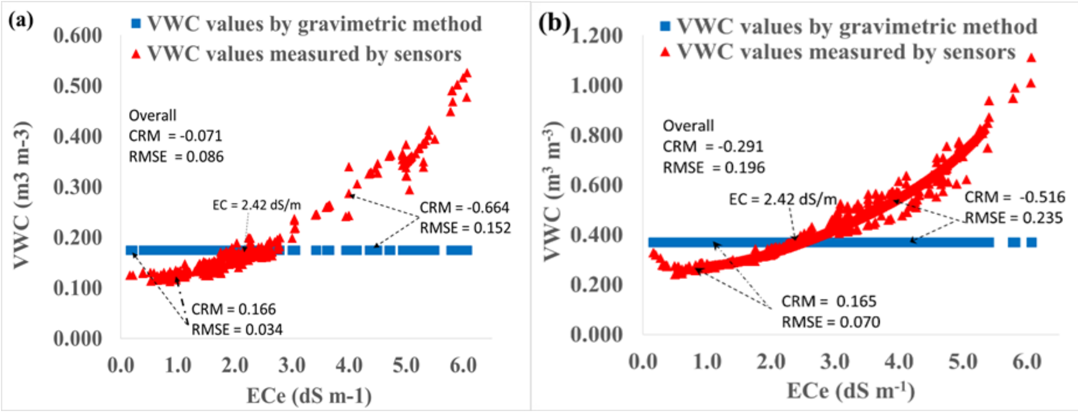


Figure 3. Effect of EC on sensor measurements at (a) field capacity and (b) saturated points.

Effect of soil temperature on sensor measurement accuracy.

The sensor measurement accuracy of VWC under different soil temperatures (from 0 to 50°C) at a fixed EC of 2.42 dS m^{-1} was tested. Figure 4 shows that the measured VWC-value line crossed the real-value line when the soil temperature was about 25°C. However, the sensor readings were under- or overestimated when the soil temperature was lower or higher than 25°C, respectively. This result agreed with many studies (Rosenbaum et al., 2011).

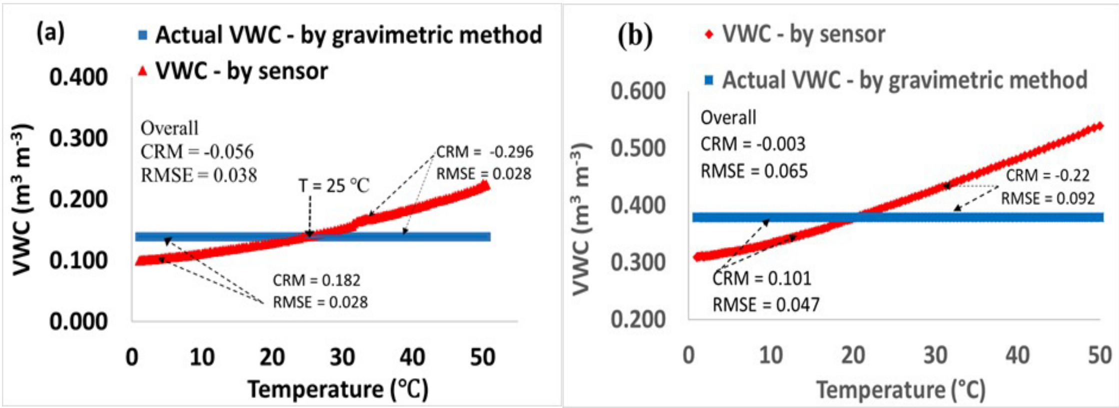


Figure 4. Effect of soil temperature on sensor measurements at (a) field capacity and (b) saturated points.

The results of water balance method showed that the relationship between the data of Terra Sen Dacom sensors and direct method (Gravimetric Method) in Figure 2 for a period of two months for the three sites is a linear relationship with r^2=0.90-0.93, and the results of Table 4 show that the water consumed is 3604.31 and 3515.25 m^3 ha^{-1} $year^{-1}$ for Qassim and Al Jouf, respectively. The amount of rainfall for Qassim and Al Jouf during the season were 92.85 and 434.99 m^3 ha^{-1} $year^{-1}$, respectively. The water balance methods showed that water consumption for the two sites was very low compared to ETc estimation by P-M or water added to field. This reduction in total amount of water consumption is mainly due to short depth of the sensor installed in the site (120 cm). It seems that 50% of water added to date palm tree considered as leaching water.

Table 4. Compared the amount water applied in the different methods sites and increase water ratio (%) compared to the Penman-Monteith method.

Sites	Water requirements of different methods (m^3 ha^{-1} $year^{-1}$)				The increase water ratio (%) compared to Penman-Monteith method.	
	Penman-Monteith method	Water balance method	Applied irrigation water			
			Field study	Farmer adjacent	Field study	Farmer adjacent
Medina	9495.24	-	11305.0	13717.00	16.0	30.8
Tabuk	7340.18	-	9463.9	12277.00	22.4	40.2
Makkah	7298.93	-	9692.0	12220.00	24.7	40.3
Al Jouf	8913.59	3515.25	11252.8	13340.00	20.8	33.2
Riyadh	8614.96	-	10007.4	12050.00	13.9	28.5
Qassim	8568.68	3604.31	10035.0	12880.00	14.6	33.5
Hail	7996.99	-	10272.5	12620.00	21.2	36.6
East Region	8510.72	-	10082.8	12610.00	15.6	32.5

CONCLUSIONS

This present study was conducted in eight different date palm regions of Saudi Arabia to estimate monthly and annual irrigation water requirements. The regions that were selected are located in Medina (Al Ula), Tabuk (Teimaa), Makkah (Al Jumum), Al Jouf (Sakakah), Riyadh (Sodos), Qassim (Riyad Al Khabra, Hail (Al Kaedh), and East Region (Al Ahsa). The results of the study showed that the crop evapo transpiration, ETc (mm $year^{-1}$), without taking shaded area $tree^{-1}$ of Medina, Tabuk, Al Jouf, Riyadh, Qassim, Hail, and Al Ahsa were 2418.75, 1940.51, 1837.76, 2259.03, 2139.23, 2207.41, 2032.09, and 2144.87 mm $year^{-1}$, respectively. The irrigation water requirements (m^3 ha^{-1}) after taking into account the proportion of cultivated area for each year were 9495.24, 7340.18, 7298.93, 8913.59, 8614.96, 8568.68, 7996.99, and 8510.72 m^3 ha^{-1}, respectively, 100 palms ha^{-1}, and the annual total irrigation water requirements (m^3 $tree^{-1}$) in these regions were 95, 73.4, 73, 89, 86, 85.7, 80, and 85 m^3, respectively as the radius of shaded area per tree was 3.5 m. The decrease of the CRW in all sites of study to around 8000 m^3 ha^{-1} was mainly attributed to percentage of shaded area of date palm tree. Therefore, the practice of a distance between trees to 10×10 m should be changed to 7×7 m in order to reduce the estimation of CRW of date palm trees. The water balance methods showed that water consumption for the two sites were very low compared to ETc estimation by P-M or water added to field. This reduction in total amount of water consumption was believed to be mainly due to short depth of the sensor installed in the site (120 cm). It seems that 50% of water added to date palm tree is considered as leaching water. Furthermore, it was found that the sensitivity of the sensors decreased when temperatures rose in high-salinity soil (exceeding 6 dS m^{-1}).

Literature cited

Al-Amoud, A.I., Mohammed, F.S., Saad, A.A., and Alabdulkader, A.M. (2012). Reference evapo transpiration and date palm water use in the Kingdom of Saudi Arabia. Int Res J Agric Sci Soil Sci *2* (*4*), 155–169.

Allen, R.G., Pereira, L.S., Raes, D., and Smith, M. (1998) Crop Evapotranspiration Guidelines for Computing Crop Water Requirement. Irrig. and Drain. Paper 56 (Rome, Italy: FAO).

Dewidar, A.Z., Ben Abdallah, A., Al-Fuhaid, Y., and Essafi, B. (2015). Lysimeter based water requirements and crop coefficient of surface drip- irrigated date palm in Saudi Arabia. Int. Res. J. Ag. Sci. Soil Sci. *5* (*7*), 73–183.

Doorenbos, J., and Pruitt, W.O. (1977). Crop Water Requirement. FAO Irrig. and Drain. Pap. No.24 (Rome, Italy: FAO), p.144.

General Authority for Statistics. (2015). Statistical Report (Riyadh, Saudi Arabia).

Hellman, E. (2010) Irrigation Scheduling of Grapevines with Evapotranspiration Data (Texas: Texas A&M University, Texas AgriLife Extension Service: College Station), http://winegrapes.tamu.edu/grow/irrigationscheduling.pdf.

Ismail, S.M., Al-Qurashi, A.D., and Awad, A.A. (2014). Optimization of irrigation water use, yield, and quality of Nabbut-Saif date palm under dry land conditions. Irrig. Drain. *63* (*1*), 29–37 https://doi.org/10.1002/ird.1823.

Mihoub, A., Helimi, S., Mokhtari, S., Kharaz, E., Koull, N., Lakhdari, K., Benzaoui, T., Bougafla, A., Laouisset, M., Kherfi, Y., and Halitim, A. (2015). Date palm (*Phoenix dactylifera* L.) irrigation water requirements as affected by salinity in Oued Righ conditions, north eastern Sahara, Algeria. Asian J. Crop Sci. *7* (*3*), 174–185 https://doi.org/10.3923/ajcs.2015.174.185.

Ministry of Water and Electricity. (2015). Statistical Annual Report of the Ministry (Riyadh, Saudi Arabia).

Rosenbaum, U., Huisman, J.A., Vrba, J., Vereecken, H., and Bogena, H.R. (2011). Correction of temperature and electrical conductivity effects on dielectric permittivity measurements with ECH$_2$O sensors. Vadose Zone J. *10* (*2*), 582–593 https://doi.org/10.2136/vzj2010.0083.

Designing and manufacturing an automatic knife for date palm tree frond cutting operations by frequency theory cutting

A.A. Jasim[1,a], S.M. Abbood[2] and M.R. Abbood[2]

[1]Dept. of Agricultural Machines and Equipment, College of Agriculture, University of Baghdad, Baghdad, Iraq; [2]Dep. of Horticulture, College of Agriculture, University of Baghdad, Iraq.

Abstract

Date palm fronds cutting requires a lot of man power and workers, thus to do this operation easily and reduce effort, time and costs, an automatic knife was designed and manufactured to cut the palm tree fronds by frequency cutting technique. The equipment included the following parts: 1) engine; 2) clutch; 3) flexible and fixed shaft; 4) bevel gear case; and 5) cutting knife. The experiment was conducted for designing and manufacturing a vibration motorized cutter used for date palm fronds pruning. Three cutting angles (45, 60 and 90°), were included which were A1, A2 and A3, respectively. Three designs of knives were symbolized by B1 (L-shaped) and B2 (L-shape is obtuse-headed) and B3 in the form of a letter (C). The height of palm trees studied was 2 and 4 m and were symbolized by H1 and H2 in this experiment. Three rows of fronds cutting time, and a comparison between designed equipment were studied. The results showed that angle A3 gave the highest placement time of 2.72 min, significantly more than the angles A1 and A2. Two-m high palm required 2.53 min compared with 2.6 min for 4 m height. The B3 knife resulted in the highest laying time of 4.04 min compared to the knives B1 and B2, which required 1.74 and 1.76 min, respectively. The design and manufacturing of equipment used for cutting date palm fronds from the base was successful and can be used by the growers.

Keywords: date palm, frond, cutting time, equipment, machines

INTRODUCTION

Date is considered as one of the oldest known fruit crops and has been cultivated in northern Africa and middle east for 5000 years (Morton, 1987; Al-Baker, 1972). For maintaining the date palm crown, there are many operations that must be performed during various times of the year. Each palm must be climbed several times in a year, to do many hand operations (Anonymous, 2002). Cutting dry and dead date palm leaves can be considered as an important date palm crown service because the fronds hinder the worker to climb the palm trunk to do several operations like pruning, pollination, thinning and harvesting (Abood et al., 2018). Pruning is a significant practice to remove the date palm dry fronds and fronds bases, the fiber, spines and offshoots. These operations can be done manually or mechanically using hydraulic lift or ladders (Akyurt et al., 2002). Base of fronds are strongest parts of the palm leaves irrespective of the contents of moisture and maturity (Jayaslena and Wan Ishak, 2012; Alnuami et al., 2015).

The date palm tree grows about 30 cm a year and it may reach a height of 35 m at an age about of 100 years (Morton, 1987). Pruning is one of the important operation practices to remove the palm dry and dead leaves and leaf bases. This operation eliminates insects and spreading of diseases and also makes the palm trunk graded to make it easier to climb. The operation can be done manually or mechanically by using a hydraulic lift or ladders (Jasim et al., 2017; Owda, 2014). Pruning is the most difficult operation and it needs more human energy especially when using traditional tools like knives and saws (Jelani, 2008). The knife shape and types have significant influence to cut the base of palm fronds (Persson, 1987;

[a]E-mail: raz55iq@gmail.com

Ahmad et al., 2000). The angle of cutting plays a big role to do the cutting operation (Jelani et al., 1998). Due to the importance of mechanical pruning by using different types of knives under the different cutting angle this study was conducted.

MATERIAL AND METHODS

The experiment was conducted for designing, manufacturing and testing a vibration motorized cutter used for palm fronds pruning. Three cutting angles which were used were 45, 60 and 90°, represented by A1, A2 and A3, respectively. Three designs of common knives were used in the installation and pruning of palm fronds, symbolized by B1 – L-shaped, and B2 – L-shape is obtuse-headed, and B3 – in the form of a letter (C), and two heights of palm trees were included: 2 and 4 m which were symbolized by H1 and H2. Three rows (lines) of fronds cutting time and a comparison between designed equipment and conventional one was measured in this experiment. Nested design under randomized complete block design (RCBD) with three replications was used in this study. The lest significant difference (LSD) and 0.05 levels were used to compare the means of the treatments.

A new motorized vibrator cutter was manufactured and assembled at the local mechanical workshop in the Department of Agricultural Machines and Equipment, College of Agricultural Engineering Sciences, University of Baghdad. The motorized vibrator palm frond cutter consists of the following parts (Figure 1):

1. Engine, it has 2 hp, gasoline fuel, 3000-5000 rpm;
2. Flexible connection, it consists of rubber tube and rotary flexible shaft 0.5 cm rectangle section;
3. Fixed connection, consist of rotor circle section shaft with radius of 6.8 mm inside of aluminum pore with 2.5 cm diameter;
4. Bevel gear case, used to convey the rotary motion to vibration motion and cutting tool, it is considered as a rigged curved knife. And the motorized vibrator palm frond cutter characteristics by the following:
   - Equipment type: date palm fronds cutting equipment;
   - Engine type: single piston engine;
   - Fuel type: gasoline
   - Horsepower: 2 HP, 3500-5000 rpm;
   - Operating system: manual;
   - Fuel tank capacity: 1.1 L petrol
   - Equipment weight: 6.5 kg;
   - Cooling type: air.

Figure 1. Motorized vibration date palm fronds cutter.

Knives were manufactured by hot forged local workshop using the (ASTM579) alloy, this material is considered to have high durability. All knives have 2 mm thickness and 0.5 mm sharp edge.

Knives were forged by using an oven at 790°C for 30 min then cooling in water to give them good hardness, and the knife samples hardness was tested by using the Rockwell test, it stood at 65 HR (Figure 2).

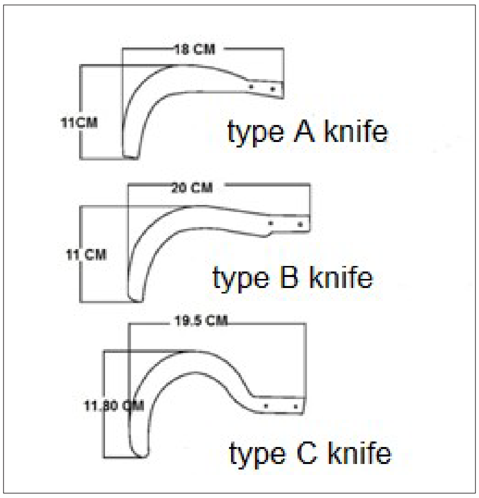


Figure 2. Types of knives.

As a result shown above, manufacturing of equipment used for cutting palm fronds from the base was successfully done with a 45° knife cutting angle (Figure 3).

Figure 3. Using the equipment for palm frond cutting.

The equipment was field tested after performing all the manufacturing and installation operations of the equipment to appear in its final form. It was initially tested without a load to ensure the safety of the parts' work and the handling of cases that need to be re-adjusted, and then it was field tested and thus became ready to carry out the installation operations effectively, and an experiment was conducted to find out the time of cutting one palm with three lines.

RESULT AND DISCUSSION

The results in Table 1 show that there are significant differences in the cutting angles in the time of planting one palm tree in three stress roles or lines, as the angle A3 gave the highest placement time of 2.72 min, with a significant difference from the angles A1 and A2, which gave 2.40 and 2.43 min, respectively.

Table 1. The effect of cutting angle and knives type on frond cutting time for three lines date palm min^{-1}.

Cutting angle (A)	Type of knife (B)			Mean of A
	B_1 (L) shape ordinary	B2 (L) shape obtuse	B3 (C) shape	
A_1 (45°)	2.40	3.83	1.69	1.68
A_2 (60°)	2.43	3.91	1.69	1.69
A_3 (90°)	2.72	4.40	1.91	1.86
LSD 0.05		0.114		0.173
Mean of knives type (B)	4.04	1.76	1.74	
LSD 0.05	0.1005			

The type of knife had a significant effect, and the use of the B3 knife resulted in the highest laying time of 4.04 min compared to the two knives B1 and B2, which recorded 1.74 and 1.76 min, respectively.

The effect of the interaction between the cutting angle and the type of knife, indicated that the interference treatment A1 B1 recorded the least application time and amounted to 1.68 min, while the interaction treatment A3-B3 gave the highest application time of 4.40 min.

Table 2 shows the effect of palm height. The results indicate that there is a significant effect in the time of devouring one palm, as the 2 m height date palm got 2.53 min compared with 2.6 min for 4 m height. The results of the interaction between the height of the palm tree and the type of knife showed the lowest application time for a single palm amounting to 1.71 min in the H1-B1 interference treatment, while the highest application time was 4.27 min in the H2-B3 intervention treatment.

Table 2. The effect of palm height and knives type on frond cutting time for three lines date palm min^{-1}.

Palm height (H)	Type of knife (B)			Mean of A
	B_1 (L) shape ordinary	B2 (L) shape obtuse	B3 (C) shape	
H_1 (2 m)	2.53	4.11	1.77	1.71
H_2 (4 m)	2.60	4.27	1.79	1.75
LSD 0.05		0.146		0.167
Mean of knives type (B)	4.04	1.76	1.74	
LSD 0.05	0.1005			

Table 3 shows the interaction between type of knives, cutting angle and the height of date palm tree and the interference between the cutting angle and the height of the palm tree. The results in the same table indicate the superiority of the treatment of A1-H1 by giving it the least application time and it reached 2.35 min, while the treatment of the interaction A3-H2 was the highest in the time of application, giving it a time of 2.76 min.

The results of the triple interaction between the treatments indicated that the least application time for one palm was 1.63 min in the treatment of interference A1-H1-B1, with a significant difference from the treatment of interference A3-H2-B3, which recorded the highest application time of 4.50 min.

The results also showed, in Table 4, that the average time of afflicting one palm tree with three stress roles is 2.5 min for the designed equipment, compared to 5.5 min using the traditional or conventional method.

Table 3. The effect of cutting angle, palm height and knives type on frond cutting time for three lines date palm min^{-1}.

Cutting angle (A)	Palm height (H)	Type of knife (B)			Mean of A×B
		B_1 (L) shape ordinary	B2 (L) shape obtuse	B3 (C) shape	
A_1 (45°)	H1 (2 m)	1.63	1.68	3.76	2.35
	H2 (4 m)	1.73	1.71	3.90	2.44
A2 (60°)	H1 (2 m)	1.67	1.65	3.89	2.40
	H2 (4 m)	1.74	1.74	3.93	2.47
A_3 (90°)	H1 (2 m)	1.85	1.90	4.31	2.68
	H2 (4 m)	1.87	1.92	4.50	2.76
LSD 0.05			0.173		0.155
Mean of knives type (B)		1.74	1.76	4.04	
LSD 0.05			0.1005		

Table 4. Average of frond cutting time for three lines date palm per minute for the designed equipment and the conventional method.

Operation	Mean
Frond cutting time for 3 lines of date palm min^{-1} for the designed equipment	2.5
Frond cutting time for 3 lines of date palm min^{-1} for the conventional method	5.5

Table 5 shows a comparison between the perforation with the manufactured equipment and the traditional method in terms of effort, costs, time and number of workers. It became clear that the manufactured equipment was superior in all the comparison paragraphs.

Table 5. A comparisons between designed equipment and conventional implement.

Categories	Designed equipment	Conventional implements
Potential	Little potential	More potential
Costs	Reduced costs	Increased costs
Time	Needs little time	Needs a lot of time
Number of laborers	Needs one worker	Needs more than one worker
Health	Good	bad

The advantages of the equipment are as follows:

1. The equipment is used to cut the date palm and fronds;
2. Ease of manufacture, ease of use and maintenance;
3. The cost of manufacturing and operating is low when compared to other implements;
4. Ease of assembly, linkage, opening and replacement of equipment parts;
5. It is easy to complete the work and does not require much effort because it weighs less than 7 kg;
6. The speed of completion of work;
7. You only need one time to operate it.

CONCLUSION

Manufacturing of equipment used for cutting date palm fronds from the base was successfully done in the treatment of interference A1-H1-B1 with less time.

The design and manufacturing of a vibration motorized cutter used for palm fronds pruning is recommended.

Literature cited

Abood, S.M., Abood, M.R., and Jasim, A.A. (2018). Manufacturing and testing of date palm vibration motorized fronds cutter. Int. J. Agric. Sustain. *49* (*3*).

Ahmad, D.S., Roy, K., and Jaelani, A.R. (2000). Evaluation of design parameters of cycle cutter and claw cutter for cutting oil palm frond. AMA Journal *31* (*2*), 55–60.

Akyurt, M., Rehbini, E., Bogis, H., and Aljanabi, A.A. (2002). A survey of mechanization efforts on date palm crown operations. Paper presented at: 6th Saudi Engineering Conference, KFUPM (Daharan).

Al-Baker, A. (1972). The Date Palm; a Review of Its Past and Present Status; and the Recent Advances in Its Culture, Industry and Trade (Baghdad: Al-Ani Press).

Alnuami, W., Janius, R.B., Ahmad, D., Akhir, M.D., and Buthainah, A. (2015). Factors affecting the mechanical strengths oil palm fronds. Journal of Purity *4* (*3*), 138–194.

Anonymous. (2002). UAE farmers ready for date with mechanical pollination. http://www.uaenteract.com/uaeint_main/newsreport/20010122.htm#UAE%20FARMERS%20.

Jasim, A.A., Abbood, M.R., and Abbood, S.M. (2017). Effect of cutting angle for a locally assembling motorized vibration cutter on some operational characteristics used for date palm fronds cutting. Elixir Mech. Engg. *109*, 47879–47881.

Jayaslena, H., and Wan Ishak. (2012). Manipulator automation for fresh fruit bunch Harvesting. Int. J. Agric. Biol. Eng. *5* (*17*).

Jelani, A.R. (2008). Cantas™ - a tool for the efficient harvesting of oil palm fresh fruit bunches. J. Oil Palm Res. *20*, 548–558.

Jelani, A.R., Ahmad, D., Ahmad, H., Azmi, Y., and Johari, J. (1998). Force and energy requirements for cutting oil palm frond. J. Oil Palm Res. *10* (*2*), 10–24.

Morton, J. (1987). Date *Phoenix dactylifera.* In Fruits of Warm Climates (Miami: Creative Resource Systems, Inc., Winterville).

Owda, A. (2014). Date Palm Tree, Cultivation, Service, Technical Care and Manufacturing (Kingdom of Bahrain: Educational Essa Center).

Persson, S. (1987). Mechanics of Plant Cutting Material (Michigan: American Society of Agricultural Engineers).

Preparation and quality evaluation of date jam at different storage temperatures

S.G. Khaskheli[a], A.H. Soomro, A.A. Panhwar, A.A Khaskheli and D. Khan

Institute of Food Science and Technology, Sindh Agriculture University, Tandojam, Sindh, Pakistan.

Abstract

The present research was carried out to study the production and processing of date jam at the Institute of Food Sciences and Technology, Faculty of Crop Production, Sindh Agriculture University, Tandojam, Pakistan. Results of the storage period showed that physical and sensorial properties of date jam significantly changed at refrigeration temperature (5+2°C) for the 28 days storage period as compared to ambient 28±2°C temperature. Jam made with date was found to have highest acceptability on the basis of sensory evaluation. Moreover, the results from this work revealed essential information that could promote the commercialization of date jam.

Keywords: date jam, physiochemical, organoleptic taste, storage

INTRODUCTION

Date palm is one of the oldest fruit trees in the world and is mentioned in the Qur'an and Bible. Not only does date palm have an effective role in the ecosystem of the desert, but it has several agricultural and animal husbandry uses. Date is an important fruit in the world and plays a significant role in the economic life and diets of people in date growing regions. The date fruit is marketed all over the world as a high value confectionery and fruit crop (Abdelouahhab, 2006). Many products, including date syrup, date powder, different types of bread, marmalade, sweet candy, chocolate, date paste, and others, can be obtained from date (Ashraf and Hamidi-Esfahani, 2011). Pakistan is among the top ten date producing countries. In 2019, the country was number 6 compared to other countries in dates production with 483,071 t (GOP, 2008). In Sindh province date palm occupies an area of 30,000 ha and production is more than 200,000 t. Due to lack of processing and packaging facilities in the country, these highly valuable fruit do not fetch their real worth in the export market. Beside direct consumption of the whole dates the fruits could be traditionally used to prepare a wide range of different products such as date juice concentrates (spread, syrup, jam and liquid sugar), fermented date products (wine, alcohol, vinegar, organic acids) and date pastes in different bakery and confectionary uses.

As there is less demand for table dates, new trends are developing in the date consumption as a component of new food products. Food industries are now producing different types of date products including date bars, date-paste, date-syrup, date-honey, date-jam, date vinegar, date cookies, date wafers, date squares (Ahmed and Ramaswamy, 2005). The amount of sucrose can be replaced with date-paste in many food products and used as filler in food formulations (Alhamdan and Hassan, 1999). Jam is a fruit preserve with a stable shelf life that depends on high sugar content (68-72%) combined with the fruits acidity that prevents microbial growth. A good jam is in fact a complex product that requires precise balance between sugar level, acidity and pectin content. In Pakistan no date jam has been developed/available in the market. Therefore, there is a need to preserve date products with high added value such as date jam with different pectin concentrations which will help the food industry to produce new products.

[a]E-mail: sgkhaskhali@sau.edu.pk

MATERIAL AND METHODS

Collection of samples

The study was conducted to prepare date jam. The date cultivar 'Aseel' was purchased from a local market and was brought to the laboratory of the Institute of Food Sciences and Technology, Faculty of Crop Production, Sindh Agriculture University, Tandojam.

Preparation of jam

The date samples were washed, cleaned, and processed for jam processing. Date pieces were homogenized and a mixture was prepared by blending the flesh in a blender. The obtained flesh was weighed and placed in a stainless-steel pan and heated. The mixture was allowed to boil and then pectin powder (0.2 g) was added to it while stirring it continuously so as to prevent pectin from clotting. When the pectin was dissolved completely, the remaining sugar was added to the heating mixture and allowed to boil. The endpoint of jam preparation was determined by cooling a small amount of sample and testing its TSS using a refractometer until reaching the consistency of 66-68 °Brix TSS, the heat was turned off. The jam was quickly transferred into pre-sterilized glass bottles to prevent contamination. Upon cooling the jars were closed and stored at refrigeration temperature (5+2°C) and ambient 28±2°C temperature for the 30 days storage period. The sample was taken every 7 days interval from the prepared jam bottle for analysis (Shahnawaz and Shiekh, 2011).

Chemical analysis

The moisture content, ash and total soluble solid of jam products were determined according to the methods of AOAC (2010).

Sensory evaluation

Sensory evaluation of date jam was performed according to the method as reported by Ali and Anjum (2014). The colour, taste, aroma, texture, appearance, and overall acceptance of the jams were evaluated using the 9-point hedonic scale (1 = extremely poor, 2 = very poor, 3 = poor, 4 = below fair above poor, 5 = fair, 6 = below good above fair, 7 = good, 8 = very good, 9 = excellent).

Statistical analysis

All the experimental data were subjected to statistical analysis using SPSS version 20 for windows.

RESULT AND DISCUSSION

The influence of different storage temperatures on physiochemical and sensorial attributes of date jam during storage was studied during 2020-2021. Two different temperatures were used for date jam storage: a) refrigerator (5±1°C), and b) room temperature (28±2°C). Moreover, the data on the colour, flavour, texture, taste and overall acceptability were also evaluated. In addition to this the shelf life/storage was also evaluated for up to 30 days. Moisture content is generally associated with the genetic variation of cultivars, drought and stressed climatic conditions, field management (water irrigation, nutrients) and postharvest storage conditions.

The results regarding the moisture, ash content and TSS of the jams are listed in Table 1. The results indicated that maximum moisture was recorded (31.75%) at ambient temperature (28±2°C) after 14 days of storage which was 18.10% under refrigerator temperature (5±2°C) after 21 days. While lowest moisture (17.30%) was noticed in refrigerator temperature (Table 1). Our results are in agreement with the findings of Al-Hooti et al. (1995). Ash content showed no significant difference and the results indicated that the maximum ash content was (1.12%) under ambient temperature (28±2°C) while 0.87% of ash content was recorded under refrigerator temperature (5±2°C). The ash content values are close to those obtained by other researchers (Ahmed and Ahmed, 1995; Besbes et al., 2009). TSS content of the jams was found to be in the range of 68.03-71.05 °Brix, recorded during a storage period up to 30 days. The maximum total soluble solids was recorded 71.05 under ambient temperature (28±2°C) whereas 69.26 was recorded under refrigerator temperature (5±1°C). The results are in agreement with the findings of Al-Hooti et al. (1995) who reported that the values of total soluble solids (65 °Brix) were similar during 28 days and there was no variation observed in these parameters.

Table 1. Influence of storage on the nutritional composition of date jam.

Storage time (days)	Storage temperature (°C)	Moisture (%)	Ash content (%)	Total soluble solids (°Brix)
0	28±2	20.07	1.20	68.03
0	5±2	20.05	1.22	68.05
7	28±2	21.30	1.09	69.02
7	5±2	19.64	0.98	68.10
14	28±2	31.75	1.12	70.72
14	5±2	18.30	0.88	68.65
21	28±2	30.75	1.08	70.90
21	5±2	18.10	0.86	69.20
28	28±2	29.66	0.97	71.05
28	5±2	17.30	0.87	69.26

Reducing, non-reducing and total sugar

The reducing, non-reducing and total sugar contents of date jams during storage refrigerator (5±2°C) and at room temperature (28±2°C) are presented in Table 2. The average reducing sugars among all date jam samples ranged from 26.40 to 17.22%. The reducing sugars ranged from 27.50 to 17.22% during storage in refrigerator whereas at ambient temperature it varies from 26.40 to 15.22%. The non-reducing sugars contents ranged from 18.25 to 10.44 during storage. The average non-reducing sugars decreased sequentially on the 7th, 14th, 21st, and 28th days of storage, respectively (Table 2). The average total sugars in all date jam ranged from 44.75 to 25.26%. Different researchers worked on the fruit jams and our results are supported by Hussain and Shakir (2010) while working on apricot diet jam and apple jam, respectively.

Table 2. Influence of storage on the reducing, non-reducing, and total sugar composition of date jam.

Storage time (days)	Storage temperature (°C)	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)
0	28±2	44.57	26.40	18.17
0	5±2	45.75	27.50	18.25
7	28±2	36.68	20.24	16.44
7	5±2	43.45	25.80	17.65
14	28±2	38.66	24.30	14.36
14	5±2	37.28	22.14	15.14
21	28±2	30.13	17.25	12.88
21	5±2	34.39	20.18	14.21
28	28±2	25.66	15.22	10.44
28	5±2	30.88	17.22	13.66

Sensory evaluation

The effect of storage and temperature on the sensory parameters of date jam are presented in Table 3. The maximum colour score was recorded (5.42) under ambient temperature (28±2°C), as compared to the colour 6.10 under refrigerator temperature

(5±2°C). The lowest score of colour (3.54) was noticed in ambient temperature (28±2°C) after 28 days of storage, whereas storage period showed better results in colour score (5.50) after 28 days refrigerator temperature (5±2°C). The maximum score of texture was recorded (5.88) under refrigerator temperature (5±2°C), compared with 5.81 recorded under ambient temperature (28±2°C). The lowest texture score (3.47) was noted in ambient temperature (28±2°C) after 28 days of storage. In case of storage period the maximum score of texture (5.88) was recorded on 0 day followed by the 7th day (6.00), respectively, whereas the minimum score of texture (3.47) was observed on the 28th day. The results showed the influence of different temperatures on the score of texture of date jam which was statistically significantly ($P<0.05$) affected by different temperatures.

Table 3. The sensory analysis of date jam under different temperatures and storage periods.

Storage time (days)	Storage temperature (°C)	Colour	Texture	Taste	Overall acceptability
0	28±2	5.42	5.81	5.75	5.44
0	5±2	6.10	5.88	5.90	5.95
7	28±2	5.34	5.60	5.57	5.80
7	5±2	6.00	5.85	5.80	5.77
14	28±2	5.11	5.00	5.22	5.50
14	5±2	6.00	5.70	5.60	6.10
21	28±2	4.64	4.57	4.37	5.00
21	5±2	6.00	5.80	5.75	5.90
28	28±2	3.54	3.47	3.44	4.00
28	5±2	5.50	5.30	5.12	6.00

CONCLUSIONS

The study concluded that the date jam remained acceptable for 30 days after storage. However, it may be concluded that storage temperature has effect on the quality of date jam. Based on the present investigation, it was concluded that refrigerator temperature (5±2°C) and storage duration of 28th days showed better results in physiochemical properties of date jam. The storability study revealed that the laboratory scale developed date jam has better shelf life and can be kept for at least 30 days at refrigerator temperature without affecting its quality attributes. The successful formulation of dates for jam manufacturing increases its marketing value and thus interest of industry to remain in working mode throughout the year.

Literature cited

Abdelouahhab, Z. (2006). World date industry: situation, challenges and opportunities. Paper presented at: International Conference on Date Palm Production and Processing Technology (Muscat, Oman).

Ahmed, I.A., and Ahmed, A.W.K. (1995). Chemical composition of date varieties as influenced by the stage of ripening. J. Food Chem. *54* (*3*), 305–309 https://doi.org/10.1016/0308-8146(95)00051-J.

Ahmed, J., and Ramaswamy, H.S. (2005). Effect of temperature on dynamic rheology and color degradation kinetics of date paste. Food Biop. Proc. *83* (*3*), 198–202 https://doi.org/10.1205/fbp.04312.

Al-Hooti, S., Jiuan, S., and Quabazard, H. (1995). Studies on the physico chemical characteristics of date fruits of five UAE cultivar at different stage of maturity. Arab Gulf J. Sci. Res. *13*, 553–569.

Alhamdan, A.M., and Hassan, B.H. (1999). Water sorption isotherms of date pastes as influenced by date cultivar and storage temperature. J. Food Eng. *39* (*3*), 301–306 https://doi.org/10.1016/S0260-8774(98)00170-8.

Ali, Z., and Anjum, F.M. (2014). Study of sensory parameters of naan using lactic acid produced from corn cobs. Pak. J. Food Sci. *24* (*1*), 28–36.

AOAC. (2010). Official Methods of Analysis, 18th edn (Washington, USA: Association of Official Analytical Chemists).

Ashraf, Z., and Hamidi-Esfahani, Z. (2011). Date and date processing: a review. Food Rev. Int. *27* (*2*), 101–133 https://doi.org/10.1080/87559129.2010.535231.

Besbes, S., Drira, L., Blecker, C., Deroanne, C., and Attia, H. (2009). Adding value to hard date (*Phoenix dactylifera* L.):

compositional, functional and sensory characteristics of date jam. J. Food Chem. *112* (*2*), 406–411 https://doi.org/10.1016/j.foodchem.2008.05.093.

GOP. (2008). Fruit, Vegetable and Condiments. Statistics of Pakistan (Islamabad: Ministry of Food Agriculture and Livestock, Economic Wing).

Hussain, I., and Shakir, I. (2010). Chemical and organoleptic characteristics of jam prepared from indigenous varieties of apricot and apple. World Journal of Diary and Food Sciences *5*, 73–78.

Shahnawaz, M., and Shiekh, S.A. (2011). Analysis of viscosity of jamun fruit juice, squash and jam at different compositions to ensure the suitability of processing applications. Int. J. Plant Physiol. Biochem. *3* (*5*), 89–94.

Consumer perception of yogurt flavoured with Moroccan date syrup

Y. Noutfia[1,a] and N. Turkmen[2]

[1]Department of Agro-food and Quality, National Institute of Agricultural Research, Rabat, Morocco; [2]Department of Dairy Technology, Ankara University, Faculty of Agriculture, Ankara, Turkey.

Abstract

This study aims to support efforts of women cooperatives to diversify their range of dairy products through the formulation of a new flavoured yogurt fortified with date syrup. At laboratory scale, eight batches of yogurt were produced from pasteurized and sterilized cow milk by dissolving 2 and 5% of date fruit syrup. The produced yogurt was assessed for its sensory quality on the basis of four attributes: odour, flavour, appearance and texture. Based on sensory quality, the result demonstrated that all of the yogurt categories produced from sterilized milk had higher scores than the flavoured pasteurized yogurt. Yogurt obtained from sterilized milk containing 5% of date palm syrup was very appreciated by consumers especially in terms of flavour and texture properties.

Keywords: yogurt, syrup, date, fortification, sensory quality

INTRODUCTION

Moroccan oases are characterized by the diversity of date palm genotypes (*Phoenix dactylifera* L.): 453 cultivars of dates have been identified; in addition to khalts (hybrids from natural sowing) which represent about 55% of the *Phoenix* cultural heritage (INRA, 2011). All this palm date heritage is used mainly for self-consumption and consumed directly after harvest, for supply of cold storage units (Noutfia et al., 2019) before commercialization in local, national and international markets.

However, the valorization of date palm fruits remains insufficient and is currently limited to its transformation into by-products such as syrup, jam, juice, and dough (Harrak et al., 2018). However, other potentialities of valorization are presented as the incorporation of this fruit into dairy products.

This is justified by the existence of women cooperatives units operating in processing of goat's and cow's milk (Figure 1). These units are limited to the valorization of milk into cheese despite the dairy surplus which is moving towards self-consumption (Noutfia et al., 2016).

Figure 1. Yogurt samples produced at laboratory scale.

The aim of this study was to develop a yogurt flavoured with Moroccan date syrup and to determine the sensory quality and acceptability of this dairy product.

[a]E-mail: noutfiaa@yahoo.fr

Acta Hortic. 1371. ISHS 2023. DOI 10.17660/ActaHortic.2023.1371.47
Proc. VII International Date Palm Conference
Eds.: A. Zaid, G.A. Alhadrami and S. Mitra

MATERIALS AND METHODS

Syrup processing

The main producing steps are: coring, juice extraction (in water with a rate of 1:3), filtration and concentration of the juice at 90°C.

Yogurt manufacturing

Eight batches of yogurt samples were prepared from pasteurized and sterilized cow milk, following a procedure developed at laboratory scale.

Yogurt samples were produced by dissolving 2 and 5% of date palm syrup. Six different yogurt samples were obtained as shown in Table 1.

Table 1. Yogurt samples produced at laboratory scale.

Acronym	Yoghurt
PT	Yogurt made from pasteurized milk
P2	Yogurt made with pasteurized milk and flavored with 2% of the date syrup
P5	Yogurt made with pasteurized milk and flavored with 5% of the date syrup
UT	Yogurt made from sterilized milk
U2	Yogurt made from sterilized milk and flavored with 2% of the date syrup
U5	Yogurt made from sterilized milk and flavored with 5% of the date syrup

Quality of syrup and sensory evaluation

Total soluble sugar (TSS) and ash contents were carried out according to AOAC methods in triplicate (AOAC, 2012). Hunter colour parameters (L*, a*, and b* values) and viscosity were determined in ten replicates in specific apparatus.

For sensory evaluation, ten semi-trained panellists consisting of technicians and staff of the National Institute of Agronomic Research of Errachidia were recruited and instructed on how to perform sensory evaluation. Yogurt samples were presented in small plastic cups under fluorescent light.

The panellists rated appearance, odour, firmness (texture or body) and flavour, using a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely).

Statistical analysis

Data analysis was conducted using XLSTAT Software. Sensory data were statistically tested using ANOVA to determine if a statistical difference existed ($P \leq 0.05$) and Tukey test was used for means comparison.

RESULTS AND DISCUSSION

Characteristics of date palm syrup

Table 2 shows some physical and chemical properties of date palm syrup produced and analysed at laboratory.

Sensory quality of yogurt fortified with date palm syrup

Flavoured yogurt with date palm syrup had a significant effect on all sensory properties except odour.

Yogurt made from sterilized milk was highly appreciated by panellists compared to the other samples in terms of appearance properties. The acceptance rating of the samples decreases while the level of fortification increase.

Considering the flavour scores, all types of yogurt samples were similar except P2 and U5 which had the lowest and the highest rates, respectively (Table 3).

Table 2. Quality characteristics of date palm syrup.

Parameter	Mean ± SEM
Total soluble sugars (TSS)[a] (%)	77.2±1.6
Ash (%)	2.08±0.31
a_w (water activity)	0.762±0.00
Viscosity (cp)	7315±6.97
Colour	
L*	4.18±0.02
a*	7.69±0.04
b*	7.04±0.03

[a]TSS content (major component) of syrup is included in the range of results obtained by El-Nagga and Abd El–Tawab (2012) for date syrup extracted by different methods. For ash content, our result is higher than 1.75 to 1.84% obtained by the same authors.

Table 3. Sensory quality of yogurt flavoured with date palm syrup (n=10).

Yogurt/attribute	Appearance	Odor	Texture	Flavor
PT	3.70 (a,b)	3.60 (a)	3.77 (a,b,c)	5.81 (a,b)
P2	3.41 (b,c)	3.54 (a)	3.53 (c)	5.33 (b)
P5	3.16 (c)	3.46 (a)	3.70 (b,c)	5.95 (a,b)
UT	4.18 (a)	3.80 (a)	3.97 (a,b,c)	6.10 (a,b)
U2	3.86 (a,b)	3.68 (a)	4.03 (a,b)	6.01 (a,b)
U5	3.76 (a,b)	3.70 (a)	4.22 (a)	6.68 (a)

Means within a column followed by different letters are statistically different ($P \leq 0.05$).

To better visualize the results of Table 3, Figure 2 represents the scores obtained for each category of yogurt on a Kiviat diagram.

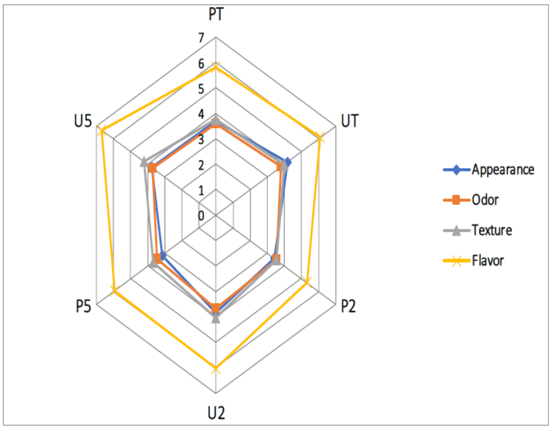


Figure 2. Kiviat diagram for sensory analysis of yogurt.

In general, the flavour attribute was highly rated (scores between 5.33 and 6.68) compared to appearance, odour and texture attributes (maximum score up to 4.22).

CONCLUSIONS

This study showed that all of the yogurt categories produced from sterilized milk had higher scores than the yogurt categories produced from pasteurized milk in terms of sensory characteristics. Yogurt obtained from sterilized milk containing 5% of date palm syrup was very appreciated by consumers especially in terms of flavour and texture properties.

According to the results of this study, it is possible to produce a new dairy product by enriching yogurt with date syrup.

Literature cited

El-Nagga, E.A., and Abd El-Tawab, Y.A. (2012). Compositional characteristics of date syrup extracted by different methods in some fermented dairy products. Ann. Agric. Sci. *57* (*1*), 29–36 https://doi.org/10.1016/j.aoas.2012.03.007.

Harrak, H., Hamouda, A., and Nadi, M. (2018). Évaluation et amélioration de la qualité des pâtes traditionnelles de dattes, produits du terroir des oasis. Cah. Agric. *27* (*1*), 15001 https://doi.org/10.1051/cagri/2017057.

INRA. (2011). Atlas du Palmier Dattier au Maroc (Rabat, Morocco: INRA), pp.197.

Noutfia, Y., Zantar, S., Alem, C., and Ibnelbachyr, M. (2016). Chaîne de commercialisation du fromage de chèvre dans la zone oasienne de la vallée de Drâa du Sud-est Marocain. In The Value Chains of Mediterranean Sheep and Goat Products. Organisation of the Industry, Marketing Strategies, Feeding and Production Systems, H. Ben Salem, J.P. Boutonnet, A. López-Francos, and D. Gabiña, eds. (Zaragoza: CIHEAM).

Noutfia, Y., Alem, C., and Filali Zegzouti, Y. (2019). Assessment of physico-chemical and sensory properties of two date (*Phoenix dactylifera* L.) cultivars under commercial cold storage conditions. J. Food Process. Preserv. *43* (*12*), e14228 https://doi.org/10.1111/jfpp.14228.

Evaluation of antioxidant, antibacterial potential, nutrition value and acute toxicity study of Libyan date palm pollen

R.B. Ashour[1], A.A. Auzi[2] and R.A. Lahmer[2,a]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya; [2]Department of Food Science and Technology, Faculty of Agriculture, University of Tripoli, Tripoli, Libya.

Abstract

Date palm (*Phoenix dactylifera* L) belongs to the *Arecaceae* family and grows widely in many areas including the Middle East and North Africa. Date palm has nutritional, medicinal, economic and ecological benefits as it could be used as a treatment of various health diseases and disorders for its antimicrobial, anti-oxidative, anti-inflammatory, anti-toxicant, and anti-cancer properties as well as its hepato-protective activities. It is also considered as an enhancer of fertility in both males and females. This research aims to assess the phytochemical screening of the date palm cultivated in Libya, test its antioxidant and antibacterial effects, and perform acute toxicity tests. The antioxidant activities of the extracts were screened using DPPH assay. The results indicated that the water-extract of palm pollen grains showed the best DPPH scavenging activity (IC_{50}=0.0005). The DPPH scavenging activities recorded for date palm pollen grain (DPP) methanol extract were IC_{50}=0.233 mg mL^{-1} and IC_{50}=0.224 mg mL^{-1} at 70 and 45°C, respectively. The methanol extracts of palm pollen grain (at concentrations of 12.5, 25, 50, and 100 mg mL^{-1}) were tested against 5 different strains of standard bacteria (ATCC) by cup cut method. The result showed no remarkable inhibition of bacterial growth. The phytochemical screening of date palm pollen revealed the presence phytosterols, flavonoids, coumarins, tannins, phenolic compounds, amino acids, protein, a small amount of saponin, and fats. The proximate nutritional value of palm pollen grains was also analyzed. The results obtained showed that the pollen contained 18.19% moisture, 5.41% ash, 67% crude fiber, 7.32% crude fat, and 27.36% crude protein. The acute toxicity test of methanol extract showed neither mortality among the graded dose groups of animals nor behavioral changes at the highest dose of 5,000 mg kg^{-1}.

Keywords: pollen, phytochemical screening, antioxidant, antimicrobial

INTRODUCTION

Pollen of date palm (DPP) has been traditionally used in Egyptian and Chinese folk medicine from ancient times and is recently used in Arab countries for the treatment of various body disorders in form of herbal preparations (Al-Samarai et al., 2016). The important traditional use of DPP was focused on improving male and female fertility, sexual hormonal balance (Hassan, 2011) in dry and wet cough, support stomach function, restore skin youth, improves healthy nails and hair, anti-diabetic and anti-oxidant (Ben Said et al., 2017). Pollen is a strong product known for male and female fertility treatment as a food supplement due to its contents of essential components such as amino acids, fatty acids, flavonoids, saponins, and estrole (Tahvilzadeh et al., 2016). Considering prostate diseases, which are age-related diseases that can mimic prostate adenocarcinoma and inflammatory prostatic cells and cause an imbalance between prostate cell growth and apoptosis (Elberry et al., 2011) and it was reported that the intake DPP acts as a remedy to prostatic diseases. Besides the treatment of infertility, the palm pollen extract was also used as anti-coccidial activity with mice earlier infected with *Eimeria papillata* responsible for increasing apoptosis cells in intestines

[a]E-mail: r.lahmer@uot.edu.ly

(Metwaly et al., 2014). DPPs give better results with cytotoxic activity when tested by Abed El-Azim et al. (2015). Moreover, DPPs are effective health treatment of mouth ulcer induced by chemotherapy in carcinogenic animals. The protective effect to date palm pollen on oral mucosa is achieved by blocking oxidative free radicals. It also prevents DNA damage. Daoud et al. (2015) investigated the content of date palm pollen and reported the presence of polyphenols, flavonoid contents and others molecules namely gallic acid, catechin, caffeic acid epicatechin, vanillic acid, coumarin, quercetin and rutin, proteins, ascorbate, b-carotenen, a-tocopherol and lycopene, all of which are characterized with a high antioxidant activity (Daoud et al., 2015). In a study conducted by Farouk et al. (2015), 21 oils were identified in date palm pollen grains using gas chromatography and mass spectrometry. The oils were subjected to antioxidant activity measurement and the results showed strong antioxidant properties. Basuny et al. (2013) estimated the antimicrobial activity of pollen of olive grain and palm date against different types of bacteria. They showed the effectiveness of pollen in the inhibition of bacteria growth and its antibacterial effect due to different phenolic content. In addition, a study conducted by Abed El-Azim et al. (2015) reported the pollen's effect against two types of fungi and its ability to inhibit some strains of bacteria zones. The nutritional value refers to the quantity and quality of nutrients present in a food item. Nutrients are the food ingredients necessary to maintain optimum metabolic functions of the body. Knowing the right nutrition helps to understand our daily calorie requirements, to feed the body with minerals, vitamins and phytonutrients, to minimise obesity and risk of developing health problems as coronary heart disease, stroke, diabetes, and cancer, and to maintain vital processes such as growth, breathing, digestion, and secretion. Pollen preparations are used worldwide for dietary purposes as diet supplement. The present study was conducted to evaluate the nutrient contents of date palm pollen grains and also to evaluate the acute toxicity of methanolic extract of date palm pollen grains. We have also access to the antioxidant activity of methanolic and aqueous extract of date palm pollen grains and antibacterial activity of methanolic extracts of date palm pollen grains on different bacteria strains at different temperature degrees.

MATERIALS AND METHODS

Materials

1. Plant material.

Date palm pollen was collected from Tripoli in March 2017. After the collection process, the pollen was air-dried and there after the powdered material was stored in -18°C until further use. The sample was authenticated by Dr. Mohamed Abo Hadra. It carries voucher number specimen (683611) from the herbarium of the botany department a plant taxonomist at the Department of Botany, Faculty of science, University of Tripoli, Libya.

2. Bacterial strains

The antibacterial activity of pollen extract was assessed against five bacterial species: Gram positive, methacillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumonia* ATCC13883, Gram-negative *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 9027.

3. Experimental animals.

Nineteen female rats were obtained from the Pharmacology Department, Faculty of Pharmacy, University of Tripoli, Libya.

Methods

1. Plant material preparation.

Collection of plants.

Date palm pollen was collected at the end of March of the date fields in Tripoli, Libya. This area is known for its palm cultivars. A total of about 310 mg of pollen was collected.

Cleaning of plants.

After the collection of the plants, saving process and collection of powder must be performed to get better results.

Drying.

The main purpose of drying is to remove the water content from the powder of pollen, before the plants are stored. Drying can be done by oven at 25°C overnight, and then it should be kept in the freezer for future analysis.

2. Extraction of plant material.

The methanolic pollen extracts were subjected to phytochemical screening, antioxidant, antibacterial activity and toxicity study, were prepared by a slight modification of the methods according to Banu and Catherin (2015). Ninety g of dried powder of date palm pollen was divided in to two parts and extracted by using methanol (99.9%) at two temperatures; in 70 and 45°C in a water bath for 30 min, then filtered. The solvent extracts were concentrated by rotary evaporator at 70 and 45°C, under reduced pressure. The extracts were weighted and stored for later use. The aqueous extract was subjected to antioxidant activity; 30 g of dried powder of date palm pollen was extracted by water maceration for 24 h, then filtered through filter paper. The filtered solution was freeze-dried. The dry extract and stock solutions were kept until further analysis.

$$\%\text{ Extraction yield} = \frac{\text{weight of the dry extract}}{\text{weight the sample used for extraction}} \text{x}100 \qquad (1)$$

3. Preliminary phytochemical screening.

Detection of alkaloids.

Few mg of each extract (date palm pollen) was boiled in 1 mL of hydrochloric acid in a water bath for 5 min, and two drops of mayres reagent were added (potassium mercuric iodide solution), of Dragendorff's reagent (potassium bismuth iodide solution), Wagner's reagent (potassium iodide and iodine) were added separately along the side of test tubes. Formation of precipitate indicate the presence of alkaloids (Pandey and Tripathi, 2014).

Detection of flavonoids.

Few drops of sodium hydroxide solution 20% were added to the extract. The formation of an intense yellow color, which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoid (Tiwari et al., 2011).

Detection of carbohydrates.

- Fehling's test: the extracts were treated with 2 drops of dilute HCl in test tube, neutralized with alkali then Fehling's A & B solutions were added and heated on a water bath for 10 min. The formation of a red precipitate indicates the presence of reducing sugars (Tiwari et al., 2011).
- Molisch's test: the extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube and was mixed. Two drops of concentrated sulphuric acid were added. The formation of the violet ring at the junction indicates the presence of carbohydrates (Pandey and Tripathi, 2014).

Detection of tannins.

Few mg of the extract was treated with 1% gelatin solution plus sodium chloride, the appearance of white precipitate indicates presence of tannins (Harborne, 1998).

Detection of phenols.

A few mg of the extract were treated with 3 drops of ferric chloride solution. The appearance of greenish or dark blue color indicates presence phenols (Harborne, 1998).

Detection of saponins.

A few mg of the extract were shaken with about 5 mL of distal water for 10 min, foam formation indicates presence of saponin (Tiwari et al., 2011).

Detection of cumarins.

Two drops of the extract were dissolved in 2 mL sodium hydroxide, spotted on Wattmans filter paper and examined under long UV, The appearance of blue florescence indicates the presence of cumarins.

Detection of phytosterols.

- Salkowskis test: a few mg of the extract were treated with color form then filtrated. Few drops of concentrated sulfuric acid added to the filtrate, shaken, and allowed to stand. The formation of a golden yellow color indicates presence of triterpens (Tiwari et al., 2011).
- Libermann-Burchard's test: a few mg of the extract were treated with chloroform and filtered. The filtrates treated with few drops of acetic anhydride, boiled and cooled, concentrated sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols (Pandey and Tripathi, 2014).

Detection of amino acid and proteins.

- Ninhydrin test: a few mg of the extract were treated with 2 drops of ninhydrin solution. The appearance of purple color indicates the presence of amino acids (Harborne, 1998).
- Xanthoproteic test: a few mg of the extract were treated with few drops of concentrated nitric acid. The appearance of a yellow color indicates the presence of proteins (Tiwari et al., 2011).

Detection of glycosides.

- Modified Borntrager's test: a few mg of the extracts boiled with dilute sulfuric acid then filtered. The filtration cooled and added equal volumes of benzene. The benzene layer separated and treated with ammonia solution. Change of the color into rose-pink in the ammoniacal layer indicates the presence of anthranol glycosides (Tiwari et al., 2011).
- Killer Killiani test: a few mg of extract treated with few mL of glacial acetic acid containing ferric chloride, concentrated sulphuric acid was added on the wall of the tube forming a red ring at the interface and the upper layer converted to blue greenish color indicating the presence of glycosides (Ashokkumar and Ramaswamy, 2014).

Detection of fixed oils and fats.

- Spot test: a few mg of extracts were pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils (Pandey and Tripathi, 2014).
- Infrared spectroscopy: IR spectra of dried powder plant materials were measured by FTIR analysis. Ten mg of the dried powder was compressed in 100 mg of KBr pellet to prepare sample in capsule form discs into 80KN by using a mold and press. The thin disc of plant specimen produced was loaded in FTIR spectroscope, with a scan range from 400 to 4000 cm with a resolution of 4 cm. One spectrum takes about 3 min to be recorded. The reference standard was injected and recorded (Sudhira et al., 2015).

4. Determination of antibacterial activity.

In vitro antibacterial screening of methanolic extracts of pollen grain of date palm was carried out using cup cut diffusion method (well diffusion method). The standard bacterial strains were activated and cloned mellur hinton agar and incubated in 37°C for 24 h, stored in refrigerator for future experimental tests.

Cup-cut diffusion method.

Antibacterial susceptibility was tested using cup-cut diffusion method (Daoud et al., 2015). The pollen extracts were dissolved in 2% (v/v) DMSO solution to get different concentration 100, 50, 25 and 12.5 mg mL^{-1} concentration (125 mg mL^{-1} DMSO), then filtered using 0.2-µ mL millipore filter for sterilization. DMSO was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. This method was performed using freshly prepared Mueller Hinton agar with overnight culture of bacteria (107 CFU mL^{-1}). Approximately 20 mL of the agar were poured into 10-cm diameter in Petri dishes and allowed to solidify. On each plate wells were made by sterile cork borer (5 mm), each well was filled with 100 µL of each pollen extract of different concentration. The controls were prepared by using the same solvent without extract, the plates were left for 30 min at room temperature for diffusion of extract into agar, then incubated at 37°C for 24 h. The diameter of the zones of inhibition was measured to the nearest mm.

5. Determination of antioxidant activity (DPPH assay).

Quantitative DPPH assay.

The free radical scavenging capacity of the date palm pollen was determined using DPPH method. A solution was prepared in 99% methanol. The extracts of date palm pollen were mixed with solvent to prepare the stock solution (10-250 µg mL^{-1}). Freshly prepared DPPH solution (0.001% w/v) was taken into the cuvettes, then date palm pollen were added to every cuvette so that the final volume was 1 mL and after 30 min, the absorbent was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and was dissolved in methanol. The control was prepared containing the same volume without any extract and reference ascorbic acid.

Percentage scavenging of the DPPH free radical was measured by using the following equation:

$$\%\ \text{scavenging Activity} = \frac{\text{Absorbance of the control- Absorbance of the test sample}}{\text{Absorbance of the control}} \text{X100} \qquad (2)$$

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation. IC_{50} value was determined from the graph obtained by using standard ascorbic acid following the formula "y=mx+c" from the slope of the graph. The IC_{50} represents the concentration where 50% inhibition of the DPPH radical is obtained. The purity and the contents of the standard ascorbic acid were confirmed using HPLC (Njoku et al., 2014).

6. Proximate analysis of nutritional value (AOAC method).

Moisture content.

The dry Petri-dish and lid was placed in oven to dry at 105°C for 3 h, then transferred to desiccator to cool and 3 g of the sample was placed in a pre-weighed Petri dish, the sample spread to the uniformity and then placed in an oven to dry at 105°C for 3 h. The dish and dry sample were transferred to a desiccator to cool at room temperature reweigh the dish and it is sample, the experiments were repeated until constant weight was obtained (Soylak et al., 2004).

$$\% \text{ Moisture content } = \frac{\text{weight before drying-weight after drying}}{\text{weight before drying}} \times 100 \tag{3}$$

Ash content.

Place crucible in muffle furnace at 550°C, overnight, to ensure that impurities on surface crucible are burnt and placed in a desiccator to cool. Five g of the sample was weighed into a crucible in a muffle furnace and heated at 550°C for 6 h until it became gray ash. The crucible was removed from the muffle furnace using a crucible tong and placed in a desiccator to cool. Re-weighed and the weight of ash was obtained by difference (Soylak et al., 2004).

$$\% \text{ Ash content } = \frac{\text{weight of Ash}}{\text{weight of sample}} \times 100 \tag{4}$$

Fat content.

The bottle was washed and dried in oven at 105°C overnight and then placed in a desiccator to cool. Place the bottle in the apparatus, 3 g of the dried sample were taken and weighed accurately into labeler thimbles. The extraction thimble was plugged with a cotton and transferred to a soxhelt apparatus. The bottle was filled with 200 mL of petroleum ether and boiled at 40-60°C. The soxhlet apparatus was allowed to reflux for 1 h. The thimble was removed carefully, and the petroleum ether on top of the container was collected and drained into another container for reuse. The flask was removed and boiled for an hour at 105°C. Finally transferred from the oven into a desiccator to cool before weighing (Soylak et al., 2004).

$$\% \text{ Fat content } = \frac{\text{weight of fat}}{\text{weight of sample}} \times 100 \tag{5}$$

Fiber content (Weende's method)

Two g of the sample was weighed into a 250-mL conical flask and 200 mL of 1.25% H_2SO_4 was added and the mixture was boiled under reflux for 30 min. The solution was filtered with Whatman filter paper; the residue was rinsed thoroughly with hot water until it was no more acidic when tested using pH paper. The residue was transferred into a 250-mL beaker and 200 mL of 1.25% NaOH was added and boiled for 30 min in a digestion apparatus after which it was filtered and rinsed with distilled water until the filtrate was neutral when tested with pH paper. The residue transferred into a crucible and placed in electric oven at 100°C for 8 h to dry. Then removed and placed in a desiccator to cool before weighing. After weighing, the sample cooled in a desiccator and re-weighed. Fiber × 100.

Protein content (Kjeldahl method)

The total nitrogen was determined and multiplied by a conversion factor of 6.25 to obtain the protein content. Weighed 1 g of the sample added tablet catalyst tablet (10 g of $NaSO_4$, 1 g of CuSO) into a Kjeldahl digestion flask, with 12 mL of H_2SO_4, and heated at digestion stand at 420°C for 1 h until chemical decomposition was complete and changed to blue green color, let digestion flask to cool. Twenty mL of deionized water was added. Then 25 mL of 40% of NaOH and placed in the Kjeldahl distillation apparatus. The mixture was distilled until a total of 50 mL distillate was collected into 250 mL conical flask containing boric acid and titrated with 0.1 N HCl. The end point of the titration was observed when the color of the distillate changed to the initial color of the mixture of boric acid and screen methyl red indicator which was light pink (Soylak et al., 2004).

$$\% \text{ Protein } = \frac{\text{Volume of standard acid X 0.1N X14.007M.Wt x 6.25}}{\text{Wt of sample (mg)}} \times 100 \tag{6}$$

7. Elemental analysis.

Preparation of sample by wet digestion.

0.5 g of pollen sample was weighed and placed in a 25 mL volumetric flask, and 5 mL of concentrated HNO_3 was added. The volumetric flask was covered then placed on hot plate and heated for 2 h at 120°C until brown fumes. After cooling 1 mL of H_2O_2 was added and heated to become clear. The volumes were completed by 25 mL of ultra-pure water and filtered with 0.45-µ mL millipore membrane filter (Jothy et al., 2011).

Mineral content.

The minerals were determined by using Inductively coupled plasma-optical emission spectrometry (ICP-OES). Ten minerals (Ba, Cd, Cu, As, Cr, Fe, Pb, Mn, Ni and Zn) were quantified, the calibration standards were prepared from multi elemental standard after dilution.

8. Acute toxicity study.

Target animals.

Healthy Wister rats weighing between 120 and 200 g were obtained from the local animal house of the Pharmacology Department, Faculty of Pharmacy, University of Tripoli, Libya. Those rats were distributed into three groups, three animals in each group i.e., treated groups were given a dose of 500 mg kg^{-1} as a low dose whereas another group received 5000 mg kg^{-1} in the high dose, the control group received distilled water only. The animals were randomly selected and marked on the tail for individual identification. All rats must be maintained on a 12-h dark place and located at room temperature approximately 23°C with constant humidity before experiment. The animals were maintained in laboratory conditions for a week before starting the experiment. The animals must be fasting 12 h prior to the treatment. The body weight of the rats were determined after the fasting period, and the dose calculated to body weight as the volume of the extracts solution given to the mice is 10 mL kg^{-1} body weight.

Procedure for acute oral toxicity study.

The acute toxicity study was conducted in accordance procedure of the Organization for Economic Co-operation and Development (OECD)(Shaheen et al., 1986). It was conducted by using a total of nine male rats. The animals were divided into 3 groups of 3 mice each. The first group was given 500 mg kg^{-1}, the second was given 5000 mg kg^{-1} body weight of sterile the extract, to possibly establish the range of doses producing any toxic effect. In addition, the third group of rats was set up as the control group, the animals were given only sterile distilled water. The animals were observed in detail for any indications of toxic effect within the first 6 h after the treatment period, and daily further for a period of 14 days. The surviving animals were weighed and monitored daily for visual behavioral changes as well as mortality.

9. Determination of pollen viability (staining test).

The viability of pollen grains was determined by spreading pollen on a slide and staining by using one to two drops of 1% aceto carmine solution. Pollen viability was examined under a light microscope, if the pollen grains are able to staining with red color, that means it is viable whereas colorless grains were considered non-viable (Serrano et al., 2010).

10. Scanning electron microscopy (SEM).

Pollen grains of male date palm sample were weighed and prepared to remove fat by soxhlet extraction before the injection in SEM. The dried material was mounted directly on stubs using double-sided conductive tapes, coated with 40-60 nm of gold (a thin layer) and then observed in the microscope (Tabbiruka et al., 2014).

11. Thermo-gravimetric analysis (TGA).

Thermo-gravimetric and differential thermal analysis.

TGA method was used to determine the weight loss percentage. Three mg of pollen grains sample was weighed and put in the sample cell in the TGA-DTA machine which was then operated and the thermogram was obtained by heating gradually from ambient temperature at a scanning rate of 25°C 5 min^{-1} up to 100°C and the gas was switched to N_2 at 20 mL min^{-1}. Different components present in the sample were decomposed at different temperatures and the resulting percent of weight loss was recorded (Sharif et al., 2010).

RESULTS AND DISCUSSION

Extraction yields

The extraction of finely dried date palm pollen from 45.12 g using methanol yielded 30.47% at 70°C and 45.41 g at 45°C yielded 27.75% of DPP crude extracts. The yield color at 70°C was a strong yellow, while it was a pale yellow at 45°C (Table 1). Methanol is a highly active solvent which facilitates the extraction of more bio-active polyphenols, tannins, saponins, flavon, terpenoids and anthocyanin compounds compared with other solvents. It is characterized by its strong ability to penetrate the cell wall of plant material and extract the intracellular components (Harborne, 1998).The difference between the extract yields and the colors from the same plant materials in the present study could be due to the temperature levels used. The increase in coloring substances and yields weight follows the increase in temperature until 70°C. Temperatures higher than 70°C may cause the degradation of the color of plant materials.

Table 1. Extraction yield and colors of DPP grains.

Methanol solvent	Extraction yield (%)	Color
At temperature 70°C	30.47	Dark yellow
At temperature 45°C	27.75	Pale yellow

Preliminary phytochemical screening of *P. dactylifera* pollen

The medicinal properties of plants are due to the presence of several active components which are exclusively accumulated in different part of the plant. These active components protect against many disease as they are characterized by their antibacterial and antioxidant properties, among others. The phytochemical screening of the palm pollen extracts revealed the presence of phytosterols, flavonoids, coumarins, tannins, phenolic compounds, amino acids, and protein along with the presence of small amounts of saponin and fats. The screening also revealed the absence of alkaloids and anthra quinone glycosides. The ratios are reported in Table 2. A study by Al-Samarai et al. (2016) on the phytochemical screening of ethanol extracts of Iraq palm pollen showed the presence of alkaloids, carbohydrate, protein, amino acides with trace amounts. The pollen was reported to be rich in flavonoids, phytosterol. No coumarins, quinones, other glycosides and saponin were found. Another study by Al-Samarai et al. (2016) reported that methanol extracts of palm pollen grain contained many types of flavonoids, consistent with Abed El-Azim et al. (2015) who found that the palm pollen grain contains high quantities of lipids and steroids. DPP is consider an excellent food and drug resource among natural products. The phytosterols in DPP is a potential male fertility enhancer as it is a gonadal stimulant and can improve lipid metabolism. Polyphenolic compounds, which have growth promoter properties and a strong antimicrobial activity, can assist in curing inflammatory diseases and have powerful antioxidant and anticancer effects. Some flavonoids, such as quercerin and rutin, have hepato protective activities. Besides, the bio-active fat and lipids contents in palm pollen increase the immune response activity (Kostova et al., 2011). Coumarins have the ability to act as anticancer, antiviral, excellent antioxidant, anticoagulant agents. Therefore, it is possible to use palm pollen in natural pharmaceutical formulations (Bentrad et al., 2017), as well as an anti-inflammatory treatment of chronic infections (Rohini and Srikumar, 2014).

Table 2. Phytochemical screening for two extracts of *P. dactylifera* pollen.

Phytochemical tests	Reagents	Methanol 70	Methanol 45
Phytosterol	Salkowski	+++ve	++ve
	Libermann burchard	+ve	+ve
Flavonoids	Alkaline reagent	+++ve	+++ve
Tannins and phenolic compounds	Ferric chloride	+++ve	+++ve
Alkaloids	Dragendorff	-ve	-ve
	Mayer	-ve	-ve
	Wagner	-ve	-ve
Glycosides	Killer killiani	++ve	+ve
	Modified Brontrager s	-ve	-ve
Saponin	Foam test	+ve	+ve
Cumarins	Sodium hydroxide	+++ve	++ve
Amino acids and protein	Xanthoprteic	+++ve	+++ve
Oil and fats	Spot test	+ve	+ve
Carbohydrate	Fehling	+++ve	++ve
	Molishes	+++ve	+++ve

high (+++), medium (++), low (+), none (-).

FTIR spectroscopy analysis

The infrared spectra of date pollen were recorded by (FTIR) and run under infrared region of 400-4000 cm^{-1} range. The present study was undertaken with a view to identify the functional groups present and purity of sample. The compatibility of Libyan DPP sample with standard are 97.1% as show in Figure 2. FTIR spectrum of DPP were obtained and depicted in Figure 1 which confirmed the presence of functional group. The intense bands occurring at 3437.15, 3414, 2924.09, 1546.91, 1639.49, and 1056.99 cm^{-1} corresponding to N-H/O-H (carboxillic acid)/O-H str/aromatic ring/C=O/C-O str/stretching. This confirms the presence of functional groups in DPP like carboxylic acids, amines, amides, phenol, etc. A strong absorption band was observed around 3437.15 cm^{-1} indicating the presence of amines and amides. A strong absorption band was observed near 2854.65 cm^{-1} showing C-H symmetric stretching of methylene group. Strong absorption band observed near 1639 cm^{-1} was due to the presence of C=O stretching. Finally the sample was in a good agreement with standard.

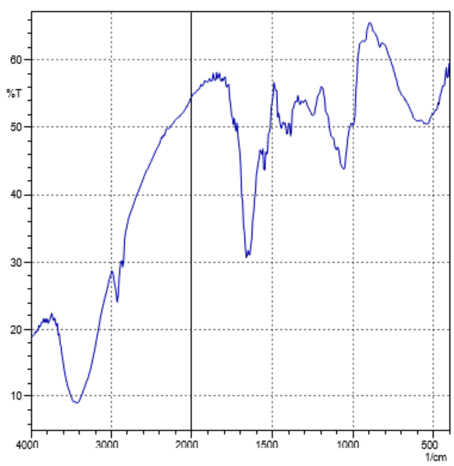


Figure 1. FTIR spectrum of date palm pollen sample.

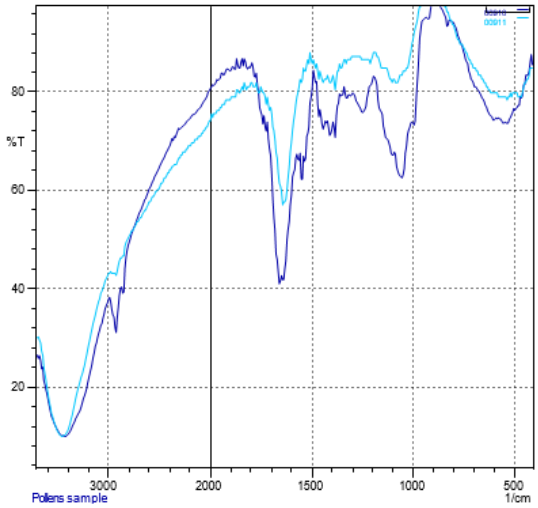


Figure 2. FTIR spectrum of standard and sample of date palm pollen.

Quantitative antioxidant activity palm pollen extracts

1. DPPH free radical scavenging activity method.

The DPPH radical scavenging assay is a simple, quick, and sensitive method used for the screening and evaluation of the antioxidants activity and free radical scavenging ability of plant extracts. The extracts of date palm pollen were quantitatively investigated for antioxidant activity and the values are compared to the ascorbic acid used as reference standard. The results are presented in Tables 3 and 4, and in Figures 3 and 4, where the activity was expressed by IC_{50} values. The values refer to the sample concentration required to scavenge DPPH radical by (50%), the lower IC_{50} reflect greater antioxidant activity of the property (Daoud et al., 2015).

Table 3. In vitro DPPH antioxidant activity of methanolic extract of DPP grains.

Extracts	IC_{50} (mg mL^{-1})
Methanol extract at 70°C	0.233
Methanol extract at 45°C	0.224
Ascorbic acid references	0.05

Table 4. In vitro DPPH antioxidant activity of the aqueous extract of DPP grains.

Extracts	IC_{50} (mg mL^{-1})
Aqueous extract	0.00075
Ascorbic acid references	0.05

Data are displayed with mean values.

The finding revealed that methanol extract at 70°C has IC_{50} value (0.233 mg mL^{-1}) and another methanol extract at 45°C has IC_{50} value (0.224 mg mL^{-1}) less than IC_{50} value ascorbic acid (0.05 mg mL^{-1}). These findings are similar to data obtained by Daoud et al. (2015) on the DPPH scavenging activity of IC_{50} value of Tunisian date palm pollen ethanol extract (0.144 mg mL^{-1}). The results shows few difference in IC_{50} between the two. The change in the IC_{50} may be because the use of heat in extraction in the present work produces little alteration in antioxidant activity of date palm pollen compounds. In the thermal stability work the results showed loss of weight might be related to loss of moisture and volatile components. These volatile compounds in another study by Farouk et al. (2015) possess IC_{50} of 0.89 mg mL^{-1}. The excellent result was shown in the aqueous DPP extract which has IC_{50} (0.0007 mg mL^{-1}) potent with 100 times of ascorbic acid. This activity may be attributed to extra concentration of active compounds extracted with more polar solvent as water.

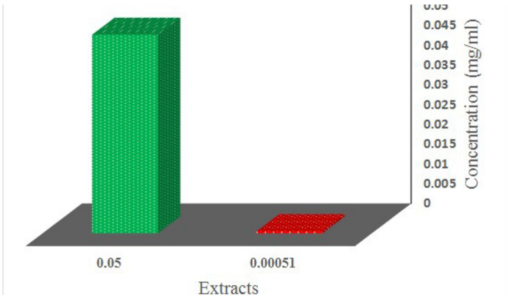


Figure 3. DPPH radical scavenging capacity (IC_{50}) of DPP methanolic extracts and standard.

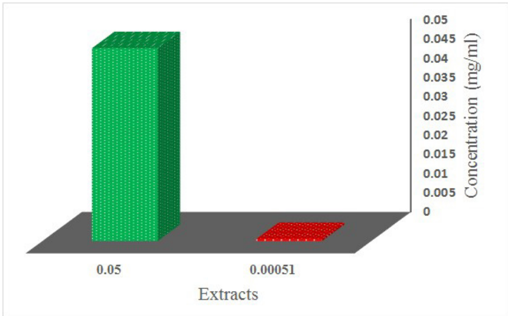


Figure 4. DPPH radical scavenging capacity (IC_{50}) of DPP aqueous extract and standard.

Antibacterial activities of the DPP grains against bacterial strains

Table 5 shows the anti-bacterial activity of date palm pollen extracts by cup cut method against five bacteria strains. The potency of each strain was evaluated based on the diameters of the inhibition zones surrounding the wells; if there is no inhibition, it assumed that there is no antibacterial activity. As in Table 5, negative results were obtained against all types of Gram positive and Gram negative bacteria for two extracts and clear zones of inhibition were produced by positive control. The results of this experiment were different from the prior literature findings, which implies that the extract of palm pollen grain may exhibit antibacterial activity depending on the concentration of the extract and the target bacterial strain as in Banu and Catherin (2015), who reported that palm pollen grain ethanol extracts resulted in inhibition zones with diameters of 2.5, 3.5, 3, and 7 mm against *Klebsiella pneumaniae* at concentrations 60, 70, 80, and 90%, respectively, zones with diameters of 2.5 and 1.5 mm with concentrations of 60 and 70% of *Pseudomonas aeruginosa*, and negative results against *Staphylococcus aureus*. Another study of the ethanol extracts of palm pollen grains from two Tunisian cultivars by Daoud et al. (2015) reported zones with diameters of 12 and 14 mm against *Staphylococcus aureus*, 10 and 10.5 mm diameter zones against *E. coli*, and negative effects to one extract, while the other extract resulted in a 8-mm diameter inhibition of zone against *Klebsiella Pneumaniae*. However, prior literature on palm pollen extracts reported a low to moderate antimicrobial activity especially against human pathogens and fungi. An inhibition zone with a diameter of 10 mm suggests that the antibacterial activity is low, while a diameter between 10 and 15 mm suggests that the antibacterial activity is moderate (Bentrad et al., 2017).

Table 5. Zone of inhibition of date palm pollen in two extracts.

Extracts	Methanol extract at 70°C						Methanol extract at 45°C					
Bacterial strains	Inhibition of zone (mm)						Inhibition of zone (mm)					
Concentration of extract	100	50	25	12.5	DMSO*	Cip	100	50	25	12.5	DMSO*	Cip
Staphylococcus aureus	-	-	-	-	-	35	-	-	-			25
Klebsiella. pneumoniae	-	-	-	-	-	40	-	-	-			32
Pseudomonas aeruginosa	-	-	-	-	-	30	-	-	-			26
E. coli	-	-	-	-	-	34	-	-	-			23
MRSA[a]	-	-	-	-	-	35	-	-	-			25

[a]Methicillin-resistant *Staphylococcus aureus.*
Data are displayed with mean values ± SD (*n*=3) The reference standard of bacteria (ciprofloxacin 5 μg).
- absent
DMSO* = 2%.

Determination of the nutritional value of the *P. dactylifera* pollen

Previous chemical analyses showed pollen grains are different from other pollens collected from different places as the principles of chemical analysis focus on moisture, ash, crude fiber, crude fat, crude protein, carbohydrates, vitamins, and minerals. In this study, the pollen grains were examined for their contents of moisture, ash, crude fiber, crude fat, crude protein and minerals and the obtained data are reported in Table 6. The moisture content of *P dactylifera* pollen is calculated at 18.19%. This value was lower than the values of 28.80 and 29.00% obtained by Hassan (2011) and Banu and Catherin (2015), respectively, and higher than the value of 8.041% obtained by Al-Samarai et al. (2016). The decrease in moisture content leads to the decrease in the growth of microorganisms and helps in a safe long period storage. The protein content in the *P dactylifera* pollen was calculated at 27.36%, a value lower than 39.80% obtained by Banu and Catherin (2015). The value of crude protein content was within the values (19.45-31.1%) obtained by Hassan (2011). Proteins are essential for human health and are needed to maintain the vital function of body cells and growth. They are also required by pregnant and breastfeeding mothers. The protein quantity in the pollen grain are mainly more than the 19% found in shrimp as reported by Stein (2014), and more than the 22.90% in chicken breasts reported by Petracci et al. (2014). The total ash content in palm pollen grains is 5.41%, nearly compatible with the 5.58%, estimated by Al-Samarai et al. (2016). Furthermore, Banu and Catherin (2015) reported a higher content of 6.20%, while Hassan (2011) found only 4.57%. The total ash refers to amount of minerals beneficial to human health along with some toxic metals. Fats are an excellent source of energy. Palm pollen grains contain 7.32% of the crude fat, consistent with the value of 7.67% reported by Al-Samarai et al. (2016). Other studies reported different results: 20.7 4% reported by Hassan (2011) and 31.50% by Basuny et al. (2013). The value obtained for fiber content was 0.68%. This value was within the range estimated by Al-Samarai et al. (2016), Banu and Catherin (2015), and Hassan (2011). The variation in type and content may be owing to many factors such as the differences in the collection time and seasons, maturation age, location of cultivates, and conditions of handling and storage after collection Hassan (2011). The pollen are valuable natural food sources rich in protein and minerals, and contain well-balanced essential amino acids, vitamins and over 100 kinds of enzymes and cofactors Hassan (2011). Nutritionally, the pollen are high in protein, low in saturated fat and calories, and have a neutral flavour, which make pollen a natural potential additive in pharmaceuticals formulations, also in salads, cakes, and soups.

Table 6. Proximate nutritive values of DPP.

Parameter (%)	Mean ± S.D.
Moisture	18.19±0.003
Crude fiber	0.67±0.001
Total ash	5.41±0.001
Crude fat	7.32±0.007
Crude protein	27.36±0.003

Elemental analysis

The date palm pollen possessed a variety of metals which play a crucial role in the development of biological functions and growth of the human body such as Mn, Fe, Zn, Cr, Cu, Mo, and Ni. The results of the elemental analysis obtained by AAS techniques are shown in Table 7 in ppm of dry weight of the palm pollen grains with a precision of about±99%. The predominant mineral was zinc (36.8), followed by iron (27.5), manganese (24.1), copper (4.73), nickel (1.20), and chromium (0.29). This study's findings vary in the concentration of the quantified elements compared with other studies. In Al-Samarai et al. (2016) and Hassan (2011), zinc contents were 2.810 and 2.799, iron contents 2.410 and 8.500, manganese 2.840 and 19.600, copper 3.196 and 3.658, and nickel 3.024 and 1.698, respectively. Some plants may be contaminated with non-essential metals for human body like Hg, Pb, As, and others. The presence of these elements is dangerous as they have toxic effects and widely present in plants (Leal et al., 2013). Date palm pollen is free from heavy metals which are usually below the detection limit. The ratio of arsenic, cadmium, and lead as observed in Table 7 are with values below 0.02, 0.002, and 0.03, respectively. Metals may accumulate from soil, water, and the variation of the flora and growth state: the soil and geographic origin of pollen can cause a large difference in the composition and concentration of minerals in the pollen. These elements are essential for life and the deficiency of vital elements in the human body can cause many diseases. The presence of Mg may decrease coma, neurological disturbances and diabetes mellitus. The body also requires sufficient amounts of zinc to produce testosterone, maintain a functional male reproductive system, prevent hair loss, and overcome free radicals. Iron also helps in protecting the body from anemia, regulating hemoglobin blood levels, while a copper deficiency causes brain disease in infants, anemia in adults. Chromium helps patients with diabetic mellitus to regulate blood sugar, supports muscle and burns fat in the human body, supports healthy bones by inhibiting the loss of calcium and increasing the rate of milk production by pregnant and lactation mothers (Zafar et al., 2010).

Table 7. Mineral composition of DPP grains.

Mineral	Concentration (ppm)
Zn	36.8
Ba	0.54
Cu	4.73
Fe	27.5
Mn	24.1
Ni	1.20
Cr	0.29
Metals	
As	<.02
Cd	<.002
Pb	<.03

1. Acute toxicity study.

The acute toxicity effect of the of methanolic extract of date palm pollen at 70°C on male rats on the appearance and the general behavioral pattern are shown in Table 8. No toxic symptoms or mortality were observed in any rats, which lived up to 14 days post extract administration at dose level 500 and 5000 mg kg^{-1} body weight. The behavioral patterns of animals were observed first 6 h and followed by 18 h in both vehicle treated (water) and extract-treated (extract) groups and the animals were normal and did not show any difficulty in breathing, or loss of appetite, muscular tremors, or abdominal cramps, sweating and general weakness. There are no previous reports on any toxic effect to palm pollen grain. Thus the LD_{50} of the of date palm pollen being greater than 5000 mg kg^{-1} body weight. It is thought to be safe as food or for medicinal purpose (Shaheen et al., 1986).

Table 8. Acute toxicity effect of methanolic extract of DPP at difference doses.

Dose	500 mg kg^{-1}				5000 mg kg^{-1}			
Observation	6h	18-h	daily	control	6-h	18-h	Daily	Control
Skin	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N
Breathing	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N	N
Mortality	None	None	None	None	None	None	None	None

N = normal; None = not observed.

Thermo-gravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) method was applied to investigate and understand the thermal stability of the natural products, pharmaceuticals and others, so some phytochemical compounds are very sensitive to heat and can easily decompose and degrade. Therefore, the thermal constancy properties of any medicinal plant play a vital role in pharmacological stability of compounds when manufactured as drug form during processing, formulation, packaging, and storage. The estimation of thermal treatments also are frequently used in the industry of food materials whose antioxidant efficiencies can be affected by heat (Farhoosh and Nyström, 2018). The findings of thermal stability was measured by thermos-gravimetric analysis to date palm pollen before extraction. The TGA curve was plotted between temperature and weight of the sample in Figure 5. These results show that little change of weight at 22-100°C was observed and 15.8% of weight loss was exhibited which might be associated with the loss of water and volatile molecules which is consistent with Nadiye-Tabbiruka et al. (2014). There are 20 one volatile compounds detected in DDP according to Farouk et al. (2015). DDP shows minimum initial temperature decomposition which might be related to its stability but further HPLC studies are required to confirm the stability.

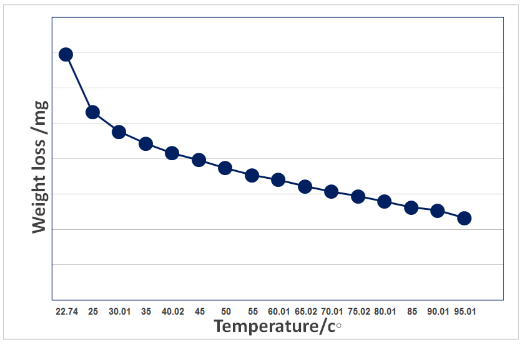


Figure 5. TGA of weight loss curves of the thermal degradation of date palm pollen.

Pollen grains viability

Viability of pollen grains of the males are high in most of the pollen grains under investigation, the viability showed most pollen grain colored with red color.

Description of palm pollen grains under scanning electron microscopy (SEM)

The mature pollen grain has a double wall, thin inner wall called intine, and a tough outer wall called exine. The exine is variously sculptured, this character of the markings is often of value for identifying genus, species. Also the other morphological characteristics of pollen grains have been categorised unique as pollen units, symmetry, shape, surface, size, apertures, polarity (Soliman and Al-Obeed, 2013). Figure 6 describes the pollen grains of males of *Phoenix dactylifera* and their shape is monad, symmetric mono sulcate, elliptical-oblate shaped, the aperture of palm pollen has one deep line in the polar surface, usually opened with rounded tips slightly shorter than polar view long axis positioned centrally in polar face of pollen grain (simple aperture). The surface is reticulate with irregular semi-circular pores and appear as roughness spongy form. The size of DPP pollen grains is between 20 to 75 microns and they have a diameter of about 21-27 microns.

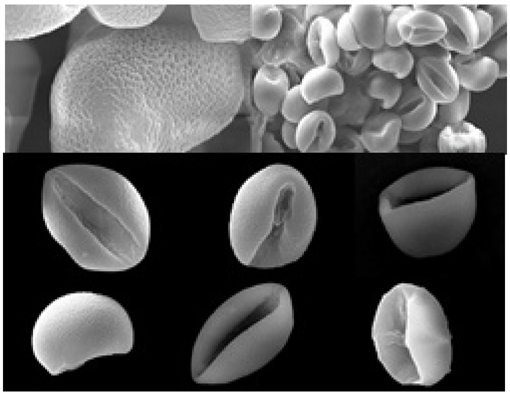


Figure 6. DPP under scan electrical microscope.

CONCLUSIONS

To conclude, the study revealed that the preliminary phytochemical analysis in present work shows DPP contain phytosterols, flavonoids, coumarins, tannins, phenolic compounds and amino acids and protein and the presence of small amount of saponin, and fats. In this study the importance of aqueous date palm pollen extract possessing higher antioxidant activity is underlined. This activity of extract may be attributed to polyphenolics compounds presented in DPP, whereas the methanolic extracts of DPP exhibited low IC_{50}. This may be related to the use of heat in extraction. During examination of thermal stability of DPP by thermo gravimetric analysis 15% of loss of weight was shown related to loss of water and evaporation of volatile matter of DPP. These volatile components of DPP in other previous studies mentioned refer to an IC_{50} of 0.89 mg mL^{-1}. In addition, the DPP contain huge nutritive components like minerals substance, protein, fiber, fatty acid which are a good economic nutritional source. It can be incorporated in human food supplements system, body care, and also as a natural pharmaceutical product. Furthermore the methanolic extract at 70°C of DPP can be considered safe, as long as the LD_{50} is greater than 5000 mg kg^{-1} body weight and without any apparent in vivo toxicity in animal model. The DPP extracts have no antibacterial activity against strains of bacteria used. The importance of DPP was mentioned in the Holy Quran several times. Beside the previous researches and traditional medicine book have shown many effects of date palm pollen for the recovery and rejuvenation of the body as a whole. Therefore, it is necessary to encourage the cultivation of pollen palm trees in the Libyan farms and how to consume them for the benefit of the human being, especially since the safety of its use was confirmed. Further research is needed on the identification and isolation of steroidal active compounds in DPP and testing for fertility problems. Date palm pollen are more efficient, safe, low cost and rich with many composition that have been formulated to a plant steroidal compound, a gonadotrophic hormone, a carotenoid, a bioflavonoid, a triterpenoid, and/or an antioxidant. Study of the DPP extract should focus on the examination of the anticancer, anti-diabetic and anti-hypertensive activities. Date palm pollen can be made available in the daily diet due to the nutritional properties of pollen. It can also be consumed with honey or cooked with daily meals. It can be added to bread or cake as well. The DPP could be introduced in pharmaceutical preparations that may be formulated for topical use in methods of treatment or prophylaxis of various skin conditions and skin aging.

Literature cited

Abed El-Azim, M.H., El-Mesalamy, A.M.D., Yassin, F.D., and Khalil, S.A. (2015). Identification phenolic and biological activities of methanolic extract of date palm pollen (*Phoenix dactylifera*). Microb and Biochem Technol *7* (*1*), 47–50 https://doi.org/10.4172/1948-5948.1000180.

Al-Samarai, A.H., Al-Salihi, F.G., and Al-Samarai, R.R. (2016). Phytochemical constituents and nutrient evaluation of date palm (*Phoenix dactylifera* L.) pollen grains. Tikrit J Pure Sci *21*, 1813–1662.

Ashokkumar, R., and Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. Int. J. Curr. Microbiol. Appl. Sci. *3*, 395–406.

Banu, S.K. and Catherin, L. (2015). General techniques involved in phytochemical analysis. Int J Adv ResChem Sci *2*, 25–32.

Basuny, A.M., Arafat, S.M., and Soliman, H.M. (2013). Chemical analysis of olive and palm pollen: antioxidant and antimicrobial activation properties. Herald Journal of Agriculture and Food Science Research *2*, 91–97.

Ben Said, R., Hamed, A.I., Mahalel, U.A., Al-Ayed, A.S., Kowalczyk, M., Moldoch, J., Oleszek, W., and Stochmal, A. (2017). Tentative characterization of polyphenolic compounds in the male flowers of *Phoenix dactylifera* by liquid chromatography coupled with mass spectrometry and DFT. Int J Mol Sci *18* (*3*), 512 https://doi.org/10.3390/ijms18030512. PubMed

Bentrad, N., Gaceb-Terrak, R., Benmalek, Y., and Rahmania, F. (2017). Studies on chemical composition and antimicrobial activities of bio-active molecules from date palm (*Phoenix dactylifera* L.) pollens and seeds. Afr J Tradit Complement Altern Med *14* (*3*), 242–256 https://doi.org/10.21010/ajtcam.v14i3.26. PubMed

Daoud, A., Malika, D., Bakari, S., Hfaiedh, N., Mnafgui, K., Kadri, A., and Gharsallah, N. (2015). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two Tunisian cultivars. Arab. J. Chem. *4*, 1–12.

Elberry, A.A., Mufti, S.T., Al-Maghrabi, J.A., Abdel-Sattar, E.A., Ashour, O.M., Ghareib, S.A., and Mosli, H.A. (2011). Anti-inflammatory and antiproliferative activities of date palm pollen (*Phoenix dactylifera*) on experimentally-induced atypical prostatic hyperplasia in rats. J Inflamm (Lond) *8* (*1*), 40 https://doi.org/10.1186/1476-9255-8-40. PubMed

Farhoosh, R., and Nyström, L. (2018). Antioxidant potency of gallic acid, methyl gallate and their combinations in sunflower oil triacylglycerols at high temperature. Food Chem *244*, 29–35 https://doi.org/10.1016/j.foodchem.2017.10.025. PubMed

Farouk, A., Metwaly, A., and Mohsen, M. (2015). Chemical composition and antioxidant activity of date palm pollen grains (*Phoenix dactylifera* L. *Palmae*) essential oil for Siwe cultivar cultivated in Egypt. Middle East JrAppl Sci *5*, 945–949.

Harborne, J. (1998). A Guide to Modern Technique of Plant Analysis, 3rd edn (United Kingdom: Chapman and Hall Publications), p.278.

Hassan, M.M. (2011). Chemical composition and nutritional value of palm pollen grains. Global J Biotechnol & Biochem *1*, 01–07.

Jothy, S.L., Zakaria, Z., Chen, Y., Lau, Y.L., Latha, L.Y., and Sasidharan, S. (2011). Acute oral toxicity of methanolic seed extract of Cassia fistula in mice. Molecules *16* (*6*), 5268–5282 https://doi.org/10.3390/molecules16065268. PubMed

Kostova, I., Bhatia, S., Grigorov, P., Balkansky, S., Parmar, V.S., Prasad, A.K., and Saso, L. (2011). Coumarins as antioxidants. Curr Med Chem *18* (*25*), 3929–3951 https://doi.org/10.2174/092986711803414395. PubMed

Leal, A.S., Prado, G., Gomes, T.C.B., Sepe, F.P., and Dalmázio, I. (2013). Determination of metals in medicinal plants highly consumed in brazil. Braz. J. Pharm. Sci. *49* (*3*), 599–607 https://doi.org/10.1590/S1984-82502013000300022.

Metwaly, M.S., Dkhil, M.A., and Al-Quraishy, S. (2014). Anti-coccidial and antiapoptotic activities of palm pollen grains on *Eimeria papillata* induced infection in mice. Biologia *69* (*2*), 254–259 https://doi.org/10.2478/s11756-013-0297-9.

Nadiye-Tabbiruka, M.S., Ddamba, W., Tsheko, K., Kenewang, Z., and Salamula, E.J. (2014). Thermal-gravimetric, calorimetric and chemical analytical characterization of coal. Int. J. Mater. Sci. Appl. *3* (*6*), 325–330 https://doi.org/10.11648/j.ijmsa.20140306.18.

Njoku, C.N., Ehiri, R.C., and Offor, I.F. (2014). Proximate nutritional analysis and heavy metal composition of dried *Moringa Oleifera* leaves from Oshiri Onicha L.G.A., Ebonyi State, Nigeria. J Environ Sci. Toxicol Fd Technol *8*, 57–62.

Pandey, A., and Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J. Pharmacogn. Phytochem. *4*, 115–119.

Petracci, M., Mudalal, S., Babini, E., and Cavani, C. (2014). Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. *13* (*1*), 179–183 https://doi.org/10.4081/ijas.2014.3138.

Rohini, K., and Srikumar, P.S. (2014). Therapeutic role of coumarins and coumarin-related compounds. Jr Thermodynamics & Catalysis *5* (*1*), 1–3 https://doi.org/10.4172/2157-7544.1000130.

Serrano, R., da Silva, G., and Silva, O.M.D. (2010). Application of light and scanning electron microscopy in the identification of herbal medicines. Chapter 4. In Microscopy: Science, Technology, Applications and Education, 4th edn (Formatex), p.182-190.

Shaheen, M.A., Nasr, T.A., and Bacha, M.A. (1986). Date palm pollen viability in relation to storage conditions. Paper presented at: Second Symposium on Date Palm (Al-Hassa: King Faisal University).

Sharif, A., Saim, N., Jasmani, H., and Ahmad, W.Y.W. (2010). Effect of solvent and temperature extraction of colorant from onion (*Allium cepa*) skin using pressurized liquid extraction. Asian J Appl Sci *3*, 262–268 https://doi.org/10.3923/ajaps.2010.262.268.

Soliman, S.S., and Al-Obeed, R.S. (2013). Investigations on the pollen morphology of some date palm males (*Phoenix dactylifera* L.). Aust. J. Crop Sci. *7*, 1355–1360.

Soylak, M., Tuzen, M., Narin, I., and Sari, H. (2004). Comparison of microwave, dry and wet digestion procedures for the determination of trace metal contents in spice samples produced in Turkey. Jr Fd and Drug Anal *12*, 254–258.

Stein, S.N. (2014). Analysis of the mineral composition of Louisiana wild caught shrimp by ICP-OES and classification of geographical origin. M.Sc. thesis (Department of Nutrition and Food Sciences, Faculty of the Louisiana State University and Agricultural and Mechanical College).

Sudhira, L., Venkateswara Rao, S., and Kamakshamma, J. (2015). Phytochemical screening, antioxidant and antibacterial activity of *Strychnos colubrina* L. as an important endangered medicinal species in eastern Ghats. Jr Pharmaceutical Sci Res *7*, 242–247.

Tabbiruka, M.N., Ddamba, W., Tsheko, K., Kenewang, Z., and Salamula, E.J. (2014). Thermal-gravimetric, calorimetric and chemical analytical characterisation of coal. Int. J. Mater. Sci. Appl. *3* (*6*), 325–330 https://doi.org/10.11648/j.ijmsa.20140306.18.

Tahvilzadeh, M., Hajimahmoodi, M., and Rahimi, R. (2016). The role of date palm (*Phoenix dactylifera* L) pollen in fertility: a comprehensive review of current evidence. J Evid Based Complementary Altern Med *21* (*4*), 320–324 https://doi.org/10.1177/2156587215609851. PubMed

Tiwari, R., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. (2011). Phytochemical screening and extraction: a review. Int Pharmaceutical Sci *1*, 98–106.

Zafar, M., Khan, M., Ahmad, M., Jan, G., Sultana, S., Ullah, K., Khan, S.M., Farooq, A., Jabeen, A., Nazir, A., et al. (2010). Elemental analysis of some medicinal plants used in traditional medicine by atomic absorption spectrophotometer (AAS). Jr Med Pl Res *4*, 1987–1990.

Physico-chemical and sensorial properties of biscuits supplemented with date powder

A.A. Panhwar, S.G. Khaskheli[a], S.A. Sheikh and A.H. Soomro

Institute of Food Science and Technology, Sindh Agriculture University, Tandojam, Sindh, Pakistan.

Abstract

The effects of date powders on physico-chemical and sensorial characteristics of biscuits were studied at laboratory of Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam during 2019-20. The treatments included: T1 = 0% without date powder (control), T2 = 20% date powder and T2 = 30% date powder. Sensory analysis indicated that biscuit supplemented with 20% date powder was found excellent. The results regarding the chemical components showed an increase in moisture content, starch, ash and fibre content of biscuits supplemented with date powder. The higher results were recorded with T2: 5.50% moisture, 2.88% ash content, 18.94% total soluble solids, 13.98% fat, 15.98% protein and 55.64% carbohydrate in biscuit supplemented with date powder. The addition of date powder provides good response in increase in the minerals content as well. It is concluded from the study that date powder treatment of 20% showed better effect on quality of biscuits, its physiological and mineral content. Our findings determined that the biscuits supplemented with 30% date powder had the lowest acceptability, whereas the biscuits supplemented with 10%, were found to be excellent and not affected by the sensorial parameters. It is concluded that date palm powder can be used and incorporated in bakery products up to 20%, it is recommended to use dates powder in the manufacturing of biscuits.

Keywords: date powder, supplementation, sensory

INTRODUCTION

Fruits of date palm (*Phoenix dactylifera* L.) are consumed throughout the world and are a vital component of the diet in most Arabian countries. Biscuits are a popular, attractive food product due to their delightful and coarse texture, efficiency, convenience, and cost effectiveness. Biscuit has lower moisture as compared to other bakery items like cake and bread and are safe from microbiological damage, hence its shelf life is also long. Bakery products are rich in carbohydrate, fat, and calorie, but low in fibre content (Mishra and Chandra, 2012). Usman et al. (2015) stated that biscuits because of its high in calories do not fall in the healthy food group especially for old, diabetic, and overweight people, and contain a significant amount of fat which is not good for people with diabetes. Date is an important fruit of hot climatic regions, nutritious and favourite fruit all over the country. They are mostly eaten fresh and in numerous value-added products viz., pickles, dry dates, jam, chantey, etc. Date fruit is rich in amounts of sugar, iron, potassium, calcium and nicotinic acid and small amounts of protein, copper, magnesium, chlorine, sulphur and vitamins. The pulp of mature date fruits contains about 80% sugars on dry weight basis, which are easily digested and provide a ready source of energy to the human body. The date is considered as a healthy fruit. Date fruits are characterized by their higher content of essential nutrients such as carbohydrates, total sugar, minerals, phytochemicals i.e., sterols, phenolic components, carotenoids and flavonoids, which increase the nutritional and sensory properties of palm dates. In Pakistan, date palm fruits are harvested from mid-June to August because of the lack of early maturing cultivars. The flesh of a fully ripe date consists of two-third sugar and one-quarter water, the rest being mainly cellulose, pectin, ash and vitamins. Research has indicated

[a]E-mail: sgkhaskhali@sau.edu.pk

the clear contribution of dates to human health when consumed with other food constituents, such as minerals and vitamins. The objective of our study was to evaluate the physico-chemical composition of date supplemented with date powder as a replacement with sugar at different ratios (20 and 30%).

MATERIALS AND METHODS

Date palm (*Phoenix dactylifera* L.) cultivar 'Aseel' was collected from the Khairpur market and ingredients for biscuits dough (wheat flour, egg, salt, baking powder and sugar) were purchased from a local market of Hyderabad and brought to the laboratory of the Institute of Food Science and Technology, Sindh Agriculture University, Tandojam.

Preparation of 'Aseel' date powder

Date was washed to remove any adhering dirt, followed by removing of the seeds and the pulp was then oven-dried at 65±1°C until constant weight. The dried date was milled using laboratory milling. The powder was packed in polyethylene bags and stored at room temperature until it was used.

Preparation of biscuits

Biscuit samples were prepared using date powder as sugar replacement at ratios of 20 and 30% according to Table 1.

Table 1. Ingredients used and methods of preparation each product (T1, T2 and T3).

Ingredient	Quantity		
	Replacing 0%	Replacing 40%	Replacing 50%
Egg	1	1	1
Sugar	50 g	-	-
Dates powder	-	40 g	50 g
Butter	133 g	133 g	133 g
Vanilla powder	2 g	2 g	2 g
Baking powder	1 1/2	1 1/2	1 1/2
Maida	200 g	200 g	200 g
Milk powder	10 g	10 g	10 g
Salt	3 g	3 g	3 g

Preparation of samples for physico-chemical and sensorial analysis

The prepared biscuits were analysed for moisture (%), ash (%), TSS, pH, protein (%), total carbohydrates (%), fat (%),using the official methods of AOAC (2000). The prepared biscuits were coded and evaluated for sensorial analyses such as flavour, taste, texture, colour consistency and overall acceptability.

Statistical analysis: the data were subjected to an analysis of variance (ANOVA) described by Steel et al. (1997).

Volume and specific volume

Volume of biscuits was determined according to the AOAC (2000), using the seed displacement method by using rapeseed. Specific volume was calculated by using the following equation:

$$\text{Specific volume} = \text{volume (mL)}/\text{weight (g)}$$

Sensory evaluation of products

The produced biscuit samples were subjected to sensory evaluation test. Ten semi-trained panelists were evaluated (numerical scoring) the samples for (taste, mouth feel, crust colour, crumb colour, texture and odour, general appearance, and overall acceptability) following the method described by Kulp et al. (1985).

Statistical analysis: the sensory evaluations of the products were statistically analysed by analysis of variance (ANOVA) according to the method of SAS Program (1998).

RESULTS AND DISCUSSION

Chemical composition of raw materials

The results regarding the effect of different ratios on chemical composition of biscuits supplemented with 'Aseel' date powder are shown in Table 2. The higher results were recorded with T3 5.50% moisture, 2.88 ash content, 18.94 total soluble solids, 13.98% fat, 15.98% protein and 55.64% carbohydrate. Respectively, these results are supported by the Doweidar (2001) who studied the chemical characteristics of wheat flour. The high content of moisture is due to high sugar content in 'Aseel' date which binds water in fortified biscuits. However, the minimum moisture (2.44%) was observed with 0% without date powder (control).

Table 2. Chemical composition of raw materials used for biscuits (on dry weight).

Parameters	Wheat flour	Date powder (Aseel)
Moisture (%)	2.70±0.13	4.43±0.01
Ash content (%)	0.51±0.015	1.40±0.05
Protein (%)	9.87±0.17	1.62±0.10
Crude fat (%)	1.11±0.01	1.62±0.22
Carbohydrate (%)	60.03	82.62

Effect of date powder supplementation on the physical characteristics of biscuits

The results regarding the effect of different ratios on physical parameters of biscuits supplemented with 'Aseel' date powder are shown in Table 3. The higher results were recorded with T1 5.50 volume %, weight 4.18, specific volume 2.63, density 0.38, hardness 13.26, respectively. Biscuits prepared with date powder replacement levels of 0, 20 and 30% were evaluated for various physical parameters. Addition of date powder decreased the specific volume ($cm^3\ g^{-1}$) of the biscuits produced from 11.0 for control to 10.40 ($cm^3\ g^{-1}$) for biscuit with date powder (Adiba et al., 2011). On the other hand the density ($g\ cm^{-3}$) increased from 0.38 for control to 0.67 ($g\ cm^{-3}$) date powder shown in (Table 3). The hardness value decreased from 13.26 for control to 12.59 and 12.33 for biscuits with date powder, respectively. As seen from the results in Table 3 biscuits became softer because of increasing the moisture content due to the date powder supplementation. The results were in accordance with Fahloul et al. (2010) who mentioned that by increasing of date powder levels the biscuit hardness values was decreased. Khouryieh and Aramouni (2012) mentioned that the biscuit hardness is related to the development of gluten and the interaction with flour ingredients in the formula. The possible reason for this result was due to date powder or date syrup by virtue of having more sugar content diluting the gluten, affecting the interaction of gluten and other ingredients (Alsenaien et al., 2015).

Table 3. Effect of date powder supplementation on the physical characteristics of biscuits.

Sample	Volume (cm^3)	Weight (g)	Specific volume ($cm^3\ g^{-1}$)	Density ($g\ cm^{-3}$)	Hardness (N)
T1	11.00±1.9	4.18±0.2	2.63±0.2	0.38±0.01	13.26±0.33
T2	10.80±0.9	6.10±0.21	1.77±0.11	0.56±0.011	12.59±0.12
T3	10.40±2	7.08±0.4	1.47±0.12	0.68±0.012	12.33±0.1

T1 = 0% without date powder (control); T2 = 40% date powder; T3 = 50% date powder.

Effect of different ratio of date powders on sensory evaluation of biscuit

The effect of date powder on the sensory characteristics of the biscuits is presented in Table 4. The results indicated that the addition of date powder to the biscuits as a sugar substitution led to improve the organoleptic quality of the product till 30% replacement ratio. The results indicated that there were no significant ($p<0.05$) differences in the sensory evaluation values between the biscuit samples with date powder replacement up to 20% for the general appearance, but the scores were significantly ($p<0.05$) reduced for the control and the samples with 30% date powder replacement. As shown in Table 4, the organoleptic quality of biscuits had low scores with increasing the level of the date powder substitution above 30% and the biscuits produced had dark crumb colour and unacceptable texture compared with the control one. The taste and mouth feel of the biscuits were affected at levels 20 and 30%, respectively. Biscuits had a coarse mouth feel at level of 30%; meanwhile at 0% supplementation the qualities of the biscuits were not adversely affected. From the above evaluation, it could be concluded that date powder could be incorporated up to 30% in the biscuits formula without affecting their sensory quality. The obtained results are in an agreement with those of El Sharnouby et al. (2012), who reported that highly acceptable biscuits could be obtained by incorporating 30% of date powder in the formula.

Table 4. Effect of different ratio of date powders on sensory evaluation of biscuit (mean of 10 values).

Treatments	General appearance (20)	Odor (10)	Taste (20)	Texture (15)	Mouth feel (10)	Crust colour (10)	Crumb colour (15)	Overall acceptability (100)
T1	16.70c±0.67	10.0 a±0.0	17.90bc±0.57	11.50c±1.43	8.70ab±1.25	6.90f±0.88	14.70a±0.67	86.40c±1.84
T2	7.60b±0.84	9.50b±0.41	17.75c±0.68	13.0a±0.78	5.80bc±0.62	8.00cd±0.67	12.10d±1.2	86.40c±1.89
T3	14.60e±1.58	15.90d±1.2	9.90d±0.91	5.30cd±1.23	4.80e±1.15	9.10e±2.18		68.30d±4.97

T1 = 0% without date powder (control); T2 = 40% date powder; T3 = 50% date powder.

Minerals content of date powders enriched biscuit

The results shows that the effect of different ratios on mineral content of biscuits supplemented with 'Aseel' date powder are shown in Table 5. the elements analysis of the date sample showed that it was characterized by higher contents of phosphorus (149-162 mg 100 g^{-1}), potassium (129-173 mg 100 g^{-1}), sodium (27-31 mg 100 g^{-1}), followed by magnesium (28-34 mg 100 g^{-1}) and calcium (29-43 mg 100 g^{-1}) as shown in Table 5. These minerals are an important factor for health. These results are in agreement with those reported by El-Sohaimy and Hafez (2010) and Ashraf and Hamidi-Esfahani (2011).

Table 5. Mineral's content of date powders enriched biscuit.

Mineral contents	Macro-elements (mg 100 g^{-1})		
	Control	T1 biscuit	T2 biscuit
Calcium (Ca)	29.83	41.36	43.57
Magnesium (Mg)	28.16	32.84	34.92
Sodium (Na)	27.29	31.11	29.15
Potassium (K)	129.65	172.24	173.00
Phosphor (P)	149.49	162.52	159.53

CONCLUSIONS

It is concluded from the study that the biscuits prepared with (T-2) 20% date powder showed better effect on quality of biscuits, and physico-chemical parameters. Our findings determined that the biscuits supplemented with 30% date powder had the lowest acceptability, whereas the biscuits supplemented with 20% were found to be excellent and not affected regarding sensorial parameters. It is concluded that date palm powder can be used and incorporated in bakery products up to 20%. It is recommended to use date powder in the manufacturing of biscuits.

Literature cited

Adiba, B.D., Salem, B., Nabil, S., and Abdelhakim, M. (2011). Preliminary characterization of food tablets from date (*Phoenix dactylifer*a L.) and spirulina (*Spirulina* sp.) powders. Powder Technol. *208* (*3*), 725–730 https://doi.org/10.1016/j.powtec.2011.01.016.

Alsenaien, W.A., Alamer, R.A., Tang, Z.-X., Albahrani, S.A., Al-Ghannam, M.A., and Aleid, S.M. (2015). Substitution of sugar with dates powder and dates syrup in cookies making. Adv. J. Food Sci. Technol. *8* (*1*), 8–13 https://doi.org/10.19026/ajfst.8.1455.

Ashraf, Z., and Hamidi-Esfahani, Z. (2011). Date and date processing: a review. Food Rev. Int. *27* (*2*), 101–133 https://doi.org/10.1080/87559129.2010.535231.

Doweidar, M.M. (2001). Chemical and physical studies on some natural resources used in improving bakery products. Ph.D. thesis (Egypt: Biochemistry Department, Fac. of Agri., Cairo University), pp.212.

El-Sharnouby, A.G., Aleid, S.M., and Al-Otaibi, M.M. (2012). Nutritional quality of biscuit supplemented with wheat bran and date palm fruits (*Phoenix dactylifera* L.). Food Nutr. Sci. *3* (*3*), 322–328 https://doi.org/10.4236/fns.2012.33047.

El-Sohaimy, S.A., and Hafez, E.E. (2010). Biochemical and nutritional characterizations of date palm fruits (*Phoenix dactylifera* L.). J. Appl. Sci. Res. *6* (*6*), 1060–1067.

Fahloul, D., Abdedaim, M., and Trystram, G. (2010). Heat, mass transfer and physical properties of biscuits enriched with date powder. J. Appl. Sci. Res. *6*, 1680–1686.

Khouryieh, H., and Aramouni, F. (2012). Physical and sensory characteristics of cookies prepared with flaxseed flour. J Sci Food Agric *92* (*11*), 2366–2372 https://doi.org/10.1002/jsfa.5642. PubMed

Kulp, K., Chung, H., Martinez-Anaya, M.A., and Doerry, W. (1985). Fermentation of water ferments and bread quality. Cereal Chem. *62* (*1*), 55–59.

Mishra, N., and Chandra, R. (2012). Development of functional biscuit from soy flour and rice bran. International Journal of Agricultural and Food Science *2* (*1*), 14–20.

SAS Program. (1998). SAS/STAT User's Guide Release 8.2 edition.

Steel, R., Torrie, J., and Dickey, D. (1997). Principles and Procedures of Statistics. A Biometrical Approach, 3rd edn (New York, NY: McGraw Hill Book Co.).

Usman, G.O., Ameh, U.E., and Babatunde, R.M. (2015). Proximate composition of biscuits produced from wheat flour and maize bran composite flour fortified with carrot extract. J. Nutr. Food Sci. *5*, 1–2 https://doi.orj/10.4172/2155-9600.1000395.

Chemical, nutritional and biological evaluation of nutritional supplement prepared with germinated date palm seeds powdered and other traditional food items

E.A. Abdelrasoul[a] and A.A. Metwalli

Food Technology Research Institute, Agricultural Research Center, Egypt.

Abstract

Germinated date palm seeds powder was used, together with soy bean flour, sugar, banana meal and maize meal for the preparation of a nutritional supplement. 12-day-old male Wister rats were fed diets containing the supplement as a protein source, both with and without soy bean flour. Casein diets with 10 or 7% protein served as respective controls. Protein efficiency ratio (PER), net protein utilization (NPU), net protein retention (NPR) and digestibility were determined. Blood biochemical parameters (triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol) were also measured in the animals and showed that all rats were in good health condition at the end of experiment. The obtained results for PER, NPU and NPR indicated that the supplement prepared with germinated date palm seeds powder was a good protein source, especially when soy bean flour was added.

Keywords: germination, data palm seeds, biological evaluation, supplement

INTRODUCTION

The prevalence of malnutrition among low-income populations in the world has led to the utilization of “alternative” food ingredients, such as powdered cassava and sugar beet leaves, date palm seeds, wheat and rice bran and powdered egg shell, among others, as nutritional supplements (Pellet and Young, 1980). Egypt is considered one of the most important countries in the production of dates globally, as it produces about 1,049,646 t annually, according to FAO (2005). The Egyptian date represented about 16.5% of the total world production. Date seeds represent about 10-15% of the fruits. There are currently 16 factories for the manufacture of dates, in addition to some factories that are under construction (Salah, 2005). All these factories have a mass production of date palm seed which comes as waste product during date processing. If these seeds are exploited well, it could play a good role in the national income. Some studies were conducted on date palm seeds (Sumianah et al., 1984) that studied the effect of germination at 35-36°C for 22 and 52 days on three cultivars (‘Razaz’, ‘Khalas’ and ‘Beshi’). It was found that crude protein, fats, total carbohydrates and starch decreased by germination but crude fibre, ash, total soluble carbohydrate and reducing sugars increased during germination. Also, the germination for 52 days was useful as a pretreatment of date palm seeds for animal feeding. Devshony et al. (1992) evaluated four cultivars of date palm kernels on the basis of dry weight and got the following results: protein 5.60%, oil 2.15%, fibre 16.13%, ash 1.13%. Analysis of the mineral elements in the ash were: Ca, 1.55%; Na, 0.97; Mg, 8.07%; K, 27.60%; Fe 0.73%; Cu, 0.13% and Mn, 0.08%. It was found that the diet containing 1.5% of date seed fibre is the most appropriate because of its effect in reducing low-density lipo-protein cholesterol, total cholesterol and triglycerides in experimental rats (Salah, 2005). However, such level (1.5%) of date seeds fibre had no effect in HDL-cholesterol level.

The aim of this study is to use cultivar date palm kernels to prepare a food supplement.

[a]E-mail: abdelrasoul63@yahoo.com

MATERIALS AND METHODS

Date palm seeds were obtained from the dates factory in Borg El Arab, Alexandria, Egypt. Soy flour was obtained from the Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Sugar, banana flour and corn flour were obtained from the local market in Kafr El-Sheikh Governorate. All chemicals were obtained from the branch of El Gomhouria Chemicals Company in Tanta, Egypt.

Date seeds germination

Date palm seeds were collected, washed, dried in air oven then wetted by water, covered by a wet cloth and then left at ambient temperature for 40 days keeping the cloth wet. The germinated seeds with the same characteristics were washed, dried by fan oven, then crushed and ground to powder. Every fraction was placed in a jar and stored in a deep freezer (-18°C) for chemical analysis.

Nutritional supplements

The proposed supplements were prepared by mixing thoroughly its ingredients and their composition is presented in Table 1.

Table 1. Composition of the germinated date palm seeds powder supplements with (A) and without (B) soy bean flour (values in % w/w).

Ingredients	A	B
Germinated date palm seeds powder	45	60
Soybean flour	15	0
Sugar	10	10
Banana meal	20	20
Maize meal	10	10
Total	**100**	**100**

Chemical analysis

Moisture, protein, fat, ash and crude fibre content were determined according to (AOAC, 1990). Reducing sugars were extracted by ethanol 80% and determined by arsino molybdates and Somogi copper reagent as described by Somogyi (1952) and Nelson (1944). The starch was determined as described by Ranganna (1997). Total free phenols were determined by using Folin-Denis reagent as described by Swain and Hillis (1959). Amino acids were fractionated by high performance amino acid analyser. Anthocyanidin was measured as reported by Barreveld (1993).

Biological assay

1. Animals and diets.

A 21-day-old male Wister rat (weighing 80-90 g) was obtained from the experimental animal house of the Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

2. Experimental design.

Laboratory mice were randomly divided into five groups (20 mice each). Groups were:

A) Rats fed on a diet containing 10% date seeds;
B) Mice fed on a diet containing 7% date seeds;
C) Rats fed a diet free of protein;
D) Rats fed on a soy bean meal;
E) Rats fed a ration without soy bean flour.

All groups were housed in individual wire cages and fed the diets shown in Table 2. Diets were approximately isocaloric and the protein contents of the control diets were in consonance with those of the experimental ones, that is 10 and 7% w/w.

Table 2. Diets composition.

Ingredients	A	B	C	D	E
Casein[a]	12.50	8.75	-	-	-
Salt mixture[b]	5.00	5.00	5.00	5.00	5.00
Vitamin mixture[b]	1.00	1.00	1.00	1.00	1.00
Soybean oil	8.00	8.00	8.00	6.00	6.00
Choline	0.04	0.04	0.04	0.04	0.04
Cellulose	100	1.00	1.00	1.00	1.00
Corn starch	72.46	76.21	84.96	11.21	-
Supplement	-	-	-	75.75	86.96

[a]Casein contained 80% w/w protein.
[b]According to AOAC (1975).

One of the diets of the control groups contained soy flour and the other not. The chemical composition of the diets is shown in Table 3.

Table 3. Chemical composition of diets.

Component	A	B	C	D	E
Protein	10.24	7.25	0.26	9.62	6.64
Ash	5.31	5.19	5.11	6.87	6.67
Fat	7.99	8.11	8.06	7.30	7.34
Moisture	10.69	10.49	10.31	6.81	6.68
Carbohydrate	64.81	67.87	75.50	68.13	71.43
Fibre	1.03	1.02	1.02	1.20	1.25

Biological parameters

Biological parameters were determined as described by Pellet and Young (1980). Food consumption and weight gain for all experimental mice were recorded weekly.

The net protein utilization (NPU) was calculated based on the difference between nitrogen in the ration eaten and nitrogen in the left hind leg of a rat after slaughter from each group 10 days after the start of the experiment. Nitrogen was determined using the following equation:

$$\text{Net protein utilized (NPU)} = \frac{\text{Nitrogen retained} \times 100}{\text{Nitrogen intake}} \quad (1)$$

Protein efficiency ratio (PER) was calculated by the ratio of weight gain (g)/protein intake (g), after 28 days for each of the remaining animals.

Digestibility (D) from the 14th to 21st day of experiment, the feces were collected daily, dried at 105°C, weighted and its nitrogen content determined. D was calculated by using the following equation:

$$D = \text{nitrogen intake-fecal nitrogen/nitrogen intake} \times 100 \quad (2)$$

Biochemical examination

Total cholesterol contents of plasma (mmol L^{-1}) were determined enzymatically (Richmond, 1973) using Huan kits (Human Gesell schaft für Biochemical and Diagnostica mbh, Germany). The HDL-cholesterol, LDL-cholesterol and triglycerides (mmol L^{-1}) were also measured enzymatically (Trinder, 1969), with kits (Human Biochemical Co, USA).

Statistical analysis

Data generated were expressed as means + significant differences among the groups were determined by one-way analysis of variance using the SPSS statistical analysis program. Statistical significance was considered at P<0.05.

RESULTS AND DISCUSSION

Chemical composition of date palm seeds before and after germination

Table 4 shows that reducing sugars of germinated seeds increased, while the starch decreased. This increase and decrease resulted from the effect of specific enzymes of special substances (amylase on starch and invertase on sucrose). The crude protein, oil, ash and crude fibre were decreased to 9.5, 4.6, 1.2 and 4.6%, respectively. Total free phenols were decreased after germination, which might be related to consumption of simple phenols through the formation of other complicated and high molecular weight compounds, which have a good role in the new parts of seed growth during germination. Sumianah et al. (1984) reported similar results as they found the anthocyanidin content decreased from 1.23 to 0.68% after germination.

Table 4. The changes of some the important components in date palm seeds before and after germination (% dry wet).

Component	Before	After
Starch	18.31	16.94
Reducing sugars	5.53	6.15
Protein	7.56	6.84
Fat	10.21	9.74
Ash	0.84	0.83
Crude fibre	16.66	15.93
Total free phenols	3.54	2.30
Anthocyandin	1.23	0.68

Palm seed protein content of amino acids

Amino acids were fractioned (Table 5), more than the recommended amount of (FAO/WHO, 1973), this amino acid decreased by germination to 2.00 g 100 g^{-1} protein. Valine increased by germination to be near the requirements, on the other hand cysteine and methionine together were more than the requirements during germination. Isolleucine content was more than half of the requirements in dried date seeds and during germination. Leucine increased by germination. The leucine, (tyrosine and phenylalanine) and lysine contents in dried and germinated date seed represented more than 70.66 and 70% of requirements (FAO/WHO, 1973). Generally, glutamic, aspartic and arginine the non-essential amino acids represented the maximal percentage of total protein.

Food ingestion, weight gain and feces weight of experimental animals' groups

Data relative to food ingestion, weight gain and feces weight are depicted in Table 6. Animals fed the diets containing the supplements, both with and without soy bean, ingested as much food as did the control 10% protein group and a bit more than the 7% protein control. On the other hand, weight gain was statistically similar when experimental groups were compared with their respective controls. Nevertheless, when food efficiency (weight gain/food ingestion ×10) was considered, animals receiving the supplements showed values comparable to their respective control (24.7, 22.1, 15.5, and 11.9% for control 10%, experimental with soy bean, control 7% and experimental without soy bean). The amount of feces excreted by animals that ingested the diets containing the supplements was higher than their respective controls. The weekly records of food consumption and weight gain showed that the biological assay proceeded without abnormalities. Food efficacy of the diets containing the proposed supplements was similar to their respective control. As expected, feces weight was dependent on the amount of fibre in the diets (Table 3) and on food ingestion (Table 6), with the animals consuming the diet with soy bean flour showing the highest value.

Table 5. Amino acids composition of date palm seeds protein before and after germination (g 100 g^{-1}).

Amino acid	Before	After	FAO/WHO (1973)
Essential amino acids			
Lysine	4.31	4.86	5.5
Threonine	4.96	2.00	4.0
Valine	3.71	4.81	5.0
Methionine	2.87	1.98	
Cysteine	2.86	1.79	3.5
Isoleucine	2.81	2.84	
Leucine	5.71	5.94	4.0
Phenylalanine	3.21	3.61	7.0
Tyrosine	0.71	0.66	6.0
Trptophan	-	-	-
Non-essential amino acids			
Asparatic acid	10.51	12.73	-
Serine	5.23	3.53	-
Glutamic acid	26.00	27.32	-
Proloine	-	-	-
Glysine	5.21	5.00	-
Alanien	4.11	4.86	-
Histidine	1.73	1.89	-
Arginine	10.91	8.93	-

Table 6. Food ingestion, weight gain and feces weight of rats.

	A (g)	B (g)	C (g)	D (g)	E (g)
Food ingestion	328.5±21.8 a	278.0±42.5 b	219.3±9.1 c	358.1±31.0 a	323.3±7.3 a
Weight gain	81.3±10.9 a	43.1±14.1 b	18.3±5.3 c	79.3±1.0 a	38.5±6.0 b
Feces weight	15.7±1.8 a	13.2±2.0 a	4.3±0.6 d	54.9±6.4 b	44.1±4.3 c

Protein quality

Table 7 shows the results obtained from the biological quality assessment of the protein. Protein efficiency ratio (PER) values were similar for all groups, except for the one fed the diet containing supplement without soy bean flour. On the other hand, rats on all diets present statistically equal values for NPU. In relation to NPR, animals receiving the diet made using the supplements with or without soy bean flour showed patterns statistically similar to their respective (10 and 7% protein) controls. As regard the digestibility, casein diets were statistically similar to each other and the same happened with the experimental diets. It must be stressed that the effect of soy bean flour addition was relevant since this item represented only about 11% w/w of the diet but raised the PER value in about 30%. This effect was confirmed by NPR that, taking into account the weight loss by the non-protein group, made similar the values of the experimental animals with their respective controls. As expected, the digestibility of the 10 and 7% control diets were similar to each other but higher than that of the experimental ones, probably due to the fibre content of date palm seeds, banana and maize employed in the preparation of the supplements (Eggum, 1995).

Table 7. Protein efficiency ratio (PER); net protein utilization (NPU); net protein ratio (NPR) and digestibility (D) of rats.

	A	B	D	E
PER	2.32±0.19 a	2.12±0.61 a	2.31±0.13 a	1.72±0.32 b
NPU	103.31±2.61 a	116.20±27.66 a	94.83±5.61 a	95.88±4.31 a
NPR	3.89±0.51 a	2.61±1.35 b,c	3.82±0.41 a,c	1.93±0.71 b
Digestibility	90.92±0.70 a	91.33±1.62 a	73.61±2.13 b	70.10±14.3 b

Blood biochemical parameters

Table 8 shows the biochemical characteristics found in the blood of the animals fed with various diets. It was observed that values were statistically similar in all parameters, except for total cholesterol and LDL-cholesterol, which were slightly less in the groups that received diet containing supplement without soy bean flour as compared to its control (10 and 7% protein). These results due to high containing of fibre in the diets.

Table 8. Blood biochemical parameters of rats.

	A	B	C	D	E
Triglycerides (mmol L^{-1})	0.952±0.31a	924±0.41a	0.923±0.33a	0.891±0.24a	0.869±0.21a
Total cholesterol (mmol L^{-1})	1.611±0.13a	1.631±0.11a	1.50±0.12a	1.491±0.22ab	1.231±0.12b
LDL-cholesterol (mmol L^{-1})	0.509±0.31a	0.491±0.22a	0.489±0.15a	0.471±0.13ab	0.363±0.24b
HDL-cholesterol (mmol L^{-1})	1.125±0.21a	1.122±0.20a	1.28±0.31a	1.121±0.18a	1.211±0.20a

CONCLUSIONS AND FUTURE WORK

In conclusion, the results obtained in the present work indicated that germinated date palm seeds showed a good potential for utilization in nutritional supplements, especially when associated with soy bean flour.

Literature cited

AOAC. (1975). Official Methods of Analysis, Association of Official Analytical Chemists (Washington, DC), https://onlinelibrary.wiley.com/doi/ abs/10.1002/jps.2600650148.

AOAC. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists (Arlington, Virginia), https://www.scirp.org/(S(czeh2tfqyw2orz553k1w0r45))/journal/paperinformation.aspx?paperid=72668.

Barreveld, W. (1993). Date Palm Product. Agricultural Services Bulletin 101 (FAO). http://www.fao.org/docrep/ t0681e/t0681e00.htm#con.

Devshony, S., Eteshola, E., and Shani, A. (1992). Characteristics and some potential applications of date palm (*Phoenix dactylifera* L.) seeds and seeds oil. J. Am. Oil Chem. Soc. *69* (*6*), 595–597 https://doi.org/10.1007/ BF02636115.

Eggum, B.O. (1995). The influence of dietary fibre on protein digestion and utilization in monogastrics. Arch Tierernahr *48* (*1-2*), 89–95 https://doi.org/10.1080/17450399509381831. PubMed

FAO. (2005). FAO Production Year Book, Vol. 51. https://doi.org/10.4060/cb4477en.

FAO/WHO. (1973). Energy and Protein Requirements. FAO Nutritional Meeting Report Services. No. 52. Technical Report Series N. 522 (Rome, Italy: Food and Agriculture Organization), https://www.fao.org/3/y5686e/ y5686e.pdf.

Nelson, N. (1944). A photometric adaptation of the Somogi methods for determination of glucose. J. Biol. Chem. *153* (*2*), 375–380 https://doi.org/10.1016/S0021-9258(18)71980-7.

Pellet, P., and Young, V. (1980). Evaluation of protein quality in experimental animals. In Nutritional Evaluation of Protein Food (Tokyo: The United Nations University), p.41–57.

Ranganna, S. (1997). Manual of Analysis of Fruit and Vegetable Products (New Delhi: Tota McGraw-Hill Publishing Company Limited).

Richmond, W. (1973). Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem *19* (*12*), 1350–1356 https://doi.org/10.1093/clinchem/19.12.1350. PubMed

Salah, A. (2005). Effect of date palm (*Phoenix dactylifera*) seed fibres on plasma lipids in rats. J. King Saudi Univ. *17* (*2*), 117–123.

Somogyi, M. (1952). Notes on sugar determination. J Biol Chem *195* (*1*), 19–23 https://doi.org/10.1016/S0021-9258(19)50870-5. PubMed

Sumianah, G., Makki, Y., and Rumne, T. (1984). Changes in the chemical composition of three cultivars of date palm seed during germination. Date Palm J. *3* (*2*), 395–407.

Swain, T., and Hillis, A. (1959). The phenolic constituents of *Prunus domestica*. I.: the quantitative analysis of phenolic constituents. J. Sci. Food Agric. *10* (*1*), 63 https://doi.org/10.1002/jsfa.2740100110.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. *6*, 24 https://doi.org/10.1177/000456326900600108.

Development and characterization of starch/LDPE active films incorporated with date palm seed extract

R. Radfar[1,2,a], H. Hosseini[1,3,b] and A. Nabizadeh[4]

[1]Food Sciences & Technology Department, National Nutrition & Food Technology Research Institute, Faculty of Nutrition Sciences & Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Department of Agriculture and Food Policies, Agricultural Planning, Economic and Rural Development Research Institute (APERDRI), Tehran, Iran; [3]Food Safety Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [4]HTA office, Iran Food and Drug Administration, Iran.

Abstract

Date palm (*Phoenix dactylifera* L.) seed extract was incorporated into LDPE-starch to develop an active packaging film. The mechanical, antioxidant, and antimicrobial properties of the films were investigated. The LDPE-starch film without date seed extracts was used as the control film. However, films with higher extract contents exhibited lower tensile strengths (TS values), which ranged from 9.5 to 4.3 MPa. Date seed extracts significantly ($p<0.05$) improved the antioxidant properties of the films which were measured by the DPPH test. The film's antimicrobial activities also increased significantly ($p<0.05$) after the incorporation of the seed extract. *Staphylococcus aureus* (*S. aureus*) was found to be the most sensitive bacterium to the active film, followed by *Escherichia coli* (*E. coli*). The largest inhibition zone (11.8 mm) was observed for *S. aureus* around the film incorporated with 5% (w/w) extract. These results revealed that films containing seed extract have great potential as an active film with antioxidant and antimicrobial properties, and thus it can help maintain the quality and prolong the shelf life of food products.

Keywords: date palm seed, LDPE, starch, active packaging

INTRODUCTION

Active packaging is a new form of packaging that protects the safety, quality and freshness of food via different mechanisms (Bodaghi et al., 2013). Active substances present in these films such as antioxidants and/or antimicrobial agents could prohibit undesirable reactions in the foods during storage (Kapetanakou and Skandamis, 2016). The antimicrobial and antioxidant films are two major types of active packaging, which have great importance in the food industries (Bhatia and Bharti, 2015). Due to the adverse and harmful effects of chemical additives in food products, the trend toward the use of natural additives especially those obtained from plant resources has increased dramatically in recent years (Karabagias et al., 2011; Mir et al., 2018). Many studies have shown that date seed extracts are rich in phenolic and flavonoids substances, which have notable antioxidant properties (Al-Farsi et al., 2007; Al Juhaimi et al., 2018; Radfar et al., 2019). It has also been shown that these extracts have antimicrobial activities, especially against Gram-positive bacteria. The antibacterial activity of the seed extracts could be strengthen against Gram-negatives by means of EDTA as chelating agents (Gadang et al., 2008; Sivarooban et al., 2008).

Low-density polyethylene is one of the most widely used synthetic polymers in the food packaging industries. However, the widespread use of it has raised many global concerns about environmental pollution. The partial replacement of the polymers with the natural bio-polymers (e.g., starch) and production of partially biodegradable films is one of the effective ways to solve this issue (Júnior et al., 2015).

The main disadvantage of the replacement method is the reduction of mechanical properties of the produced films due to the incompatibility between the hydrophobic polymer

[a]E-mail: rr_date@yahoo.com
[b]E-mail: hedayat@sbmu.ac.ir

and hydrophilic starch; the use of compatibilizers e.g., PE-g-MA and thermoplastic starch are two approaches for the improvement of the mechanical properties of the films (Sabetzadeh et al., 2012). Therefore, in the present study for the first time, we developed active packaging films based on LDPE/starch with date seed extracts.

MATERIAL AND METHODS

Materials

Film grade LDPE (LDPE0200) was provided as granules from Bandar Imam Petrochemical Co. (Tehran, Iran); corn starch (consisting of 70% amylopectin and 30% amylose wt/wt) was obtained from Glucozan Co. (Tehran, Iran); glycerol was provided by Dr. Mojalali Co. (Tehran, Iran); PE-g-MA, containing 2 mol% maleic anhydride groups and MFI equal to 2 g 10 min^{-1}, was purchased from Grankin Co. (Tehran, Iran). EDTA and Mueller-Hinton agar were purchased from Merck Co. (Darmstadt, Germany). The studied bacteria, including *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* O157:H7 (ATCC 35218), were supplied from PTCC (Persian Type Culture Collection).

Preparation of date seed extracts

Date palm seed extracts (from the 'Kabkab' cultivar) were prepared according to the methods of Radfar et al. (2019) and Al-Farsi and Lee (2008). Date seeds were crushed into fine powders, after drying at room temperature. Powdered seeds were mixed with ethanol and then stirred at 30°C for 3 h. After the centrifugation of the obtained mixture at 2800 g for 15 min at room temperature, the collected supernatants were filtered through No. 2 Whatman filter papers to remove the remaining insoluble debris. Finally, the solvent was removed by a rotary evaporator (HS 2005s Hahnshin Scientific, South Korea) at 40°C.

Preparation of the films

All ingredients, including corn starch, LDPE, EDTA, and LDPE-g-MA were dried in an oven at 50°C for 24 h. Gelatinized starch was produced by mixing starch and glycerol (30 wt% of starch) in an internal mixer (Haake, SYS 90, USA) at 120°C, for 5 min with the screw speed of 60 RPM. The thermo-plasticized starch (TPS), then, was melt blended with other ingredients including LDPE, PE-g-MA, C20A, and antimicrobial agents (EDTA and date seed extract) respectively, using a Brabender internal mixer (Plastic order, W50EHT, Germany). The processing temperature and rotor speed were set at 140°C and 60 RPM, respectively (Radfar et al., 2020). The formulation of the blends is given in Table 1. Finally, the prepared blends were hot-pressed to obtain the desired films.

Table 1. The formulation of the blends.

Sample code	LDPE (%)	TPS (wt%)	PE-g-MA (wt%)	EDTA (%)	Extract (%)
LDPE/TS/EXT0 (control)	75	20	3	2	0
LDPE/TS/EXT1	74	20	3	2	1
LDPE/TS/EXT3	72	20	3	2	3
LDPE/TS/EXT5	70	20	3	2	5

Properties of the prepared films

1. Antimicrobial activity of the LDPE/starch active films.

The antibacterial activity of the produced films was evaluated by the agar disc diffusion method as described by Shojaee-Aliabadi et al. (2013).

Firstly, 1 mL of 10^8 CFU mL^{-1} of studied bacteria was inoculated to Mueller-Hinton agar in Petri dishes. The produced films were cut into 6-mm discs and then placed on the prepared cultures. After incubation of cultures at 37°C for 24 h, the diameters of inhibition zones were determined. Three replicates of each film were examined and the average value of each measurement was reported.

2. Mechanical properties of films

The mechanical properties of the films, including tensile strength (TS, MPa), and elongation at break (EB, %), were measured according to ASTM- D882 standard using a SANTAM (model STM-20, Tehran, Iran) mechanical testing machine (ASTM International, 2012). The dimension of the film strips along with the cross head speed were 2×10 cm^2 and 50 mm min^{-1}, respectively. Three replicates were performed for each experiment.

3. Determination of antioxidant activity.

The antioxidant activity of the film samples after 2 days of storage was evaluated using DPPH free radical scavenging assay as described by Noronha et al. (2014) with some modifications. Briefly, 0.2 g of films were cut into small pieces and immersed in 10 mL ethanol at 40°C for storage time (2 days) in the dark. One mL of the obtained supernatant was mixed with 1 mL of ethanol solution of DPPH (0.1 mM). The mixture was vortexed and left for about 30 min at 25°C under dark conditions. Absorbance was measured at 516 nm on a Shimadzu UV-1800 UV-visible spectrophotometer (Kyoto, Japan). The antioxidant capacity of films was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = 100\times\left(\frac{Abs_{blank}-Abs_{sample}}{Abs_{blank}}\right)$$

Statistical analysis

All analyses were carried out in three replicates and expressed as means ± SD (standard deviation). One way analysis of variance was used to compare the groups and significance of differences was reported using Duncan's post hoc test at P≤0.05. All the analyses were carried out using SPSS Software v.10.1 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Mechanical properties

1. Tensile strength (TS).

The tensile strength, which is a measure of the resistance to direct pull, is of importance in machine ability and packaging applications. The tensile strength of control film (10.5 MPa) decreased to 4.3 MPa (about 59.2% reduction) with the addition of 5% extract to the blends (Table 2). Increasing the concentration of the seed extracts in the films decreased the tensile strength. The negative impact of the adding extract on tensile strength could be explained by the inherent incompatibility of the present phases between hydrophobic polyethylene and the hydrophilic segment of the films including date seed extract (Toumi et al., 2019). Therefore, agglomeration and uneven distribution of the hydrophilic components in the LDPE matrix generate stress concentration areas and subsequently micro-voids and cracks inside films during deformation tests such as tensile strength test. The incompatibility can also cause poor inter-facial adhesion between the film-forming phases (Majid et al., 2009).

As the amount of hydrophilic components of the composite (date seed extract) increases, the intensity of this effect also increases. Sung et al. (2014) reported that the incorporation of antimicrobial agents, especially at high concentrations, adversely affects the tensile strength of low-density-polyethylene/ethylene-vinyl-acetate copolymer (LDPE/EVA) films.

2. Elongation at break (EB).

Elongation at break is a measure to express the stretchability of a polymeric film and demonstrates the ability of a film to deform under pressure. As Table 2 shows the EB of the films decreased from 65 to 38.2% when the amounts of antimicrobial component (date seed extract) increased. In the blends of synthetic polymers with a ductile behavior, adding an immiscible and incompatible secondary phase has previously been shown to significantly reduce the elongation at break (Pedroso and Rosa, 2005). In such polymers, EB is highly related to the inter-facial interaction between constituent phases (St-Pierre et al., 1997).

Table 2. Mechanical and antibacterial properties of the films.

Sample code	Mechanical properties[a]		Antibacterial properties[a] Inhibitory zone[b] (mm)	
	Tensile strength (MPa)	Elongation at break (%)	*S. aureus*	*E.coli*
LDPE/TS/EXT0 (control)	10.5±0.08[a]	65±0.88[a]	0	0
LDPE/TS/EXT1	9.5±0.18[b]	50±0.06[b]	6.8±2.5[a]	4.3±1.1[a]
LDPE/TS/EXT3	8.6±0.5[c]	43±0.15[c]	10.9±0.77[b]	8.8±0.1[b]
LDPE/TS/EXT5	4.3±0.6[d]	38.2±1.5[d]	11.8±0.5[c]	9.4±0.5[c]

[a]Each value is the mean of three measures ± SD.
[b]Inhibitory zone indicates the antimicrobial activity against *E. coli* and *S. aureus*.
Means in the same column with the same letters are not significantly different ($p>0.05$).

Similarly, Xia et al. (2015) and Park et al. (2010) reported that the addition of antimicrobial agents to the plastic films decreased the elongation at break properties.

Antimicrobial activities of the film

The antimicrobial activities of active films with different concentrations of date seed extracts toward *S. aureus* and *E.coli* are presented in Table 2. Film samples exhibited excellent antimicrobial activities against Gram-positive bacteria such as *S. aureus* and Gram-negative bacteria such as *E.coli*. The result showed that in the control film no zone of inhibition was observed. The films with higher concentrations of the date seed extracts, from 1 to 5%, showed significantly ($p<0.05$) increased antimicrobial activities. Among the tested samples, the highest antibacterial activity was recorded against *S. aureus*, and in this case, the film with 5% date seed extract caused an inhibition zone 11.8 mm in diameter. According to the obtained results, the extract of date palm seeds has significant effects on the inhibition of *S. aureus* growth. With the increase in the loading of the extract, the antibacterial activity of the films increased.

According to the previous studies, the major components that exhibit antibacterial activity in date seed extract are phenolic compounds. These compounds, with mechanisms such as the disruption of the function of the bacterial cytoplasmic membrane, enzyme inactivation, and binding to the proteins, retard the growth of the bacterial cells (Emam-Djomeh et al., 2015; Walsh et al., 2003; Yuan et al., 2015).

Antioxidant activity

DPPH radical-scavenging assay was used to determine the antioxidant activity of the films. The scavenging capacity of the antioxidants was indicated by the degree of discoloration. Figure 1 shows the radical-scavenging activity of the active films with different date extract concentrations. Free radical scavenging of control film was constant at zero. Films containing date seed extracts showed significantly higher radical scavenging activities ($p<0.05$). The addition of 1% extract increased the DPPH radical-scavenging activity to 46%. The DPPH radical-scavenging activity of the films with 5% extract was the highest at 78%. The date seed extract is known to be a rich source of bio-active compounds such as phenolic compounds, which are potent antioxidants. However, the major component contributing to the antioxidant capacity of date seed is cinnamic acid compounds, which are present in the seeds (Radfar et al., 2019).

CONCLUSIONS

Environmentally friendly active films prepared from LDPE/starch incorporated with date seed extract were successfully developed. Date seed extract contributed to the excellent antioxidant and antimicrobial properties of the films. The incorporation of seed extract decreased the tensile strength of the films due to incompatibility of the hydrophobic polymer and hydrophilic components. However, it also led to increase the antimicrobial and antioxidant capacity of the films.

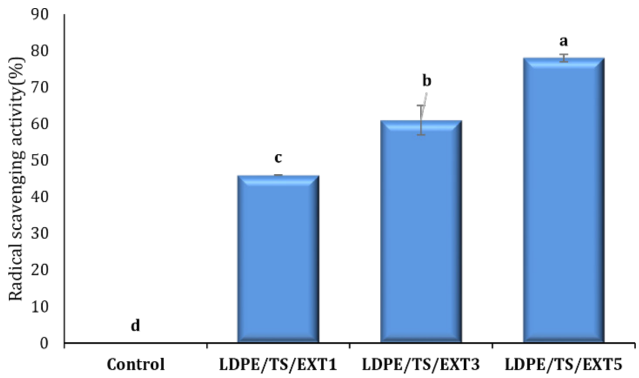


Figure 1. DPPH radical-scavenging activity (%) of the activated films with date palm seed extract. Values are expressed as the means of three replicates and error bars represent standard deviation (SD) (n=3). Means with a different superscript letter in each column are significantly different at P≤0.05.

Thus, the results of this study proposed that date seed extract has great potential as an additive for LDPE/starch film to create an active packaging material with strong antioxidant and antimicrobial properties. However, it is crucial to find ways to improve the mechanical properties to enhance the performance of the films.

Literature cited

Al-Farsi, M.A., and Lee, C.Y. (2008). Optimization of phenolics and dietary fibre extraction from date seeds. Food Chem *108* (*3*), 977–985 https://doi.org/10.1016/j.foodchem.2007.12.009. PubMed

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., and Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem. *104* (*3*), 943–947 https://doi.org/10.1016/j.foodchem.2006.12.051.

Al Juhaimi, F., Özcan, M.M., Adiamo, O.Q., Alsawmahi, O.N., Ghafoor, K., and Babiker, E.E. (2018). Effect of date varieties on physico-chemical properties, fatty acid composition, tocopherol contents, and phenolic compounds of some date seed and oils. J. Food Process. Preserv. *42* (*4*), e13584 https://doi.org/10.1111/jfpp.13584.

ASTM International. (2012). ASTM D882-12, Standard Test Method for Tensile Properties of Thin Plastic Sheeting.

Bhatia, S., and Bharti, A. (2015). Evaluating the antimicrobial activity of Nisin, Lysozyme and Ethylenediaminetetraacetate incorporated in starch based active food packaging film. J Food Sci Technol *52* (*6*), 3504–3512. PubMed

Bodaghi, H., Mostofi, Y., Oromiehie, A., Zamani, Z., Ghanbarzadeh, B., Costa, C., Conte, A., and Del Nobile, M.A. (2013). Evaluation of the photocatalytic antimicrobial effects of a TiO_2 nanocomposite food packaging film by in vitro and in vivo tests. Lebensm. Wiss. Technol. *50* (*2*), 702–706 https://doi.org/10.1016/j.lwt.2012.07.027.

Emam-Djomeh, Z., Moghaddam, A., and Yasini Ardakani, S.A. (2015). Antimicrobial activity of pomegranate (*Punica granatum* L.) peel extract, physical, mechanical, barrier and antimicrobial properties of pomegranate peel extract-incorporated sodium caseinate film and application in packaging for ground beef. Packag. Technol. Sci. *28* (*10*), 869–881 https://doi.org/10.1002/pts.2145.

Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G., and Owens, C. (2008). Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a Turkey frankfurter system. J Food Sci *73* (*8*), M389–M394 https://doi.org/10.1111/j.1750-3841.2008.00899.x. PubMed

Júnior, A.V., Fronza, N., Foralosso, F.B., Dezen, D., Huber, E., dos Santos, J.H.Z., Machado, R.A.F., and Quadri, M.G.N. (2015). Biodegradable duo-functional active film: antioxidant and antimicrobial actions for the conservation of beef. Food Bioprocess Technol. *8* (*1*), 75–87 https://doi.org/10.1007/s11947-014-1376-9.

Kapetanakou, A.E., and Skandamis, P.N. (2016). Applications of active packaging for increasing microbial stability in foods: natural volatile antimicrobial compounds. Curr. Opin. Food Sci. *12*, 1–12 https://doi.org/10.1016/j.cofs.2016.06.001.

Karabagias, I., Badeka, A., and Kontominas, M.G. (2011). Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. Meat Sci *88* (*1*), 109–116 https://doi.org/10.1016/j.meatsci.2010.12.010. PubMed

Majid, R.A., Ismail, H., and Taib, R.M. (2009). Effects of PE-g-MA on tensile properties, morphology and water absorption of LDPE/thermoplastic sago starch blends. Polym. Plast. Technol. Eng. *48* (*9*), 919–924 https://doi.org/10.1080/03602550902995018.

Mir, S.A., Dar, B.N., Wani, A.A., and Shah, M.A. (2018). Effect of plant extracts on the techno-functional properties of biodegradable packaging films. Trends Food Sci. Technol. *80*, 141–154 https://doi.org/10.1016/j.tifs.2018.08.004.

Noronha, C.M., de Carvalho, S.M., Lino, R.C., and Barreto, P.L.M. (2014). Characterization of antioxidant methylcellulose film incorporated with α-tocopherol nanocapsules. Food Chem *159*, 529–535 https://doi.org/10.1016/j.foodchem.2014.02.159. PubMed

Park, S.I., Marsh, K.S., and Dawson, P. (2010). Application of chitosan-incorporated LDPE film to sliced fresh red meats for shelf life extension. Meat Sci *85* (*3*), 493–499 https://doi.org/10.1016/j.meatsci.2010.02.022. PubMed

Pedroso, A.G., and Rosa, D.S. (2005). Mechanical, thermal and morphological characterization of recycled LDPE/corn starch blends. Carbohydr. Polym. *59* (*1*), 1–9 https://doi.org/10.1016/j.carbpol.2004.08.018.

Radfar, R., Farhoodi, M., Ghasemi, I., Mousavi Khaneghah, A., Shahraz, F., and Hosseini, H. (2019). Assessment of phenolic contents and antioxidant and antibacterial activities of extracts from four varieties of Iranian date palm (*Phoenix dactylifera* L.). Seeds *6*, 12.

Radfar, R., Hosseini, H., Farhoodi, M., Ghasemi, I., Średnicka-Tober, D., Shamloo, E., and Khaneghah, A.M. (2020). Optimization of antibacterial and mechanical properties of an active LDPE/starch/nanoclay nanocomposite film incorporated with date palm seed extract using D-optimal mixture design approach. Int J Biol Macromol *158*, 790–799 https://doi.org/10.1016/j.ijbiomac.2020.04.139. PubMed

Sabetzadeh, M., Bagheri, R., and Masoomi, M. (2012). Effect of corn starch content in thermoplastic starch/low-density polyethylene blends on their mechanical and flow properties. J. Appl. Polym. Sci. *126* (*S1*), E63–E69 https://doi.org/10.1002/app.36329.

Shojaee-Aliabadi, S., Hosseini, H., Mohammadifar, M.A., Mohammadi, A., Ghasemlou, M., Ojagh, S.M., Hosseini, S.M., and Khaksar, R. (2013). Characterization of antioxidant-antimicrobial κ-carrageenan films containing *Satureja hortensis* essential oil. Int J Biol Macromol *52*, 116–124 https://doi.org/10.1016/j.ijbiomac.2012.08.026. PubMed

Sivarooban, T., Hettiarachchy, N.S., and Johnson, M.G. (2008). Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. Food Res. Int. *41* (*8*), 781–785 https://doi.org/10.1016/j.foodres.2008.04.007.

St-Pierre, N., Favis, B.D., Ramsay, B.A., Ramsay, J.A., and Verhoogt, H. (1997). Processing and characterization of thermoplastic starch/ polyethylene blends. Polymer (Guildf.) *38* (*3*), 647–655 https://doi.org/10.1016/S0032-3861(97)81176-7.

Sung, S.-Y., Sin, L.T., Tee, T.-T., Bee, S.-T., and Rahmat, A.R. (2014). Effects of *Allium sativum* essence oil as antimicrobial agent for food packaging plastic film. Innov. Food Sci. Emerg. Technol. *26*, 406–414 https://doi.org/10.1016/j.ifset.2014.05.009.

Toumi, N., Guessoum, M., and Nekkaa, S. (2019). Biocomposites based on date palm flour reinforced (70/30) polypropylene/thermoplastic starch blend: effects of flour treatment and selective dispersion. J. Adhes. Sci. Technol. *33* (*19*), 2071–2092 https://doi.org/10.1080/01694243.2019.1626538.

Walsh, S.E., Maillard, J.-Y., Russell, A.D., Catrenich, C.E., Charbonneau, D.L., and Bartolo, R.G. (2003). Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. J Appl Microbiol *94* (*2*), 240–247 https://doi.org/10.1046/j.1365-2672.2003.01825.x. PubMed

Xia, G., Reddy, K.O., Maheswari, C.U., Jayaramudu, J., Zhang, J., Zhang, J., and Rajulu, A.V. (2015). Preparation and properties of biodegradable spent tea leaf powder/poly (propylene carbonate) composite films. International Jr Polymer Analysis and Characterization *20* (*4*), 377–387 https://doi.org/10.1080/1023666X.2015.1019257.

Yuan, G., Lv, H., Yang, B., Chen, X., and Sun, H. (2015). Physical properties, antioxidant and antimicrobial activity of chitosan films containing carvacrol and pomegranate peel extract. Molecules *20* (*6*), 11034–11045 https://doi.org/10.3390/molecules200611034. PubMed

The consumption of date palm fruits as a source of bioactive compounds in patients with type 2 diabetes: a cross sectional study

M.Q. Al-Mssallem[1,2,a]

[1]Department of Food &Nutrition Sciences, College of Agriculture and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia; [2]Ministry of National Guard Health Affairs, Eastern Region, Al-Ahsa, Saudi Arabia.

Abstract

It is commonly believed by the public and health care providers that patients with diabetes should restrain or even avoid consumption of date palm fruits (DPFs). However, DPFs are a good source of nutritive and non-nutritive bio-active compounds. This study aimed to assess the nutritive and non-nutritive bio-active compounds of consumed DPFs and examine their relationship with glycaemic control in patients with type 2 diabetes mellitus (T2DM) using oral anti-diabetic medications (*n*=199) and insulin treatments (*n*=205). Blood glucose levels (fasting and random), glycated hemoglobin (HbA1c), total cholesterol, high-density lipo protein, low-density lipo protein, and triglycerides were retrieved from the medical records of the patients. Date palm fruit consumption was assessed, and the bio-active compound indices of consumed DPFs were calculated. The results revealed that the consumption of DPFs was higher in patients with oral medications than in those on insulin treatments, but the difference was not significant. The bio-active compound content of the consumed DPFs ranged from 4.80±0.18 g day^{-1} for non-starch polysaccharides (NSPs) to 178±6.83 µg day^{-1} for selenium. However, the selenium index (273%) was the highest bio-active compound index in the consumed DPFs, followed by NSP and phenolic indices (20%). Moreover, this study has detected a weak but significant correlation between the level of bio-active compounds of consumed DPFs and HbA1c levels among patients with T2DM. In conclusion, the study has revealed that the bio-active compound content of DPFs is negatively associated with lower HbA1c levels among patients with T2DM. An intervention trial is required to support this finding.

Keywords: anti diabetic, antioxidant, bio-active, oxidation, radicals, constituents

INTRODUCTION

Bio-active compounds are a natural part of the food chain. Most bio-active compounds are not required for the daily function of organisms, and they are produced as secondary metabolites (Biesalski et al., 2009; Murthy et al., 2014). It is well-documented that the main sources of bio-active compounds are fruits, vegetables and whole grain foods (Biesalski et al., 2009; Guaadaoui et al., 2014). Bio-active compounds are effective against oxidative species and play an important role in scavenging any intermediates of free radicals (Porrini and Riso, 2008; Guaadaoui et al., 2014). Free radicals are basically generated through cellular oxidation reactions, and then chain reactions are continuously commenced by these free radicals, leading to serious damage to cells in the human body (Valko et al., 2006).

In date palm (*Phoenix dactylifera* L.) fruits (DPFs), the bio-active compounds refer to phenolic components (Bouhlali et al., 2015; Hamad et al., 2015; Septembre-Malaterre et al., 2018), and some nutrients are regarded to be biologically active in addition to their nutritional properties, such as soluble and insoluble NSPs, vitamin C, β-carotene, α-tocopherol, and selenium (Al-Farsi and Lee, 2008; Septembre-Malaterre et al., 2018). Bio-active compounds in DPFs exert anti-radical, anticancer, anti-mutagenic, antimicrobial, and anti-diabetic properties (Kris-Etherton et al., 2004; Cushnie and Lamb, 2005; Valko et al., 2006; Stan et al.,

[a]E-mail: mmssallem@kfu.edu.sa; mmssallem@gmail.com

2008; Daayf et al., 2012; Gupta et al., 2013; Al-Alawi et al., 2017; Zhang et al., 2017). These beneficial health effects are mostly attributed to their ability to scavenge any intermediates of free radicals resulting from oxidative species and offer protection against oxidative stress-related diseases (Porrini and Riso, 2008; Al-Turki et al., 2010; Guaadaoui et al., 2014).

It is still unclear whether regular consumption of DPFs escalates the risk of diabetes. In Saudi Arabia, where the consumption of DPFs is commonly practiced, patients with diabetes are usually advised to limit or even abstain from consumption of DPFs (Rock et al., 2009).

This guidance actually refers to the fact that DPFs contain a high content of mono-saccharides such as glucose and fructose (Al-Farsi and Lee, 2008). However, consumption of DPFs has not been found to have deleterious effects on glycated hemoglobin or fasting blood glucose (Rock et al., 2009; Al-Mssallem et al., 2022).

The main objective of this observational study was to assess the nutritive and non-nutritive bio-active compounds of consumed DPFs and examine their association with glycaemic control among patients with type 2 diabetes mellitus using oral medications and insulin injections.

PATIENTS AND METHODS

Patients with type 2 diabetes on oral medication (n=199) and insulin injection (n=205) were included in this observational, cross-sectional study. The study was carried out in the Diabetic Clinic of the National Guard Health Affairs, Eastern province, Al-Ahsa, Saudi Arabia. Informed written consent was obtained from all participants. The study was approved by the Institutional Research Board (IRB), Ministry of National Guard Health Affairs (Ref. No. IRBC/0666/19). Weight, height, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by trained nurses at the time of arrival. Body mass index (BMI) was estimated as the weight in kg divided by the square of the height in m. Fasting blood glucose (FBG), random blood glucose (RBG), glycated hemoglobin (HbA1c), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG) were retrieved from patient electronic medical records that were available at the time of the interview.

The amount of consumed DPF and frequency of DPF consumption (rutab and tamer) were obtained from the patients during a face-to- face interview with a dietitian using a modified validated questionnaire. The amount of consumed DPFs was multiplied by the number 9 (assuming that the average weight of a date = 9 g), and then it was described on a daily basis by dividing the amount in g by the number of days (Al-Mssallem, 2018; Al-Mssallem et al., 2019, 2022). The indices of energy, total carbohydrates, non-starch polysaccharides (NSPs), and bio-active compound concentrations of consumed DPFs were calculated (Al-Mssallem et al., 2022).

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS software, Version 26.0). Means were compared using an independent samples test based on DPF consumption and the Pearson correlation between DPF consumption, the content of bio-active compounds and HbA1c, with a two-tailed significance level of 5% (p value<0.05).

Simple descriptive analysis was applied to analyze the data, and the results are expressed as a percentage (%) and means ± one standard error of the mean (SE) unless stated otherwise.

Simple equations were also applied to calculate the nutrient indices as follows:

$$IX = (ACX/ARX)\times 100 \tag{1}$$

where IX = index of energy, carbohydrates, non-starch polysaccharides, β-carotene, vitamin C, selenium, carotenoids, anthocyanins, phenolics, or antioxidant; ACX = average content of energy, carbohydrates, non-starch polysaccharides, β-carotene, vitamin C, selenium, carotenoids, anthocyanins, phenolics, or antioxidant; ARX = average recommended of energy, carbohydrates, non-starch polysaccharides, β-carotene, vitamin C, selenium, carotenoids, anthocyanins, phenolics, or antioxidant.

RESULTS AND DISCUSSION

Patients' characteristics

The study population was patients with T2DM; 51% were male, and 49% were female. They were divided into two groups, the oral anti diabetic medication group (*n*=199) and insulin injection group (*n*=205), and the two groups had similar mean ages of 54 and 55, respectively (Table 1). Both groups had a BMI greater than 30 kg m^{-2}, indicating that the participants suffered from obesity. Moreover, BMI was significantly higher in the insulin injection group than in the oral medication group ($p<0.05$). Additionally, the mean FBG, RBG, and HbA1clevels were also significantly higher in the insulin injection group than in the oral medication group ($p<0.0001$). However, the mean blood pressure (MBP) was within the normal level for both groups (<110 mm/Hg).

Table 1. General characteristics of the patients with type 2 diabetes with respect to oral medication (*n*=199) and insulin therapy (*n*=205).

Measurement	Patients on oral antidiabetic (mean ± SD)	Patients on insulin (mean ± SD)	p-value
Age (y)	54±9	55±9	0.40
Weight (kg)	85.49±16.89	89.12±16.17	0.02
Height (m)	1.60±0.09	1.60±0.09	0.87
BMI[a] (kg m^{-2})	33.16±6.18	34.42±5.98	0.039
DBP[b] (mm Hg)	73.85±10.48	71.3±10.47	0.015
SBP[c] (mm Hg)	138.25±17.15	139.07±19.29	0.65
MBP[d] (mm Hg)	95.31±11.16	93.89±11.51	0.20
HbA_{1c}[e] (%)	7.67±1.46	8.79±1.37	0.00
FBG[f] (mmol L^{-1})	8.9±3.08	10.55±3.97	0.00
RBG[g] (mmol L^{-1})	10.32±4.12	12.09±4.3	0.00
TC[h] (mmol L^{-1})	4.49±0.87	4.32±0.96	0.05
HDL[i] (mmol)	1.06±0.27	1.03±0.21	0.22
LDL[j] (mmol L^{-1})	2.79±0.78	2.62±0.82	0.037
TG[k] (mmol L^{-1})	1.71±0.95	1.61±0.91	0.27

[a]BMI: body mass index;-[b]DBP: diastolic blood pressure; [c]SBP: systolic blood pressure; [d]MBP: mean blood pressure; [e]HbA_{1c}: glycated hemoglobin; [f]FBG: fasting blood glucose; [g]RBG: random blood glucose; [h]TC: total cholesterol; [i]HDL: high-density-lipoprotein; [j]LDL: low-density-lipoprotein; [k]TG: triglycerides.

Date palm fruit content

The average DPF intake reached 2.24±0.11 servings day^{-1}, and it was higher in patients on oral medication than in those on insulin injection, but this difference was not significant (Table 2). Consequently, there were no significant differences between the two groups in DPF energy, mono-saccharides, NSPs, β-carotene, vitamin C, selenium, carotenoids, anthocyanins, phenolic or antioxidant content.

The NSPs (4.80±0.18 g day^{-1}) of consumed DPFs were the most nutritive bio-active compounds and covered approximately a fourth of the recommended daily allowance. However, selenium had the highest bio-active compound index, with a value of 273%, followed by phenolics (20%) (Figure 1). In contrast, β-carotene (0.32%) had the lowest, followed by anthocyanins (0.92%).

Moreover, a low level of HbA1c was associated with higher DPF bio-active compound content ($r=-0.13$, $p<0.01$).

Table 2. Consumed date palm fruits and their content (mean ± SE) of energy, monosaccharides, nutritive and non-nutritive bio-active compounds among patients with T2DM.

Date palm fruit consumption and content	Patients in oral medication (*n*=199)	Patients in insulin injection (*n*=205)
Date palm fruits (serving day^{-1})	2.31±0.12	2.17±0.11
Energy (calorie day^{-1})	181.30±10.14	173.21±9.20
Monosaccharides (g day^{-1})	36.88±2.06	35.25±1.87
NSPs (g day^{-1})	4.97±0.27	4.64±0.24
β-carotene (µg d^{-1})	14.82±0.82	14.03±0.74
Vitamin C (mg day^{-1})	2.42±0.13	2.29±0.12
Selenium (mg day^{-1})	0.18±0.14	0.17±0.13
Carotenoids (µg day^{-1})	596.20±33.22	565.55±30.01
Anthocyanins (mg day^{-1})	0.80±0.68	0.09±0.007
Phenolics (mg day^{-1})	148.11±9.33	131.65±7.20
Antioxidant (µmol day^{-1})	1138.35±62.92	1076.17±57.12

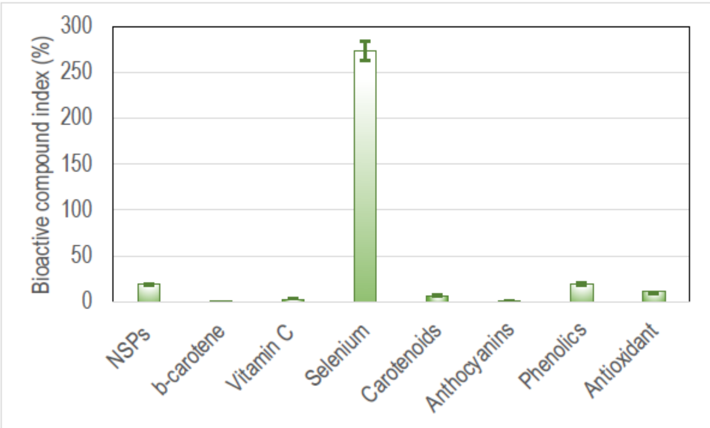


Figure 1. Nutritive and non-nutritive bio-active compounds indices of consumed date palm fruits among patients (*n*=404) with T2DM.

Diets rich in bio-active compounds play a vital role in human health and preventing or improving some health conditions. This study aimed to assess the bio-active compounds of consumed DPFs among two groups of patients with T2DM (oral anti-diabetic medication and insulin injection). Although both groups were obese (BMI >30 kg m^{-2}), BMI was significantly lower in the oral anti-diabetic medication group than in the insulin injection group ($p<0.05$). Obesity is strongly associated with developing T2DM, and obese people with a BMI greater than 30 kg m^{-2} are up to 80 times more likely to be at risk of developing T2DM than those with normal BMI (Al-Quwaidhi et al., 2013; Reis et al., 2013).

Date palm fruits are considered an excellent source of both nutritive and non-nutritive bio-active compounds. Examples of nutritive bio- active compounds in DPFs are selenium and NSPs. The results have shown that the consumed DPFs can provide 273% of the recommended daily allowance of selenium. Selenium, as a trace mineral, is required in a small amount, and most people can efficiently obtain their requirement of selenium from well-balanced and healthy diets. Selenium has attracted attention due to its antioxidant properties. The effective role of selenium as an antioxidant is accomplished by fighting free radicals, which are closely related to chronic degenerative diseases. In addition to this protective effect of selenium against oxidative stress, selenium plays an important role in the prevention of T2DM development, as it possesses anti-diabetic and insulin-mimetic properties (Rayman and

Stranges, 2013; Fontenelle et al., 2018). However, high-dose selenium supplementation is not recommended due to its interference with glucose homeostasis and insulin action leading to impaired glucose metabolism among patients with T2DM (Bleys et al., 2007; Stranges et al., 2007; Rayman and Stranges, 2013; Zhou et al., 2013; Faghihi et al., 2014; Fontenelle et al., 2018).

This study also found that patients can obtain approximately a fifth of their requirements for NSPs and phenolics from DPFs. It is well documented that a high intake of dietary NSPs is associated with improving insulin sensitivity and reducing the risk of developing T2DM (Sluijs et al., 2010; McRae, 2018). Phenolics, as non-nutritive bio-active compounds, also have anti-diabetic effects. Phenolics exert a significant role in inhibiting the activity of hydrolysis enzymes of carbohydrates, such as α-amylase and α-glucosidase, leading to reduced availability of glucose to be absorbed and utilized (Ranilla et al., 2008; Phillips et al., 2009).

Other nutritive bio-active compounds that the consumed DPFs can provide are vitamin C and β-carotene (vitamin A precursor). This study found that DPFs can cover only 3% and 0.32 of the requirements for vitamin C and β-carotene, respectively. The role of vitamin C and β-carotene as antioxidants is well known. The benefits of vitamin C and β-carotene as antioxidant supplements in the management of diabetes are marginal (Abdali et al., 2015). On the other hand, patients with T2DM can benefit from a high intake of vitamin C to reduce the risk of developing number of diabetic complications (Jacob and Sotoudeh, 2002). Additionally, high dietary intake of β-carotene was associated with a reduced risk of developing T2DM in healthy adults (Sluijs et al., 2015). However, the association between the intake of α-tocopherol or β-carotene and the risk of developing T2DM is still controversial (Park and Park, 2021). There is limited evidence to confirm the long-term safety of these vitamin supplements and their essential role in the management of diabetes (Hasanain and Mooradian, 2002; Cuerda et al., 2011; Zhao et al., 2017; ADA, 2019).

In this study, there were several potential limitations, including the ethnicity of the patients, who were all Saudi citizens. Therefore, the results may not be applicable to other races. Although the study has used a validated questionnaire for collecting daily intake of DPFs, it may be exposed to recall bias. Therefore, patients were interviewed face-to-face by well-trained dietician to minimize recall bias. Additionally, as this study is a cross-sectional observational study, it can only exhibit relationships.

CONCLUSIONS

This study has shown that DPFs are excellent sources of selenium and NSPs as nutritive bio-active compounds. In addition, DPFs are a good source for non-nutritive bio-active compounds such as phenolics. These bio-active compounds, as antioxidants, possess the potential to reduce oxidative stress. Increasing attention should be given not merely to the concentrations of antioxidants in particular foods but also to the daily intake of the food. Further intervention studies are required to investigate the possibility of including DPFs in diabetic diets as a source of bio-active compounds, particularly for those who consider DPFs to be an essential part of their daily food intake.

ACKNOWLEDGEMENTS

The author extends her appreciation to the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia for funding this research through the project No.GRANT1135. Many thanks go to the Ministry of National Guard Health Affairs, Eastern Region, Al-Ahsa in Saudi Arabia for the facilitation of the process to conduct this study.

Literature cited

Abdali, D., Samson, S.E., and Grover, A.K. (2015). How effective are antioxidant supplements in obesity and diabetes? Med Princ Pract *24* (*3*), 201–215 https://doi.org/10.1159/000375305. PubMed

Al-Alawi, R.A., Al-Mashiqri, J.H., Al-Nadabi, J.S.M., Al-Shihi, B.I., and Baqi, Y. (2017). Date palm tree (*Phoenix dactylifera* L.): natural products and therapeutic options. Front Plant Sci *8*, 845 https://doi.org/10.3389/

fpls.2017.00845. PubMed

Al-Farsi, M.A., and Lee, C.Y. (2008). Nutritional and functional properties of dates: a review. Crit Rev Food Sci Nutr *48* (*10*), 877–887 https://doi.org/10.1080/10408390701724264. PubMed

Al-Mssallem, M.Q. (2018). Consumption of dates among Saudi adults and its association with the prevalence of type 2 diabetes. Asian J. Clin. Nutr. *10* (*2*), 58–64 https://doi.org/10.3923/ajcn.2018.58.64.

Al-Mssallem, M.Q., Elmulthum, N.A., and Elzaki, R.M. (2019). Nutrition security of date palm fruit: an empirical analysis for the Al-Ahsa region in Saudi Arabia. Sci. J. KFU *20*, 47–54.

Al-Mssallem, M.Q., Al-Qarni, A.A., and Al-Jamaan, M. (2022). Dietary pattern of patients with type 2 diabetes mellitus including date consumption. J. Public Health (Berl.) *30* (*2*), 301–307 https://doi.org/10.1007/s10389-020-01270-7.

Al-Quwaidhi, A.J., Pearce, M.S., Critchley, J.A., and O'Flaherty, M. (2013). Obesity and type 2 diabetes mellitus: a complex association. Saudi J. Obes. *1*, 49–56 https://doi.org/10.4103/2347-2618.128627.

Al-Turki, S., Shahba, M.A., and Stushnoff, C. (2010). Diversity of antioxidant properties and phenolic content of date palm (*Phoenix dactylifera* L.) fruits as affected by cultivar and location. J. Food Agric. Environ. *8*, 253–260.

American Diabetes Association. (2019). Standards of medical care in diabetes-2019. Diabetes Care *42* (*Suppl 1*), S173–S181 https://doi.org/10.2337/dc19-S015. PubMed

Biesalski, H., Dragsted, L.O., Elmadfa, I., Grossklaus, R., Muller, M.R., Schrenk, D., Walter, P., and Weber, P. (2009). Bioactive compounds: definition and assessment of activity. Nutritio *25*, 1202–1205.

Bleys, J., Navas-Acien, A., and Guallar, E. (2007). Selenium and diabetes: more bad news for supplements. Ann Intern Med *147* (*4*), 271–272 https://doi.org/10.7326/0003-4819-147-4-200708210-00177. PubMed

Bouhlali, E.T., Ramchoun, M., Alem, C., Ghafoor, K., Ennassir, J., and Zegzouti, Y.F. (2015). Functional composition and antioxidant activities of eight Moroccan date fruit varieties (*Phoenix dactylifera* L.). J. Saudi Soci. Agri. Sci. *16*, 257–264.

Cuerda, C., Luengo, L.M., Valero, M.A., Vidal, A., Burgos, R., Calvo, F.L., and Martínez, C. (2011). [Antioxidants and diabetes mellitus: review of the evidence]. Nutr. Hosp. *26* (*1*), 68–78. PubMed

Cushnie, T.P.T., and Lamb, A.J. (2005). Antimicrobial activity of flavonoids. Int J Antimicrob Agents *26* (*5*), 343–356 https://doi.org/10.1016/j.ijantimicag.2005.09.002. PubMed

Daayf, F., El Hadrami, A., El-Bebany, A.F., Henriquez, M.A., Yao, Z., Derksen, H., El-Hadrami, I., and Adam, L.R. (2012). Phenolic compounds in plant defense and pathogen counter-defense mechanisms. In Recent Advances in Polyphenol Research, S. Quideau, V. Cheynier, and P. Sarni- Manchado, eds. (Oxford, UK: Wiley-Blackwell).

Faghihi, T., Radfar, M., Barmal, M., Amini, P., Qorbani, M., Abdollahi, M., and Larijani, B. (2014). A randomized, placebo-controlled trial of selenium supplementation in patients with type 2 diabetes: effects on glucose homeostasis, oxidative stress, and lipid profile. Am J Ther *21* (*6*), 491–495 https://doi.org/10.1097/MJT.0b013e318269175f. PubMed

Fontenelle, L.C., Feitosa, M.M., Morais, J.B.S., Severo, J.S., de Freitas, T.E.C., Beserra, J.B., Henriques, G.S., and Marreiro, D.N. (2018). The role of selenium in insulin resistance. Braz. J. Pharm. Sci. *54* (*1*), e00139 https://doi.org/10.1590/s2175-97902018000100139.

Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., and Hamal, A. (2014). What is a bio-active compound? A combined definition for a preliminary consensus. Int. J. Nutr. Food Sci. *3* (*3*), 174–179 https://doi.org/10.11648/j.ijnfs.20140303.16.

Gupta, A.D., Bansal, V.K., Babu, V., and Maithil, N. (2013). Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). J. Genet. Eng. Biotechnol. *11* (*1*), 25–31 https://doi.org/10.1016/j.jgeb.2012.12.001.

Hamad, I., AbdElgawad, H., Al Jaouni, S., Zinta, G., Asard, H., Hassan, S., Hegab, M., Hagagy, N., and Selim, S. (2015). Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules *20* (*8*), 13620–13641 https://doi.org/10.3390/molecules200813620. PubMed

Hasanain, B., and Mooradian, A.D. (2002). Antioxidant vitamins and their influence in diabetes mellitus. Curr Diab Rep *2* (*5*), 448–456 https://doi.org/10.1007/s11892-002-0110-6. PubMed

Jacob, R.A., and Sotoudeh, G. (2002). Vitamin C function and status in chronic disease. Nutr Clin Care *5* (*2*), 66–74 https://doi.org/10.1046/j.1523-5408.2002.00005.x. PubMed

Kris-Etherton, P.M., Lefevre, M., Beecher, G.R., Gross, M.D., Keen, C.L., and Etherton, T.D. (2004). Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. Annu Rev Nutr *24* (*1*), 511–538 https://doi.org/10.1146/annurev.nutr.23.011702.073237. PubMed

McRae, M.P. (2018). Dietary fiber intake and type 2 diabetes mellitus: an umbrella review of meta-analyses. J Chiropr Med *17* (*1*), 44–53 https://doi.org/10.1016/j.jcm.2017.11.002. PubMed

Murthy, H.N., Dandin, V.S., Zhong, J.J., and Paek, K.Y. (2014). Strategies for enhanced production of plant secondary metabolites from cell and organ cultures. In Production of Biomass and Bio-Active Compounds Using Bio-Reactor Technology, K.Y. Paek, H. Murthy, and J.J. Zhong, eds. (Dordrecht: Springer).

Park, S., and Park, S.Y. (2021). Can antioxidants be effective therapeutics for type 2 diabetes? Yeungnam Univ J Med *38* (*2*), 83–94 https://doi.org/10.12701/yujm.2020.00563. PubMed

Phillips, K.M., Carlsen, M.H., and Blomhoff, R. (2009). Total antioxidant content of alternatives to refined sugar. J Am Diet Assoc *109* (*1*), 64–71 https://doi.org/10.1016/j.jada.2008.10.014. PubMed

Porrini, M., and Riso, P. (2008). Factors influencing the bioavailability of antioxidants in foods: a critical appraisal. Nutr Metab Cardiovasc Dis *18* (*10*), 647–650 https://doi.org/10.1016/j.numecd.2008.08.004. PubMed

Ranilla, L.G., Kwon, Y.I., Genovese, M.I., Lajolo, F.M., and Shetty, K. (2008). Antidiabetes and antihypertension potential of commonly consumed carbohydrate sweeteners using in vitro models. J Med Food *11* (*2*), 337–348 https://doi.org/10.1089/jmf.2007.689. PubMed

Rayman, M.P., and Stranges, S. (2013). Epidemiology of selenium and type 2 diabetes: can we make sense of it? Free Radic Biol Med *65*, 1557–1564 https://doi.org/10.1016/j.freeradbiomed.2013.04.003. PubMed

Reis, J.P., Hankinson, A.L., Loria, C.M., Lewis, C.E., Powell-Wiley, T., Wei, G.S., and Liu, K. (2013). Duration of abdominal obesity beginning in young adulthood and incident diabetes through middle age: the CARDIA study. Diabetes Care *36* (*5*), 1241–1247 https://doi.org/10.2337/dc12-1714. PubMed

Rock, W., Rosenblat, M., Borochov-Neori, H., Volkova, N., Judeinstein, S., Elias, M., and Aviram, M. (2009). Effects of date (*Phoenix dactylifera* L., Medjool or Hallawi variety) consumption by healthy subjects on serum glucose and lipid levels and on serum oxidative status: a pilot study. J Agric Food Chem *57* (*17*), 8010–8017 https://doi.org/10.1021/jf901559a. PubMed

Septembre-Malaterre, A., Remize, F., and Poucheret, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: changes in bioactive compounds during lactic fermentation. Food Res Int *104*, 86–99 https://doi.org/10.1016/j.foodres.2017.09.031. PubMed

Sluijs, I., van der Schouw, Y.T., van der A, D.L., Spijkerman, A.M., Hu, F.B., Grobbee, D.E., and Beulens, J.W. (2010). Carbohydrate quantity and quality and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition-Netherlands (EPIC-NL) study. Am J Clin Nutr *92* (*4*), 905–911 https://doi.org/10.3945/ajcn.2010.29620. PubMed

Sluijs, I., Cadier, E., Beulens, J.W.J., van der A, D.L., Spijkerman, A.M.W., and van der Schouw, Y.T. (2015). Dietary intake of carotenoids and risk of type 2 diabetes. Nutr Metab Cardiovasc Dis *25* (*4*), 376–381 https://doi.org/10.1016/j.numecd.2014.12.008. PubMed

Stan, S.D., Kar, S., Stoner, G.D., and Singh, S.V. (2008). Bioactive food components and cancer risk reduction. J Cell Biochem *104* (*1*), 339–356 https://doi.org/10.1002/jcb.21623. PubMed

Stranges, S., Marshall, J.R., Natarajan, R., Donahue, R.P., Trevisan, M., Combs, G.F., Cappuccio, F.P., Ceriello, A., and Reid, M.E. (2007). Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Ann Intern Med *147* (*4*), 217–223 https://doi.org/10.7326/0003-4819-147-4-200708210-00175. PubMed

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact *160* (*1*), 1–40 https://doi.org/10.1016/j.cbi.2005.12.009. PubMed

Zhang, C.R., Aldosari, S.A., Vidyasagar, P.S.P.V., Shukla, P., and Nair, M.G. (2017). Health-benefits of date fruits produced in Saudi Arabia based on in vitro antioxidant, anti-inflammatory and human tumor cell proliferation inhibitory assays. J. Saudi Soc. Agric. Sci. *16* (*3*), 287–293 https://doi.org/10.1016/j.jssas.2015.09.004.

Zhao, L.G., Shu, X.O., Li, H.L., Zhang, W., Gao, J., Sun, J.W., Zheng, W., and Xiang, Y.B. (2017). Dietary antioxidant vitamins intake and mortality: A report from two cohort studies of Chinese adults in Shanghai. J Epidemiol *27* (*3*), 89–97 https://doi.org/10.1016/j.je.2016.10.002. PubMed

Zhou, J., Huang, K., and Lei, X.G. (2013). Selenium and diabetes–evidence from animal studies. Free Radic Biol Med *65*, 1548–1556 https://doi.org/10.1016/j.freeradbiomed.2013.07.012. PubMed

Functional and sensory properties of 'Mozawati' and 'Aseel' dates

A. Shahwani, T.F. Miano[a] and A.H. Soomro

Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam, Sindh 70060, Pakistan.

Abstract

The present study was designed to analyze the functional properties of fresh and dehydrated 'Mozawati' and 'Aseel' dates. The sample of 'Mozawati' and 'Aseel' dates were randomly collected from Quetta Baluchistan, and Khairpur Sindh Provinces of Pakistan. The fresh date samples were dehydrated at 60-65°C for 18 h in a cabinet dehydrator. Further the samples were rehydrated in distilled water to observe the physical changes. Each sample was analyzed for functional and sensory properties for dates flesh weight (g), stone weight (g), pH value, length (cm), width (cm), rehydration %, moisture %, TSS (°Brix), total sugars (%) and total carbohydrate content. The results obtained for date samples showed statistically significant difference at $p \leq 0.05$ probability level for moisture loss 46.2%, weight of dates 10 g, width of date 2.2 cm and length 3.4 cm analyzed, respectively. Further the observation of date samples were recorded for pH value (6.4), total soluble solids (81.49 °Brix) reducing sugar obtained (67.1%) and carbohydrate (84.9%). The data recorded for sensory properties of dehydrated date samples revealed a better score for 'Mozawati' dates in color, flavour, texture, and taste. It was concluded from the present study that a cabinet dehydrator is effective for dehydration, as the dates retained their functional and sensory properties without loss after rehydration.

Keywords: dates, dehydration, rehydration, functional properties, organoleptic properties

INTRODUCTION

The date fruit *Phoenix dactylifera* (L.) is a major fruit crop belonging to the genus *Phoenix* and *Arecaceae* (formerly *Palmae*) family, grown in subtropical and tropical climatic conditions (Zaid and de Wet, 2002). High nutrition, high profit and environmental advantages make date palm become the next generation of high-quality food. The contents of carbohydrate total sugar (44-88%), fat (0.2-0.5%), protein (2.3-5.6%), pectin (0.5-3.9%), dietary fiber (6.4-11.5%), 15 kinds of salt, at least 15 kinds of minerals and 6 kinds of vitamins in the fruit of date palm are higher. The oil content of date palm pulp is (0.2-0.5%), and the oil content of seed is 7.7-9.7% (Dada et al., 2012). The pulp and seeds contain fatty acids, the seeds also have different proportions of aluminum, cadmium, chloride, lead, sulfur and oleic acid. In many ways, dates have been considered near-ideal, which provides a broad range of basic nutrients and potential health benefits. In areas where dates are not used as food rather than delicacy, they have the potential to improve the diet of a large number of people (Al-Shahib and Marshall, 2003). In Arab countries, dates are considered to be one of the main fruit crops. Due to the environmental conditions at that time, the number of date palm trees and the production and consumption of date palms vary from country to country/region (Kader and Hussein, 2017). Date palm juice contains 19.00% of water, 2.20% ash, 1.70% protein, 1.90% fat (ether extract) and 73.5% reducing sugars as inverted sugar. Date palm contains 70% carbohydrates, most of which exist in the form of sugar. In most cultivars, the content of sugar is almost completely transformed sugar, and the human body will quickly absorb transformed sugar (Karra et al., 2020). Date palm juice produces more quality jaggery than sugarcane and may be an alternative source of sugar to supplement the increased demand of sugar. The physical and chemical properties of date-determined cultivars have always been a

[a]E-mail: tahseenmiano@sau.edu.pk

Acta Hortic. 1371. ISHS 2023. DOI 10.17660/ActaHortic.2023.1371.53
Proc. VII International Date Palm Conference
Eds.: A. Zaid, G.A. Alhadrami and S. Mitra

characteristic of researchers. Compared with date production in other parts of the world, date cultivars produced in the Middle East are considered to be high-quality cultivars. However, the coastal areas of Balochistan and upper Sindh also produce high-quality fruit cultivars. In the Middle East, dried date are found to have good quality in color and taste and are popular with consumers; the khalal stage of bent 'Aisha' date can be processed into jam with good taste (El-Din, 2016). Pakistan exports 10% of its date on average, 90% of which is consumed or wasted locally. Increasing food production to feed its rapidly growing population is a major policy goal of Pakistan. In Pakistan, date is an important livelihood crop, especially in its vast desert areas. However, dates are also sold nationwide as a highly regarded fruit and confectionery product, and are used during the Muslim feast of Ramadan and the Hindu celebration of Diwali. Dates rich in minerals, carbohydrates and vitamins are not only an excellent source of food for humans, but the low-grade part of the harvest can also be used as a feed supplement for livestock when they are scarce. Date trees are dioecious in nature (Hassan et al., 2006). The dates have important traditional significance in the Islamic world. As the population of these countries increases, date provides not only food but also a large number of products, which are widely used in the rural areas and urban areas of Pakistan. In Balochistan, district Kech (Turbat) and Panjgoor; in Sindh, Khairpur, Sukkur and Naushahro Feroze while in KPK, Dera Ismail Khan are major date palm producing areas of Pakistan. Unfortunately, its actual output at the farm level is low compared to its potential output. Considering the above facts, a thorough investigation has been made to produce and planned to functional properties of 'Muzawati' and 'Aseel' dates with the following objectives.

MATERIALS AND METHODS

The study was conducted on functional properties of 'Muzawati' and 'Aseel' dates. The research was carried out in 2021 in the laboratory of the Institute of Food Science and Technology (IFST), Sindh Agricultural University in Tandojam.

Sample collection

'Muzawati' was purchased from the market of Quetta, Balochistan and 'Aseel' was purchased from a local market of Hyderabad, Sindh and samples were brought to laboratories of IFST, Sindh Agriculture University, Tando Jam. The experiment was designed according to complete randomized design, the dates are divided randomly for dehydration and rehydration. The following observations were recorded: 1) functional properties moisture (%), pH (value), total soluble solids, total sugar (%), total carbohydrate (%); 2) physical observations e.g., size of fruit (cm), rehydration properties, weight of fruit (g), flesh (g), stone (g).

Dehydration and rehydration of date sample

Fresh date samples were randomly selected from 'Muzawati' and 'Aseel' dates. Dates samples were dried in a cabinet dehydrator at 55°C for about 48 h. Dehydrated samples were rehydrated by placing each sample in a 30-mL filled with distilled water, samples were placed at room temperature (25±2°C) for 10, 20 and 30 min, respectively.

The functional properties for moisture (%) of 'Aseel' dates was determined according to the method described by AOAC (2000). pH value of 'Aseel' dates was determined according to the method of AOAC (2005) by using a digital pH meter. Total soluble solids (°Brix) of 'Aseel' date samples was determined according to the method described by Mazumdar and Majumder (2003). The total sugar was determined according to method described by AOAC (2000). Total carbohydrates were determined according to the method described by James (1995) and calculated by the following formula: Carbohydrate % = 100 – (% moisture + % Ash + % Fat + % Protein + % Fiber).

Sensory evaluation of date samples

Sensory evaluation evaluated color, texture, flavour/taste, chewing ability and overall acceptability through the described methods i.e., Lawless and Heymann (1998), using a 9-point hedonic scale (1 = like extremely, 2 = like very much, 3 = like moderately, 4 = like slightly, 5 = neither like or dislike, 6 = dislike moderately, 7 = dislike very much, 8 = dislike slightly, 9 = dislike extremely).

Rehydration properties

The dates from 'Muzawati' and 'Aseel' were selected randomly for physical observations e.g., fruit weight in g, flesh weight in g, and stone weight in g which were measured with digital weight balance and width and length of fruit were measured with the help of a Vernier caliper.

Statistical analysis

The data obtained were tabulated and analyzed according to statistical procedure of analysis of variance (ANOVA) and significant differences of the mean were further computed by the method as described by Gomez and Gomez (1984) using least significant difference (LSD) at 0.05% level of significance.

RESULT AND DISCUSSION

Result regarding physical observation of date fruit samples are presented in Table 1. The weight of fruit results are statistically significant in all three samples i.e., fresh dates fruit samples, dehydrated dates, and rehydrated date samples. The highest weight 9.6±0.83, 8.4±0.81, and 9.6±0.83 g for 'Muzawati' date was compared with 'Aseel' with the values of 8.4±0.63, 7.9±0.59, and 8.6±0.61 for fresh, dehydrated, and rehydrated, respectively. The flesh weight of fruit and stone weight g 'Muzawati' was observed with a greater value when compared to 'Aseel'. While, witnessing at the length in cm, not much difference was recorded regarding both cultivars. An average length of all the samples recorded was 3.2 cm for 'Muzawati' samples with highest value width 2.1±0.0602, 2.0±0.0979, and 2.2±0.09 in comparison to the length observed of 1.9±0.07, 1.7±0.07, and 1.8±0.09 for 'Aseel' fruit. The results showed more or less similar results in accordance with work done by Fatima et al. (2017) who reported that fruit length to width ratio of 'Muzawati' fruit were the same. The overall comparison of physical properties of selected cultivars reveals that 'Muzawati' dates were statistically significantly different in physical properties compared to 'Aseel' fruit.

Table 1. Physico chemical analysis of 'Muzawati' and 'Aseel' dates.

Cultivar	Treatments	Moisture	pH value	TSS	Total sugar	Carbohydrate	Weight	Flesh weight	Stone weight	Length	Width
Muzawati	Fresh	21.70± 0.4899	6.4± 7.698E-03	42.2± 0.4481	64.0± 5.9697	43.8± 3.8470	9.6± 0.8273	8.6± 0.7001	1.0± 0.1256	3.3± 0.0715	2.1± 0.0602
	Dehydrated	13.17± 0.3432	5.9± 0.00112	69.7± 1.3019	70.0± 5.3533	84.4± 2.9788	8.4± 0.8165	8.3± 0.3048	0.9± 0.1161	3.1± 0.0758	2.0± 0.0979
	Rehydrated	18.53± 0.7798	6.2± 0.0584	61.1± 0.1103	66.6± 5.6612	64.6± 3.9918	9.6± 0.8235	8.7± 0.7189	1.0± 0.0680	3.3± 0.0763	2.2± 0.0953
Aseel	Fresh	20.20± 0.6080	5.3± 8.165E-03	37.8± 0.2698	50.3± 5.0126	42.0± 3.7774	8.4± 0.6226	7.6± 0.4885	0.8± 0.0812	3.3± 0.0707	1.9± 0.0755
	Dehydrated	10.80± 0.2735	5.6± 0.0667	81.3± 4.2873	59.7± 5.5967	84.5± 4.2151	7.9± 0.5940	7.5± 0.1301	0.7± 0.0738	3.2± 0.1640	1.7± 0.0743
	Rehydrated	16.80± 0.7108	5.3± 0.0168	59.1± 0.1464	56.1± 6.0801	52.5± 5.1648	8.6± 0.6061	7.7± 0.4846	0.9± 0.1065	3.3± 0.1160	1.8± 0.0985

TSS: total soluble solids.

The results presented in Table 1, for moisture % for 'Muzawati' fruit samples were statistically highest, 21.70±0.49, 13.17±0.34, and 18.53±0.78 observed for fresh fruits, dehydrated dates, and rehydrated date samples, respectively. The highest moisture % was recoded for 'Aseel' fruit samples showing 20.20±0.61, 10.80±0.27, and 16.80±0.71%. Results regarding pH of 'Muzawati' fruit samples recorded 4±0.001 in comparison to 5.3±0.008 for 'Aseel' as control sample. However, increase in pH of 'Muzawati' fruit was also noted in dehydrated and rehydrated samples. The TSS (Brix) value results presented in Table 1 for fresh dates and rehydrated samples of 'Aseel' fruit were found to be with greater values with 81.3±4.29 for 'Aseel' in comparison to 69.7±1.31 for 'Muzawati'. Fatima et al. (2017) also reported that dates from DI Khan were high in TSS. Result presented in Table 1 for total sugars show significant difference between the fruits of ‘Muzawati’ and ‘Aseel’ dates. The highest TSS 70.0±5.35 was recorded in the dehydrated samples for ‘Muzawati’ and TSS of 59.7±5.59 was recorded in ‘Aseel’ fruit, respectively. The results presented in Table 1 for total carbohydrate showed statistically significant difference in rehydrated date samples, 64.6±3.99 for ‘Muzawati’, and 52.5±5.16 for ‘Aseel’ dates. A study regarding dates was conducted by Khan et al. (2015) on the nutritional value of the date fruit. Their findings concluded that carbohydrate level was at highest in the date fruit. The overall functional properties of both the date samples found for ‘Muzawati’ date samples with highest functional properties compared to ‘Aseel’ date samples. Manaa et al. (2013) presented research on functional properties of date palm in Algeria and showed results more or less in accordance with our research findings. Another study conducted on date palm functional properties was conducted by Jamil et al. (2010). The results showed similar parameters as the current study results (Mrabet et al., 2008).

The result for sensory analysis for ‘Muzawati’ and ‘Aseel’ date samples are presented in Table 2 for color, flavour, texture, and taste scores. The results regarding color score for all date samples as control, dehydrated and rehydrated samples showed statistically significant difference for ‘Muzawati’ dates followed by the ‘Aseel’ date sample. There was no significant difference observed in color recorded for ‘Muzawati’ and ‘Aseel’ in control samples followed by color of ‘Muzawati’ and ‘Aseel’ rehydrated and in dehydrated samples. The results regarding color score after dehydration showed minimum color score 3 and 2 for ‘Muzawati’ and ‘Aseel’ after rehydration. The flavour was noted in the control group. As well as in treated dehydrated and treated rehydrated. Maximum flavour 2 and 1 was recorded for ‘Muzawati’ and ‘Aseel’ in control samples followed by flavour of ‘Muzawati’ and ‘Aseel’ 3 and 4 after dehydration and minimum flavour score of ‘Muzawati’ and ‘Aseel’ were recorded after srehydration. The taste score of dehydrated and rehydrated fruit sample was maximum 3 and 4 for ‘Muzawati’ and ‘Aseel’ in control samples followed by taste of ‘Muzawati’ and ‘Aseel’, 2 and 4 after dehydration and minimum taste score 3 is presented in Table 2. The maximum texture score recorded 8 for dehydrated and minimum texture score of 7 observed in ‘Muzawati’ and ‘Aseel’ after rehydration. The overall acceptability score 4 after dehydration of dates samples is presented in Table 2. Karra et al. (2020) showed similarities in results in research conducted on dates.

Table 2. Sensory evaluation ‘Muzawati’ and ‘Aseel’ dates.

Cultivar	Color			Flavor			Taste			Texture			Overall acceptability		
	Fresh	Dehy-drated	Rehy-drated	Fresh	Dehy-drated	Rehy-drated	Fresh	Dehy-drated	Rehy-drated	Fresh	Dehy-drated	Rehy-drated	Fresh	Dehy-drated	Rehy-drated
	Mean scores														
Muzawati	3	1	2	1	4	2	4	4	1	2	7	7	2.5	4	3
Aseel	1	2	3	2	3	3	3	2	3	1	8	6	1.75	3.75	3.75

1 = like extremely, 2 = like very much, 3 = like moderately, 4 = like slightly, 5 = neither like or dislike, 6 = dislike moderately, 7 = dislike very much, 8 = dislike slightly, 9 = dislike extremely.

CONCLUSIONS

It is concluded from the study that ‘Muzawati’ and ‘Aseel’ dates have good functional and physical properties. The fresh dates subjected for dehydration result in good sensory properties for color, flavour, texture, and overall acceptability score for ‘Muzawati’ dates. The sample of ‘Muzawati’ dates for showed functional and sensory properties for dates flesh weight (g), stone weight (g), pH value, length (cm), width (cm), rehydration %, moisture %, TSS (°Brix), total sugars (%) and total carbohydrate content. The sensory properties of dehydrated date samples revealed better score for ‘Muzawati’ dates in color, flavour, texture, and taste. It is concluded from the present study that a cabinet dehydrator is effective for dehydration, as the dates retained their functional and sensory properties without loss after rehydration.

Literature cited

Al-Shahib, W., and Marshall, R.J. (2003). The fruit of the date palm: its possible use as the best food for the future? Int J Food Sci Nutr *54* (*4*), 247–259. PubMed

AOAC. (2000). Official Method of Analysis of the Association of the Analytical Chemists (Virginia, USA).

AOAC. (2005). Official Method of Analysis of the Association of the Analytical Chemists (Virginia, USA).

Dada, M., Nwawe, C.N., Okere, R.A., and Uwubanmwen, I.O. (2012). Potentials of date palm tree to the Nigerian economy. World J. Agric. Sci. *8* (*3*), 309–315.

El-Din, A.E.M.M. (2016). Effect of some technological treatments on ripening and drying of soft date (Bent Aisha variety). Egypt Journal of Agriculture Research *76* (*1*), 247–259.

Fatima, G., Wiehle, M., Khan, I.A., Khan, A.A., and Buerkert, A. (2017). Effects of soil characteristics and date palm morphological diversity on nutritional composition of Pakistani dates. Exp. Agric. *53* (*3*), 321–338 https://doi.org/10.1017/S0014479716000399.

Gomez, K.A., and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research, 2nd edn (John Wiley & Sons).

Hassan, S., Bakhsh, K., Gill, Z.A., Maqbool, A., and Ahmad, W. (2006). Economics of growing date palm in Punjab, Pakistan. Int. J. Agric. Biol. *8* (*6*), 788–792.

James, C.S. (1995). Analytical Chemistry of Food (London, UK: Blackie Academic and Professionals), p.256–257 https://doi.org/10.1007/978-1-4615-2165-5.

Jamil, M.S., Nadeem, R., Hanif, M.A., Ali, M.A., and Akhtar, K. (2010). Proximate composition and mineral profile of eight different unstudied date (*Phoenix dactylifera* L.) varieties from Pakistan. Afr. J. Biotechnol. *9* (*22*), 3252–3259.

Kader, A.A., and Hussein, A.M. (2017). Harvesting and Post-Harvest Handling of Dates (International Center for Agricultural Research in the Dry Areas, ICARDA).

Karra, S., Sebii, H., Yaich, H., Bouaziz, M.A., Blecker, C., Danthine, S., Attia, H., and Besbes, S. (2020). Effect of extraction methods on the physicochemical, structural, functional, and antioxidant properties of the dietary fiber concentrates from male date palm flowers. J Food Biochem *44* (*6*), e13202 https://doi.org/10.1111/jfbc.13202. PubMed

Khan, H., Nouroz, F., Khan, M.F., and Rizwan, S. (2015). Nutritional values of selected date palm varieties in Pakistan. American-Eurasian J. Agric. & Environ. Sci. *15* (*5*), 764–768.

Lawless, H., and Heymann, H. (1998). Sensory Evaluation of Food Science Principles and Practices, 2nd edn, Chapter 1 (Ithaca, New York).

Manaa, S., Younsi, M., and Moummi, N. (2013). Study of methods for drying dates; review the traditional drying methods in the region of Touat Wilaya of Adrar - Algeria. Energy Procedia *36*, 521–524 https://doi.org/10.1016/j.egypro.2013.07.060.

Mazumdar, B.C., and Majumder, K. (2003). Methods on Physico-Chemical Analysis of Fruits (India: College of Agriculture, Calcutta University), p.108–109.

Mrabet, A., Ferchichi, A., Chaira, N., Mohamed, B.S., Baaziz, Z., and Penny, T.M. (2008). Physico-chemical characteristics and total quality of date palm varieties grown in the southern of Tunisia. Pak J Biol Sci *11* (*7*), 1003–1008 https://doi.org/10.3923/pjbs.2008.1003.1008. PubMed

Zaid, A., and de Wet, P.F. (2002). Climatic requirements of date palm. In Date Palm Cultivation. Food and Agriculture Organization Plant Production and Protection Paper no. 156, A. Zaid, ed. (Rome, Italy: Food and Agriculture Organization of the United Nations), p.57–72.

Determination of amino acids and fatty acids levels in date palm kernel flour in Sudan

R.S.M. Abdalla[1], M.A. Eltayeb[2], A.M.E. Hassan[2,a] and A.M. El Hussein[3]

[1]Sudanese Nuclear and Radiological Regulatory Authority, Ministry of Higher Education and Scientific Research, Khartoum 11111, Sudan; [2]Sudan Atomic Energy Commission, Ministry of Higher Education and Scientific Research, Khartoum 11111, Sudan; [3]Central Laboratory, Ministry of Higher Education and Scientific Research, Khartoum 11111, Sudan.

Abstract

The aim of this study was to determine the various levels of amino acids and fatty acids in date palm kernel (DPK) for enhancing production performance of animals natural feed in Sudan, in addition to protecting the environment from waste. The material used is flour of date seeds from the following date cultivars: 'Berkawi', 'Gandilla', 'Tamouda', 'Klmah', 'Mashreq Wad Khatib', 'Mashreq Wad Lagi'. This research was conducted at the following institutions: Geological Research Authority of Sudan, preparation of the DPK; Institute Penyelidikan Produk Halal of University Putra-Malaysia, determination of amino acids using high performance liquid chromatography mass spectroscopy mass spectroscopy (HPLC MSMS) fluorescence; University of Medical Sciences and Technology-Sudan, determination of fatty acids using gas chromatography-mass spectrometry (GC-MS). Seeds were collected from various areas of Sudan and from factories. All seeds were mixed together and they weigh 8 kg. Samples preparation includes five steps, namely 1) washing, 2) soaking, 3) flushing, 4) drying, 5) grinding. The flour was then used to determine its contents of amino acids by high performance liquid chromatography-fluorescence detector (HPLC-FLD) and fatty acids by GC-MS. Concentrations of the following amino acids were determined in the sample (percentages in w/w are indicated between parenthesis): hydroxyprofine (0.224), aspartic acid (7.029), serine (6.350), glutamic acid (1.745), glycine (3.183), histicline (0.705), arginine (7.262), threonine (ND), alanine (15.558), proline (4.302), cysteine (34.326), tyrosine (2.070), voline (0.402), methionine (2.989), lysine (4.723), isoleucine (2.203), leucine (4.485), phenylalanine (2.442) while the determined fatty acids are: lauric acid (11.765), tetradecanoic acid (14.214), methyl palmitoleate (16.235), palmitic acid(16.431), cis-10-Heptadecenoic acid, methyl ester (17.253), margaric acid (17.464), linoleic acid (18.183), oleic acid (18.236), stearic acid (18.449), methylester (20.106), and arachidic acid (20.308). The seeds of the dates are found to be rich in amino acids as well as fatty acids, which means they can be used as natural feed for animals. These seeds are now dealt with as waste so using them as source of nutrition means protecting the environment and also reducing the cost of animal feed.

Keywords: date palm kernel, solid waste, nutritional value, amino acids, fatty acids

INTRODUCTION

Date fruit (*Phoenix dactylifera* L.) has become an important fruit in some countries as a source of nutrition and economics (Nancib et al., 1997; Briones et al., 2011). Date fruit consists of 73-79% carbohydrates; 14-18% total dietary fibers, 2.5% ash, 2.1-3.0% protein (Elleuch et al., 2008) and 2.0-3.2% fat (Al-Farsi et al., 2007), depending on the cultivar of the date fruit. The world production of dates has increased from 4.6 million t in 1994 to 7.2 million t in 2009, and 9.4 million t in 2020 (Statista, 2022). Date palm is an important fruit crop produced abundantly in tropics and subtropics and it also grows in some areas of Sudan. The previous use of date palm seed or kernel has not been reported in livestock feed particularly and animals example poultry in Sudan. The use of fruit waste products was helpful for declination

[a]E-mail: ammarmehassan@gmail.com

of dependence on pricey raw materials used for feed composition, which could manage with worldwide policies of depending on agro-industrial by-products for animal feed to reduce competition with man for cereals and to facilitate diminishing environmental pollution. Date pits stones, kernels or seeds of the date fruit which can reduce the cost of animal feed when used as replacement of the expensive additives. Date pits are cheap by-products with high energy content that can provide a potential alternative for conventional energy feeding ingredients in the poultry industry and natural animal feed. The date palm (*P. dactylifera* L.) is cultivated in dry and semi-arid regions in the world and is an important member of the family *Palmaceae* (Saafi et al., 2008; Al-Jasser, 2009). Palm trees are abundant all over the world, particularly in the Arab Gulf area. It is considered to be the most important tree in most of the Arabian countries (Jamil et al., 2010; Mustafa et al., 1983). On the other hand, fruit exploitation of date palm trees represents an important economic support for indigenous populations (Reynes et al., 1994). Dates are rich in certain nutrients and provide a good source of rapid energy due to their high carbohydrate content (70-80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body (Myhara et al., 1999; Mrabet et al., 2008). In most cases, date seeds end up as waste products of many date fruit-processing plants. Date seeds are also ground and added to the feed.

Traditionally, much attention has been directed justifiably to the role of essential amino acids in animal nutrition. They are defined as either those amino acids whose carbon skeletons cannot be synthesized by animals or those that are inadequately synthesized in animals relative to needs and that must be provided from the diet to meet requirements for maintenance, growth and reproduction. Recently, there has been growing interest in non-essential and conditionally essential amino acids because of their unique, versatile functions in metabolic regulation and physiology. Conditionally essential amino acids are those that normally can be synthesized in adequate amounts by animals, but which must be provided from the diet under conditions where rates of utilization are increased relative to rates of synthesis. Non-essential amino acids are the amino acids whose carbon skeletons can be synthesized in adequate amounts by animals to meet requirements. One can argue that animals have conserved the ability to synthesize amino acids during the thousands of years of evolution because these nutrients are indispensable for survival and reproduction of sows.

Amino acids and fatty acids display remarkable metabolic and regulatory versatility. They serve as essential precursors for the synthesis of a variety of molecules with enormous importance, and also regulate metabolic pathways and processes vital to the health, growth, development, reproduction, and functional integrity of animals. The current sow feeding program aims at providing amino acids for optimum protein synthesis. However, in view of the crucial regulatory roles of functional amino acids, their supplementation to the sow' side can be highly beneficial for enhancing production performance.

One of the most interesting findings in recent lipid nutrition research is the role of omega-3 fatty acids (O3FA) in both humans and animals. Their potential benefits in improving health and preventing certain diseases have now been widely recognized. However, unlike omega-6 fatty acids (O6FA), smaller amounts of O3FA are found in the typical grain-based animal feeds. Although studies with livestock have been limited, dietary supplementation with O3FA holds great promise in improving the reproductive performance of sows.

The aim of this study was the determination of various levels of amino acids and fatty acids in date palm kernel (DPK) flour for enhancing production performance of animals when used as natural feed in Sudan, in addition to protecting the environment from waste.

MATERIALS AND METHODS

Seeds were collected from various areas of Sudan and from factories. All seeds were mixed together that weigh of 8 kg. The method for sample preparation include five steps, namely: 1) washing, this step aims to clear up residuals and dirt from the seeds by washing the seeds with tap water; 2) soaking, seed has been cleaned; 3) flushing, this step aims to clear up the seed after soaking process; 4) drying, the kernel was dried in the shade for 3 days and was not exposed to sunlight so as not to lose any part of its components; 5) grinding, and then was broken by a simple checker to ease it in the German milling machine known as Retsch (RS

200), Retsch GmbH, Haan, Germany. The seeds were converted to flour and turning 1 kg of the seeds into flour took 2 min.

Determination of amino acid concentrations

Determination of amino acids profile was done using high performance liquid chromatography-fluorescence detector (HPLC-FLD), Simadzu, Tokyo, Japan and this is done to determine types of amino acids and how many of each composes a protein identification and quantisation. Only the amino acid composition is determined, not the order or sequence of amino acid residues. Amino acid analysis involves four basic steps:

1. Hydrolyse a protein to individual constituent amino acids;
2. Label amino acids with a detectable ultraviolet (UV)-absorbing or fluorescent marker;
3. Separate different types of amino acids by chromatography;
4. Measure relative amounts of each amino acid type based on intensity of the detectable marker associated with the emergence of each type of amino acid from the chromatographic system.

Basically, the procedure involves extracting amino acids from the sample by hydrolyzing with dilute hydrochloric acid under total hydrolysis condition. A small portion of the amino acids solution was derivatized with AccQ-Fluor Reagent together with amino butyric acid as internal standard. The amino acid derivatives were then analyzed on an HPLC-FLD. Amino acid amount (ng) was determined by external standard calibration with α-amino butyric acid (AABA) used as internal standard to compensate for variation in derivatization between samples. Weight-percent (w/w%) of each amino acid was calculated from the total amount of the amino acids in the extract.

Preparation of the hydrolysate

Five mL of 6 N HCl was added to 0.2 g of the sample and heated at 110°C for 24 h, 4 mL of 2.5 mM ABBA was added as internal standard. The hydrolysate was marked with water in a 100-mL volumetric flask/1.5 mL of the hydrolysate was filtered using 0.45 pm polytetra fluoroethylene (PTFE) syringe.

Derivatization of amino acids

Seventy µL borate buffers were put into an Eppendorf tube, 10 µL of the hydrolysate sample/standard was added and vortexed, then 20 µL of AccQ Flour reagent was added and vortexed. The mixture was left to stand for 1 min at room temperature then transferred into vials and heated at 55°C for 10 min. Finally the mixture was placed in the instrument for analysis (Table 1).

Table 1. Chromatographic conditions of amino acid analysis kernel date.

Column	AccQ tag column (3.9×50 mm)
Equipment used	High performance liquid chromatography (HPLC) Brand: Waters (Alliance e2695) with fluorescence detector (2475-waters)
Mobile phase A	Eluent A, Accco Tag Eluent A: Deionised water (I:10)
Mobile phase B	Acetonitrile
Mobile phase C	Water
Column temperature	36+1°C
Flow rate	1 mL min^{-1}
Detection wavelength	E_x: 230 nm, E_m: 395 nm
Injection volume	10 µL

Determination of fatty acids

Determination of fatty acids was done by using gas chromatography-mass spectrometry (GC-MS) to identify the available fatty acids (qualitative) and their concentrations (quantitative). The used GM-MS technique model was GC-MS-QP2010-Ultra with serial number 020525101565SA and capillary column (Rtx-5 ms-30 m × 0.25 mm × 0.25 µm), Simadzu, Tokyo, Japan. The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 mL min^{-1}, the temperature program was started from 60°C with rate 10°C min^{-1} to 300°C as final temperature degree with 3-min hold time, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of 40-500 (m/z) charges to ratio and the total run time was 27 min. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST), and results were recorded.

RESULTS AND DISCUSSION

Amounts of amino acids

Amount of amino acids detected (ng) is obtained directly from result output of the HPLC run based on the internal standard and single point calibration as shown in Table 2 and Figure 1.

Table 2. Amino acid compound analysis date palm kernel (DPK).

Amino acid compound	Amount	
	Compound weight (ng)	Compound percentage (w/w)
Hydroxyprofine	4.519	0.224
Aspartic acid	141.775	7.029
Serine	28.070	6.350
Glutamic acid	35.88	1.745
Glycine	64.191	3.183
Histicline	4.227	0.705
Arginine	146.467	7.262
Threonine	ND	-
Alanine	313.791	15.558
Proline	86.776	4.302
Cysteine	692.323	34.326
Tyrosine	41.759	2.070
Voline	8.08	0.402
Methionine	60.292	2.989
Lysine	95.257	4.723
Isoleucine	44.422	2.203
Leucine	90.461	4.485
Phenylalanine	49.259	2.442

$$\text{Weight-percent of amino acid (\%w/w) in sample} = \frac{\text{Amount of amino acid injected ng}}{\text{Total amount of amino acid injected ng}} \times 100$$

The following amino acids were determined in the sample: hydroxy-profine (0.224), aspartic acid (7.029), serine (6.350), glutamic acid (1.745), glycine (3.183), histicline (0.705), arginine (7.262), alanine (15.558), proline (4.302), cysteine (34.326), tyrosine (2.070), voline (0.402), methionine (2.989), lysine (4.723), isoleucine (2.203), leucine (4.485), phenylalanine (2.442), and these results agreed with what was found by Bouaziz et al. (2008), as they found 17 types of amino acids namely: glutamic acid (Glu) was the predominant amino acid, followed by arginine (Arg), aspartic acid (Asp), leucine (Leu), lysine (Lys), valin (Val), glycine (Gly), alanine (Ala) and phenylalanine (Phe).

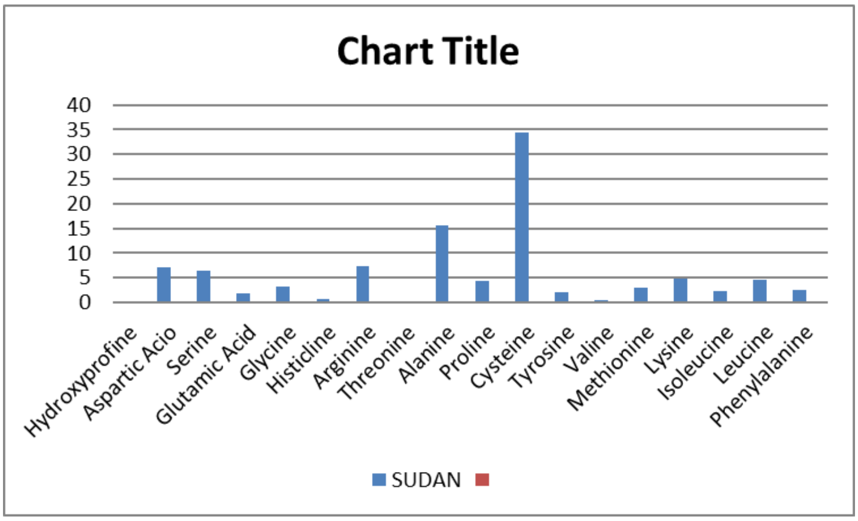


Figure 1. Concentrations of amino acids in% (w/w).

DPK was found to be very rich with cysteine (34.326%) and alanine (15.558%) while Bouaziz et al. (2008) found glutamic acid presented the largest amount, varying from 17.83% for 'Deglet-Nour' seeds to 16.77% for the 'Allig' seeds.

Concentrations of fatty acids

The determined fatty acids are: lauric acid (11.765), tetra-decanoic acid (14.214), methyl palmitoleate (16.235), palmitic acid (16.431), cis-10-heptadecenoic acid, methyl ester (17.253), margaric acid (17.464), linoleic acid (18.183), oleic acid (18.236), stearic acid (18.449), methyl ester (20.106) and arachidic acid (20.308) as shown in Figures 2-4 and Table 3. Sawaya et al. (1984) found that oleic (44.25%), lauric (17.35%), myristic (11.45%), palmitic (10.30%) and linoleic (8.45%) were the major fatty acids in date seed oil.

Table 3. Results of gas chromatography-mass spectrometry (GC-MS) for determination of fatty acids.

ID#	Name	Ret. time (min)	Area (nm^2)	Area (%)
1	Dodecanoic acid, methyl ester	11.765	13,126,354	16.26
2	Methyl tetradecanoate	14.214	9,195,668	11.39
3	9-Hexadecenoic acid, methyl ester, (Z)-	16.235	30,518	0.04
4	Hexadecanoic acid, methyl ester	16.431	9,652,436	11.96
5	cis-10-Heptadecenoic acid, methyl ester	17.253	37,283	0.05
6	Heptadecanoic acid, methyl ester	17.464	52,994	0.07
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.183	6,176,772	7.65
8	9-Octadecenoic acid (Z)-, methyl ester	18.236	38,820,113	48.07
9	Methyl stearate	18.449	2,264,621	2.81
10	cis-11-Eicosenoic acid, methyl ester	20.106	569,861	0.71
11	Eicosanoic acid, methyl ester	20.308	312,497	0.39

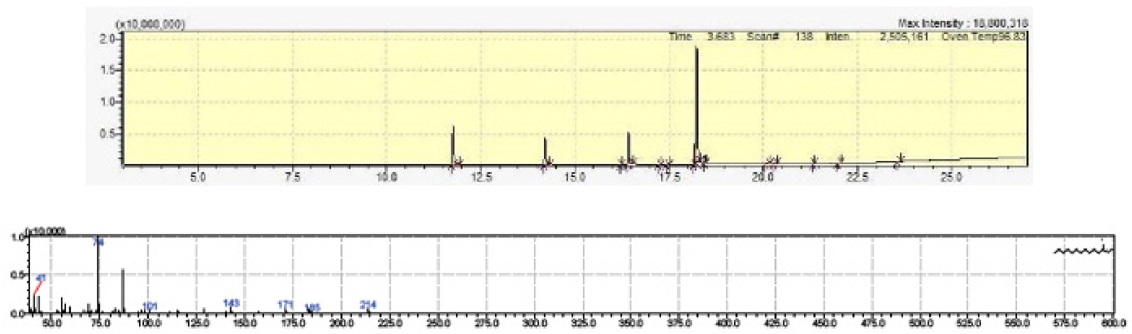


Figure 2. Chromatogram of fatty acids by gas chromatography-mass spectrometry (GC-MS).

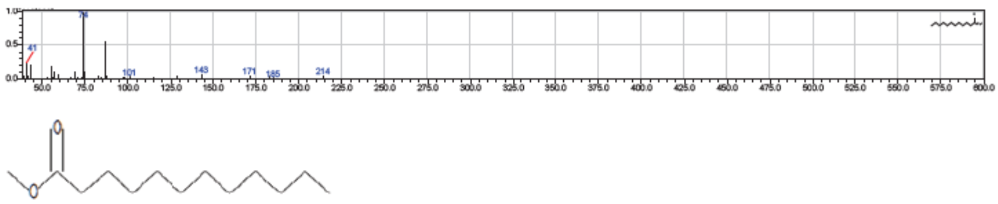


Figure 3. Mass spectra of lauric acid (molecular weight: 214, formula: $C_{13}H_{26}O_2$).

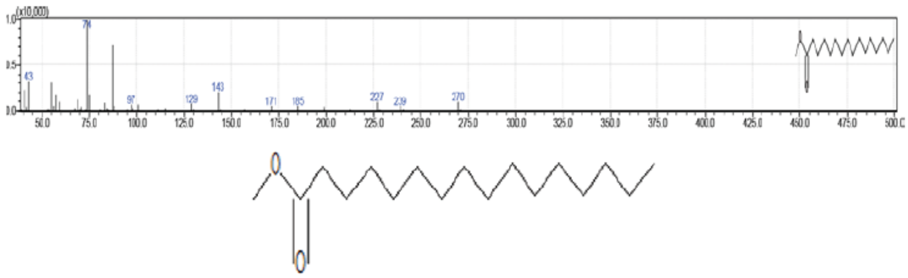


Figure 4. Mass spectra of palmitic acid (molecular weight: 270, formula: $C_{17}H_{34}O_2$).

CONCLUSIONS

From the above results, it can be concluded that seeds of dates can be used as sources of natural feed especially when compared with the food used in the poultry industry. The performance of chicken broilers fed with 2 and 3% DPK as additives showed excellent results (unpublished results). So the recommendation is to use the DPK as replacement in poultry industry which reduces the cost of the production and at the same time safe the environment from huge amount of waste.

Pits of date palm or DPK flour could be an excellent source of functional food components.

DPK can be used in animals natural feed as an inexpensive source of dietary food and other functional components, enhancing production performance of animals natural feed in Sudan.

Literature cited

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., and Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem. *104* (*3*), 943–947 https://doi.org/10.1016/j.foodchem.2006.12.051.

Al-Jasser, M.S. (2009). Physico chemical composition of date fruit (*Phoenix dactyifera* L.) from offshoots and cultured cells at different stages. J. Food Technol. *7* (*4*), 102–105.

Bouaziz, M.A., Besbes, S., Blecker, C., Wathelet, B., Deroanne, C., and Attia, H. (2008). Protein and amino acid profiles of Tunisian Deglet Nour and Allig date palm fruit seeds. Fruits *63* (*1*), 37–43 https://doi.org/10.1051/fruits:2007043.

Briones, R., Serrano, L., Younes, R.B., Mondragon, I., and Labidi, J. (2011). Polyol production by chemical modification of date seeds. Ind. Crops Prod. *34* (*1*), 1035–1040 https://doi.org/10.1016/j.indcrop.2011.03.012.

Elleuch, M., Besbes, S., Roiseux, O., Blecker, C., Deroanne, C., Drira, N.E., and Attia, H. (2008). Date flesh: chemical composition and characteristics of the dietary fibre. Food Chem. *111* (*3*), 676–682 https://doi.org/10.1016/j.foodchem.2008.04.036.

Jamil, M.S., Nadeem, R., Hanif, M.A., Ali, M.A., and Akhtar, K. (2010). Proximate composition and mineral profile of eight different unstudied date (*Phoenix dactylifera* L.) varieties from Pakistan. Afr. J. Biotechnol. *9* (*22*), 3252–3259.

Mrabet, A., Ferchichi, A., Chaira, N., Salah Moha, B., Baaziz, M., and Mrabet Pen, T. (2008). Physico-chemical characteristics and total quality of date palm varieties grown in the southern of Tunisia. Pak J Biol Sci *11* (*7*), 1003–1008 https://doi.org/10.3923/pjbs.2008.1003.1008. PubMed

Mustafa, A.I., Hamad, A.M., and Al-Kahtani, M.S. (1983). Date varieties for jam production. Paper presented at: First Symposium on the Date Palm in Saudi Arabia.

Myhara, R.M., Karkalas, J., and Taylor, M.S. (1999). The composition of maturing Omani dates. J. Sci. Food Agric. *79* (*11*), 1345–1350 https://doi.org/10.1002/(SICI)1097-0010(199908)79:11<1345:AID-JSFA366>3.0.CO;2-V.

Nancib, N., Nancib, A., and Boudrant, J. (1997). Use of waste date products in the fermentative formation of baker's yeast biomass by *Saccharomyces cerevisiae.* Bioresour. Technol. *60* (*1*), 67–71 https://doi.org/10.1016/S0960-8524(97)00004-7.

Reynes, M., Bouabidi, H., Piombo, G., and Risterucci, A.M. (1994). Characterization of the principal varieties of dates cultivated in the area of Djerid in Tunisia. Fruits *49*, 289–298.

Saafi, E.B., Trigui, M., Thabet, R., Hammami, M., and Achour, L. (2008). Common date palm in Tunisia: chemicall composition of pulp and pits. Int. J. Food Sci. Technol. *43* (*11*), 2033–2037 https://doi.org/10.1111/j.1365-2621.2008.01817.x.

Sawaya, W.N., Khalil, J.K., and Safi, W.J. (1984). Chemical composition and nutritional quality of date seeds. Shipin Kexue *49* (*2*), 617–619 https://doi.org/10.1111/j.1365-2621.1984.tb12482.x.

Statista. (2022). http://www.Statista.com/Statistics/960247/dates-productionworldwide/.

The influence of date seed powder feeding on broiler performance

R.S.M. Abdalla[1,a], I.I. Hamid[2], A.A. Mohammed[2], M.A. Eltayeb[3] and A.M. El Hussein[4]

[1]Sudanese Nuclear and Radiological Regulatory Authority, Ministry of Higher Education and Scientific Research, Sudan; [2]Animal Production Research Centre (Kuku), Sudan; [3]Sudan Atomic Energy Commission, Ministry of Higher Education and Scientific Research, Sudan; [4]Central Laboratory, Ministry of Higher Education and Scientific Research, Sudan.

Abstract

This study was conducted during the period from October 18 to November 28, 2020 at the Animal Production Research Centre (APRC) to assess the effects of dietary inclusion of date seed powder (DSP) on the performance of broiler chickens reared for 35 days of age. Ninety broiler chicks (Arber Acres Hybrid) were used. Birds were divided into 3 equal groups (30 birds group^{-1}) replicated 3 times with 10 birds per replicate and were randomly allocated to the experimental diets. Three broiler diets were formulated according to the nutrients requirements outlined by NRC (1994), diet 1 was control (0.0 DSP), diet 2 (1% DSP) and diet 3 (2% DSP). All diets were isocaloric and isonitrogenous. Feed intake, body weight gain and feed conversion ratio were recorded weekly. Organoleptic test of chicken meat was conducted at the end of the experiment. The obtained data were statistically analyzed by one-way ANOVA using SPSS 22 program. The results showed that the diets containing 1 and 2% DSP gave higher weight gains and better feed conversion compared to the control; however, feed intake was significantly not affected. Furthermore, broiler meat of the group fed 2% DSP showed the best sensory evaluation, regarding color, flavour, odour, juiciness, tenderness and the overall acceptability compared to the control and those fed 1% DSP. It can be concluded that adding DSP up to 2% in broiler diet will improve broiler performance and the taste of broiler meat.

Keywords: date pits, broiler performance, panel test, final weight

INTRODUCTION

Date palm is an important and one of the oldest trees cultivated by man. It has a good tolerance to cold and dry-hot climates. Date fruit have been found effective in constipation, inflammation, chemical induced toxicity, ulcer and hypertension. Date seeds constitute between 10 to 15% of date fruit weight and contain relatively high amount of protein (5.1 g 100 g^{-1}) and fat (9.0 g 100 g^{-1}) compared to date flesh. They are very rich source of dietary fiber (73.1 g 100 g^{-1}), phenolic (3942 mg 100 g^{-1}) and antioxidants (80400 µmol 100 g^{-1} (Hussein et al., 1998)). The date seed have been used traditionally as animal feed or ground into smaller size and being roasted to turn it into caffeine-free coffee substitute (Rahman et al., 2007; Al-Farsi and Lee, 2011). At present, date seeds are used mainly for animal feed. Utilization of such waste is very important as date seeds could potentially be considered as an inexpensive source of dietary fiber and natural antioxidants.

El-Far et al. (2016) compared control, Bio-Mos (0.1% Bio-Mos), β-glucan (0.1% β-glucan), 2, 4 and 6% date crushed seeds groups.

The substantial enhancement of performance can be attributed to the immunity, and antioxidant status by date seeds supplementation of palm date seeds (Alfarsi et al., 2007; El-Far et al., 2016). Hammod et al. (2018) replaced corn by 0, 5, 10 and 15% date pits in broiler ration during the periods 0-5 weeks of age. The results showed no significant differences in live body weight and feed intake between groups, however, the group with corn gave the significantly best feed conversion ratio compared to that of date pits groups. Tareen et al.

[a]E-mail: rehabone77@yahoo.com

(2017) fed broiler chickens with diets containing 0, 1, 2, 3 and 4% levels of date palm kernel in balanced ration for 6 weeks. They found that groups fed 3 and 4% date kernel showed significantly lowest feed intake and the best growth weights and feed conversion ratio compared to 1 and 2% date kernel.

MATERIALS AND METHODS

Experimental site and duration

The experiment was carried out in a semi closed poultry house equipped with cages (1×1 m) at the Animal Production Research Centre (APRC) at Kuku, Khartoum North, Sudan. The house was cleaned, washed and disinfected then bedded with wood shavings before the arrival of the birds.

Experimental diets

The date seeds or kernels were collected from different Sudanese date cultivars (‘Berkawi’, ‘Gandilla’, ‘Tamouda’, ‘Klmah’, ‘Mashreq Wad Khatib’, ‘Mashreq Wad Lagi’) as waste of a date factory in the Northern State of Sudan. Seeds were then ground at the Ministry of Minerals Laboratory (instrument Retsch 200 grand date palm kernel). Samples of the date seeds powder (DSP) were taken for proximate chemical analysis to determine dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and (NFE). The analysis was carried out according to the methods outlined by AOAC (1995). Three experimental diets were formulated to meet the nutrients requirement for broilers starter and finisher as recommended by the National Research Council (NRC, 1994). Diet 1 (0.0% DSP) served as (control), while diets 2 and 3 contained 1 and 2% of DSP. All diets were isocaloric, isonitrogenous. Compositions and chemical analysis of the experimental starter and finisher diets are presented in Table 1. All birds were fed a commercial pre-starter diet (23% CP and 3000 Kcal kg ME) during the first week of age as adaptation period. Feed and water were offered ad libitum throughout the experiment.

Experimental birds

A total of 200 Arber Acres Hybrid day-old chicks were reared in a metal ring favored with intensive light for acclimatization in the same house. At the beginning of the experiment chicks were arranged in a complete randomized design into three groups, each group was further subdivided into 3 replicates with 10 chicks each. The studied broiler performance parameters of feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were recorded on weekly basis for each replicate. Twenty-four h day light was provided throughout the experimental period.

Sensory evaluation

At the end of the field experiment one broiler from each treatment was slaughtered, de-feathered, eviscerated, cleaned and kept in a refrigerator. Samples were then taken from the thigh of cold carcass for the sensory evaluation. This test was carried out at the lab of Food Technology Department at APRC (Kuku) where samples were cooked in an oven at 180°C for half an hour. The panellists were instructed to record their response for each attributed parameters (color, flavour, odour, juiciness, tenderness and the overall acceptability). A panel test sheet or chart was used for the evaluation of organoleptic parameters for each of the treatments, so that the number of panellists shared the same grade or degree for each of the parameters was calculated as percentage of the total number of panellists. The higher the percentage the higher the score of the panel tested parameters.

Statistical analysis

The statistical measures were handled by the SPSS programming (SPSS version 21, 2012). One-way ANOVA was used. The means were separated using the Duncan’s (1955) new multiple range test (Steel and Torrie, 1980).

Table 1. Composition and calculated analysis of the experimental broiler diets.

Ingredients (%)	Treatment					
	Starter diet			Finisher diet		
	1	2	3	1	2	3
Sorghum	69.5	68.5	67.7	76.0	75.6	75.4
Broiler concentrate	5.0	5.0	5.0	5.0	5.0	5.0
Ground nut cake	24.0	24.0	23.9	17.5	17.2	17.0
Ground date kernel	0.0	1.0	2.0	0.0	1.0	2.0
Lime stone	1.0	1.0	1.0	1.0	0.8	0.5
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Methionine	0.3	0.3	0.2	0.1	0.1	0.1
Lysine	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin premix	0.1	0.1	0.1	0.1	0.1	0.1
Antimycotoxin	0.2	0.2	0.2	0.1	0.1	0.1
Total	**100**	**100**	**100**	**100**	**100**	**100**
Chemical analysis (calculated)						
ME (kcal kg^{-1})	2978	2959	2943	3008	3000	3001
Crude protein (%)	22.3	22.3	22.3	20.0	20.0	20.0
Crude fiber (%)	4.2	4.2	4.2	3.7	3.7	3.7
Ether extract (%)	3.7	3.7	3.7	3.4	3.4	3.4
Calcium (%)	1.1	1.1	1.1	1.1	1.1	1.0
Phosphorus (%)	0.67	0.67	0.67	0.66	0.66	0.66
Lysine (%)	1.1	1.1	1.1	0.98	0.98	0.99
Methionine (%)	0.71	0.71	0.71	0.54	0.54	0.54

RESULTS AND DISCUSSION

Effect of date seed powder on the broiler performance

The experimental data collected was tabulated and statistically analyzed. The effect of feeding date seed powder on weekly feed intake, body weight gain and feed conversion are presented in Tables 2-4 and Figures 1-3. The results obtained for the whole the experimental data collected were tabulated and statistically analyzed.

The effect of the date seeds powder on the studied broilers performance parameters and feed intake

The results of this study presented that the broiler performance in terms of body weight gain and feed conversion ratio was improved by the addition of date seed powder in feed compared to the control without effect on feed intake (Table 5). This result agreed with the findings of El-Far et al. (2016) who found that broilers fed 2 and 4% date crushed seeds showed significantly higher weight gain and the best feed conversion ratio in comparison to control. The obtained results were inconsistent with that of Tareen et al. (2017) who concluded that the high levels of date palm kernel (3-4%) significantly decreased broiler feed intake and increased live body weight, growth weight compared to 1 and 2% date palm kernel groups. Al-Farsi and Lee (2011), Alfarsi et al. (2007), Rahman et al. (2007) and Hussein et al. (1998) discussed that date seeds are a very rich source of antioxidants. This might be the reason of the improved broiler performance by the addition of date seed powder in this study. This suggestion was supported by El-Far et al. (2016) and Alfarsi et al. (2007) who also attributed the improved performance of broilers to the antioxidant and immune-stimulant constituents of palm date.

Table 2. Effect of date seed powder on feed intake (g bird^{-1} week^{-1}) (mean ± SD).

Treatment	W2	W3	W4	W5
0 DSP	196.47±0.50	243.40^{a}±6.04	438.00^{b}±5.37	630.40±1.22
1% DSP	196.60±0.72	244.67^{a}±1.65	444.67^{b}±10.20	631.67±2.89
2% DSP	196.33±0.58	232.80^{b}±2.95	465.00^{a}±13.23	630.73±1.27
LS	NS	*	*	NS

LS = level of significance: NS = not significant, *Significant (p≤0.05).

Table 3. Effect of date seed powder on body weight gain (g bird^{-1} week^{-1}) (mean ± SD).

Treatment	W2	W3	W4	W5
0 DSP	190.89±23.38	264.20^{ab}±6.96	277.40^{b}±18.39	296.50^{c}±39.98
1% DSP	220.75±13.20	258.17^{b}±21.62	317.03^{a}±9.51	428.33^{b}±32.32
2% DSP	206.30±1.26	290.67^{a}±7.46	287.70^{b}±4.81	501.27^{a}±6.01
LS	NS	*	*	*

LS = level of significance: NS = not significant, *Significant (p≤0.05).

Table 4. Effect of date seed powder on feed conversion ratio (g feed g gain^{-1}) (mean ± SD).

Treatment	W2	W3	W4	W5
0 DSP	1.04±0.14	0.92^{b}±0.04	1.58^{b}±0.08	2.15^{b}±0.31
1% DSP	0.89±0.06	0.95^{b}±0.08	1.40^{a}±0.02	1.48^{a}±0.11
2% DSP	0.95±0.01	0.80^{a}±0.03	1.62^{b}±0.03	1.26^{a}±0.01
LS	NS	NS	*	*

LS = level of significance: NS = not significant, *Significant (p≤0.05).

Table 5. Effect of date seed powder on the overall broiler performance (2-5 week) (mean ± SD).

Treatment	Parameters				
	Intial body weight (g bird^{-1})	Final body weight (g bird^{-1})	Total feed intake (g bird^{-1})	Total weight gain (g bird^{-1})	Total FCR (T. feed/T. gain)
0 DSP	155.01±4.33	1184.00^{c}±17.7	1508.27±1.7	1028.99^{c}±15.8	1.47^{c}±0.0
1% DSP	155.38±0.98	1379.67^{b}±19.6	1517.60±9.0	1224.28^{b}±20.2	1.24^{b}±0.0
2% DSP	156.40±4.2	1442.33^{a}±8.7	1524.87±13.6	1285.94^{a}±12.8	1.19^{a}±0.0
LS	NS	*	NS	*	*

LS = level of significance: NS = not significant, *Significant (p≤0.05).

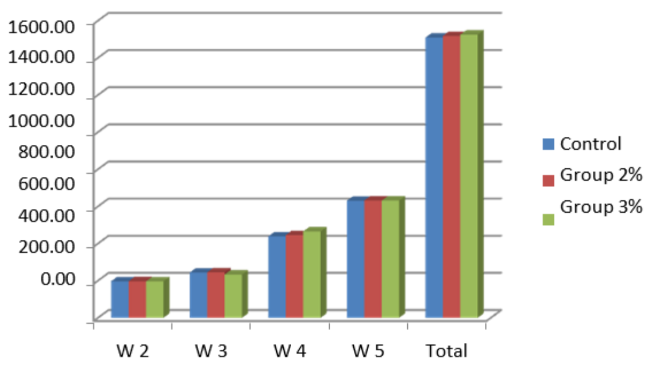


Figure 1. The weight feed intake (g bird^{-1} week^{-1}).

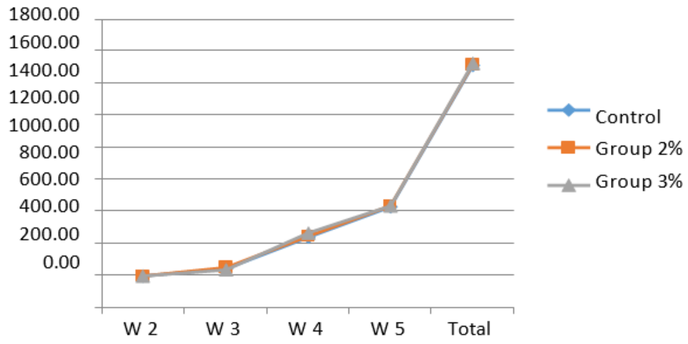


Figure 2. Standard feed intake (g bird^{-1} week^{-1}).

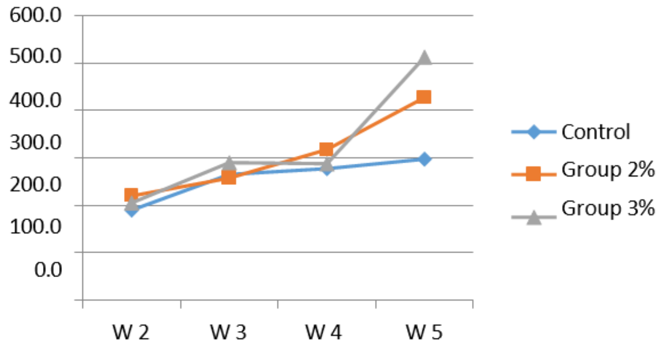


Figure 3. Effect of date kernel flour on body weight gain (g bird^{-1} week^{-1}).

RECOMMENDATION

It is highly recommended to use the date kernel as food additive in poultry industry as it increases the weight by 75% which will prevent using hormones that are now used to increase the weight of poultry.

To use the date kernel instead of the antibiotics in the poultry industry.

To use the analytical techniques in analyzing plant samples as they give accurate and precise results which are globally accepted.

CONCLUSIONS

The good findings of chemical analysis of the date kernel encourage using it as food compliment in poultry industry where excellent results were obtained in the weight, taste and shape of the poultry used in the study. The Animal Production Research Center- Kuku approved the date kernel as food additives to the poultry feed.

The addition of the date kernel to the poultry under study provide them with immunity against infectious diseases and this save the usage of antibiotics.

From the result of this research it can be concluded that adding DSP 1 and 2% in broiler diet will improve broiler performance and the taste of broiler meat, however, the meat taste of broiler fed 2% DSP is more preferred than that of 1% DSP.

Literature cited

Al-Farsi, M., and Lee, C.Y. (2011). Usage of date (*Phoenix dactylifera* L.) seeds in human health and animal feed. In Nuts and Seeds in Health and Disease Prevention, Chapter 53, V.R. Preedy, R.R. Watson, and V.B. Patel, eds. (ScienceDirect), p.447–452.

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., and Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem. *104* (*3*), 943–947 https://doi.org/10.1016/j.foodchem.2006.12.051.

AOAC (1995). Official Methods of Analysis, 15th edn (Washington, DC, USA: Association of Official Analytical Chemists).

Duncan, D.B. (1955). Multiple range test and multiple F-tests. Biometrics *11* (*1*), 1–42 https://doi.org/10.2307/3001478.

El-Far, A.H., Ahmed, H.A., and Shaheen, H.M. (2016). Dietary supplementation of *Phoenix dactylifera* seeds enhances performance, immune response, and antioxidant status in broilers. Oxid Med Cell Longev *2016*, 5454963 https://doi.org/10.1155/2016/5454963. PubMed

Hammod, A.J., Ali, N., Alkassar, A.M., and Jameel, Y.J. (2018). The effect of partial replacement of maize by date pits on broiler performance. J. Pure Appl. Microbiol. *12* (*2*), 807–813 https://doi.org/10.22207/JPAM.12.2.42.

Hussein, A.S., Alhadrami, G.A., and Khalil, Y.H. (1998). The use of dates and date pits in broiler starter and finisher diets. Bioresour. Technol. *66* (*3*), 219–223 https://doi.org/10.1016/S0960-8524(98)00054-6.

NRC. (1994). Nutrient Requirements of Poultry, 9th revised edn (Washington, DC: National Research Council, National Academy Press).

Rahman, M.S., Kasapis, S., Al-Kharusi, N.S.Z., Al-Marhubi, I.M., and Khan, A.J. (2007). Composition characterisation and thermal transition of date pits powders. J. Food Eng. *80* (*1*), 1–10 https://doi.org/10.1016/j.jfoodeng.2006.04.030.

Steel, R.G.D., and Torrie, J.H. (1980). Principles and Procedures of Statistics, 2nd edn (New York: McGraw-Hill).

Tareen, M.H., Wagan, R., Siyal, F.A., Babazadeh, D., Bhutto, Z.A., Arain, M.A., and Saeed, M. (2017). Effect of various levels of date palm kernel on growth performance of broilers. Vet World *10* (*2*), 227–232 https://doi.org/10.14202/vetworld.2017.227-232. PubMed

Production of fragrant vinegar from date wastes applying submerged acetification technique

G.S. Markhand[1], Z.P. Markhand[1], S.A. Shahzada[2,a], A.S. Ambreen[3], Y. Sakina[4] and M.E. Elahi[5]

[1]Date Palm Research Institute, Shah Abdul Latif University Khairpur, Sind, Pakistan; [2]Food Technology Section, Agriculture Research Institute, Dera Ismail Khan, Pakistan; [3]National Physical and Standards Laboratory (NPSL), PCSIR, Islamabad, Pakistan; [4]Pakistan Agriculture Research Council (PARC), Islamabad, Pakistan; [5]PARC Arid Zone Research Center, Dera Ismail Khan-29050, Pakistan.

Abstract

Vinegar plays a focal role for being an important ingredient in a great variety of industrial, medical, and domestic products. It is considered one of the best condiments with particular uses in industrial production of mayonnaise, mustard, ketchup, pickles, salad dressings, hot sauce and so on. Thus there is a great demand for good quality vinegar which requires setting up of industrial fermentation systems capable of generating a large amount of the produce. Many techniques have been developed to improve production of vinegar. The majority try to increase the speed of the transformation of ethanol into acetic acid in the presence of the acetic acid bacteria. The most common technology for industrial production of vinegar today is based on the submerged culture with diverse technical modifications trying to improve the general fermentation conditions by modifying aeration, stirring, heating, etc. techniques. The overall aim in the present study is to utilize date wastes available in abundant amount for conversion into vinegar using submerged acetification technique at the existed lab scale production setup equipped with a venturi air system. A semi continuous process is developed for the production of vinegar from date waste. The culture of vinegar bacteria was charged, and the activated biomass along with date mash was taken into submerged vinegar processing system for vinegar production optimizing air flow rate provide a desired oxygen partial pressure to the bio-reactor adjusted at 30°C. The air bubbling and quality evaluation of the product continued till 12% acidity. After taking out a suitable amount of the generated vinegar equal volume of date mash was added into the reactor to restart the acetification cycle. A variety of fragrant vinegars is prepared by adding an approved fragrant derived from various herbs and quality evaluated.

Keywords: acetification, date waste, fragrant, submerged culture, vinegar

INTRODUCTION

Vinegar is fermentation based important condiment. It plays a prominent role in a great variety of industrial, medical and domestic products, and wide applications in food processing and technological formulations for traditional and industrial production of mayonnaise, mustard, ketchup, pickles, salad dressings, hot sauce, etc. Vinegar can be made directly from sugar but is best made by first converting the sugar into alcohol and then turning the alcohol into vinegar. Therefore, there are two major fermentation steps which are being carried out independently by different microbes working under anaerobic and aerobic environment, respectively. Initially, the carbohydrates present in the material are converted to ethyl alcohol which eventually leads to vinegar production by means of acetic acid bacteria *Acetobacter*. The single major component of the vinegar is acetic acid varying from 5 to 12% along with several other minor yet important ingredients like colouring and flavouring materials of varying levels and quality which constitute the product in fermentation process. Vinegar can be produced from almost any material which contains sugar or starch. Many different raw materials having a carbohydrate source like fruits, grains, roots or even wood can be used for

[a]E-mail: sasdikpk@gmail.com

vinegar production. Fruits, unsuitable for sale as fresh, or drying and canning, etc., may often be used for vinegar making or for the manufacture of acetic acid from which to produce acetone having many industrial applications. However, quality vinegar required for specific target is produced from specific and quality grade raw material. Evidently, there is a never ending demand for quality vinegar, which requires setting up an industrial fermentation unit capable of generating a large amount of quality produce. Vinegar production methods can range from traditional methods using wood casks (Orleans process) and surface culture (generator process) to submerged fermentation (Morales et al., 2001). Most of the latest techniques are equipped with highly sophisticated equipment having in-built a variety of technological features and manipulation provisions to facilitate different operations. However, choice of the method also depends on the availability of the existing installations/facilities as well as the production requirements and the raw material under investigation. The main objective of the present study is to examine the possibility of utilizing low grade dates for vinegar production. Such type of waste is abundantly available in Pakistan and other date producing countries of the world, the potential of which has not been explored to a great deal.

MATERIAL AND METHODS

Sample preparation

Low grade dates not fit for sale fresh were taken for vinegar production. The dates were cleaned to remove unwanted materials, and then dumped in a covered drum and heated to about 70-72°C by passing steam through it. The material was cooled down to 35-40°C, and allowed to stand for overnight at room temperature. The partially fermented dates were passed through a hydraulic press to squeeze out date juice. The date juice was used for further fermentation.

Anaerobic fermentation

The date extract was then subjected to fermentation by *S. cerevisiae* yeast under anaerobic conditions with controlled temperature to favour the process to run at 29-31°C with off and on shaking, and fermentation continued till completion.

Vinegar production by submerged fermentation

Submerged vinegar processing is the latest development in vinegar processing and the most common system (De Ory et al., 1999) used by commercial manufacturers who produce high quantities of vinegar with a high content of acetic acid. The submerged vinegar processing system works with the continuous aeration of the liquid. The vinegar bacteria are floating in the liquid and do not produce a vinegar mother. The product from anaerobic fermentation is then fermented aerobically by acetic acid bacteria to form vinegar. This operation works on the development of successive discontinuous cycles of acetification. At the completion of every cycle, a given volume of acetic acid is discharged and refilled with mash.

A known amount of racked clear mash previously produced from date fermentation is taken in a metallic acetator which is equipped with venturi air system that keeps the mixture constantly turning by introducing air into the mixture to provide enough oxygen from alcoholic transformation. To this was added a measured quantity of 'mother of vinegar at 25%' bought from the market to act as a catalyst. Before the submerged process to begin, the culture was held in an incubator at 30°C for 3 days to charge it. This was done to keep the high strain bacteria culture alive, and to speed up fermentation process using vinegar forming bacteria *Acetobacter aceti*. At the end of this activation period the culture was taken from the incubator and used to initiate the submerged process.

The functioning of the process was monitored by analysing the contents periodically for acetic acid concentration. After about 20 h fermentation there appeared no increase in acetic acid beyond 11.8% concentration. At the end of the cycle, a known volume of the charge (1/3 vol) is discharged from the reactor and refilled with the same volume of initial medium (fresh alcoholic mash) to begin a new fermentation cycle. Theoretically, final acidity was calculated as 12.4%, however, experimentally 11.8% concentration was found. The vinegar is filtered through cheese cloth. The clear vinegar of dark date colour is pasteurized before storage. It stores well if properly pasteurized.

The samples were periodically taken out and were subjected to analysis for acidity and pH (AOAC, 2000a, b, 2016a, b). The soluble dry matter content of date vinegar samples was determined by a hand refractometer (Atago Refractometer N-1E, Japan) according to Haroun (2006). Alcohol determination was performed according to Taslipinar (2018). The colour determination was performed according to Voss (1992), and Unal (2007). The sensory analyses of the samples were evaluated according to Taslipinar (2018).

Flavouring of vinegar

Fragrant vinegar was made by soaking dry leaves of fresh herbs like basil (*Ocimum basilicum*), dill (*Anethum graveolens*), tej pat (*Cinnamomum tamal*) or fennel seeds. The leaves/seeds of the herbs (15-20% by wt) were taken in cheese cloth and allowed to steep in the vinegar for 3-4 days at 40-50°C with occasional shaking. The vinegars were found very appealing, tasty and fragrant.

RESULTS AND DISCUSSION

This was our preliminary study but the results are very promising. The submerged process functions highly satisfactorily. Since the acetator was equipped with aeration and agitation devices those constantly turning the mixture and allowing tiny bubbles of air to pass through the solution of ethanol offering improved oxygen transfer from the medium to the bacterial cells to keep working on top speed.

The process is very efficient but sensitive and needs good control and great care in cleanliness, maintenance of pure culture, and introducing selected yeast and bacteria (vinegar) to obtain good and standard quality every time. The acetification works ideally at 27-30°C, however, the efficiency of vinegar producing bacteria decreases rapidly on increasing temperature. Low or fluctuating temperatures slowed the process. In order to increase vinegar yield and process acceleration it needs a lot more to standardize bacterial culture, or explore new cultures as well as to improve the process further. The study is being continued.

Alcoholic fermentation

The initial sugar concentration of the date fruit mash was 110.1 g L^{-1}. The amount of sucrose was rather low in comparison with fructose and glucose (Table 1). The initial pH was 5.5 and decreased sharply to 3.8 after 24 h in both the spontaneous and inoculated alcoholic fermentations. This value remained constant throughout the alcoholic fermentation. The FAN was not a limiting factor for alcoholic fermentation, yet in all cases it was completely consumed. The inoculated alcoholic fermentation was faster than the spontaneous one because the lag phase was shorter and the fermentation rate similar. Furthermore, the alcohol concentration was 0.5% (v/v) higher in the inoculated fermentation.

Table 1. Chemical analysis of the dates before vinegar production.

Parameter	Dates
Total sugars	110.1
Fructose	44.8
Glucose	57.3
Sucrose	8
FAN (mg L^{-1})	120
pH	5.5
Titratable acidity (%, w/v)	0.6
Ethanol (%, v/v)	-

Acetification

Independent to the alcoholic fermentation, the acetification process in the dates fruit proceeded similarly. The overall acetification process finished in 30 days, and in both cases the acetic acid content was 4.5% (w/v). In both processes, the pH decreased to 3.4. The initial titratable acidity at the end of the alcoholic fermentation of the dates fruits was 0.6% (w/v) in all cases.

The fruit vinegar yield

Fruit vinegar was only obtained when fruit pulp was used. However, the pulp was still dense and had to be pressed to remove the solid debris and obtain a clear product. Despite the obvious differences between the two processes, the vinegar yields were similar. In terms of percentage of final product (liquid vinegar, in L) obtained per initial amount of fruit (in kg) the results obtained with dates were 64.8±5.4% for the inoculated process and 63.2±2.9% for the spontaneous one.

Submerged fermentation process

Nowadays the most popular as well as productive (high yielding) vinegar production method is submerged fermentation method. The speciality of this process of fermentation conditions are that it is able to be monitored viz., continued air supply, stirring, heating and many more (Tan, 2005). The submerged fermentation process is probably implemented in commercial vinegar production; it is the cheapest production method as compared with other existing methods. Mash is stimulated continuously as well as a continued supply of air is needed for completion of this process (De Ley et al., 1984). For regulating minimum temperature within the fermenter a heat exchanger is incorporated inside the fermenter through the fermentation process (De Ory et al., 1999). Irregular sequence of acetification is constituent of fermentation system, after completion of each and every sequence of acetification volume of acetic acid is released and collected for bottling purpose and remaining volume is replaced by mash (De Ory et al., 2004). Allgeier and Hildebrandt (1960) and Tan (2005) proposed 86°F (30°C) is the most common temperature for production of quality vinegar (11-12%) at commercial level. Sometimes acetic acid bacteria get scratched due to enhancement in temperature over 86°F. Fermentation conditions have an effect on acetic acid bacteria (AAB) as well as acetic acid bacterium (AAB) has impact on the final volume of acetic acid (Fregapane et al., 1999).

Venturing air system

As alternative to turbines which have near about similar specifications and are available in cheapest cost and size reduction format, a venturi air system is implemented for pumping of alcoholic liquid then it is released in a tank which is made by stainless steel. Air is immersed with involvement of air nozzle, through which air goes to the liquid. Air bubbles have similar specifications in dimensional format to turbines bubble (Haliu et al., 2012). For completion of complete treatment the venturing air system takes near about 30 times more time than a turbine system, but if looking towards quality attributes it has good sensorial as well as rich nutritional properties as compared with turbines. Because of the low amount of supply of air there is no effect on loss of flavour (Kocher et al., 2006). Vinegar obtained from these machines always has good characteristics in all kinds of manner as fruitier. The changes come after manual monitoring of acetic acid level with pump. Operation of replenishing is completed with outermost pump (Bhat et al., 2014). The machine is convenient to use, it comes in variable formats having a capacity of 20-66 L. The venturi air system is used over the world for vinegar production by those who want to take production in all scales (Tan, 2005; De Ory et al., 2004).

Submerged process 1

In the submerged process 1, alcohol was added for three weeks but the percent of acidity did not increase. The reason for failure could be that the solution was contaminated; excessive dilution of the ferment liquid by 50 mL addition of the dissolver solution per day or the bacteria may have died because of poor air supply. Following this, another fermenter was used to study the submerged acetification.

Submerged process 2

A complete diagram of all cycles in the lab scale submerged process can be seen in Figure 1. Mid-range culture solution was obtained from a commercial submerged unit Creole Fermentation Inc. (Abbeville, LA) to start the submerged process 2. The 1.87 gallons (8.5 L) of mid-range culture broth with 9.5% acidity and 3.35% of alcohol was added into the 2.2 gallon (10 L) fermenter and used at the beginning of the starting cycle.

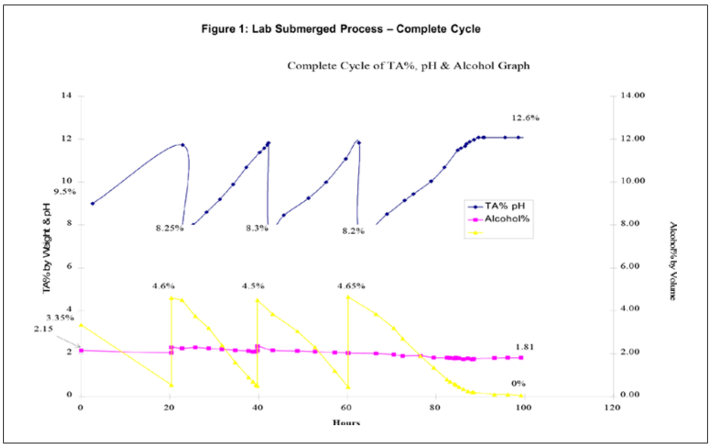


Figure 1. Lab submerged process – complete cycle.

Figure 2 shows the starting cycle of the submerged acetification. Acidity started at 9.5%, pH at 2.15 and alcohol at 3.35%. This is the mid-range of culture solution taken from the commercial submerged process tanks during the fermentation which explains why the initial alcohol content was 3.35%. The initial cycle begins with the mid-range unfiltered vinegar containing the culture source having an acidity of 8.5 to 9.5%. This ensures the bacteria are in the exponential growth phase in a suitable environment. In fact, the fermentation process continued to 12.25% acidity within 20.35 h with a pH drop to 2.05. Table 2 contains the theoretical and actual results.

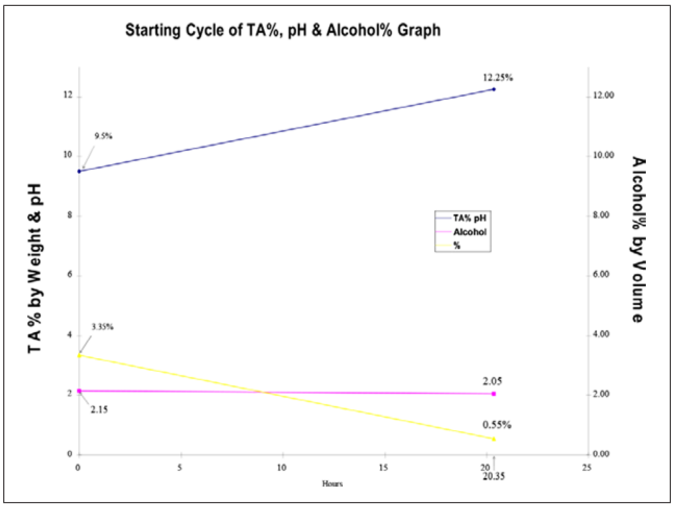


Figure 2. Starting unit vinegar fermentation submerged process (cycle begin).

Table 2. Lab submerged process physico chemical analysis.

Date	Hours	Volume (L)	TA[a] (%)	pH	Alcohol (%)	Temperature (°C)	Remark
June 6	0	8	9.5	2.15	3.35	31.5	Starting from big tank
June 7	20.35	8	12.25	2.05	0.55	31.5	Discharge 2.6
	0	8	8.25	2.29	4.6	31	
	2.4	7.8	8.5	2.25	4.5	31.2	
	5.4	7.6	9.1	2.29	3.75	31.3	
	8.4	7.5	9.7	2.25	3.2	31.3	
	11.4	7.4	10.4	2.21	2.4	31.5	
	14.4	7.2	11.2	2.16	1.6	31.5	
June 8	17.4	7	11.9	2.13	0.9	31.5	
	18.4	7	12.1	2.09	0.7	31.5	
	19.1	7	12.25	2.09	0.55	31.5	
	19.4	7	12.35	2.15	0.5	31.5	Discharge 2.6
	0	8	8.3	2.35	4.5	30	
	3.45	7.8	8.95	2.16	3.85	31.2	
	9	7.7	9.75	2.13	3.05	31.2	
	13	7.6	10.5	2.1	2.3	31.5	
	17.45	7.5	11.6	2.06	1.2	31.2	
	20.45	7.4	12.35	2.04	0.45	31.5	Discharge 2.6
	0	8	8.2	2.03	4.65	30.5	
June 9	6.3	7.9	9	2.01	3.85	31.2	
	10.3	7.8	9.65	1.96	3.2	31.2	
	12.3	7.7	9.95	1.9	2.7	31.3	
	16.3	7.5	10.55	1.9	1.9	31.2	
	19.3	7.2	11.2	1.82	1.35	31.4	
	22.3	7	12	1.81	0.8	31.4	
	23	7	12.1	1.8	0.7	31.4	
	24	6.9	12.2	1.78	0.6	31.4	Discharge 2.6
	24.3	6.8	12.3	1.81	0.55	31.4	
	25	6.7	12.4	1.79	0.45	31.4	
June 10	26	6.6	12.5	1.75	0.35	31.4	
	27	6.5	12.6	1.78	0.25	31.4	
	28	6.4	12.6	1.74	0.2	31.4	
	28.3	6.3	12.6	1.76	0.2	31.4	
	33	6.2	12.6	1.8	0.1	29.5	
	39	6	12.6	1.81	0.05	29.5	

[a]TA: titration acidity.

Theoretically, final acidity should have been 12.85% but the actual result was 12.25%. Some alcohol appears to have been lost at the end of the starting cycle which may be due to alcohol evaporating from the cap of the thermometer holder. After 20.35 h, the fermenter was discharged with 1/3 (2.6 L) of the volume being removed and replaced with fresh SM mash.

After new mash was added the acidity dropped to 8.25%, the pH rose to 2.29 and the alcohol content increased to 4.6%. The normal commercial standard of submerged process at the beginning of a cycle is 4.5 to 4.7% alcohols (Creole Fermentation, Inc. Abbeville, LA).

Figure 3 shows the first cycle of the submerged fermenter with acetic acid beginning at 8.25% and ending at 12.35% after 19.4 h. The pH dropped to 2.15 and the alcohol content dropped to 0.5%. After 19.4 h 1/3 (2.6 L) vinegar was discharged and the fermenter was recharged with another 2.6 L SM mash.

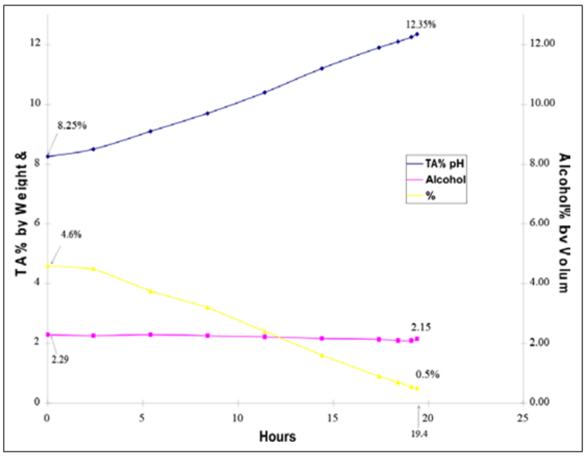


Figure 3. First cycle after the first discharged – submerged process.

Table 3 shows that theoretically final acidity should have been 12.85% but the actual result was 12.35%. The fixed leaking cap at the thermometer holder may have helped with the better recovery. The fermenter seems to be a very efficient process for vinegar production. This is because the environment is enclosed so that the fermentation is under control with little loss of volatiles. According to the results of the mass balance calculations shown in Table 3, the theoretical maximums were obtained. The second cycle is shown in Figure 4 and the results are similar to the first cycle (Table 4) but the final acidity reached 12.35% at 20.45 h. Table 5 shows the theoretical and actual results of the second cycle. The theoretical final acidity was 12.8% at the end of second cycle but the actual result was 12.35%. The alcohol dropped to 0.45% in 20.45 h. So, 4.05% alcohol had been converted to acid.

Table 3. After starting cycle, theoretically and actual result in submerged process.

Starting acidity (%)	Theoretical final acidity (%)	Actual final acidity (%)	Starting alcohol (%)	Theoretical final alcohol (%)	Actual final alcohol (%)
9.5	12.85	12.25	3.35	0.6	0.05

Table 4. First cycle, theoretically and actual result after added SM mash.

Starting acidity (%)	Theoretical final acidity (%)	Actual final acidity (%)	Starting alcohol (%)	Theoretical final alcohol (%)	Actual final alcohol (%)
8.25	12.85	12.35 at 19.4 h	4.6	0.5	0.5 at 19.4 h

Table 5. Second cycle, theoretically and actual result after added SM mash.

Starting acidity (%)	Theoretical final acidity (%)	Actual final acidity (%)	Starting alcohol (%)	Theoretical final alcohol (%)	Actual final alcohol (%)
8.30	12.8	12.35	4.5	0.45	0.45

Figure 5 shows the third cycle of the fermentation finishing in 39 h with an acidity of 12.6%. The acid did not change from 24 h on because the vinegar bacteria had exhausted the alcohol converting it to acid. The process was terminated at this point. The vinegar bacteria started to die because of lack of food supply and the liquid became clear after 39 h with the bacteria setting to the bottom of the fermenter.

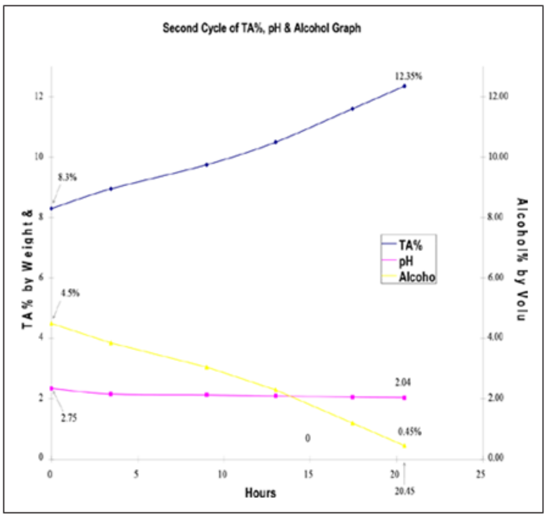


Figure 4. Second cycle of submerged process.

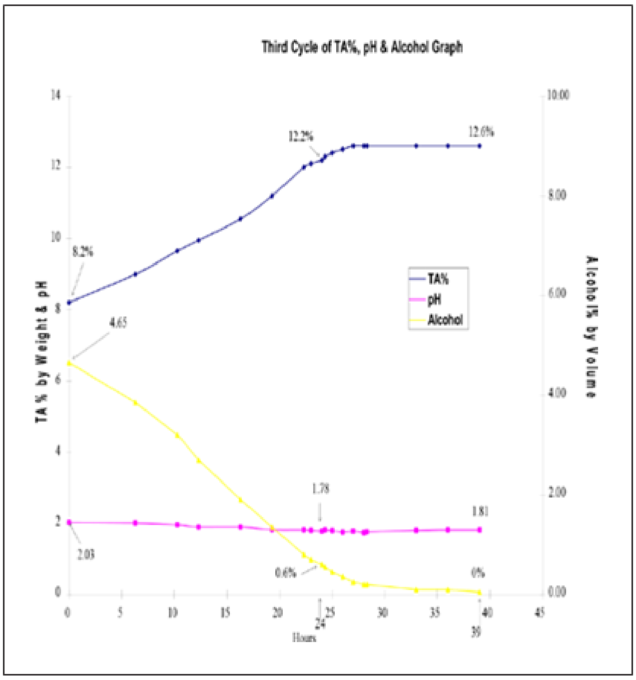


Figure 5. Third and final cycle – submerged process.

Submerged process 3

In the submerged process 3, the application of 2, 4 and 6% beech wood powder showed poor colour development and weak beech wood aroma. The final 8% beech wood powder application produced good colour and aroma and was chosen for detailed analysis. The GC-MS profile from the 8% sample was used for comparison with the lab scale submerged samples without powder and with the lab scale generator process.

CONCLUSIONS

The submerged process pilot unit was very efficient and produced vinegar with an acid strength of 12% or more. The highest acid strength produced by industry reported, so far, is 16%. In addition, this was closed process with controlled exposure of the fermenting liquid to air. This method minimizes the alcohol and acetic acid loss. The bacteria will grow easily in the aerated liquid under perfect conditions. The submerged process was easy to start compared to the other process. The yield in terms of final product (wine or vinegar) is acceptable as it was always well over 60%. We performed the whole process at the laboratory level, with such limiting factors as the strength of the press and the recovery of fruit pulp on a small scale. Scaling up to higher volumes and with industrial equipment will produce higher yields, similar to those observed in wine. The final product obtained in both cases showed good colour (yellow brown for dates) and good organoleptic characteristics. Dates vinegar had a good flavour and proved to be a very promising product. Further chemical characterization of products is under way. From the technological point of view, most of the protocols used in wine and wine vinegar production can be used to produce dates wine and vinegar. However, initial pressing of the fruit is not recommended because of the strength of the date's fruit. Although the alcoholic fermentation and acetification of dates proceeded at good rates and took a reasonable length of time, the use of selected starter cultures is recommended to shorten the time and increase the safety of the product. In our laboratory we are now analysing the possible use of native micro-biota associated to dates as starter cultures for both alcoholic fermentation and acetification.

It has been determined that date vinegar is an extremely useful food for human health from many aspects, such as lowering bad cholesterol, having antioxidant properties, preventing cancer, diabetes and cardiovascular diseases, due to phyto-chemicals and phosphorus it contains. In addition to all these, it is clear that date vinegar with functional features should be promoted in a way to reach the large masses and, necessary studies should be conducted to move the production from home conditions to industrial dimension. Furthermore, the total antioxidant and total phenolic values of date vinegar were found to be quite high. This feature is due to the fact that date fruit is rich in carotenoids, phytosterols, and group B vitamins. At present, most of the diseases are treated using chemical and synthetic drugs. These drugs have side effects that seriously threaten human health. This result directs human beings towards natural, herbal treatment methods. In particular, the antioxidant capacity of date vinegar content and high total phenolic ratio suggest that it can be used in the treatment of many diseases. Furthermore, the fact is that date vinegar is a strong source of phosphorus as determined in this study. It is known that the foods with the highest levels of phosphorus are in sea products, goat's milk, and dairy products. For people who do not consume these foods, date vinegar can be recommended as an alternative source.

Literature cited

Allgeier, R.J., and Hildebrandt, F.M. (1960). Newer developments in vinegar manufacture. Adv Appl Microbiol *2*, 163–182 https://doi.org/10.1016/S0065-2164(08)70125-4. PubMed

AOAC. (2000a). Official Methods of Analysis, 17th edn, 930.35(d) Vinegars (Washington: Association of Official Analytical Chemists).

AOAC. (2000b). Official Methods of Analysis, 17th edn, 985.19 (Washington: Association of Official Analytical Chemists).

AOAC. (2016a). Official Methods of Analysis, 20th edn, 930.15 (Washington, DC: Association of Analytical Chemists).

AOAC. (2016b). Official Methods of Analysis, 20th edn, 981.12 (Washington: Association of Official Analytical Chemists).

Bhat, S.V., Akhtar, R., and Amin, T. (2014). An overview on the biological production of vinegar. Int. J. Fermented Foods *3* (*2*), 139 https://doi.org/10.5958/2321-712X.2014.01315.5.

De Ley, J., Gillis, M., and Swings, J. (1984). Family VI. *Acetobacteriaceae*. In Bergey's Manual of Systematic Bacteriology, Vol. 1, N.R. Krieg, and J.G. Holt, eds. (Baltimore: The Williams and Wilkin Co.), p.267–278.

De Ory, L., Romero, L.E., and Cantero, D. (1999). Maximum yield acetic acid fermenter. Bioprocess Eng. *21* (*2*), 187–190 https://doi.org/10.1007/s004490050661.

De Ory, I., Romero, L.E., and Cantero, D. (2004). Operation in semi-continuous with closed pilot plant scale acetifier for vinegar production. J. Food Eng *63* (*1*), 39–45 https://doi.org/10.1016/S0260-8774(03)00280-2.

Fregapane, G., Rubio-Fernandez, H., Nieto, J., and Salvador, M.D. (1999). Wine vinegar production using a noncommercial 100-litre bubble column reactor equipped with a novel type of dynamic sparger. Biotechnol Bioeng *63* (*2*), 141–146 https://doi.org/10.1002/(SICI)1097-0290(19990420)63:2<141:AID-BIT2>3.0.CO;2-6. PubMed

Haliu, S., Admassu, S., and Jha, Y.K. (2012). Vinegar production – an overview. Food process engineering program. Brevage and Food World *3* (*2*), 139–155.

Haroun, M.I. (2006). Determination of phenolic and flavonoid profiles of some Flora land honey dew honeys produced in Turkey. Ph.D. thesis (Ankara, Turkey: Ankara University, Graduate School of Natural and Applied Sciences).

Kocher, G.S., Kalra, K.L., and Tewari, H.K. (2006). Production of vinegar from Cane juice. Paper presented at: Symposium on Food and Nutritional Security: Technological Interventions and Genetical Options (Palampur, India: HPKV).

Morales, M.L., Gustavo, A., Gonzalez, J.A., and Troncoso, A.M. (2001). Multi variety analysis of commercial and laboratory produced sherry wine vinegar: influence of acetification and aging. J Food Tech *212*, 676–682.

Tan, S.C. (2005). Vinegar fermentation. M.Sc. thesis (Baton Rouge: Louisiana State University, Dept. of Food Science), pp.101.

Taslipinar, E.S. (2018). Effect of vinegar production process on bioactivity of some traditional fruits. M.Sc. thesis (Istanbul, Turkey: Yildiz Technical University, Graduate School of Natural and Applied Sciences).

Unal, E. (2007). A study on vinegar production from Dimrit grape by different methods. M.Sc. thesis (Adana, Turkey: Cukurova University, Graduate School of Natural and Applied Sciences).

Voss, D.H. (1992). Relating colorimeter measurement of plant color to the Royal Horticultural Society Colour Chart. HortScience *27* (*12*), 1256–1260 https://doi.org/10.21273/HORTSCI.27.12.1256.

Engineering properties of date palm to design a tree lifter/climber service machine

M. Keramat-Jahromi[1,a] and A. Imani[2]

[1]Mechanical Engineering of Biosystems Department, Faculty of Agriculture, Shiraz University, Shiraz, Iran; [2]Department of Soil Science, Faculty of Agriculture, Urmia University, Urmia, Iran.

Abstract

One of Iran's most significant horticultural exports is the date fruit. The automation of date palm plants requires an understanding of the properties of date palms. The goal of this study is to examine the 'Shahani' date palm's engineering characteristics. The maximum crown height of the tree, which was determined by physical features independent of the tree's age, was found to be 3.15 m, whereas the maximum trunk height was 23.32 m. Without taking age into account, the greatest circumference was measured at the top, middle, and bottom to be 1.85, 2.5, and 2.6 m, respectively. Experimental tests revealed that the trunk's average flexural strength and flexural modulus were 269.467 and 6.917 Mpa, respectively. In the parallel compressive strength test, modulus and stress were 293.9 and 4.829 MPa, respectively, whereas the average stress in the perpendicular compressive strength test was 1.124 MPa. The hardness test's average maximum load value was determined to be 0.767 kN. Designing tree climbers and lifters may benefit from these findings.

Keywords: automation, robot, strength, date fruit

INTRODUCTION

Pruning, pollination, harvesting, de-thorning, pest control procedures, and other procedures are all necessary while caring for a palm tree. A person climbing the tree often carries out such tasks (Al-Suhaibani et al., 1988). A palm tree's height varies depending on its type and growing environment. In Jahrom, for example, the 'Shahani' type may grow to a height of 20 m (Keramat-Jahromi et al., 2007; Hashempour, 1999). Farmers often have a major labor shortage since it is risky to work at such a height (Keramat-Jahromi et al., 2008b). The automation of date palm activities may be improved with the use of an appropriate date tree service machine. Tree climbers and lifter machines are the two main categories that these devices fall under. Robots or machines that can climb are directly attached to the trunk and are using it for support. In date palm orchards when the palm is grown among other trees or where there is no specific planting design, it results in lower weight and size (Keramat-Jahromi et al., 2008b). Tree climbers are smaller, lighter, and less expensive than lifter machines that seek support from the ground since they use the trunk as support. Additionally, it makes it possible to use them in orchards with irregular planting and those where fruit trees and field crops are inter-cropped. The tree climbers can thus navigate in these circumstances due to their reduced size. The difficulty that resulted from haphazard planting, inter cropping with field crops and fruit trees, and not being able to maneuver past these machines in these orchards and limit the use of lifter machines. Despite the fact that these machines are durable, useful, and safe, they need a number of adjustments in conventional gardens, including rearranging the tree spacing and altering the irrigation system. Due to the aforementioned challenges, ideal equipment would be able to service orchards just as they are without needing any modifications (Al-Suhaibani et al., 1988). The suggested equipment must also be available at a reasonable price (Ali and Akyurt, 1998). Numerous studies have been done to create various kinds of date palm climbers and date palm lifter machines (Davis, 1997; Shamsi, 1998; Keramat-Jahromi et al., 2008b; Nourani and Pegna, 2014; Nourani et al., 2017).

[a]E-mail: mahdikeramat@shirazu.ac.ir

Ali and Akyurt (1998) suggested several machine system concepts for doing crown-related activities. Three alternative gripper robot concepts were put forward by Sclater and Chironis (2001). They demonstrated a reciprocating lever system that enables a gripper robot to pick up and drop things by opening and closing its jaws. Trends in the automation of date palm plants were examined (Nicklin, 1993; Mostaan, 2016). An essential necessity for a date palm climber or a date palm lifter machine is the ability to reach the typical height of a palm. A tree climber must also be able to carry a heavy weight (Mazlumzadeh and Shamsi, 2003). The maximum weight of a robot or tree climber relies on the highest applied stress that the tree can withstand. Additionally, tree height and diameter affect the safe weight. For trees with a diameter between 0.40 and 0.60 m. The maximum load (critical mass results due to buckling) was determined; it ranged between 144.22 and 730.10 kg, respectively (Keramat-Jahromi et al., 2008b). Providing simple and secure access to the tree top as well as ensuring the operator's safety were considered essential components of a date palm servicing machine. Several factors, including as height, inter-tree distance, trunk diameter, bunch spacing, and ground contour (without taking into account the type and age of the tree) were assessed in a research carried out in Saudi Arabia on 19 farms to create a climbing service machine (Al-Suhaibani et al., 1988). The mechanical characteristics of date palm trees were however not been extensively studied (Amirou et al., 2014; Keramat-Jahromi et al., 2008a; Kriker et al., 2005). In order to develop tree lifter machines or climbing robots/machines, this study evaluated the engineering characteristics of date palm trees using one of the tallest (highest) and most significant date palm cultivar 'Shahani'.

MATERIALS AND METHODS

The present study was conducted at Jahrom, one of Iran's most significant horticultural hubs. Regardless of the age of the trees, the physical characteristics of 100 palm trees, including tree height, diameter of a trunk in different portions, and crown height, were measured. Tree trunk samples were moved to the wood craft section to create standard samples for each investigation. Every mechanical experiment was carried out three times using the ASTM 143-Secondary technique at a constant temperature of 25°C. The Instron testing machine was used to perform trunk trials, including compressive strength tests parallel and perpendicular to the trunk fibers, flexural strength tests, and hardness tests (model 4486). For flexural strength and hardness investigations, the Instron testing machine's lowest and maximum load cell capacities were 10 and 300 kN, respectively. For each experiment, three cube-shaped samples were created in order to gauge the trunk's density and moisture content. A mass-volume connection was used to compute the cubic samples' densities. As moisture is lost, wood strength decreases (Parsapajuh, 1988). The samples were placed in an oven set at 105°C for 3 days to measure the moisture content. By using the AOAC (1984) suggested approach, weight loss during drying to a final constant weight was measured as moisture content. Cubic samples of dimension 2×2×28 cm^3 were provided to measure the flexural strength. There were two Instron supports for each sample. The samples were loaded in the center and spaced out by 24 cm. Each sample was loaded at a rate of 10 mm min^{-1}. A faint noise caused by rupture caused the loading of the samples to cease, and the load-displacement diagram was horizontal. To determine the compressive strength tests; parallel and perpendicular to trunk fibers, samples of dimension 5×5×15 cm^3 were provided. In the parallel compressive strength test, samples that were placed longitudinally between Instron supports were subjected to a compressive force at a rate of 1.2 mm min^{-1}. In the case of the perpendicular compressive strength test, the load was imposed on the 5×15 cm^2 section of the sample and the loading area was 5×5 cm^2. The loading rate was 0.3 mm min^{-1}.

The samples of dimension 5×5×15 cm^3 were provided to accomplish the hardness test. A metal shot of diameter 11.3 mm, the projected area of 1 cm^2, and the penetration rate of 6 mm min^{-1} was applied to penetrate the sample, which were subjected to increasing load. The test was stopped when the shot penetrated the sample to its radius.

RESULTS AND DISCUSSION

According to measurements, the trunk's average density and moisture content were found to be 1.165 g cm^3 and 234.48% (d.b.), respectively. Flexural strength test findings showed that the trunk's average flexural strength and flexural modulus were 6.917 and 269.467 MPa, respectively. In the parallel compressive strength test, the average elastic modulus and yield stress were measured as 293.9 and 4.829 MPa, respectively, while the stress at the proportional limit in the perpendicular compressive strength test was 1.124 MPa. The hardness test's maximum load was determined as 0.767 kN. Wood strength was inversely correlated with moisture content and decreases when moisture was lost (Parsapajuh, 1988). The trunk's highest point was 23.32 m tall (regardless of the age of the tree). The greatest and lowest values of 17.43 and 1.00 m were found on several farms in a research done in Saudi Arabia, while the average tree height (independent of variety and age of the tree) varied between 4.38 and 9.82 m (Al-Suhaibani et al., 1988). In another research, measured tree heights in orchards in Kerman Province, Iran, varied between 6.5 and 17 m, with an average value of 10.3 m, independent of the age of the trees (Mazlumzadeh and Shamsi, 2003). The average elevation for 'Shahani' palm trees 45 years old was 10.32 m (Keramat-Jahromi et al., 2007). Trees older than 100 years were usually found at higher altitudes (Rohani, 1998) and may grow up to 10.2 m tall, (Ahmed et al., 1992). When developing date palm service machines, height is a crucial consideration. At the bottom, the diameter was measured as 2.6 m, while the mid-section and top were measured as 2.5 and 1.85 m, respectively and 2.15 m was judged to be the maximum crown height. In Saudi Arabia, the highest circumference was 2.56 m (independent of the tree's cultivar or age) (Al-Suhaibani et al., 1988), and 1.72 m was recorded in another study (Ahmed et al., 1992). Another research finds that the greatest diameters of the palm kinds it picked were 85 cm on the ground level and 64 cm beneath the crown (Mazlumzadeh and Shamsi, 2003). The diameter of the palm tree trunk may be utilized as a platform for developing lifter equipment or as a support for climbers. The bottom circumference measurements varied between 1.60 and 2.50 m (without taking age into account), with 1.94 m being the reported average. The top circumference measurements varied between 1.28 and 2.40 m, with an average of 1.72 m (Keramat-Jahromi et al., 2007) and 3.15 m was the maximum crown height recorded. Ahmed et al. (1992) in their studies on the crown length which was measured in several farms reported a variation of 0.68 to 1.2 m while the crown height varies between 1.32 and 3.09 m with an average value of 2.01 m (Keramat-Jahromi et al., 2007).

CONCLUSIONS

Designing a lifter/climber machine requires thorough knowledge of both the mechanical and physical features of palm trees. The trunk's highest point was 23.32 m tall (regardless of the age of the tree). Special mechanisms must be taken into consideration in order to preserve the safety of tree climbers or climbing machines, safeguard the machine and the safety of the likely operator. The lowest leaf in the crown may be reached using a climbing platform or tree climber. A technique to reach the fruits for harvesting should be taken into consideration given that this machine can go as far as it can below the lowest leaves. The findings supported the assertion that parallel compressive strength is greater than perpendicular compressive strength. It is vital to conduct an extensive investigation on many date palm kinds since the physical and mechanical qualities of each cultivar varies from others.

Literature cited

Ahmed, A.E., Alyhassan, O.S., and Khalil, M.M. (1992). Surveying of some date palm parameters and properties to be utilized in date palm mechanization. Agricultural Mechanization in Asia and Africa and Latin America *23* (*2*), 67–69.

Al-Suhaibani, S.A., Babeir, A.S., Kilgour, J., and Flynn, J.C. (1988). The design of a date palm service machine. J. Agric. Eng. Res. *40* (*2*), 143–157 https://doi.org/10.1016/0021-8634(88)90111-4.

Ali, T., and Akyurt, M. (1998). Design of a Hi-lifter gripper for palm trees. Appl. Eng. Agric. *14* (*3*), 215–221 https://doi.org/10.13031/2013.19379.

Amirou, S., Zerizer, A., and Pizzi, A. (2014). Investigation of chemical, physical and mechanical properties of Algerian date palm wood. Mater. Test. *56* (*3*), 236–240 https://doi.org/10.3139/120.110548.

AOAC. (1984). Official Methods of Analysis, 14th edn (Washington, DC: Association of Official Analytical Chemists).

Davis, A.T. (1997). Attempt at mechanical climbing of palms with special reference to the coconut palm. J. Plant. Crops *5* (*1*), 31–35.

Hashempour, M. (1999). Dates Treasure, Vol. 1: Generalities (Tehran, Iran: Nashr-e-Amozeshe-Keshavarzi) (in Farsi).

Keramat-Jahromi, M., Jafari, A., Rafiee, S., and Mohtasebi, S.S. (2007). A survey on some physical properties of date palm tree. Agric. Technol. Thail. *3* (*2*), 317–322.

Keramat-Jahromi, M., Jafari, A., Mohtasebi, S.S., and Rafiee, S. (2008a). Engineering properties of date palm trunk applicable in designing a climber machine. Agricultural Engineering International: the CIGR Ejournal Vol. X, Manuscript FP 08 002.

Keramat-Jahromi, M., Mirasheh, R., and Jafari, A. (2008b). Proposed lifting model for gripper date palm service machines. Agricultural Engineering International: the CIGR Ejournal Vl. X, Manuscript PM 08 018.

Kriker, A., Debicki, G., Bali, A., Khenfer, M.M., and Chabannet, M. (2005). Mechanical properties of date palm fibres and concrete reinforced with date palm fibres in hot-dry climate. Cement Concr. Compos. *27* (*5*), 554–564 https://doi.org/10.1016/j.cemconcomp.2004.09.015.

Mazlumzadeh, S.M., and Shamsi, M. (2003). Evaluation and determination of mechanization engineering parameters for date palm harvesting. Paper presented at: Third Nation Congress on Agricultural Machinery and Mechanization (Shahid Bahonar University of Kerman) (in Farsi).

Mostaan, A. (2016). Framework to develop the mechanisation of date palm cultivation. Biosyst. Eng. *147*, 26–38 https://doi.org/10.1016/j.biosystemseng.2016.02.016.

Nicklin, C.D. (1993), A review of past and current date harvesting mechanization to investigate the concept of designing a palm tree climber. Unpublished B. Eng thesis (UK: Harper Adams Agricultural College).

Nourani, A., and Pegna, F.G. (2014). Proposed harvester model for palm date fruit. Agric. Technol. Thail. *10* (*4*), 817–822.

Nourani, A., Kaci, F., Pegna, F.G., and Kadri, A. (2017). Design of a portable dates cluster harvesting machine. AMA Agric. Mech. Asia Afr. Lat. Am. *48* (*1*), 18–21.

Parsapajuh, D. (1988). Wood Technology, 2nd edn (Iran: Tehran University Publications) (in Farsi).

Rohani, A. (1998). Date (Tehran, Iran: Markazenashre Daneshgahi).

Sclater, N., and Chironis, N. (2001). Mechanisms and Mechanical Devices Sourcebook (McGraw-Hill).

Shamsi, M. (1998). Design and development of a date harvesting machine. Ph.D. thesis (UK: Silsoe College, Cranfield University).

Analytics and comparative study of date fruit by-product quality

O. Laout[1,a], K. Jdaini[1], K. Guerrouj[2] and M.A. Elhoumaizi[1,b]

[1]Laboratory for Agricultural Productions Improvement, Biotechnology and Environment (LAPABE), Faculty of Sciences, University Mohammed First, BP-717, 60000 Oujda, Morocco; [2]Higher Institute of Health Professions and Nursing Skills, 60000 Oujda, Morocco.

Abstract

Different date by-products samples including syrup, jam, paste, seed, powder and seed coffee, were collected from Moroccan oases and different physico chemical properties (pH, moisture, total sugars, and dry matter) and microbiological analysis were performed. Results registered highest moisture levels among jam and paste samples, reaching 35.96±8.65 and 22.94±5.27%, respectively, while date powder, seed coffee and seed powder presented the lowest moisture levels, ranging between 2.5±1.07 and 4.65±1.59%. pH of all samples was slightly acidic and varies from 4.56±0.10 to 5.35±0.87. Date syrup samples had the highest total sugar contents, while seed coffee presented the lowest amount of total sugar. This diversion may be due to date cultivars used for production, and to different date by-products production process. Enumeration of spoilage microorganisms and pathogenic bacteria has shown that all of the samples are micro-biologically compliant. Pathogenic bacteria (coliforms and *Staphylococcus aureus*) were not detectable in all samples. Spoilage microorganisms (TVC, molds and yeasts and *Bacillus*) were indistinguishable in syrup, jam and powder. Low moisture levels, acidic pH and addition of aromas and phenolic compounds during production process provide better protection against spoilage microorganisms. However, traditional production process of date by-products in Morocco still presents hygienic defects, inciting its structure for maximal valorization of dates in Morocco.

Keywords: date, by-products, spoilage, quality, microbiological, physico-chemical

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most involved trees in crop protection and ecological system in dry lands, moreover, its fruits constitute one of the main sources of income and economic prosperity for oasian people. Morocco is among the sixth largest producer of dates. There are numerous oases, mostly distributed in the provinces of Ouarzazate, Errachidia, Tata, Tiznit, Guelmim, Figuig, Marrakech and Agadir (Hasanaoui et al., 2010) with an area allocated to its production estimated by 50,000 ha, with more than 4.8 million trees, and over 100,000 t of dates year^{-1}; in which 25% are considered of high quality ('Medjool', 'Boufeggous', 'Bouskri', and 'Aziza Bouzid'), 35% of medium quality. While the 40% remaining are classified as low-quality dates.

Valorization technology may be the best way to fully exploit low-quality dates, improve their marketing presentation and increase their consumption. Indeed, Moroccan dates are characterized by an excellent transformation aptitude, due to their physico-chemical characteristics, their various consistencies (soft, semi-soft and dry), their organoleptic properties (aromas, flavours, colors, etc.) and their quantities of sugars and fibers (Harrak et al., 2005; Harrak, 2007).

In Morocco, oasian population traditionally valorize a part of dates production into various by-products (juice, paste, syrup, powder, etc.), using their own ingenious skills. Besides being badly handled, these traditional methods are in serious danger of

[a]E-mail: ouahibalaout@gmail.com,
[b]E-mail: elhoumaizi@yahoo.fr

disappearance. Indeed, people practicing and transmitting their knowledge are increasingly rare, and modern actions to promote local products are largely deficient (Zirari et al., 2005).

The main aim of this study is to characterize different dates by products (paste, syrup, coffee and powder) collected from different oases in Morocco (Figuig, Errachidia and Tata), based on their physico-chemical and microbiological parameters. Thus, allowing future research in terms of the optimization of the traditional process.

MATERIALS AND METHODS

By-products samples

Twenty-five samples of date by-products were collected from Moroccan oases: 15 products from Figuig region (5 syrups, 3 pastes, 2 date seed coffees, 2 date seed powder and 2 date powder); 3 from Errachidia (2 jams, 1 syrup); 5 from Tata (2 syrups, 1 paste, 1 coffee, and 1 jam) and 1 paste from UAE. Samples were placed in a refrigerator at 4°C before examination.

Physico-chemical analysis

Samples' pH was measured by using an electronic pH-meter (pH211, Hanna instrument). Dry matter was obtained following the method described by the Association of Official Analytical Chemists (AOAC, 1990). Moisture was calculated by subtract dry matter percentage from 100.

Sugars extraction was performed by centrifuging 2 g of date pulp at 5000 rpm followed by a clarification and filtration. Reducing sugar content was quantified by Dinitro salicylic acid (DNS) colorimetric method (Miller, 1959). Total soluble sugars were determined according to DUBOIS method (DuBois et al., 1956). Sucrose content was calculated from sucrose hydrolysis reaction following the equation below and results were expressed on dry matter basis.

$$\% \, Sucrose = \frac{(\% \, total \, sugars - \% \, reducing \, sugars)}{0.95}$$

Microbiological analysis

Each sample was weighed into sterile stomacher bags, which were then filled with 90 mL sterile peptone water and homogenized for 30 s. Serial decimal dilutions were then made, and 0.1 mL of each dilution was spread onto solid culture medium in Petri dishes (spreading method). Plate count agar (BIOKAR, France) was used as the TVC culture medium, which was incubated at 30°C for 48 h. Total coliforms and fecal coliforms were counted on violet red bile lactose agar (BIOKAR, France), incubated at 37 and 44°C, respectively, for 48 h. For lactic acid bacteria (LAB) detection, the Man Rogosa and Sharpe medium (BIOKAR, France) was considered, and incubation was made for 72 h at 30°C. The Baird and Parker medium (BIOKAR, France) was used for enumeration of *Staphylococcus aureus* (48 h for 30°C).

Yeasts and molds were counted on Oxytetra cycline glucose agar medium (BIOKAR, France), incubated at 25°C for 72 h.

For *Bacillus* enumeration, pre-heating of samples was made at 80°C for 15 min, before serial dilutions preparation. Dilutions were poured onto PCA medium (BIOKAR, France), and incubated at 30°C for 48 h. Microbiological tests were done in triplicate, and data obtained were expressed as colony forming units g^{-1} of sample.

RESULTS AND DISCUSSION

Physico-chemical characteristics of dates by-products

The approximate composition and physico-chemical characteristics from studied date by-products are represented in Table 1. Analysis of physico-chemical parameters revealed very significant differences among studied date by-products.

Table 1. Physico-chemical characteristics.

Date by-product	pH	Dry matter (%)	Moisture (%)	Sugar (g 100 g^{-1})
Powder	5.05±0.5	97.49±1.07	2.5±1.07	42.98±0.62
Syrup	4.51±0.19	78.79±14.8	21.20±14.82	51.74±8.49
Paste	4.74±0.19	77.06±5.27	22.94±5.27	43.25±2.30
Seed coffee	4.56±0.10	95.35±1.59	4.65±1.59	3.26±1.07
Jam	4.87±0.23	64.04±8.65	35.96±8.65	19.86±6.38
Seed powder	5.35±0.87	95.08±0.16	4.92±0.169	8.45±0.07

Moisture levels in dates and date by-products are an important parameter affecting their quality. Results obtained showed high moisture values among jam and syrup samples; reaching 35.96±8.65 and 21.2±14.82%, respectively, while values below 2.5±1.07% were noticed for powder. Same findings were reported by Al-Hooti et al. (2002) and Al-Farsi et al. (2007), stating that date jam presents moisture values above 35.96±8.65%. High or low levels of by-products moisture may be correlated to moisture values of dates used for the production. In fact, date fruits are classified to soft-dates (more than 30% of moisture) and to semi-dry dates (20-30% of moisture) (Elleuch et al., 2008; Amira et al., 2011). The global stability (biochemical and microbiological) of a food product such as date by-products need a water content control, for preservation against microbial spoilage (Belarbi et al., 2000).

Dry matter content registered highest values among powder and coffee (97.49 and 95.35%, respectively), followed by syrup, paste and jam (78.79, 77.06 and 64.04%, respectively). Same results were found by Haddia et al. (2014), reporting that dry matter values of date syrup are much higher than those of dates.

pH analysis revealed non-significant differences between date by-products categories. Hydrogen potential of studied samples varied from 4.51 to 5.35. This acidity may be contributed to organic acid formation (citric, malic and oxalique acid) during storage. In fact, low range of pH allows protection of date by-products from microbial and enzymatic spoilage (Harrak et al., 2018). According to Al-Ghanam et al. (2005), acidity confers better stabilization to date pastes.

Date syrup samples present the highest total sugars contents (51.74 g 100 g^{-1}) (Table 1). Same outcomes were reported by Al-Hooti et al. (2002) and El-Nagga and El-Tawab (2012) in date syrups prepared from other cultivars, dominated by glucose and fructose. Date pastes also had high total sugar amounts (43.25 g 100 g^{-1}), but lower than those reported by Sánchez-Zapata et al. (2011), which may be attributed to cultivar characteristics and traditional preparation methods of date pastes used in Morocco. All of the date jams in the present study showed low amount of total sugar (19.86 g 100 g^{-1}) comparing to jams obtained from three Tunisian date cultivars, ranging between 82.8 and 90.7 g 100 g^{-1} for total sugar (Besbes et al., 2009). Dates powder samples present high quantity of total sugars (42.98 g 100 g^{-1}), which is very interesting if we add low pH and low moisture level (4.55 and 1.44%, respectively). In fact, such characteristics contribute widely to inhibit development of spoilage microorganisms, and increase dates powder shelf life despite presence of high amount of sugars. Seed coffee and seed powder showed the lowest amount of total sugars (3.26 and 8.45 g 100 g^{-1}, respectively) and lowest moisture values (4.65 and 4.92%, respectively), ensuring protection against spoilage microorganisms.

Microbiological quality of dates by-products

Date by-products samples were collected from different traditional production areas in Morocco. Enumeration of spoilage microorganisms and pathogenic bacteria (Table 2) showed that all samples were micro-biologically compliant. Pathogenic bacteria (coliforms and *Staphylococcus aureus*) were not detectable. Spoilage microorganisms (TVC, molds and yeasts and *Bacillus*) are indiscernible in syrup, jam and powder. According to Harrak et al. (2005), dates syrup has good microbiological quality, due to acidification and addition of flavours and phenolic compounds, having antimicrobial effects and ensuring protection against microbial spoilage during the storage.

Table 2. Microbiological analysis.

Origin	Type	TVC	Molds	Yeasts	Coliforms	*S. aureus*	*Bacillus* sp.	LAB
Figuig	Syrup	<1	<1	<1	<1	<1	<1	<1
	Powder	<1	<1	<1	<1	<1	<1	<1
	Seed powder	23	<1	<1	<1	<1	<1	<1
	Seed coffee	<1	<1	<1	<1	<1	<1	<1
	Paste	103	5	93	<1	<1	<1	<1
	Syrup	<1	<1	<1	<1	<1	<1	<1
	Seed powder	<1	<1	<1	<1	<1	<1	<1
	Syrup	<1	<1	<1	<1	<1	<1	<1
	Powder	6.4	<1	<1	<1	<1	5.2	<1
	Paste	342	<1	168	<1	<1	268	<1
	Syrup	6.4	<1	<1	<1	<1	4	<1
	Paste	15.2	12.4	<1	<1	<1	<1	<1
	Syrup	<1	<1	<1	<1	<1	<1	<1
	Powder	17.4	<1	<1	<1	<1	<1	<1
	Seed coffee	<1	<1	<1	<1	<1	<1	<1
Errachidia	Jam	<1	<1	<1	<1	<1	<1	<1
	Jam	<1	<1	<1	<1	<1	<1	<1
	Syrup	<1	<1	<1	<1	<1	<1	<1
Tata	Paste	9.4	<1	<1	<1	<1	<1	<1
	Seed coffee	<1	<1	<1	<1	<1	<1	<1
	Jam	<1	<1	<1	<1	<1	<1	<1
	Syrup	<1	<1	<1	<1	<1	<1	<1
	Syrup	<1	<1	<1	<1	<1	<1	<1
UAE	Paste	<1	<1	<1	<1	<1	<1	<1

Results are expressed as CFU g^{-1} of samples.

However, a slight growth was identified among date pastes from Figuig, which may be assigned to high moisture among pastes and probable contamination during their preparation. The Codex Stan (2013) advocate that moisture rate among dates paste should not exceed 20%. In the present study, pastes from Figuig present a moisture rate of 29.04%, which is high enough to allow the growth of non-required molds, yeasts and bacteria, especially if the product is inadequately stored.

According to Harrak et al. (2018), traditional preparation of date paste is completely simple, in which selected, washed and drained dates are vigorously kneaded by hand. At macroscopic level, authors reported that pastes contain unwanted foreign particles: date cores, sand particles and filaments. Enumeration of the micro-flora among these pastes revealed a high load of TVC and fungi ($3.32\ 10^5$ to $4.70\ 10^5$ CFU g^{-1} and $5.49\ 10^2$ to $8.00\ 10^3$ CFU g^{-1}, respectively), showing that hygienic quality of pastes traditionally prepared is insufficient, due to the non-respect of adequate hygienic and preparation conditions (Harrak et al., 2018).

CONCLUSIONS

Results obtained in this study showed that physico-chemical characteristics of dates by-products (pH, moisture and total sugar) significantly contribute in their protection against spoilage microorganisms. However, traditional process of fabrication has some defects concerning hygienic side, which can affect microbial conformity of dates by-products, especially during storage. Thus, its strongly recommended to structure traditional production process of dates by-products, which allowed maximal valorization of dates in Morocco.

Literature cited

Al-Farsi, M.A., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., and Al-Rawahy, F. (2007). Compositional and functional characteristics of dates syrups and their by-products. Food Chem. *104* (*3*), 943–947 https://doi.org/10.1016/j.foodchem.2006.12.051.

Al-Ghanam, M., Al-Arifi, I.B., and Al-Amer, R.B. (2005). Étude des variations physico-chimiques et microbiologiques dans la pâte de datte et recherche de technique pour sa conservation. Paper presented at: Symposium International sur le Développement Agricole durable des Systèmes Oasiens (Erfoud, Morocco: INRA).

Al-Hooti, S.N., Sidhu, J.S., Al-Saqer, J.M., and Al-Othman, A. (2002). Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. Food Chem. *79* (*2*), 215–220 https://doi.org/10.1016/S0308-8146(02)00134-6.

Amira, E.A., Guido, F., Behija, S.E., Manel, I., Nesrine, Z., Ali, F., Mohamed, H., Noureddine, H.A., and Lotfi, A. (2011). Chemical and aroma volatile compositions of date palm (*Phoenix dactylifera* L.) fruits at three maturation stages. Food Chem. *127* (*4*), 1744–1754 https://doi.org/10.1016/j.foodchem.2011.02.051.

AOAC. (1990). Official Methods of Analysis, 15th edn (Washington, D.C., USA: Association of Official Analytical Chemists).

Belarbi, A., Aymard, C., Meot, J., Themelin, A., and Reynes, M. (2000). Water desorption isotherms for eleven varieties of dates. J. Food Eng. *43* (*2*), 103–107 https://doi.org/10.1016/S0260-8774(99)00138-7.

Besbes, S., Drira, L., Blecker, C., Deroanne, C., and Attia, H. (2009). Adding value to hard date (Phoenix dactylifera L.): Compositional, functional and sensory characteristics of date jam. Food Chem. *112* (*2*), 406–411 https://doi.org/10.1016/j.foodchem.2008.05.093.

Codex Stan. (2013). Norme Régionale pour la Pâte de Dattes (Proche Orient): CODEX STAN 314R-2013.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. *28* (*3*), 350–356 https://doi.org/10.1021/ac60111a017.

El-Nagga, E., and El-Tawab, Y.A. (2012). Compositional characteristics of date syrup extracted by different methods in some fermented dairy products. Ann. Agric. Sci. *57* (*1*), 29–36 https://doi.org/10.1016/j.aoas.2012.03.007.

Elleuch, M., Besbes, S., Roiseux, O., Blecker, C., Deroanne, C., Drira, N.E., and Attia, H. (2008). Date flesh: chemical composition and characteristics of the dietary fibre. Food Chem. *111* (*3*), 676–682 https://doi.org/10.1016/j.foodchem.2008.04.036.

Haddia, N., Mennane, Z., Charof, R., Berny, E., Mardhy, A., and Kerak, E. (2014). Etude de la qualité d'un dérivé de dattes Marocaines (cas de Tahlaoute). IJIAS *8*, 990–998.

Harrak, H. (2007). Archivage, analyse et amélioration du savoir-faire traditionnel oasien: cas du jus de dattes. Thèse de Doctorat des Sciences Agronomiques, pp.233.

Harrak, H., Reynes, M., Lebrun, M., Hamouda, A., and Brat, P. (2005). Identification et comparaison des composés volatils des fruits de huit variétés de dattes marocaines. Fruits *60* (*4*), 267–278 https://doi.org/10.1051/fruits:2005033.

Harrak, H., Hamouda, A., and Nadi, M. (2018). Évaluation et amélioration de la qualité des pâtes traditionnelles de dattes, produits du terroir des oasis. Cah. Agric. *27* (*1*), 15001 https://doi.org/10.1051/cagri/2017057.

Hasanaoui, A., Elhoumaizi, M., Hakkou, A., Wathelet, B., and Sindic, M. (2010). Physico-chemical characterization, classification and quality evaluation of date palm fruits of some Moroccan cultivars. J. Sci. Res. *3* (*1*), 139–149 https://doi.org/10.3329/jsr.v3i1.6062.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. *31* (*3*), 426–428 https://doi.org/10.1021/ac60147a030.

Sánchez-Zapata, E., Fernández-López, J., Peñaranda, M., Fuentes-Zaragoza, E., Sendra, E., Sayas, E., and Pérez Alvarez, J.A. (2011). Technological properties of date paste obtained from date by-products and its effect on the quality of a cooked meat product. Int. Food Res. J. *44* (*7*), 2401–2407 https://doi.org/10.1016/j.foodres.2010.04.034.

Zirari, A., Chetto, A., Harrak, H., Lalaoui, Y., and Outlioua, K. (2005). Diagnostic participatif de la diversité génétique du palmier dattier et de son utilisation dans le Drâa: cas de l'oasis de Fezouata. Paper presented at: Symposium International sur le Développement Agricole Durable des Systèmes Oasiens (Erfoud, Morocco: INRA).

Status of the date palm industry in the United States

G.C. Wright[a]

Yuma Mesa Agriculture Center, University of Arizona, Yuma, AZ, USA.

Abstract

Despite being ranked 16th in world date production, the United States has a vibrant and progressive date palm industry. In 2020, almost 57,000 MT were produced, mostly of the 'Deglet Noor' and 'Medjool' cultivars, valued at almost USD$ 200,000,000. Domestic date consumption is slowly increasing, while a significant portion of the crop is exported. Imports play a key role as well. This presentation will include the most up-to-date production, consumption, import and export statistics. Details of the actions that US date producers are taking to decrease production costs and increase sustainability will be presented. Also, results from a survey of consumer preferences and subsequent measures that are being launched to increase domestic consumption of fresh fruit and value-added products will be highlighted. Finally, the status of several insect and disease pests that threaten the industry will be detailed.

Keywords: cultivars, horticultural practices, insect pests, diseases, trade, marketing

HISTORY, CULTIVARS AND EXTENT

Spanish priests were the first to introduce the date palm to the US in the late 1700s (Toumey, 1898; Trent and Seymour, 2010). Palms were planted across both California and Arizona wherever the climate was favorable (Wright, 2016). These early plantings came from seed. However, US date consumption in the late 1800s and early 1900s exceeded the domestic supply, so most dates were imported(Hopper, 2013). As early as the 1820s American ships carried US goods to ports on the Arabian Peninsula, and in exchange they picked up dates and other commodities. By 1925, US date imports exceeded 8,000 MT.

In response to the demand and the need to find suitable crops for the desert, the United States Department of Agriculture (USDA) organized importations of thousands of date palm offshoots, chiefly from Algeria, Morocco, Tunisia, Egypt and Iraq to California and Arizona (Toumey, 1898; Nixon, 1950; Hilgeman, 1972). Private individuals also imported thousand more. Many cultivars were imported by 1910, including 'Barhi', 'Deglet Noor', 'Fard', 'Hayany', 'Khadrawy', 'Sayer' and 'Zahidi'. 'Medjool' was imported in 1927 (Wright, 2012).

Today, the US date industry encompasses almost 6,700 bearing ha, all in California and Arizona (USDA, 2021). This author estimates that there are at least another 1000 ha of non-bearing trees. Over 90% of the acreage is 'Deglet Noor' and 'Medjool', with lesser amounts of 'Barhi', 'Khadrawy', 'Zahidi' and other imported and local cultivars. About 75% of the US date palms are grown in California, where about 55% of the palms are 'Deglet Noor', 40% are 'Medjool', and 5% other cultivars. Arizona produces exclusively 'Medjool' and comprises the other 25% of US palm production.

Horticultural practices

Dates are planted on both alluvial river-bottom soil and sandy upland soil. Since both 'Deglet Noor' and 'Medjool' make many offshoots, tissue culture-derived palms are not common. Traditional planting of dates is about 9×9 m square, but newer plantings are being established at a density of 8×8 m, and 7.5×7.5 m. One male palm is planted for every 49 females. There are no generally accepted male palms identified as superior pollinators.

Growers employ either flood or pressurized drippers or micro-sprinklers to irrigate the palms. Irrigation rates vary by tree size and season and can be as much as 500 to 600 L tree^{-1} day^{-1} in the summer, and as much as 200 m^3 annually tree^{-1}. For flood, irrigation timings range

[a]E-mail: gwright@ag.arizona.edu

from once every 11 days to once a month, depending on the soil type, age of the tree and time of year. For pressurized irrigation, timings range from every day to once a week depending on the factors noted above (Takele et al., 2005). Since the western US is suffering an ongoing drought, there is a research project at the University of Arizona to quantify the daily water needs of mature dates.

Conventional fertilizers are applied through the irrigation water. Most growers consistently apply nitrogen, potassium, and boron. Some also apply magnesium. Other macro- and micro-nutrients are not as commonly applied. Organic dates receive only composted or liquid chicken or composted steer manure. Cover crops, such as 'Lana' vetch (*Vicia villosa*) are used in the winter in California. Where weeds are a problem, they are disked or controlled with glyphosate.

Workers clean the 'Medjool' palms in January, removing thorns and old leaves. 'Deglet Noor' palms may be cleaned in January or in July. Male palms bloom in early March, and once the spathe cracks, the male flowers and pollen are extracted and dried with fans and heat. Growers may dilute pollen with flour, talc, or cornstarch. Female palms bloom in mid-to late March, and a mature palm will produce 20 to 25 bunches. Each emerging bunch is forced to curve downward by tying them to leaves below. Bunches are pollinated as many as four times a season using cotton balls, squeeze bottles, blow pipes, or modified air blowers.

Thinning the 'Medjool' fruit begins in April. In areas with higher humidity, such as Arizona, workers remove about 70% of the fruit, leaving fruit spaced at about 2-3 cm apart on the strands. Where humidity is less of a problem, the strands are cut so that six to ten fruit remain. Thirty-five to 40 strands remain on the bunch after the center strands are removed. Thinning is the most expensive operation in a 'Medjool' orchard before harvest. The University of Arizona has active research projects on using growth regulators to thin date fruit, and on using various potassium salts to improve fruit size.

Workers support the increasingly heavy bunches by tying them to nearby leaf petioles for support. Some 'Medjool' growers limit the number of bunches to no more than 20. 'Deglet Noor' fruit are not typically thinned, but weaker bunches are removed.

Workers cover the 'Medjool' bunches with cotton or nylon bags in late July. These bags retain fruit that might drop early, protect the fruit from birds, insects, and animals, protect the ripening fruit from rain and provide ventilation to the bunch. Some growers also insert metal rings into the bunches to spread out the strands, improve ventilation and reduce the chance of fermentation. Bag type and mesh size vary depending on the soil type of the orchard. 'Deglet Noor' dates are covered with craft paper or nylon bags. These bags have the same function as those for 'Medjool' but are not always tied close at the bottom.

Insect pests and diseases

'Medjool' dates in Arizona and California currently have no significant pest problems. Carob moth (*Ectomyelois ceratoniae*) infestations are eliminated by the bagging, drying and freezing steps noted above.

Because use of enclosed bags is not as common, and the carob moth can survive better where they can hide in bunches with high densities of fruit, carob moth infestation is a problem in 'Deglet Noor'. Recommended treatments include removal of waste dates from the orchard floor that can support the insect, dislodging abscised immature dates from the bunches during August, cutting the center strands, using enclosed bags as with 'Medjool'. There are three registered insecticides that can be used against carob moth, spirodiclofen, methoxy fenozide and malathion. Registration of the first two insecticides has resulted in growers no longer using Malathion. An alternative treatment is mating disruption using pheromones (Mafra-Neto et al., 2013; Elmer, 1965).

Other insect pests of 'Deglet Noor' include Banks grass mite (*Oligonychus pratensis*), almost exclusively on 'Deglet Noor' (Elmer, 1965), and the giant palm borer (*Dinapate wrightii*), a secondary pest attacking dead and dying plants (Carpenter and Elmer, 1978).

The palm weevil (*Rhynchophorus vulneratus*) has been eliminated from the US (Hoddle et al., 2016) although the South American palm weevil (*Rhynchophorus palmarum*) has been advancing northward in the coastal areas of Mexico and southern California since 2010

(Hoddle et al., 2021). Date growers and Agriculture Department officials are monitoring for the arrival of this pest. Black scorch (*Thielaviopsis punctulata*) is an occasional problem and the Bayoud disease has been kept out because of phytosanitary regulations. No date palm offshoot has been imported to the US since 1929.

Harvest and packing

'Medjool' harvest begins in late August; each tree is harvested three to four times, every 10 to 14 days as the fruit do not all mature uniformly. Harvest is finished by October. Each mature palm can produce as much as 80 to 100 kg of fruit. At the packinghouse, unmarketable fruit are eliminated, then the rest are gently washed then sorted by maturation. This sort allows the fruit to be segregated according to the amount of time needed to dry them to 16 to 21% moisture content. Fruits are dried from one to seven days at about 65°C. Most dates are dried using forced hot air, but a few are dried traditionally in the sun. Following drying, the dates are washed again then sorted according to size and external appearance. Finally, they are packaged and kept refrigerated or frozen until sold. Because of the use of the bags, the heat applied during drying, and the frozen storage, no additional fumigation is necessary.

'Deglet Noor' harvest begins in October and continues through December. Each mature tree can produce 115 to 135 kg of fruit (Takele et al., 2005). Entire bunches are cut off and lowered to the ground, where the mature dates are shaken off into a container. Dates are dry brushed and graded in the packinghouse, then packed.

Date production, trade and consumption

The 2020 value of the US date industry was about USD$ 189,000,000, based on about 31,000 MT (USDA, 2021). This author estimates that 50 to 60% of that total is 'Deglet Noor', and the remainder is mostly 'Medjool', since the USDA does not distinguish between the two cultivars. USDA statistics show that US dates are exported to Canada, Australia, Mexico, and the UK. Dates are imported into the US from Tunisia, Algeria, Israel, and Mexico (Agricultural Marketing Resource Center, 2018).

Date Pac is a large grower cooperative in Yuma Arizona that produces conventional, pesticide-free, and organic 'Medjool' dates under the "Natural Delights" label (https://www.naturaldelights.com). Date Pac claims that their dates are sold in 90% of all the supermarkets in the US. There are other smaller date packinghouses in Arizona who distribute their fruit to local, regional, and international markets, including: Martha's Gardens (https://www.marthasgardens.com), Southwest Medjool (https://southwestmedjools.com/), Naked Dates (https://healthynakeddates.com), and Date land (https://www.dateland.com).

Producers in California, who market both 'Deglet Noor' and 'Medjool', include: Hadley's Date Gardens (https://www.hadleys.com), Sun Date (https://sundateusa.graphtek.com), Double Date Packing (https://www.doubledatepacking.com), Joolies (https://joolies.com), Alexandra Dates (https://www.alexandradates.com/home2.html), Oasis Date Gardens (https://oasisdate.com), and Brown Date Gardens (https://www.browndategarden.com), Oasis Date Gardens and Brown Date Gardens are unique in that they sell the 'Barhi' and other less common dates. This is not an exhaustive list, as there are others.

US table date fruit consumption (not processed) was just 100 g person^{-1} year^{-1} in 2020 (consumption calculated based on data from the United States Census Quick Facts (2020) and Statista (2022)). This level of consumption represents a 50% increase over 2012. Because the level of consumption in the US is still low, and there is an increasing amount of product on the market, it has become clear that there needs to be a better understanding of the date eating consumer and how marketing will increase sales.

Date consumers and marketing

A recent survey showed that Arizona consumers are willing to pay a premium for 'Medjool' dates labeled as "Grown in Arizona". Moreover, they are willing to pay a premium for dates labeled as "pesticide-free", but not as likely to pay a premium for dates labeled as "GMO-free" (Grebitus and Hughner, 2021). As for price, most consumers would pay between USD$ 4 and $5 for 225 g of dates. The survey also found that younger consumers (age 21-30), males and families with children were more likely to buy greater amounts of dates.

Currently, dates are most often marketed in the US as whole fruit, with pit and pitted, both conventionally grown and organic. Dates are also found in energy bars and occasionally in breakfast cereals.

'Deglet Noor' dates are typically sold in small bags or containers ranging from 200 to 600 g. They may be pitted or chopped. Often, this cultivar is sold in the supermarket alongside dried or canned fruits, or alongside, flour, sugar, and other items needed for baking. Smaller 'Deglet Noor' fruit are often ground and formed into "rolls", then coated with coconut or nuts.

Historically, whole 'Medjool' dates have been marketed in 5 kg containers, often next to the fresh fruit. At the supermarket, these containers were opened, and consumers could choose individual dates to purchase. In response to these studies indicating that consumers preferred smaller packaging, and in response to the COVID-19 pandemic, it is now unusual to see bulk 'Medjools' in the US supermarket. Boxes weighing from 1 to 2 kg are sold individually to meet the needs of customers who purchase more dates at Ramadan.

Currently, 'Medjool' dates are most often sold in plastic containers or bags where the customer can see, but not touch the fruit. Sometimes these containers are made of recycled plastic. An oncoming trend is to sell dates in even smaller containers. Joolies sells three pitted dates in a "snack pack", weighing just 40 g each. Natural Delights is now grinding smaller 'Medjool' dates into a paste and forming date "nuggets", each about 10 g each. These are then rolled in coconut, cacao, pecans, almonds, pistachio, or cranberries, and marketed as "Mini Medjools" in 280 g packages. The package indicates that the product is non-GMO, organic, began and a useful source of fiber.

Meanwhile, date marketers are changing their strategies in the hopes of increasing consumption. It is easy to find some date producers on Facebook, Instagram, and Twitter. These producers also have blogs, where date recipes can be found, consumers can join date information email lists. Coupons and point of sale sampling are helping to drive sales at the grocery store.

Literature cited

Agricultural Marketing Resource Center. (2018). https://www.agmrc.org/commodities-products/fruits/dates

Carpenter, J., and Elmer, H. (1978). Pests and Diseases of Date Palms (Washington, DC: USDA), pp.10.

Elmer, H. (1965). Banks grass mite, *Oligonychus pratensis*, on dates in California. J. Econ. Entomol. *58* (*3*), 531–534 https://doi.org/10.1093/jee/58.3.531.

Grebitus, C., and Hughner, R.S. (2021). Consumer demand and preferences for Medjool dates grown in Arizona. Arizona Food Industry Journal *June 2021*, pp.7.

Hilgeman, R. (1972). History of date culture and research in Arizona. Date Grower's Institute *49*, 11–14.

Hoddle, M., Kabashima, J., Millar, J., and Dimson, M. (2016). The palm weevil *Rhynchophorus vulneratus* is eradicated from Laguna Beach. California Agric. *71* (*1*), 23–29.

Hoddle, M.S., Hoddle, C.D., and Milosavljević, I. (2021). Quantification of the life time flight capabilities of the South American palm weevil, *Rhynchophorus palmarum* (L.) (*Coleoptera*: *Curculionidae*). Insects *12* (*2*), 126 https://doi.org/10.3390/insects12020126. PubMed

Hopper, M.S. (2013). The globalization of dried fruit - transformations in the eastern Arabian economy, 1860s-1920s. In Global Muslims in the Age of Steam and Print, J.L. Gelvin, and N. Green, eds. (Berkeley, CA: University of California Press), p.158–181.

Mafra-Neto, A., de Lame, F., Fettig, C., Munson, A., Perring, T., Stelinski, L., Stoltman, L., Mafra, L., Borges, R., and Vargas, R. (2013). Manipulation of insect behaviour with specialized pheromone and lure application technology (SPLAT®). In Pest Management with Natural Products, J. Beck, J. Coats, S. Duke, and M. Koivunen, eds. (Washington, D.C.: Am. Chemical Society Publications), p.31–58.

Nixon, R. (1950). Imported varieties of dates in the United States. USDA Circular *834*, 144.

Statista. (2022). Annual consumption of table dates in the United States from 2012 to 2020. https://www.statista.com/statistics/936362/ date-consumption-us/.

Takele, E., Mauk, P., and Sharabeen, I. (2005). Sample costs to establish a date palm orchard and produce dates in the Coachella Valley, Riverside County 2005–2006 (Davis, California: University of California Cooperative Extension Press).

Toumey, J.S. (1898). The date palm. Arizona Agric. Experiment Stn. Bulletin *29*, 105–150.

Trent, H., and Seymour, J. (2010). Examining California's first palm tree: the Serra Palm. J. San Diego Hist. *56* (*3*), 105–120.

United States Census Quick Facts. (2020). https://www.census.gov/quickfacts/fact/table/US/POP010220

USDA. (2021). Non-Citrus Fruits and Nuts - 2020 Summary. https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/sf269213r/6t054c23t/ncit0521.pdf.

Wright, G. (2012). Date cultivation in Arizona and the Bard Valley. J. of the Am. Pomological Society *66* (*3*), 110–117.

Wright, G. (2016). The commercial date Industry in the United States and Mexico. HortScience *51* (*11*), 1333–1338 https://doi.org/10.21273/HORTSCI11043-16.

Current status of date palm sector in Eritrea

B. Mussie Fekadu[1,a] and A. Ben Abdallah[2]

[1]National Agriculture Research Institute (NARI), Eritrea; [2]Consultant for Date Palm (NAP/IFAD), Project, Eritrea.

Abstract

The contribution of the agricultural sector plays a vital role in the rural economy of Eritrea. The mode of agricultural production in the rural areas is subsistence and in most cases leaves little or no surplus for income generation. However, an immense effort was initiated to reverse the situation and hence improve the livelihoods of the farmers. One of the recent initiatives by the Ministry of Agriculture is the provision of an integrated package for farmers to diversify farm production by the introduction of fruit crops into their farming operations. Date palm has been identified as a suitable crop, taking into consideration a huge potential for dates on the national market. A survey was conducted to study the current situation of date palm in the Northern Red Sea (NRS) and Southern Red Sea (SRS) regions of Eritrea. The result indicated only 21,250 date palm trees are existed (55.3% in NRS and 41.5% in SRS). The majority of them are originated from in vitro plants (19,000 date palm trees) and the rest are from seeds and offshoots. 252 farmers have been engaged in date palm cultivation in both regions. Farmers are well trained on Good Agriculture Practices (GAP) of date palm cultivation. Four years after plantation, the results of growing and fruiting of date palm are showing a real success. One of the factors contributing to good growth is the rich soil along the red sea coast. The recent date palm sector culture in Eritrea is achieving the first steps with success but will need more efforts and investment to establish a date palm industry in the country as an important opportunity for Eritrea to fight poverty and to improve its economy.

Keywords: survey, dates, vitro-plants, cultivation, GAP, NRS, SRS

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest fruit crops cultivated in the Middle East and North Africa (MENA). Eritrea is situated in the Horn of Africa and in sub-Sahelian African countries. Eritrea lies between latitudes 12 and 18°N, and longitudes 36 and 44°E. It is bordered to the northeast and east by the Red Sea, Sudan to the west, Ethiopia to the south and Djibouti to the south east. The country is virtually bisected by a branch of the East African Rift. It has fertile lands to the west and descending to desert in the east. The Northern and Southern Red Sea regions of Eritrea that include the coastal area are the most suitable regions for date palm cultivation with possibility of underground water. In the NRS and SRS regions of Eritrea, hot season extends from April to October (maximum temperature 50°C) and cold season from November to March (minimum temperature 15°C). The climate is hyper-arid with little or no rainfall, the altitudes ranges between 0 to 500 m a.s.l. and it has rich fertile soil.

The Ministry of Agriculture in Eritrea has identified date palm as suitable crop in the Northern and Southern Red Sea Region not only to improve the livelihoods of the farmer's household budget but also taking into account the big potential for dates on the national and international market. It has exerted a great effort to establish viable date production sector by introducing new cultivars and raising awareness of GAP to farmers & extension agents. The introduction of commercial date palm cultivars into Eritrea aimed at benefiting the middle- and low-income producers (Ben Abdallah, 2017). As international consultant of date palm (FAO, 2015) stated that, "the introduction of date palm cultivars to Eritrea will have a tremendous economic and environmental gains by: a) ensuring food security, b) increasing

[a]E-mail: f.mussiela@gmail.com

nutritional value, c) diversifying crop in the desert area, d) reclaiming land, e) creating job opportunities, f) generating income to the farm community, g) earning foreign currency, and h) protecting the environment and reducing the impact of climate change".

The Strategic Plan of date palm production in Eritrea has three levels: a) household family, b) subsistence farmer, c) commercial date palm producer. Private farmers and the government are making big efforts and investments to cultivate date palm in the Northern and Southern Red Sea regions. Many household families have been working in date palm cultivation in the NRS and SRS regions supported by the previous TCP/FAO and IFAD projects. Date palm household family farming is part of on-going scheme the "Minimum Integrated Package" program of the Ministry of Agriculture that deals with subsistence livelihoods of rural farmers.

The National Agricultural Research Institute (NARI) has made a major intervention in the NRS and SRS regions with the introduction of improved tissue culture raised date palm cultivars as farming activities. However, dates have not been produced for real commercial purposes. The date fruit was produced under natural conditions is locally consumed and no dates are channelled into any formal markets.

The present survey was to study the current situation of date palm cultivation in Eritrea and to investigate potential expansion of date palm cultivation in both Northern and Southern Red Sea regions of Eritrea.

MATERIALS AND METHODS

The research survey was conducted by National Agricultural Research Institute (NARI) in the year of 2021 in both Northern and Southern Red Sea regions. Date palm plantation sites were pointed in the map of Eritrea (Figure 1) using GPS instrument in both NRS and SRS regions of the country. Data were collected by interviews and group discussions (Figure 2) with farmers and extension agents of Ministry of Agriculture, visiting in the date palm plantation areas. Six group discussions were conducted with farmers and extension agent in the NRS region of six districts (Gindae, Foro, Massawa, Shieb, Gahtelay and Afabet). Similarly, three group dissection were conducted in SRS region (Assab, Afambo and Tyio). Interviews were carried out for 15 farmers and eight extension agents of the ministry. Finally, the obtained data were analyzed using SPSS software and cross-check.

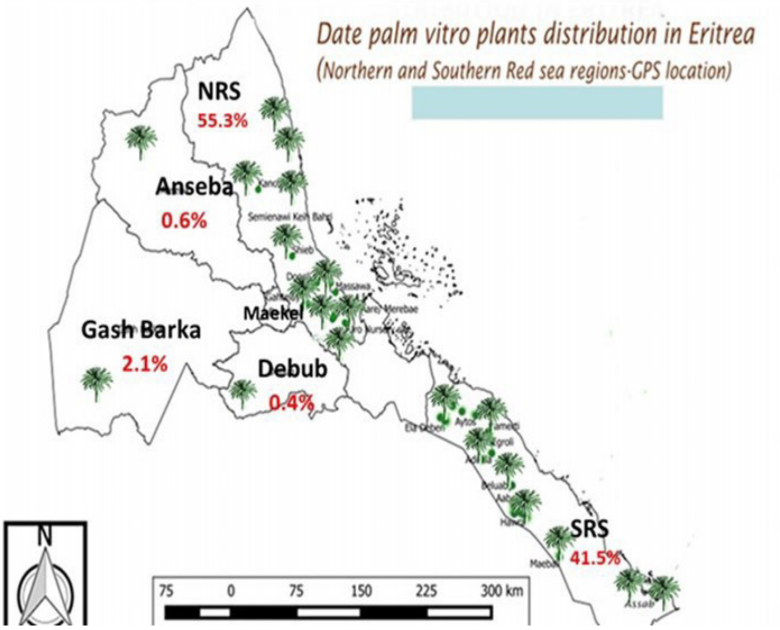


Figure 1. A map of Eritrea showing date palm coverage area (%) in the country.

Figure 2. Group discussion with date palm growers at Afabet, NRS region.

RESULTS AND DISCUSSION

Date palm cultivation areas in Eritrea

In the last four years, a major intervention has been made in the NRS and SRS regions with the introduction of date palm cultivars as a farming activity. Date palm before 2016 was a relatively new crop in Eritrea, with only less than 1000 date palm trees (MoA report, 2016). It is estimated that about 252 farmers have been engaged in date palm farming in both regions. A total available number of date palms in the Northern and Southern Red Sea regions reached 21,250 during the period of 2017-2021, with the total date palm production covers an area of 136 ha. During this period, about 19,000 vitro-plants of date palm of 11 international cultivars ('Medjool', Barhee, Khalass, Oum Dahan, Seggai, Nawader, Sheishi, Abumaan, Zahidi, Hilali and male 'Ghanami') have been introduced from UAE (Figure 3).

Figure 3. Introduced vitro-plants of date palm cultivars into Eritrea from UAE.

The rest date palm trees were propagated by seeds and offshoots. The ministry of Agriculture in collaboration with FAO and IFAD has been promoting date palm plantation in different part of the country since 2017 (NARI, 2019). Four years after plantation of vitro-plants, the results of growing and fruiting of date palm have been showing a real success story (Figures 4 and 5).

Figure 4. Date palm plantation area in Massawa-Gedem (NRS region), four years after plantation.

Figure 5. Date palm plantation area in Assab-Abo (SRS region), four years after plantation.

Northern Red Sea (NRS) Region

A total production area of date palm covers 75.3 ha, with a total number of 11,745 date palm trees (Table 1). These have scattered in five districts areas (Gindae, Shieb, Afabet, Foro and Massawa). From the total number of date palm trees in the NRS region, 89.8% was from tissue culture plant materials and the rest 10.2% was propagated from seeds. The tissue culture date palm raised seedlings have distributed to 170 farmers and majority of them are the household family. Currently, date palm from tissue culture have started fruit bearing at the age of four years after plantation. Statistical data indicated that 55.3% (Figure 1) from the total number of date palm in the country has found in the Northern Red Sea region.

Table 1. Estimated number of date palm in NRS Region.

District	No. of date palms	No. of farmers
Gindae	1059	10
Shieb	704	3
Afabet	511	13
Foro	285	14
Massawa	9,186	130
Total	**11,745**	**170**

Southern Red Sea (SRS)Region

Four districts potential areas were identified by the Ministry of Agriculture for date palm plantation in SRS region namely Araeta, Assab, Southern Denkalia and Central Denkalia (Table 2). A sum of date palm in SRS region has reached 8,830 trees that covers 56.6 ha of land with tissue culture raised plants and offshoots, 98 and 2%, respectively. Eighty-two farmers and household families have benefited from tissue culture raised date palm. Presently, tissue culture raised date palm and offshoots have started fruit bearing at the age of four years after plantation. The data showed that 41.5% (Figure 1) of date palm plantation has found in the Southern Red Sea region out of the total available date palm in the country.

Table 2. Estimated number of date palm in SRS Region.

District	No. of date palms	No. of farmers
Araeta	200	26
Assab	1500	9
Southern Denkalia	6500	28
Central Denkalia	630	20
Total	**8,830**	**82**

Current availability of date palm in the country can be limited to reach dates in the formal market. If a Good Agricultural Practice is properly implemented, the country could easily reach around 200,000 trees in just five to seven years (Ben Abdallah, 2022).

Awareness raising about Good Agricultural Practices (GAP) of date palm

The information obtained from interviews and group discussions have indicated that farmers and extension agent of Ministry of Agriculture had lack of awareness (fertilization, irrigation, pollination, bunch management, harvesting, handling, sorting, packing and storage of dates for the various cultivars) on date palm cultivation since 2017. A few cultivars have produced in a handful of orchards, and with limited know-how of propagation and pollination (Ben Abdallah, 2017). Currently, these problems have solved through intensive theoretical and practical training (Figure 6) by an international consultant of date palm. The Ministry of Agriculture, Monthly News Letter January, 2022 reported that "600 farmers and extension workers have benefited from theoretical and practical training programs on GAP of date palm".

Figure 6. Awareness raising on Good Agricultural Practice by an expert of date palm.

As a conclusion, the key for a successful production of dates should have an intervention of extension agents of Ministry of Agriculture to farmers during the pollination period and fertilization in both regions of Red Sea of the country. The availability of date palm in the country should be increased to fulfil the gap through introducing of tissue culture vitro-plant cultivars, producing vitro-plants in the local tissue culture laboratory and offshoots propagation. Awareness for GAP to farmers and extension agents should be continued. Small packing house should be established to avoid post-harvest loss of dates.

ACKNOWLEDGEMENTS

We are very grateful to the National Agricultural Research Institute (NARI) of Eritrea for providing the necessary facilities to implement the research work. For the financial support, we would like to thank NAP/IFAD projects in Eritrean. We are grateful to H.E. Minister of Agriculture for encouragement, motivation and constant support. Our special appreciation and acknowledgements are also extended to our colleagues Mr. Simon Eyob (NARI), Mr. Daniel Kesete (MoA, NSR) and Mr. Mohammed Abdurrahman (MoA, SRS) for their kind support.

Literature cited

Ben Abdallah, A. (2017). Mission Report on Date Palm in Eritrea /Project: FAO-TCP/ERI/3503 (Asmara, Eritrea: FAO), pp.6.

Ben Abdallah, A. (2022). Mission Report on Date Palm Technical Assistance in Eritrea (International Fund for Agricultural Development (IFAD), National Agricultural Project (NAP) of MoA), pp.32.

FAO. (2015). TCP/ERI/3503 Micro-propagation of Date palm cultivars Using Tissue Culture Techniques (Eritrea: FAO), pp.55.

Ministry of Agriculture Newsletter. (2022). Eritrea: Date Palm Plantations Progressing to Semi-Commercial Stage (Asmara, Eritrea: Public Relation Division, MoA), pp.8.

Ministry of Agriculture Report. (2016). Eritrea: Annual Progress Report on Horticulture Unit of Agricultural Extension Department (Asmara, Eritrea), pp.108.

NARI. (2019). Report. (Asmara, Eritrea: International Fund for Agricultural Development (IFAD), National Agricultural Project (NAP) of MoA), pp.12.

The economic feasibility of byproducts as a part of the value chain of the date palm sector

N. Abdulwahab Abdul Razzak[a]

International Consultant (Investment & Agriculture Economics), Sultanate of Oman.

Abstract

The article presents date palm and date palm by products added value efforts and opportunities in the NENA region. Some of FAO's, Egyptian's, and Oman's efforts and achievements were used as success stories and lessons to learn from and to enhance. Cost-benefit and competitiveness analysis (CBCA) for date palm value added (DPVC) as developed by FAO experts, its approach, methodology, indicators been used, and some related issues, were presented. The article considers the traditional by-products of date palm (which may reach net wet weight 60-100 kg palm^{-1} annually depending on their age, cultivar, environment, and local agriculture practices) in addition to low-quality dates, not of proper market value, and all that can be produced by the date palm and industries related to those products as renewable resources. The Ministry of Agriculture and Fisheries (MAF) of the Sultanate of Oman had conducted for Technical and Economic Feasibility Studies for four projects to use date palm and date palm by-products, all showed reasonable (economic, social, development, and environmental), impacts on stakeholders and societies. The study recommends FAO to join hands with (KIAAI) to focus a training program to all related stakeholders of the date palm sector in the NENA countries producing dates to adopt an action plan to establish a series of value-added commercial projects program may present conceptual theoretical back ground and pre-feasibility studies of added value commercial projects suitable for the related country.

Keywords: cost-benefit, sustainability, environment friendly, economic returns

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest fruit crops grown over 5000 years ago and is a major crop in the Near-East and North Africa (NENA) region. There are more than 3000 species of date palms. Since ancient times, dates have been an important crop for the livelihood of the population and provide many benefits and services to the population of date-producing countries. Historically, despite the fact that the main objective of date palm farming is the nutrition value of dates to humans and their animals, creative local communities benefited from most parts of the palm. For example, many hand-crafted industries, as rural house appliances, date's storage and local marketing containers, building poles and roofs, animal feed, and many other useful by-products as raw materials for industries. Many important enhancements for the livelihoods of farmers and rural people such as providing new job opportunities, an additional income, increase family economic performance efficiency and a multiple impact on the economy, on the micro, mezzo, and macro levels.

In 2019 world production of dates reached 9.1 million t. Countries of the NENA produce about 90% of the world's production of dates.

In 2019 the top 5 date producing countries by volume were: Egypt, Saudi Arabia, Iran, Algeria and Iraq. The value of global date exports in 2019 amounted to about 2.0 billion US dollars (FAOSTAT, 2021). Some references showed that number of date palms across the world exceeded 100 million trees, more than 90% of them in the NENA countries (https://nakhlaty.com). It is crucial information to help in better understanding the scope of

[a]E-mail: nashwan57@gmail.com

work needed by related stakeholders.

CHALLENGES FACING THE DATE PALM SECTOR

The last three decades showed a significant increase in the cultivated area of palms and production of dates in many countries in the region, but experts believe that the expected development of the date palm sector still faces many obstacles, technical, institutional and environmental constraints, and some emphasize on lack of strategic support for VC development at the national and regional levels. The performance of the date palm sector and the efficiency of its VC is also affected by the lack of effective interdependence of the VCs of the entire date sector, and other factors that go beyond direct production, postharvest circulation, marketing and related activities to include factors such as: systems and regulations, economic policies, investment climate, institutional support and values social and human resource efficiency at various stages of the date palm VC (DPVC) and many other areas related to the enabling environment for the sector (Yaseen, 2021).

Strengthening VC linkages enhances stakeholders' ability to work with each other to achieve common economic interests and sector development.

FAO support for the dates palm sector in the NENA region

FAO has implemented more than 30 projects in more than 25 countries in Asia and Africa over the past 30 years in the areas of sector development projects, training activities, technical advisory, publications, risk communication, early warning, seminars and technical workshops, and many more.

The Regional Office for the Near East of the FAO established a Date Palm Technical Working Group to assist Member States to develop DPVC in the NENA region to support smallholder farmers, sustainable use of natural resources, date palm pest and disease management, use of by-products, build resilience to climate change and identify key stakeholders in DPVC in producing countries and their involvement in technical consultations on the main challenges and opportunities to improve and expand the DPVC products in the region with interest in the participation of youth of both sexes and the establishment of a regional strategic framework to guide and combine efforts for the development of the DPVC products in the region.

The organization works to support the development of the date palm sector through work and coordination within many entities, alliances and partnerships with international and regional organizations interested in developing and rehabilitating the date palm sectors one of them is (KIAAI).

Cost-benefit and competitiveness analysis (CBCA) of DPVC

Economic development experts, including many experts from reputable regional and global organizations such as (FAO, which has taken an institutional interest in the topic and has issued several publications, training and implementation programs) use the CBCA approach for the DPVC, as part of the broader analytical and strategic work to:

1. Providing stakeholders with a view of the DPVC in the framework of the country's strategic development;
2. Diagnose the VC by identifying its bottlenecks, on technical, socio-economic and environmental grounds, its challenges and opportunities;
3. Diagnose and evaluate "select policy" options for chain development based on previous (expected) evidence of its potential (social, economic and environmental) impacts;
4. Presenting policy recommendations to stakeholders, each according to his/her competence.

This tool (CBCA) needs to be essential part of the extension program of date palm stakeholders.

1. Approach and methodological foundations.

a) The analysis begins by examining the context in which the VC exists, the physical and monetary flows that describe "representative agents" and the entire chain, the activities they undertake, and the exchanges that take place;

b) Under CBCA, the "production and income accounts" are calculated first for each agent, then for the entire VC;
c) The production account determines the A V of each factor as the difference between the value of the total output and the costs of the intermediate inputs used in the production process. The income account instead specifies how V A is distributed among the factors that provide factors in the production process, such as employment, financial resources, land, capital services, and entrepreneurial skills;
d) It conveys information about the profitability of the VC;
e) Income and production accounts are usually calculated on an annual basis for a standard period;
f) The different margins that characterize the agents in the different sectors of the VC are studied and compared and compiled with a "picture" of the economic situation of the agents as well as of the entire chain;
g) Analysis of the accounts of agents and the chain as a whole, as well as a deep analysis of the role and functions of the VC in the broader socio-economic and environmental context, allows the analyst to carry out a "diagnosis" of the VC, i.e., identifying and discussing the main problems and opportunities of the chain.
h) This is the basis for selecting policy options to address problems and exploit opportunities, and policy options are then broadly defined and described;
i) The costs of implementing those changes are estimated, and the expected changes they are likely to bring on prices or quantities of inputs and outputs that affect the agents' production and income accounts are estimated as well;
j) The CBCA adopts counter factual policy options: after a benchmark is built, it is intended to represent the situation in which no policy change has occurred, and one or more options are made which reflect the effects of one or more of the public policy measures;
k) The reference option (without policy) and the options are then compared including policy simulations (with policy) to assess differences in terms of total value production, VA and profits created by each agent and the entire VC;
l) The analysis is carried out from two different perspectives: the 1st of the private agent(s) using market prices and the 2nd of society as a whole using reference prices;
m) When calculating reference prices, goods and services are ranked based on the existence of a price outside the economic system that would approximate the alternative opportunity cost to society of producing an output or consuming an input;
n) This classification distinguishes between trade able goods (importable or exportable) and non-internationally trade able goods on one hand, and the inputs and outputs of local production processes on the other hand.

2. Indicators to understand added value (AV) and profitability.

a) Private cost ratio (PRC): domestic worker costs to AV created at market prices;
b) Private value-added ratio (PVAR): activity contribution to create local AV at market prices;
c) Domestic resource cost ratio (DRCR): local factor costs to AV generated at reference prices;
d) Social value-added ratio (SVAR): activity contribution to create local AV at reference prices.

3. Protection indicators can be checked such as.

a) Nominal protection coefficient (NPCO) which compares revenue generated using market prices and prices obtained using reference prices;
b) Nominal protection factor on negotiable inputs (NPCI) which compares costs obtained using market prices with those obtained using reference prices;

c) Effective protection coefficient (EPC) which calculates the ratio between the added value expressed at market price and the reference price.

4. Other indicators that have been investigated include.

a) Domestic factor ratio (DOFAR) the ratio between factor costs expressed at market prices and those at reference prices;

b) Support to the private sector ratio (SURPA) measures the net transfer to the private sector as a percentage of total revenues at reference prices.

The results from the comparison of the various options and levels of analysis provide the basis for making recommendations on preferred policy measures that should be implemented in order to achieve the objectives set.

5. Primary and secondary data.

VC analyzes are based on a set of data that can be presented in two categories:

1. Primary data.
2. Secondary data.

Modeling and analysis are carried out using computer programs for VC analysis developed by (FAO) as the FAO VCA-Tool.

Report model: cost-benefit analysis of policy options for the date palm VC

As a contribution to building the capacities of young professionals, female and male, working in the fields of VC in Sudan, a "Report model on cost-benefit analysis of policy options for the DPVC" was prepared, as a part of the literature of the project "Development of date palm products and by-product VC in Sudan" Reference number (TCP/SUD/3703), implemented by (FAO) in 2021. It is a simplified practical model, presented in Arabic (the appropriate language for the vast majority of stakeholders, especially farmers). A summery is attached for further details.

SMALL SCALE ADDED VAUES ACTIVITIES

Date palm by-products (DPbyP's) defined as the additional products that were not targeted by the main activity of palm cultivation. The main product is dates. As for the DP leaves, midribs, leaflets, kernel, coir, spadix, stem, trunk, and pollen grains Therefore, maximizing the returns of natural resources contribute to increasing revenues as they represent additional resources without a significant cost. As long as the innate human behavior seeks to benefit from all available resources, and to maximize their environmental and economic benefits, whether for his/her self, family and society, or his/her economic activity. The concept of "added value" came to establish a systematic work that documents these benefits, and highlights "lost opportunities" as a result of the insufficient employment of human creativity in the development and sustainability of resources. Experts, local and international organizations such as (FAO) had an influential experience need to be under focus and learn from.

The definition of by-products of date palm for the purposes of this article includes all of the above, in addition to low-quality dates, less desired, not of proper market value, and all that can be produced by the date palm and industries related to those products as renewable resources. Industrial innovations have proven that "all palm products without distinction can achieve a beneficial added value for the individual and society".

We, in this part of the planet, have deep and influential success stories accompanying the march of the indigenous people of our countries from the date palm during more than six thousand years, and this heritage has accomplished many products using all possible technologies and techniques that are appropriate for their time and human needs. It was of value to their lives, starting with preserving dates in their daily food basket and their traveling to their shelter with abundant beautiful ceilings, passing through the treatment of some stomach diseases, malnutrition and other needs. Table 1 shows some details. Numbers may vary between date palms depending on their age, cultivar, environment, and local agriculture practices.

Table 1. Some by-products of date palm quantities and availability during the year.

Item	No. in a tree	No. annually	Dried weight (kg ear^{-1})
DP leaf	30-150	12-15	15
DP midrib			
DP leaflet	120-240		
Thorns (20 cm long)			
Spadix stem (Arjun or Sebata)		8-20	8-20
DP coir			1.56
DP petiole		12-15	12-15
Total			36.56-51.56

The wet weight of these remains is 60-100 kg palm^{-1}.
Trunk (10-30 m depending on the cultivar)
DP kernel represents about 10% of the fruit's mass, fibers and phenolic acids, depending on the cultivar.

REDISCOVERING PALM BY-PRODUCTS: INNOVATIVE RESEARCH FINDINGS AND EXPERIENCES.

Some Egyptian scientist have remarkable achievements of many applied scientific research aimed at re-discovering the by-products of dates. Table 2 shows some of their main research titles:

Table 2. Some of the main applied research titles that achieved applied results in Egypt. Source: Al-Mosili (2021).

Field of research	From of the most prominent applications/results
Using palm leaf as a substitute for wood	Depending on the cultivar, environmental conditions and agricultural operations, a palm annually make 12-15 new leaves, stay for 3-7 years, its length 3-6 m. In the annual pruning process, dry leaves are removed to facilitate access to the head, ease harvest the crop, and to provide healthy conditions for the fruits
The specific weight of DP leaf	Averages 0.66 at a moisture content of 8%, and these values correspond to beachwood (0.65) and spruce wood (0.35)
The dimensional stability of leaves	It is of high ability to swell when immersed in water compared to wood. The % of volume swelling of 4 cultivars ('Siwi', 'Mujahl', 'Tamr', 'Zokor') after being immersed in water for 38 h were 980% for 'Zokor' and 176% for 'Siwi, compared to beachwood, spruce wood 13 and 29%, respectively
The mechanical properties of leaf	Mechanical specifications close to those of wood according to ASTM specifications. The modulus of rupture in curvature was 70 N mm^{-2} for 'Ommahat', 76 for 'Baladi' and 40 for 'Siwi'. The compressive strength is 34, 36 and 40 N mm^{-2}, tensile strength is 66, 71 and 75 N mm^{-2}, respectively
Anatomical structure of leaves	It belongs to the family *Monocotyledons*, there is no transverse support in the section and the fibro-vascular bundle is the structural unit of the section
The mass of pruning	Last column of Table 1. Study results for a single palm 'Siwi' cultivar
Super strong materials from palm leaf	The leaf is characterized by higher density and lower diameter fibro-vascular bundles. It has a tensile strength of 25 kg mm^{-2} (commercial steel analog), while its specific tensile strength is 4.3 times that of commercial steel. This encourages the investigation of the success of its uses in polymer compositions, production of fibers for craft products and textile fibers
Modern furniture from palm leaves	The flaps (bulges) were transformed from palm leaves into panels of regular thickness, highlighting the aesthetics of fibro-vascular bundles distribution in the form of modern furniture pieces. The project won the Khalifa International Award for Date Palm in 2013

Egypt, has about 15 million productive palm trees, so the mass of the annual pruning process reaches 810,000 t (air-dried weight), this is a sustainable wealth that is useful for establishing industries on it. It is expected that the natural products of leaf will use the opportunity currently offered by the green market globally: there are segments of environmentally sensitive and socially conscious consumers who prefer products produced naturally. It opens the way for the expansion of green products as the preferential system of the EU gives preference to products produced in the countries of the south in an environmentally friendly manner: rather, palm leaf products are natural materials do not need a certificate of origin when participating in international furniture fairs, other than products made of wood, which need to prove that the wood used in the industry obtained from a sustainably managed forest – and the slogan that Al-Mosli (2021) suggested for marketing these products is: tree free products.

SOME IMPORTANT PRACTICAL EXPERIENCES

1. Charcoal from date palm midribs. The project achieved a calorific value of 86% according to the specifications of FAO;
2. The production of organic fertilizer (compost). For example, the production of compost in the village of Fares – Kom Ombo – Aswan, It has about 300 thousand trees, of which the fruit is about 80% of them. Usually, pruning products are burned in the field annually (up to 3768 t – air-dried weight). Considering that the pruning rate is 30%, the by-products of the date palm were estimated as follows: a) coir 1.5 kg × 0.30×240000 = 108 t; b) leaflet 30 sheets per palm × 0.5×0.3×240000 = 1080 t; c) petiole 30×0.75×0.3×240000 = 1620 t. The cost t^{-1} was estimated at EgP 260, and selling at EgP 300, profit t^{-1} EgP 40 (up to 20% on the invested capital);
3. Manufacturing MDF panels from palm pruning products. The technical and economic feasibility study showed the most important profitability indicators were rate of return on invested capital 39.4%, revenue: costs rate 1.43:1, capital payback period 3.6 years, net present value EgP 92.9 million, and internal rate of return (IRR) was 36.2%;
4. Using palm pruning products in animal nutrition. The most important of these remnants are: date kernels, sorting dates, palm fronds, and palm leaves.. Studies have proven that it contains a high percentage of food energy, equivalent to approximately 3760 kg-calories, which is equivalent to the energy content of traditional fodder grains such as corn, barley and others. It is also possible to add crushed dates to heaps of green silage as a good and cheap source of energy at 25% of the weight of the green fodder. The diet has a positive effect on the rates of weight gain of fattening animals due to growth hormones in dates. It was also noted that the date nuclei (kemel) have a hormonal effect on the performance of reproductive processes in female animals, as it helps to alert the uterine muscles and facilitate the delivery process (25% of the total concentrated ration of the animal). The study proposed a diet of 14% protein + 25% sorted dates + 35% date kernels + 20% olive pomace + 18% cake (soy or sesame) + 1% mixed minerals and vitamins + 1% crushed limestone (Al-Mosli, 2021);
5. Palm leaflet products project. Leaflet products often come in the form of mats or pie plates, or in the form of bowls, or bags. One of the prominent projects in Egypt is an industrial use of by-products, which was established by the "Egyptian Association for Self-Development" using traditional techniques and technologies developed by them. The project initiated by a team of women, who were trained and specialized in wicker industries in the village. They used their innovative imagination, hand craft, and working from home during their spare time without spoiling their social lifestyle or negatively affecting their care for the home and family members. Women were so happy by their achievements and additional income they got from their own work.

AGRI-BUSSINESS COMMERCIAL PROJECTS MAXIMIZE VC BENEFITS

Having more than 100 million trees of date palms, produce more than 9 million t of dates worldwide, it is very important to think about large scale commercial Agri-business projects using date palm by-products as inputs. The Ministry of Agriculture and Fisheries (MAF) of Oman, had conducted technical and economic feasibility to highlight the added value from financial, economic, social, and environmental impacts indicators. All studies showed reasonable impacts (economic, social, development, and environmental), on stakeholders and societies.

Producing wood from date palm by-products project

The project objective is to produce annually: 1) medium density fiber board (MDF): 30,000 t; 2) wood pallets: 600,000; and 3) wood plastic composite products (WCP) doors: 8,000 t.

The amount of wooden raw materials was estimated by 52,134 t annually. The financial analysis showed some results summarized in Table 3.

Table 3. Gross margin and net profit, and their percentages at Year 10 of the project's life.

	Product	Gross margin (US$)	Gross margin (%)	Net profit (US$)	Net profit (%)
1.	MDF	4,966,881	39	2,813,111	22
2.	Wood pallets	2,001,524	64	1,140,321	37
3.	WCP doors	1,148,734	58	286,189	14
Total average		**8,117,140**	**45**	**4,239,622**	

Pallets have the highest gross margin of 64% and WPC doors came second. All products have a positive net profit, the most profitable was pallets (37%). It is recommended to consider all products to provide product diversity and reduce risk. After all, the market should be the determining factor in terms of production.

Total investment cost of the project's full development estimated at US$ 15.08 million. The cash flow analysis for estimating NPV was US$ 9.041 million and internal rate of return (IRR) was 37.7%.

The specific economic impact of the project viewed in terms of: 1) business output (sales volume); 2) the AV of the wood industry or the gross national product; 3) wealth (including assets value); 4) personal income; and 5) new jobs. The social impact, development effect: such a project is environmentally friendly using up to date and innovative technologies. It could be a good opportunity to the country, or to the district, to process the date palm leaves and convert them to wood. Some of those residuals (including some shrubs as mesquite) are established as an invasive weed in some countries. Measurements need to be taken to contain the trees by cutting or burning.

Derived date project (DDP)

Concept: the fact of most main date producing countries is having important quantities of low-grade or medium-grade date cultivars, especially in non-commercial projects, and for social and moral reasons the farmers are not willing to replace them with new cultivars of high quality or suitable market prices. In some counties this category of dates represents about 25-45% of date annual production. Also, the dates seeds (cores) and other by-products may be of viable AVs.

Project objectives: the main objective of the project is to produce high-quality date derived products, such as: 1) liquid sugar; 2) ethanol (industrial and medical); 3) vinegar; 4) useful and marketable by-products. Local market and export potential for DDP. Production of AV products which are economically feasible. Participating in the socio-economic development of date farming community. The diversity is promising. Raw material, total amount of dates required at full capacity of all lines was 48,000 t (Table 4).

From financial analysis average gross margin ranges 47-48% during the first 10 years. Total investment cost for the full development of the project is estimated at US$ 30.134 million. Cash flow showed the estimated net present value (NPV) of the project at a discount rate of 10% was estimated at a positive US$ 12.22 million and the internal rate of return (IRR) was 14.7%. The project has positive accumulated net cash flows for the ten years of operation. The accumulated net cash flow over 10 year was over US$ 23.4 million.

Table 4. Initial total investment costs (US$).

Investment cost	Year 1 (US$)	Investment cost	Year 1 (US$)
A. Fixed structures:		B. Equipment and related costs	
Alcohol plant building	297,000	Alcohol plant building	2,423,898
Vinegar plant building	66,000	Vinegar plant building	146,432
Liquid sugar plant building		Liquid sugar plant building	1,898,534
Common buildings	1,334,300	Common equipment	1,984,603
Total fixed structures	2,357,300	Total equipment cost	6,453,467
C. Initial working capital	2,468,709	D. Pre-investment costs	314,160
Total initial investment			**11,593,636**

This project has specific impacts such as: it creates VA products which have economical value in the food and pharmaceutical industry. The actual operational viability is giving a 44% gross margin. It generates direct jobs for about 224 persons, supporting the farmers income, improving standard of living of the communities in villages where dates are grown. One of the main elements of environmental protection is by upgrading the value and use of low grades dates (many evidence showed leaving date in farm been subject for fermentation and losses).

Date feed processing plant (DFPP)

The project produces: 1) roughage replacement feed 6 mm pellets with minimum 77.5%, and 2) high fiber feed 6 mm pellets with minimum 50%. Both include treated DPL, date processing by-products and additional protein, minerals and vitamins. Its major ingredient is date palm leaves (DPL).

The study assumptions:

a) The availability of 5.2 million date palms;
b) Each palm may produce 15-20 kg of leaves annually;
c) About 78.5 to 104.7 t of leaves will be available annually;
d) Four different collection zones, the average price for the DPL stacked at the DFPP is US$ 75.4 Mt^{-1} including handling, chopping and treatment;
e) The cost of the treated DPL is US$ 88.4 Mt^{-1}.

In general, based on the marketing study, the market opportunities and realistic sales prices the volume of sales and revenues can be estimated for the final scenario for each country or district at the time of the study. Different reports and studies showed there is a suitable potential for such product at competitive market prices. The project required employing (65) persons.

Financial analysis of the project showed:

a) The gross margins of the two different end products varies from 51% for RRF and 40% for HFF with an average over the total production of 45%;
b) The total investment costs for the full development of the project is US$ 8,158,012;
c) The total cumulative working capital over the first 5 years is US$ 1,038,588;
d) The project has a net present value (NPV) of plus US$ 3,861174 as well as a positive IRR of 14.8% and is economical viable.

Social impact is showing that the project will enhance and diversify the usage of DPL and other DP by-products to an AV feed resource. Provide direct employment of 64 persons. Development impacts showing that the project enhances DP producers' life. Provide a proper level of technology to produce palletized feeds using local unused raw material. The environmental impact shows the project utilizes about 23,500 M t of DPLs annually, mostly been burned. Technically the DPLs to be a valuable crude fiber rich raw material comparable to reasonable quality roughage. The project's outputs are to replace local or imported roughages hay and straw.

Economic impact of the three projects

Economic effects are effects on the level of economic activity in a particular area, district, or the whole country. The three projects, in addition to the specific effect of each one, are of common contribution to the economic growth of the country as they will:

a) Increase the production of agri-industrial sector and become a profitable business;
b) Require capital investments for infrastructure, equipment and salaries (Table 5);
c) Expenses on day-to-day operations will become an important cost item;
d) Be an investment in the local or regional economy and creates many economic (direct, indirect or induced) effects;
e) The projects are considered to be main agri-industrial models;
f) They utilize the surplus and low-grade dates which have very low value in the market;
g) They increase the date palm farmers revenue, especially those of low income;
h) They will enhance other related sectors and activities;
i) The end products as well as the by-products values are much higher than its original;
j) The infrastructure, new buildings, equipment and logistics are considered AV to the national economy and the GDP of the country;
k) The projects' outputs are to replace some imported goods;

The three projects have common social impacts as follows:

a) The projects will provide more than (388) jobs as direct employment (100+224+64). Most of them can be as sustainable career for local citizens;
b) Additional indirect job will be generated in other related sectors and sub-sectors;
c) Improve standard of living of the communities in villages where dates are grown;
d) The business opportunities created thereafter will also lead to "spin-offs" in other related industries and sectors both upstream and downstream.

Table 5. Summary of establishment investment costs (fixed assets of the project) (US$).

Total estimated cost of	US$	Total estimated cost of	US$
Date palm processing plant	776,685	Staff canteen	10,920
Finished product store	218,400	External additional works	220,311
Quality control lab building	46,410	Elec. and electro mech. works	451,815
Office block	109,200	Warehouse for raw materials in bags	383838
Total			**2,217,579**

Development impacts of the three projects can be summarized as follows:

a) By establishment such multi products projects, a new type of business will be developed that will utilize the by-product as AVs;
b) Enhance DP producers' life as new source of income, new jobs, and more of their products as industry inputs required;
c) Optimize national natural resources allocations among the best economic and financial opportunities;
d) Proper level of technology using local unused raw material;
e) Factories may use an innovative, or modern technology to develop business which lead to nation's creativity and better life.

Last but not least the three projects have common environmental impacts as life cycle assessment studies consider the environmental impact of materials over their entire life, from extraction or harvest of raw materials through manufacturing, transportation, installation, use, maintenance and disposal or recycling. These three projects consistently show that positive use of by-products instead of being a waste burden on environment, and environment friendly techniques to be used in the processing, treatment and manufacturing units.

CONCLUSIONS

a) We, in this part of the planet, have deep and influential success stories accompanying the march of the indigenous people of our countries from the date palm during more than six thousand years, and this heritage has accomplished many products using all possible technologies and techniques that are appropriate for their time and human needs. This heritage needs enhancement and simulate the digital future challenges;
b) Most of dates neither been processed nor been manufactured. The highest

percentage been marketed as whole dates. AV activates are the engine of development;

c) A total annual amount surplus date used as animal feed, very low-tech processing (if any!) and postharvest losses reached 25-40% of world production;
d) There is a very good potential for dates as raw material for producing VA derived products through extraction, fermentation and other processes;
e) Large commercial projects, or SMEs crafted base projects can be a good model for countries, districts, villages to adopt such approach;
f) Provide incentives to producer's associations, local investors, and SME's traders to study and engaged in such projects.
g) It is highly important to build the capacities of young professionals, female and male, working in the fields of date palm using CBCA model and other FAO materials;
h) Despite the appreciated work of countries, national centers, NGOs, and regional and international organizations to develop date palm sector, FAO and (KIAAI) are the leading institutions that can motivate others for more sustainable work;
i) Emphasize on such projects in the agricultural extension programs, chambers of commerce, and governments of the objective countries.

RECOMMENDATIONS

There are many and varied success stories, from countries or institutions and organizations, most notably of FAO, that stakeholders can benefit from and achieve sectoral and national goals such as economic efficiency, sustainability and food security. FAO as a leading word wide specialized organization with such experience, in establishing the theoretical basis for DPVC and practical achievements, is the eligible institution to join hands with (KIAAI), after such successful cooperation in numerous date palm activities. The two association may have a focus training program to all related stakeholders of date palm sector in the main countries producing dates to adopt an action plan (funded by a special fund) to establish a series of value-added commercial projects (may start next date festivals in the 6 Countries, UAE, Egypt, Jordan, Sudan, Morocco, and Mauritania, in addition to Iraq, Oman, and KSA). The program may present conceptual theoretical back ground and pre-feasibility studies of AV commercial projects suitable for the related country.

ACKNOWLEDGEMENTS

I thank, FAO, and FAO RPW eradication program, institutions and personnel, for organizing and inviting me to this session of "DPVC development and bio-regional collaboration" at the 7th International DPC, Abu Dhabi, UAE. March, 2022, and for their covering my travel expenses to participate.

My thanks are extended to the 7th International DPC's Organizing Committee, and Khalifa Int'l Award for Date Palm & Agricultural Innovation, the Award's General Secretariat, institutions and personnel, for their appreciated developmental efforts towards agriculture, and for their covering the hotel accommodation, in-country transportation, and all other support.

Literature cited

Al-Mosli, H.I. (2021). Benefiting from the by-products of date palm. Sustainable practices to improve the date palm value chain in Sudan (in press).

Ministry of Agriculture and Fisheries. (2016). Feasibility Studies for Date Palm Feed Processing Plant (DFPP) (Sultanate of Oman), pp.133–138, 151–156.

Ministry of Agriculture and Fisheries. (2017a). Feasibility Studies for Date Derived Products Processing Complex (DDPPC) (Sultanate of Oman), p.129–137.

Ministry of Agriculture and Fisheries. (2017b). Feasibility Studies for Date Palm Wood Products Processing Complex (DPWPPC) (Sultanate of Oman), p.152–169.

Yaseen, T. (2021). Dates palm sector in the world and the role of the Food and Agriculture Organization in promoting the sector in the NENA. Sustainable practices to improve the date palm value chain in Sudan (in press).

The current situation and future prospects for manufacturing date products and palm waste in Sudan

H.D. Dawoud[a] and A.A. Fatima[b]

Horticultural Crop Research Center, Khartoum, Sudan.

Abstract

Palm trees are considered the focus of agriculture in many different states of Sudan, where they produce fruits and large quantities of agricultural by-products that can be used for many purposes. This study aims to raise the economic value of the palm by making use of all palm products and waste. We hope to provide a model for development thinking coupled with science fiction to provide sustainable employment opportunities in the countryside and facilitate a local and sustainable material base for many industries in the cities and capacity building of rural youth to address this change. This paper also aims to preserve the national palm wealth and reduce the burden on the state's balance of payments as a result of importing timber and its products. The Forest Research Center has made many contributions to the production of compressed wood. This project presents a model for the development of villages in all palm states in Sudan. Dawoud and Fatima (2020) conducted a survey of palm orchards, and random samples of Sudanese and imported date cultivars, in an average length of 3 m and age of 12-15 years, showed that one palm produces around 26.19 kg of by-products annually, and in total Sudan could produce approximately 497,800 t of by-products based on the total number of palms of 19 million. The second research objective is to develop manufacturing at traditional rural levels and large industries to maximize the return on value-added and encourage families to produce small-scale businesses such as honey, pastries and others. The third message of this survey is spreading consumer awareness of date products and raising productivity and high-quality production to meet the demand for food locally, regionally and globally. To achieve such objectives, a collaboration and interplay between all the stakeholders in Sudan must be established through the government and private sector.

Keywords: manufacturing, date products, Sudan

INTRODUCTION

As results of the uprising of this gigantic crop began to spread south from traditional area (North and River Nile states) and covered new states also according to the introduction of tissue culture offshoots from Emirate and Gulf countries (4,322,600 till 2015) and to the high agricultural potential of the Sudan, the number of date palms growth from 17,637,388 in 2016 to 19,669,910 within few years.

As a result of this horizontal increase in the number of date palms, we had considerable quantities of dates and their other by-products (Figure 1), which aggravated the rates of waste and spoilage in the produced dates, which is estimated at (35-50.5%) and the environmental pollution of the resulting waste (which is not used at all) and causes continuous fires in the palm orchard.

This study aims to:

1. Develop industrialization at rural and traditional levels and large industries to maximize the return on value-added and encourage family re-industrialization of industries such as honey, pastries and others;
2. Spreading consumer awareness of date products and raising productivity and high-

[a]E-mail: dawoudhussien@gmail.com
[b]E-mail: fatimapomology@gmail.com

quality production to meet the demand for food locally, regionally and globally;
3. Preserving the national palm wealth and reducing the burden on the state's balance of payments as a result of importing timber and its products.

Figure 1. Different products from pinnae, frond, Karook, Al-Ashmeek, trunk. Photo by H.D. Dawoud.

MATERIALS AND METHODS

Based on many experiments and surveys (Dawoud and Fatima, 2020), a survey of detailed study for date palm orchards to estimate waste and losses of Sudanese and imported date cultivars, (most of tested cultivars with an average length of 3 m and an age of 12-15 years), is shown in Table 1.

Table 1. Estimation of palm products and their waste and losses of palm products and their waste and losses products and by products per palm. Source: Dawoud and Fatima, (2020).

Palm parts (product)	**Weight palm^{-1}**	**Yield palm^{-1} (45 kg fruits palm^{-1})**	
Number of fronds pruned from the palm year^{-1} = 11.2 (frond weight = 1.7 kg)	19.04 kg	Yield tree^{-1} = 45 kg	Waste and losses of fruits %
Leaf-fiber (Al-Ashmeek)	189.5 g	45 kg × 19 Th Date palm = 855,000 t	50.5-35% = 299.3-431.8 (thousand t)
Thorns or spine palm^{-1}	26.2 g		
Karook (end of petiole) pruned palm^{-1} year^{-1}	480.8 g palm^{-1} year^{-1}		
The average wt. of the inflorescences tree^{-1} The average weight of one empty sheath is 524.5 g	3755 g palm^{-1} year^{-1}		
(inflorescence) spadex + dry sheath palm^{-1} year^{-1} The average weight of one (inflorescence) after drying is = 487 g Average number of (inflorescences) palm^{-1} = 12	5,844 kg palm^{-1} year^{-1}		
Average number of strands (inflorescences) palm^{-1} = 181.6 palm^{-1}			
The average weight of the pollen is 667.6 g tree^{-1}, including the pollen grain powder			
The number of male flowers varies with strands (27-106) with an average weight of 450 g of pollen			
The average pollen grains weight ranges between 596 and 1153 g palm^{-1}			
The average weight of the seed fruit^{-1} = 1 g, and estimated as 13-15% from the ton of fruits			

Every thousand ton of dates fruits include 130-150 kg of seeds, which contains 7-12% of a medicinal oil of high economic value.

There are 497,800 t of by-products annually (without seeds) (which is not used) and causes continuous fires in the palm orchard.

RESULTS AND DISCUSSION

Uses of dates in Sudan

1. Direct use.

Most of produce dates in Sudan is consumed fresh, especially in the month of Ramadan, social and religious events. It is worth to mention the Sudanese individual's consumption of dates varies (6.2-9.9 kg person^{-1} year^{-1}) as shown in Table 2.

Table 2. Annual consumption of dates (kg^{-1} person^{-1} year^{-1}) in some states of Sudan. Source: master's thesis, Abdullah Omar Hassanein (Sudan Academy of Sciences 2015).

	State	Annual consumption of dates kg^{-1} year^{-1}		State	Annual consumption of dates kg^{-1} year^{-1}
1	Khartoum	19.3	9	West Kordofan	46.8
2	Nile River	99.0	10	North Darfur	129.9
3	Al Jazeera	29.8	11	West Kordofan	46.8
4	Northern	109.6	12	North Kordofan	59.7
5	Sennar	37.9	13	South Kordofan	67.5
6	South Darfur	119.1	14	Central Darfur	138.7
7	White Nile	8.6	15	Kassala	149.2
8	Red Sea	158.4	16	Blue Nile	78.3

2. Indirect consumption.

Recently, with the development of the nutritional habits of the Sudanese society, the consumption of products manufactured from dates began to flourish and spread as new dietary habits, such as honey, vinegar, pickles, jams, regular or stuffed pressed dates, cakes or baked goods.

A number of food industry researchers have contributed in promoting these dietary habits and industries, their studies (Mohammed, 2015) showed that many chemical industries based on the utilization of low-quality dates can be applied simply. They reported that a ton of Sudanese dates can produce the following products as shown in Table 3 and Figures 2 and 3.

Table 3. The products of dates which produced from one ton of dates. Source: ARC-Sudan Extension pamphlet – Product and By-Product of Sudanese Dates (2021) (in Arabic).

Product name	Produced quantity (inputs %)
Chopped date	Date pulp 90-88% crush
liquid sugar	600-550 kg depending on the type of dates
Industrial alcohol	330-300 L at a concentration of 98-96, according to date cultivar
Vinegar	4.5 t PH concentration 4.5 according to the date cultivar
Citric acid	90-70% citric acid according to the cultivar
Soft drink 12%	4200 L
Honey	650 kg according to date cultivar
Pressed dates with seeds	99% pressed dates
Pressed dates without seeds	89-88% pressed dates

Industries can be divided into:

1. Simple rural industries: prepared by experienced and skilled craftsmen, in small quantity.

a) Frond industries: it is a traditional craft, a basic handicraft that has been passed down from children to parents in northern Sudan, hundreds of years ago. It consists of collecting the frond, cutting them and soaking them in water for two days, cleaning them from the appendages and then drying them for four days. Al-Karoug (base of the frond).
b) Traditionally: in the past, and before the spread of water pumps, the water pump and the "shadouf" were among the most important means of irrigation, and most of their components were made from palm parts and its fibers.
c) Fiber industries: fiber (leaf or ashmieq) is the thin tissue that covers the top of the plant and acts as an insulator that protects the top from weather fluctuations. It is used in making ropes, and filling seats, as well as a cleaning tool. The fiber is treated with water or a solution (0.05 NaoH) for a few minutes and then washed with water. It has been possible to separate long, high-strength threads used to make ropes, rugs, pillows and mats. Fiber is used in many traditional and modern industries in the Sudan: ropes, handles for baskets and walkers, manufacture of hats, beds and chairs. and fillings for seats, hand brooms mats and pillows. To manufacture it, the fiber is placed in anguish in water for two days for fermentation so that it is easy to cut the longitudinal fibers and then separate it from the knots. To make ropes the longitudinal fibers are woven manually in the form of ropes that are placed in bundles, and are marketed. The frond collected and made by hand craftsmanship by making a wide braid that narrows or expands according to production. The midrib intertwined with each other in the braid after it turns white as a result of exposure to the sun. The product is made by using a wide and long needle and thread that may be of wool. Also these products can color by using dye green, burgundy, violet and others by boiling water in a large bowl and dipping the product for five minutes in water and then put it in the shade.

   From an industry point of view there are two types of frond: soft pulp, bright white, easy to shape, small in size and used to make certain products; the rest of the palm leaves are long and coarse and must be soaked in water until they are soft and easy to shape. Working tools in the frond industry: hands and teeth first, bones and pointed stones, and awls that take the place of a needle in the second degree, cutters and the bowl in which the palm leaves are dipped. Leaflets (pinnae) (khows). Each frond contains an average of 120-240 leaf let (pinnae). The leaflets are used in several traditional and popular industries.

   The trunk: historically the trunk is used in the roofing of rural houses and making doors, grape cues, and stairs, as a source of wood for the timber industry for the manufacture of beams, supports and light bridges for pedestrians. The stem is a good source of fiber.

2. Sophisticated industries: it is done by factories or specialized units that use machines, technologies and systems, in a large quantity.
   a) Developed industries: the Sudanese knowledge in the manufacture of compressed wood from palm fronds: Forest Research Center (The Wood Technology Unit) in Soba has been able to transform palm fronds into compressed wood, which is characterized by its hardness, superior bending ability, and non-flammability, which increases the demand for its manufacture and marketing. It is used in the manufacture of plywood, counter tops, particleboard, etc. It is distinguished as one of the environmentally friendly projects, as it does not produce pollutants or waste (El-Latif et al., 2003a, b, c).
   b) Wood grain production: the Wood Technology Unit at the Forest Research Center confirmed that one plank of wood needs about 30 kg of palm leaf, in a research study that was applied on a very limited scale, using a small press (El-Latif et al., 2003a, b, c). The Forest Research Center used fronds in the manufacture of counter panels Banoh, arabesque (instead of imported beech wood), and the manufacture of granulated wood (from counter and arabesque residues).

c) Paper industry: as a result of a study by El-Latif et al. (2003a, b, c), it was found that the amount of cellulose obtained from palm fronds is less than that obtained from pine trees. One ton of palm waste produces 700 kg of paper pulp, and since the by-products in Sudan are estimated at 497,61 t that 348,327 t of paper year^{-1} can be produced.
I suggest conducting more studies and experiments to determine the most appropriate ways to exploit palm fronds in the production of certain types of paper at a lower economic cost.

Figure 2. Different industries from date waste. Photo by H.D. Dawoud.

Figure 3. Different food products from date. Photo by H.D. Dawoud.

Secondary and advanced industrial products

1. Pollen grain – Economic importance of pollen drying.

Some date palm growers extract fragrant water from the pollen wrappers by distilling the soaked ones. The resulting liquid is called "vaccine water". People use this in folklore medicine to treat diarrhea, relieve colic, and as an aphrodisiac. They keep it in their homes with glassware. Some of them re-distil it, and then it is called "refined pollen water", characterized by its good smells. It is used to treat colic and stomach disorders. It is astringent and against diarrhea. In summer, it is mixed with drinking water to give a natural and aromatic flavour.

Pollen grain contains about 17% of cane sugar, 22% of protein, 54% of calcium, vitamins C and B and minerals such as phosphorous and iron. It also contains estrogen that activates the ovary. It also contains antioxidant compounds such as vitamins E and C, beta-carotene and flavonoids.

The average weight of a palm tree = 3755 g, and the average weight of one empty sheath is 524.5 g.

How to use the pollen: it is eaten by putting it with tea, coffee or milk as hot daily drinks. It is also mixed with honey, warm water in winter and cold water in summer, and it must be used according to the instructions of the attending physician.

2. Medical and pharmaceutical manufacturing industries of dates.

Date seeds.

The average weight of the seed is equal to 1 g, and generally the weight of the kernels represents 13-15% of the weight of dates, meaning for every thousand tons of dates there are 130-150 t of seeds. The seeds contains 5-15% of medicinal oil of high economic value and in medicinal chemical preparations, For example, as a source of female hormones.

Seeds are used as animal feed, and other beneficial substances have been revealed in several studies. The most important compounds are: flavonoid, which are considered among the antioxidants and free radical scavengers in the peels of dates by Mohammed Al-Abd Al-Abid, 2003). These compounds are known for their medicinal benefits in that they: i) stimulants for the heart (small amounts of it are sufficient); ii) anti-fungal, anti-bacterial and anti-viral; iii) helps immunity and fight cancer; iv) strengthen the walls of blood vessels.

The most important compounds in the seeds: i) galactomannan compound in date seeds is important in the food, pharmaceutical, cosmetic, paper products, and paint industries, antiviral (Ishrud et al., 2001); ii) alkali-soluble heteroxylan extracted from one of the hemicellulose compounds (Ishrud et al., 2003); iii) unsaturated fatty acids are more than saturated ones, especially linoleic acid, which is required in diet meals. It can be used as edible oil and for multiple medical uses, as proven by Al-Shahib and Marshall (2003). The percentage of oil in the seeds ranged between 7 and 12%.

Caffeine-free date coffee: date coffee can be made by grinding the hard kernels to the appropriate degree and then roasting them. Some promising experiments have been carried out in this regard in the Agricultural Engineering Department, especially with the addition of some appropriate flavours. It is characterized by the fact that it is caffeine-free, which filters it to be suitable for a segment of consumers.

The date seeds are also an important source of oil and consist of the following acids as in Table 4.

Table 4. The acids % in the date palm seeds. Source: ARC-Sudan Extension pamphlet – Product and By-Product of Sudanese Dates (2021) (in Arabic).

	Acid name	%
1	Lauric acid (saturated)	18-21
2	Palmitic acid (saturated)	10
3	Citric acid (saturated	8
4	Linoleic acid (saturated)	8-15
5	Oleic acid (omega9)	39-49
6	Free fatty acids	0.5

Manufacture of date chips products and baby food.

Chip technology is one of the successful technologies in the food industry sector to produce many distinct products and it depends on many grains such as maize, wheat, sorghum, millet and rice, in addition to grains, soy bean, peanut, sesame, etc., to produce successful products such as instant breakfast cereals (corn flakes and baby food and others). Several studies have been conducted to use date flakes in the production of protein-rich food).

A mixture of wheat, chickpeas, lentils, milk powder and dates were prepared, and some vitamins, iron and methionine were added to it. Some vitamins, iron and methionine were added to it. After mixing, the nutritional value was studied in terms of protein content, fats, fiber, sugars and mineral salts. The mixture was characterized by low production costs and contained nutrients for children and school students. The experiments of storing it in polyethylene bags lined with paper at room temperature for a year were distinguished. Free from pathogenic microbes.

Making bread yeast from dates.

Bread yeast can be produced from an extracted medium consisting of date honey diluted in sufficient quantities to reduce the concentration of organic acids present in date honey, and the main beneficiaries of this product are bakeries and food factories.

Alcohol and liquor industry.

The manufacture of alcohol from dates is a traditional industry in Sudan, where two types are produced, one for human consumption, such as ethnic, wine, brandy, and others, after dilution and adding flavour and color enhancers, and the second for industrial consumption, such as ethyl alcohol. Resin industry, and the manufacture of some medicinal drugs, with specifications: i) purity: (94-96%); ii) color: colorless; iii) smell and taste: natural; iv) aldehyde: 0.2%; v) acidity: 18 mg L^{-1}; vi) esters: 100 mgL^{-1}; vii) remaining after fermentation: 10 mg 10 L^{-1}.

Manufacture of Tamer El dien.

The project aims to establish an industry based on date products; by consuming huge amounts of low-quality dates. The project is characterized by its economic and social importance; where it can provide job opportunities for young people in rural and urban areas that are famous for the cultivation of date palms, in addition to the possibility of women's employment in these projects that are characterized by the simplicity of production technology and this product is dried date pulp in the form of rolls. The product is an important means of using the poor quality commercially (returned), which is separated when the excellent varieties are graded. The idea is to produce a cheap product as an alternative to apricot apricots imported from abroad.

Ice cream industry.

Ice cream is a complete food as it contains all the fats, carbohydrates, proteins and others that a person needs. It is easy to digest and has a high calorific value. Because dates are high in sugar, their sugars can be used in the ice cream industry, where sugar represents 14-15% of its components, so liquid sugar or date paste can be used in this industry, which gave good and encouraging results in terms of taste and flavour.

Production of vinegar from dates.

Vinegar is produced from solutions of sugar or starch materials through alcoholic fermentation, which is followed by acetic fermentation or acetic oxidation. Vinegar is a solution of acetic acid (acetic acid) diluted with water.

Pickled of Tamer.

It is one of the rural industries that can be established in the areas of traditional production of dates, and each family can produce it to diversify its table or source of income. Pickled dates can be produced in the khalal stage. The product comparable to the fruits of pickled olives in taste and quality.

Caramel industry.

Caramel is formed as a result of burning sugars with the formation of a color and this color is of great importance in the food industries, especially in soft drinks, spirits and pastries.

Production of citric acid (citric acid) from dates.

Citric acid is a natural fruit acid with a low acidity and has many uses in the food, pharmaceutical and chemical industries. Its food uses include soft drinks as a flavouring substance that gives the appropriate acidity to the palatability of the drink. It is used in many industries such as:

- Confectionery industry: dates can be added to sweets for the purpose of flavouring and increasing their solubility and stability, especially for artificially sweetened materials, at a rate of (0.8-2%), while the percentage in pressed sweets and chewing gum is 0.5-1%;

- Dairy industry: citric salts are used as emulsifying agents for the manufacture of cheese products with concentrations of up to 3% in the final product. Citric acid improves the properties of the solubility properties of cheese and improves the texture of cheeses in general;
- Pharmaceutical industries: citric salts are good buffer solutions in the preparation of medicines and are used in the preparation of ascorbic acid and in the production of antacids.
- Jelly and jam industries: the acid concentration ranges in jams and jellies.

Production of dried date's powder.

It is a product extracted from heat-dried date paste after removing the pits from it. This product has the same properties as the original dates, and the percentage of impurities in the final product is low. And it is one of the most healthy types of sugar because it contains minerals and vitamins, and in one of the studies published by the British with great success, it is possible to produce dry date powders from dates distinguished by their high content of sucrose, such as the sugar dates in the Kingdom of Saudi Arabia and many types of Sudanese dry dates (Berkawi) in particular. Ms. Fathia Mohamed Hassan produced Berkawi powder (and won the Khalifa Award for Industrialization at the level of Sudan in Festival 2017). These dried powders are characterized by their long shelf life and wide possibilities for use as a rich raw material in many food products at the local and international levels.

The manufacturing processes are considered uncomplicated processes that include removing the kernels and drying the dates and turning them into powder by grinding and then packing them in moisture-impermeable packages and envelopes. It is preferable to dry dates or dry them under vacuum until the moisture content in it is 3-4%. Then the dates are ground in the form of a powder. Usually the resulting powder is fast absorbing moisture, so it must be kept in tightly closed cans. It is also quick to dissolve in water. Date powder is one of the most types of sugar. Healthy because it contains minerals and vitamins. Date powder is one of the healthiest types of sugar because it contains minerals, vitamins, and fiber found in dates. The dates were not separated from all their properties, but were only dried and ground.

Date powder is one of the healthiest types of sugar because it contains minerals, vitamins, and fiber found in dates. The dates were not separated from all their properties, but were only dried and ground. Experiments have been conducted on a number of cultivars and led to excellent results, and the best of them were the dry ones. This product is included in many products and foods because of its many benefits, as it is used as a substitute for sugar in foods and in many powders industry for various purposes, including: i) it is a substitute for sugar, especially that it is 100% natural, tricks that form the mono-saccharides glucose and fructose (about 95%), and they are quickly absorbed; ii) it is considered a natural flavour and therefore it can be used in many food industries; iii) a substance with a high nutritional value that contains minerals, vitamins, fiber and other important materials for building the body.

Promising investment opportunities for dates powder:

- It is used in the manufacture of nutritional drinks with the addition of some flavourings such as ginger, cinnamon, cardamom;
- Various dairy products, such as milk ice cream such as ice cream and others;
- Bakery, pastries, biscuits and sweets industry;
- Baby food products that are fast-digesting and of high nutritional value;
- It is used in the pharmaceutical industry as a natural sweetener;
- A natural nutritional sweetener, without any additives.

Manufacture of compost.

A layer of fronds and others date residuals is laid with a thickness of 15-20 cm, to which is added a layer of row farm manure of the same thickness, and an amount of 15 g of urea, and the layers are successively until reaching a height of 1.5 m.

Covered with agricultural nylon or shade nets to keep moisture inside and stimulate decomposition.

Folklore manufacturing of dates

There is an appreciated number of traditional meals based on dates that are prepared and produced at the level of the home and the countryside and have been consumed by the various sectors of the Sudanese since a long time ago.

1. Date juice or sharbout.

Date juice or sharbout is considered the main entry point for the majority of date industries from which the rest of the products are made, and its production process depends on extracting the largest possible amount of what dates contain from the sugars and sherbet resulting from the fermentation of date infusion (date juice) by natural yeast with the addition of spices to give the distinctive flavour and control.

The best types of sherbet are produced from the 'Khalas' cultivar, which produces 350 mL of sherbet from 0.5 kg of 'Khalas' date fruits.

It is worth mentioning that the 'Khalas' palm under irrigated conditions produces 450 kg palm^{-1} year^{-1}. In general, date juices can be produced with great success, as for soft drinks, they need more research and development efforts to study the feasibility of technical and economic production on an industrial level.

Almost every 100 g of dates contains 60 g of sugar. To obtain wine from pure dates with an alcohol content of approximately 8%, we need the following: to calculate the percentage of alcohol in dates, since each kg of dates contains approximately 600 g of sugar, i.e., each kg of dates gives 350 mL of alcohol after fermentation, and these calculations are approximate, because the percentage of sugar varies between types of dates.

2. Gorast Al-tumor (date cake).

Crushed date pieces are roasted and mixed well with thick wheat flour dough. The dough is baked on a hot, smooth metal surface in the form of a round cake, about 1 cm thick.

3. Gorast tumor el-mousafrien.

This product is a modification of the above product and is a commonly prepared product in the northern region. Mixed with some spices to prolong its preservation period without damage.

4. Al-Ajwa.

It is a traditional and historical food in northern Sudan, easily prepared by removing the seeds from the dates, and mixing them with very few ingredients to prepare date paste: i) put the dates in a pan over a medium heat, then add the oil, then the cinnamon; ii) stir the ingredients with a wooden spoon until you get a smooth and cohesive dough; iii) remove the pot from the heat, and leave the dough aside to cool; iv) then use it to prepare various types of sweets.

5. Date honey.

Date honey is one of the old transformational products of dates in northern Sudan. It is defined as the concentrated sugary liquid extracted from the fruits of some types of dates by heat and free of fiber. Sugars represent 85% of the dry weight, most of which are reducing sugars in addition to protein, mineral acids and vitamins. It is hoped that it will become one of the successful trans formative products for dates in the future and that it will open wide doors for many uses in the manufacture of sweets, pastries and various food industries such as the industries of juices, dairy products, ice cream, baby food and chip technology products.

6. Production of date jams.

Generally, jam is a mixture of fruits and sugars in a ratio of 55:45 and is concentrated with heat until the soluble solids become 65-68%. Production of some high-fiber and low-sugar food processing industries.

7. Animal feed manufacturing.

All River Nile tribes State, traditionally prefer to feed the sheep, goats and cows on the foul and the wasted sorting products of dates and seeds after soaking them in water. From their culture, these type of date residues an excellent flavour to mutton meat, as well as increases milk production in sheep and cows in general. There are many studies that show the components of the kernels, which are on average a moisture content of 5-10%, fiber, 10-20%, carbohydrates 55-65%, protein 5-7%, oil 7-10%, ash 1-2%. Foulings and screening products can also be used in the feed industry.

Manufacturing of (non-food) industries

These products are usually not suitable for human consumption, so they are converted to industrial production.

a) Activated carbon: a scientific study of the Sudan National Research Center in Khartoum has shown that there is a possibility to benefit from date seeds in preparing activated carbon that is used in wastewater purification and treatment processes;
b) A number of chemical processes of extraction;
c) It can also be used to remove the color of honey as one of the steps in converting it to liquid high-fructose sugar. This is a vital contemporary issue of utmost importance from both industrial and environmental aspects;
d) It is usually converted into a carbon product at high temperatures 300-400°C as a carbon fuel similar to the first use;
e) Carbon waste can also be used for heating;
f) Medical alcohol ethanol is used as a disinfectant and other medicinal uses.

Ethanol production (bio-fuel)

Ethanol production from renewable agricultural resources has received unprecedented global attention during the past two decades for its use as a bio-fuel to reduce the consumption of petroleum products whose prices have increased dramatically.

Ethanol can be successfully produced from date residues, according to estimation of Mohammed (2015), the amount produced from a ton of date residue comparing to sugarcane and sugar beet is shown in Table 5.

Table 5. Bioethanol produced from ton of palm waste. Source: Unpublished paper, I.A. Mohamed (2015). National Food Research Center.

Plant	Produced bioethanol (L)
Sugarcane	60
Sugar beet	116
Dates	280

Advantages of producing ethanol from dates or palm waste:

- Helps to increase economic income;
- Creating a clean and pure environment in which humans, animals and plants can live, for example when 5% of car fuel is replaced with ethanol, this will help reduce carbon monoxide from the air by 30%;
- Reducing the percentage of greenhouse gases that cause global warming, thus preserving the environment;
- Recycling ethanol manufacturing waste and using it as animal feed, and thus the area of agricultural land used to grow grains allocated to feed livestock will be reduced.

NOTE

From an economic point of view, it is recommended to collect the secondary palm waste in rural centers near palm plantations, and then transfer it to centers in the towns where Electricity, water and other industrial needs were available, these method of waste collection in rural centers lead to reduces the cost by 1-4 from the actual cost of collecting it in the traditional way.

SUMMARY AND CONCLUSIONS

All these modern and traditional activities and industries mentioned above led to the flourishing of some industries such as:

- Palm MDF industry;
- Palm particle board;
- Palm carton industry;
- Palm charcoal industry;
- Palm animal feed industry;
- Palm organic compost industry.

In addition to initiate the traditional and historical use in food technology, folklore medicinal and pharmaceutical use of dates products and their waste. The progress of all these activities leads to:

- Develop industrialization at traditional rural levels and large industries to maximize the return on value-added and encourage family re-industrialization of industries such as honey, pastries and others;
- Spreading consumer awareness of date products and raising productivity and high-quality production to meet the demand for food locally, regionally and globally;
- To preserve the national palm wealth and reduce the burden on the state's balance of payments as a result of importing timber and its products.
- The Forest Research Center has made many contributions to the production of compressed and granulated wood, and through this project we want to present a model for the development of villages in all palm states in Sudan;
- To establish a national strategic project in which all segments of Sudanese society, research centers and universities will participate, and that it is traded at the level of all date producing states in Sudan and the project implementation strategy includes the country's adoption of a palm tree afforestation program, in addition to benefiting from male date palms as a sustainable source of renewable materials.

Finally, this study summarizes the promotion of Sudan's potential and inviting foreign investors to enter the field of date production and maximum benefit from its waste, building an industrial base for the production and processing of dates, and training rural youth on modern industries and crafts (arabesques, pastries, juices, and bio-fuels) for their stability and limiting immigration.

ACKNOWLEDGEMENTS

The authors thank FAO Regional Office for the Near East and North Africa Cairo, Egypt, especially Dr. Thaer Yassin, technical director of the program of Development of Date Palm Products and By-products Value Chains in Sudan.

As well as those in charge of the Khalifa International Award for date palm and Agricultural Innovation especially Dr. Abdelouahhab Zaid, those who participated in the Seventh Palm Conference, and the dream became a tangible reality.

Literature cited

Al-Shahib, M.A., and Marshall, A.O. (2003). The percentage and oil Types in different Sudanese Date Palm Seeds Cultivars. Technical Annual Report (Shambat, Sudan: Oil Technology Unit, National Food Research Center, Agricultural Research Corporation, (ARC)).

Dawoud, H.D., and Fatima, A.A. (2020). Study for Date Products and Palm Waste of Misriq Wad Laggia, Mishriq Wad Khatiab and Barhi from Different Ecological Zones under Sudan Condition. Scientific Annual Report 2021 (Shambat, Sudan: Horticulture Research Center, Agricultural Research Corporation (ARC)).

El-Latif, M.T.A., Mohamed, A.Y., and Talp Alla, A. (2003a). How to Convert Palm Fronds into Compressed Wood. Scientific Recommendation (Soba, Sudan: Forestry Research Center, Agricultural Research Corporation, (ARC)).

El-Latif, M.T.A., Mohamed, A.Y., and Talp Alla, A. (2003b). How to Produce Wood Grain from Palm Fronds and Other Punned Waste. Scientific Recommendation (Soba, Sudan: Forestry Research Center, Agricultural Research

Corporation, (ARC)).

El-Latif, M.T.A., Mohamed, A.Y., and Talp Alla, A. (2003c). Estimated Amounts of Cellulose Produced from One Ton of Palm Fronds Waste. Scientific Recommendation (Soba, Sudan: Forestry Research Center, Agricultural Research Corporation, (ARC)).

FAO. (2022). Extension Pamphlets to improve the situation of Date palm in the Sudan (In Arabic), through Program of Development of Date Palm Products and By-Products Value Chains in Sudan. TCP/SUD/3702 (Khartoum Office with Agricultural Research Corporation).

Hassan, A.O. (2015). Economics of growing and consumption dates in Sudan. M.Sc. thesis (Sudan Academy of Science (SAS), Agricultural Research Council).

Ishrud, A.H., Ibrahim, M.A., and Azza, A.I. (2001). Chemical Compounds of Seeds of Different Cultivars of Sudanese Date Palm. Technical Annual Report (Khartoum, Sudan: National Research Center, Medicinal plants Institute).

Ishrud, A.H., Ibrahim, M.A., and Azza, A.I. (2003). Comparison Study between Saturated and Unsaturated Fatty Acids in Different Seeds of Cultivars of Sudanese Date Palm. Technical Annual Report (Khartoum, Sudan: National Research Center, Medicinal plants Institute).

Mohammed, I.A. (2015). The Amount of Ethanol Produced from Date Residues Compared with Sugarcane and Sugar Beet. Scientific Annual Report 2015 (Shambat, Sudan: National Food Research Center, Agricultural Research Corporation, (ARC)).

Mohammed, I.A. (2021). The Amount of Different Products of Dates Which Produced from One Ton of Dates Scientific Annual Report 2015 (Shambat, Sudan: National Food Research Center, Agricultural Research Corporation, (ARC)).

Prospective of modified atmosphere methods in preserving quality and storability of some Libyan soft dates

M.A. Fennir[a], S.A. Raheel and M.T. Morghem

Department of Agricultural Engineering, Faculty of Agriculture, Tripoli University, Tripoli, Libya.

Abstract

This paper reports the potential of using modified atmosphere (MA) for extending shelf-life and maintaining quality of soft dates at balah and rutab stages. First, MA of 10, 15 and 20% CO_2 each with 5% O_2 were applied at 1±0.5°C for storing 'Hellawi' and 'Hurra' dates palatable at balah stage. MA treatments were significantly different from regular air (RA) at 0.5 level; they extended shelf-life to12 weeks, maintained total soluble solids (TSS) and firmness, with no significant effects between MA treatment levels. However, MA treatments led to darker skin colour (L, a, b). They were significantly different from those measured at harvest. Second, 5 and 10% CO_2 with 5% O_2 were applied for keeping rutab of 'Bronsi' and 'Taboni' cultivars at 1±0.5°C, naturally and artificially ripe fruits were used. MA treatments led to extending shelf-life of both cultivars to 12 weeks, no significant difference among MA and ripening method were recorded in colour, firmness and taste at 0.05 level. Contrarily, RA treatment lasted for less than 4 weeks. Third, ≤5% CO_2 and O_2 were applied at 1±0.5°C for keeping dates of 'Deglet' cultivar at three ripening stages, fully ripe, partially ripe and dry. MA treatment was compared with freezing at -18°C and RA. Results showed comparable effect of MA treatment with freezing, but different from RA in colour, taste, and sensory parameters after 24 weeks. The reported findings enlightened the potential use of ≤5% CO_2 and O_2 at near 0°C for maintaining quality and extending shelf-life of dates at balah and rutab stages. MA tested conditions have shown good potential in packaging, transportation and storage of soft dates.

Keywords: soft date, modified atmosphere, quality, firmness, colour

INTRODUCTION

The last decade has witnessed a great magnitude in date palm cultivation as well as rapid increase in demand in quality dates. In 2019 world production exceeded 9 million metric t, up from 7 million t in 2017 (FAO, 2019). Worldwide, more than 100 million ha are currently cultivated with date palms. Date palm is very important in arid and semi-arid ecosystems, it is deeply tied to deserters, and its importance has gone beyond agriculture to be part of social, cultural, environmental, sustainable resources and food security aspects. To locals, it has been the symbol of generosity, glory, tolerance, strength and pride. Additionally, dates are invaluable in history, tradition, culture and religion, yet deeply attached to the livelihood of millions around the world. In North-African oases, the social status of the family is very much related to date palm, and hence owning a date palm orchard is quite important. It has been reported that about 3000 cultivars are grown worldwide (Salomon-Torres et al., 2021), found in regions with climate ranges from moderately warm, mild, to very hot and dry. Generally cultivars are classified into soft, semi-dry and dry, based upon growing conditions and fruit properties (Kader and Hussein, 2009). Dates are nutritious, contain about 70% carbohydrates; most are in simple sugars of fructose and glucose.

Libya attains the tenth place among top producers (Kader and Hussein, 2009), nearly 10 million trees are cultivated, the annual production is about 180 thousand metric t (FAO, 2019), and about 400 local cultivars have been reported (Racchi et al., 2014). Also, its climate

[a]E-mail: fennir@uot.edu.ly

ranges from southern Mediterranean mild weather round 32°N to very hot and dry desert down to 24°N, making it home of the three groups of cultivars. Soft dates are intensively grown in the coastal fertile plain from the gulf of Sirt east to the Tunisian boarder west, and the semi-dry cultivars are in the middle oases of Wahat and Jufra around 29°N, while dry dates are grown deep in Great Sahara oases up to Chad and Niger boarders. Soft cultivars are characterized by decent quality, good fruit size, taste, and moderate in sugar, making them a great autumn fresh fruit. Nonetheless, coastal dates are susceptible to high losses due to their high moisture content, warm and humid conditions at harvesting, in addition to occasional early autumn rain (Fennir and Morgham, 2019). Traditionally, drying is the main method of preservation, however, with the availability of low cost home freezers, freezing dates for household use has become popular. Although freezing maintains good appearance and reduces physiological disorder of dates (Dehghan-Shoar et al., 2010), it is blamed for affecting quality as storage advances and after thawing (Alhamdan et al., 2018); short shelf life after defrosting, necessity of efficient cold chain infrastructures for marketing and handling, in addition to high energy consumption.

Controlled and modified atmosphere (CA and MA) are efficient in storage, they have facilitated preserving and handling of highly perishable non respiring food items and respiring agricultural products (Gorris and Peppelenbos, 2007). Basically, it relies on reducing O_2 and raising CO_2 levels surrounding the commodity in addition to high relative humidity and low temperature. CA is very efficient in long-term storage of many agricultural commodities, it delays senescence, reduces respiration, slows down physiological and biochemical activities (Kader, 1992). It is extensively used in long-term storage of apples and pears (Saquet and Streif, 2017) and cabbage (Gariepy et al., 1985). Also, MA is quite efficient in shipping of banana (Kudachikar et al., 2011), extending shelf life of strawberries and figs (Colelli et al., 1991; Alturki, 2013; Matar et al., 2021). Nonetheless, despite MA potential for storing dates at research level yet its practical applications are still limited. Several research investigations have proven potential of MA in storing of fresh 'Berhi' dates (Al-Redhaiman, 2004, 2005; El-Rayes, 2009) extending shelf life of Libyan 'Hurra' soft dates (Fennir and Morgham, 2016) and enhancing quality of 'Madjool' dates (Alsmairat et al., 2013). Also, it has been recommended as the best preserving method with minimum occurrence of physiological disorders (Sarraf et al., 2021) and retaining quality attributes. This paper reports the potential of using MA treatments for keeping some Libyan soft cultivars at balah and rutab stage.

MATERIALS AND METHODS

The work was carried out in three parts, balah of 'Hurra' and 'Hellawi', rutab of 'Bronsi' and 'Taboni', and rutab of 'Deglet'. Each part has its own considerations, yet similarities in experimental procedures exist.

Plant materials

1. Balah of 'Hurra' and 'Hellawi'.

Dates of the two cultivars were collected at full maturity of balah stage from an orchard located near Tripoli. Bunches were cut early in the morning and transferred to the Postharvest Laboratory at the Department of Agricultural Engineering, Faculty of Agriculture, Tripoli University. Samples were washed, sorted, strands were cut, and unfit units were discarded. Samples were weighed and prepared for further experimental procedures.

2. Rutab of 'Bronsi' and 'Taboni'.

'Bronsi' and 'Taboni' targeted were naturally ripe and ripened by inducing method. Fruits were ripened using modified incubation method wherein fruits at the balah stage were kept in an airtight enclosure, CO_2 increased, O_2 decreases and ethylene (C_2H_4) accumulates until fruit ripening starts, details of the method can be found in a previous investigation (Fennir and Morgham, 2019). Naturally ripe fruits were collected form the same tree by shaking the cluster above a basket, harvesting was done by an expert.

3. Rutab of ‘Deglet’ Houn.

Dates of ‘Deglet’ cultivar were brought from the city of Houn in a field box; they were sorted into three categories, fully ripe, partially ripe, and dry and cull. The first three were used in the study.

Enclosures

For airtightness, 3-L glass jars and 2-L plastic bottles were used, both were equipped with threaded plastic lid. On the lid a tire valve was installed, facilitating tightness and easy air withdrawal for analysis and adjustment. Enclosures were tested at every fill for airtightness by pressurizing at 0.4 bar and submerging them in water. Samples were filled in and placed in a walk-in cold room with an on-off temperature controller was sat at 1±0.5°C.

MA conditions system

A manual MA system based on three cylinders containing CO_2, O_2, N_2 and pressurized air, each connected with liver pressure valve were the main components for MA conditions. The four air components are connected to a mixing room via rubber pipes, in the room selective entering of air constituent is made via corresponding valve. The room is equipped with a pressure gauge installed on flexible plastic pipe which is connected at its end to a threaded female connection that fits on the valve mounted on the jar lid. The system facilitates air constituent measurements and their daily adjustments to the set point whenever they deviated by ±2%. A portable gas analyzer (Model CANAL120 O_2 & CO_2 Gas Analyzer, EMCO Packaging Systems Ltd., Kent, UK) was used for air analysis. MA conditions applied for the three parts of the study were:

1. For ‘Hurra’ and ‘Hellawi’ treatments were 10, 15, and 20% CO_2 each with 5% O_2 in addition to RA as control. Treatments were kept in cold room of which the temperature was set at 1±0.5°C. Fruit firmness, total soluble solids (TSS) and skin colour were measured at harvest and repeated after 4 and 12 weeks;
2. For ‘Taboni’ and ‘Bronsi’ treatments were 5 and 10% CO_2 each with 5% O_2 and RA treatment as control. Similarly 1±0.5°C temperature was applied. Fruit firmness and colour were measured at harvesting and repeated after 12 weeks in addition to sensory evaluations.
3. For ‘Deglet’ Houn three ripening stages were used, fully ripe, partially ripe and dry. MA treatment was (<5% CO_2 and O_2) and RA treatment both at 1±0.5°C storage temperature. The same ripening stages were kept in sealed polyethylene bags in a freezer at -18°C. Colour measurements were made at harvest, after 12 weeks and after 24 weeks.

Colour measurements

Fruit skin colour was measured using hand-held Tristimulus reflectance colour meter (Minolta CR 400, Minolta Corp., New Jersey, USA). Colour was recorded using (Lab colour spaces), with (L) indicating lightness (100-0), (a) for chromaticity from green (-100) to red (+100), while (b) represents chromaticity from blue (-100) to yellow (+100).

Fruit firmness

Fruit firmness was tested using hand-held penetro meter (Model FHT 803, General Tools & Instruments™, New York, NY, USA). Fruit was divided longitudinally, surface skin and seed were removed for balah state but for rutab only seed was removed, and the sample was placed against solid surface. A 7.9-mm diameter tip size was used to penetrate fruit tissue, and maximum breaking force was recorded in Newton (N).

Total soluble solids (TSS)

TSS was measured as degrees of Brix (°Bx) for balah using digital hand-held refractometer (Model PAL-α, ATAGO Co, Ltd., Tokyo, Japan). Since most TSS of date fruit at balah stage is sugar; thus TSS was used as an indication to sugar content of fruit juice. The instrument was zeroed every measurement using distilled water. Ten fruits were blended and pressed for juice extraction using a special pressing tool, then juice was filtered and few drops were used in the measurements.

Visual inspection and sensory evaluations

Treatments were kept in transparent enclosures, facilitating easy inspections. Visual inspection was made periodically, changes such as shrivelling, fungal infections, ripening for balah (conversion to rutab) and cracking were considered as sings for quality deterioration, leading to terminating the treatment. Also, periodical weight loss was performed, and treatments were discarded when loss reached 15%.

At the end of the storage duration of each part, sensory evaluation was made. Few dates from the treatments were randomly coded, introduced to four members as non-trained panellist, a taste scale was given ranged from 1 to 4, using a 4-point hedonic rating scale, assigned as poor, good, very good and excellent, respectively.

Statistical analysis

For each part of the study, three replicates of each level of the treatment were used and for the colour analysis measurement 3-5 measurements were made. Analysis of variance was carried out using SPSS statistics software ver. 20, significance level was declared at ($p<0.05$) and comparisons among treatments were made using Tukey-Kramer pair wise comparisons.

RESULTS AND DISCUSSION

'Hurra' and 'Hellawi' at balah stage TSS

1. TSS.

ANOVA results showed no significant effect of storage duration on TSS measured as Brix of 'Hellawi' cultivars, ($F_{(1,80)}=8.45$, $p<0.001$) and mean value were 39.56(±0.3), 37.61(±0.35) and 38.1(±0.35), measured at harvest and after 4 and 12 weeks in storage, respectively. On the other hand, effect of CA treatments was insignificant on TSS ($F_{(3,80)}=0.231$, $p=0.874$) and no interaction was recorded among time and CA treatments $F_{(4,80)}=1.14$, $p=0.344$. TSS was considered as the most important quality attribute at balah stage. Although significant effect of storage duration on TSS was recorded, when storage duration of 12 weeks is taken into consideration, the loss can be considered quite low at 4.2%.

For 'Hurra' cultivar effects on time were again significant ($F_{(3,80)}=5.12$, p=0.0.008), mean values were 32.99(±0.46), 35.25(±0.53) and 33.39(±0.53) measured at harvest, after 4 and 12 weeks, respectively. However, significant increase in TSS was recorded; this may be due to high moisture loss from fresh skin or due to variations in samples. Also, no interaction was recorded between storage duration (time) and CA treatments ($F_{(4,80)}=0.75$, $p=0.561$).

'Hellawi' dates kept at regular atmosphere (RA) treatment lasted for 4 weeks; weight loss exceeded 15%; wherein the control treatment was discarded. 'Hurra' date under RA treatment was discarded after 2 weeks, fruits showed signs of deterioration, cracking and fungus infection. 'Hurra' dates are well known as very sensitive and have short shelf life; not exceeding few days under RA conditions, also susceptible to cracking, rotting and souring. However, under CA conditions, fruits lasted for 12 weeks, although some fruits exhibited cracking may be due to physiological reasons. The sensory evaluation at the end of the storage duration showed acceptable taste for all treatment ranged from good to very good. Taking into consideration variations in fruit taste, generally fruit taste was not affected by the applied treatments. Maintaining TSS indeed was attributed to low respiration rate, in addition to low temperature and high relative humidity effects that were maintained inside enclosures. Such positive effects of the current CA treatments are in good agreement with investigations reported in the literature for many fruit and vegetable items (Kader, 1992; Thompson, 2003). For dates however, the use of CA treatments led to stable sugar contents of 'Barhi' cultivar dates at balah stage (Al-Redhaiman, 2005), giving the current study fair agreement with results reported in the literature.

2. Fruit firmness.

ANOVA results for firmness showed no significant effects of CA on fruit firmness of both cultivars. For 'Hellawi' and 'Hurra' they were ($F_{(2,80)}$=0.263, p=0.77) and ($F_{(3,80)}$=0.253, p=0.859), respectively. Fruits of both cultivars retained their firmness for 12 weeks under the three CA treatments. Again, no interactions between storage duration and CA treatments were recorded ($F_{(4,80)}$=0.675, p=0.611) and ($F_{(4,80)}$=0.196, p=0.94) for 'Hellawi' and 'Hurra', respectively. Generally, mean breaking force for 'Hellawi' dates was recorded at 94.3 N. Firmness is an important physical quality attribute for most fruits. Nonetheless, such results are in good agreement with maintaining firmness of balah of 'Barhi' dates under CA conditions (Al-Redhaiman, 2005).

For 'Hurra' significant effect of time on firmness was recorded ($F_{(2,80)}$=21.49, p<0.001). Mean firmness values were 72.4±3.58, 104.8±4.13, and 106.3±4.13 at harvest, after 4 and 12 weeks, respectively. Such observation may be cultivar related or due to variability in degree of ripening among dates within the bunch. However, apart from dates, Smith and Skog (1992) and Harker et al. (2000), reported improved firmness of strawberries subjected to elevated CO_2 conditions as storage time advanced. Nonetheless, maintaining firmness of dates at balah stage is an indication of turgidity and freshness. Therefore, the two cultivars benefited from the applied CA treatments. Indeed, CA treatments improve firmness of many fruits, but for these two date cultivars, it seems that 'Hurra' dates responded positively to storage conditions, firmness increased by nearly 47%.

3. Colour.

The results showed no significant effects of on (L) of 'Hellawi' ($F_{(2,80)}$=1.56, p=0.205), also no significant effect on (a), results were ($F_{(2,80)}$=0.078, p=0.97), but CA effects on (b) were significant ($F_{(2,80)}$=7.61, p<0.001). On the other hand effect of storage duration (time) was significant ($F_{(2,80)}$=62.29, p<0.001), ($F_{(2,80)}$=4.41, p=0.015) and ($F_{(2,80)}$=100.47, p<0.001) for (L), (a) and (b) colour attributes, respectively. Effect of CA treatments and time on colour attributes (L) and (b) are presented in Figure 1. It clearly shows effects of CA treatments colour attributes values (L and b). The colour attribute (b) was selected because it represents the yellow colour, and its lower value indicates reduced yellowness. Clearly, effects of storage time can be observed, generally fruit colour became darker, converting to light brown. For 'Hellawi' and 'Hurra' cultivars a bright yellow colour is preferred. In fact, colour change was observed after 2 weeks in storage for 'Hellawi' and 'Hurra'. However, dates consumed at balah stage are preferred to be yellow, but in the current study, considerable colour changes were recorded for both cultivars. Such changes gave dissimilarity with colour attributes of 'Barhi' dates at balah stage were reported by Al-Redhaiman (2005), wherein CO_2 treatment was reported as significantly improving quality attributes of 'Barhi' dates, and 20% CO_2 gave best results. Also, elevated CO_2 levels have been suggested as an effective tool for extending shelf life of many fruits without affecting quality (Kader, 1992; El-Rayes, 2009).

For fruits other than dates, high CO_2 combined with low temperature was also reported having negative effects on tomato tissue (Deltsidis et al., 2011), physiological disorder on apples known as (scald) was also linked to high CO_2 levels (Sitton and Patterson, 1992). Nonetheless, from a physiological point of view, effect of elevated CO_2 on fruit tissue is also somehow related to O_2 level, temperature and other factors (Kader, 1992). It has been reported that molecular changes related to the development of CO_2 injury are not well explained (Johnson and Zhu, 2015).

'Taboni' and 'Bronsi' at rutab stage

1. Effect of ripening method and MA on firmness.

Fruit firmness is an important property in dates at rutab stage. ANOVA results for 'Taboni' showed insignificant effect of ripening method on firmness at 0.05 level, ($F_{(1,42)}$=0.795, p=0.381), but MA treatment significantly affect firmness ($F_{(1,42)}$=11.75, p<0.001). Similar effects of ripening on firmness were recorded on 'Bronsi' ($F_{(1,42)}$=1.731, p=0.201), but MA treatment showed significant effect ($F_{(1,42)}$=6.123, p=0.007). However, other investigations reported significant loss in firmness in ripening of 'Negaros' cultivar (Serrano et al., 2001). Generally, dates at rutab stage are subjected to substantial structure changes compared with other fruits. Reduced firmness is very much related to enzymatic activities that normally occur during ripening and may extend their activity at rutab stage (Akasha, 2014). Among MA treatments, no significant difference in hardness was recorded; indicating similarities in effects of 10 or 5% CO_2. It is worth mentioning that soft cultivar dates are quite different from dry and semi dry cultivars; their tissues are less dense and undergo enzymatic activities, exhibit elevated respiration rate and sugar changes, possibly due to their physiology and high moisture content. Indeed, reduced hardness occurs due to physiological changes, fruit maturity and senescence (Kader, 1992).

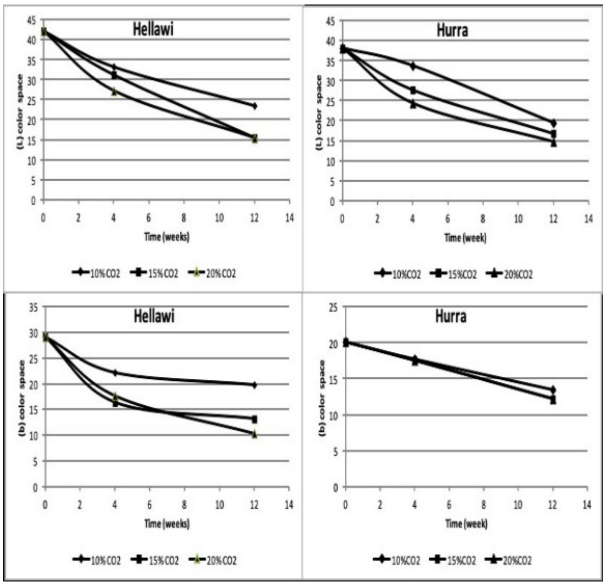


Figure 1. Colour attributes L, and b of 'Hellawi' and 'Hurra' dates measured after 4 and 12 weeks.

2. Effect of ripening method and MA on colour properties (L), (a) and (b).

A factorial ANOVA test was carried out to examine the effect of ripening method whether natural or induced on colour properties (L), (a) and (b). Results for 'Taboni' at 0.05 level were insignificant ($F_{(1,42)}$=0.18, p=0.18), ($F_{(1,42)}$=0.30, p=0.59), and ($F_{(1,42)}$=6.7, p=0.13) for the three colour properties, respectively. No interaction between ripening treatments and MA conditions were recorded for the three colour attributes. No significant differences among MA treatments were observed in colour properties after 100 days of storage, but they were different from time zero values, clearly seen by large values of the colour difference (ΔE). Results for 'Bronsi' were ($F_{(1,24)}$=0.00, p=0.995), ($F_{(1,42)}$=0.026, p=0.87) and ($F_{(1,42)}$=139.7, p<0.001) for (L), (a) and (b) properties, respectively; showing no changes in the first two colour properties, however, significant changes were recorded for the (b) colour property, but such change does not indicate noticeable overall colour changes. Therefore, such observation was considered as unimportant colour changes. No references dealt with colour changes in relation to ripening method of dates in general and soft cultivars in particular were found in the literature.

MA treatment showed significant effect on colour properties for 'Taboni' cultivar they were ($F_{(1,42)}$=41.36, p<0.001), ($F_{(1,42)}$=53.575, p<0.001) and ($F_{(1,42)}$=113.29, p<0.001) for (L), (a) and (b) properties, respectively. MA treatment showed significant effect on colour attributes at 0.05 level, values of the three attributes were lower than values obtained at time zero. On the other hand, colour analysis for 'Bronsi' was quite different, MA treatments did not affect (L) values ($F_{(1,42)}$=2.085, p=0.138), but significantly affected (a) values at ($F_{(1,42)}$=78.99, p<0.001), similar effects were recorded on (b) values ($F_{(1,42)}$=120.68, p<0.001).

As it can be observed in Table 1, similarities in effects of the two MA treatments (5 and 10% CO_2) on colour properties for the two cultivars existed, yet they were less than values recorded at the time zero. (L) values for 'Bronsi' were not affected by time and MA treatments; this may be due to the dark colour of the cultivar compared with 'Taboni' which has lighter colour. Once again, MA conditions after 100 days significantly affected (b) and (a) colour properties. Colour change in (ΔE) can be observed. Similar changes in colour of fresh dates of 'Khenaizy' cultivar had been reported as (ΔE) after 30 days under MA conditions (Aleid and Al-Saikhan, 2017).

Table 1. Effect of ripening method and MA treatment on colour properties.

Ripening method	Source	L	A	B	ΔE
		Taboni			
Natural	5% CO_2	18.42^a	3.88^a	5.04^a	17.3^a
	10% CO_2	19.48^a	3.71^a	4.88^a	16.9^a
	Time zero	28.51^b	9.61^b	17.87^b	
Induced	5% CO_2	19.71^a	7.84^a	3.51^a	14.95^a
	10% CO_2	18.44^a	2.264	3.70^a	16.65^a
	Time zero	29.59^b	7.84^a	14.73^b	
	MA 5% CO_2		1.274	1.18^a	
		Bronsi			
Natural	MA 10% CO_2	18.1^a	0.84^a	1.10^a	3.04
	Time zero	16.25^a	2.89^b	2.36^b	
Induced	MA 5% CO_2	18.35^a	1.21^a	0.25^a	4.13^a
	MA 10% CO_2	19.68 ^{a}	1.03^b	0.64^a	4.42^a
	Time zero	17.29^a	2.83^a	3.90^b	

Means with same letter in the same column are not significantly different at p<0.05 (Tukey Kramer test).
ΔE = overall change in colour, ΔE = √(ΔL2+Δa2+Δb2).

For the other two colour properties (a) and (b); lower (a) values indicate reduced redness while lower (b) positive values indicate lower yellowness. Generally, lower values of the three colour properties approaching zero means dark brown colour. Similar observations were reported for keeping 'Barhy' cultivar dates under CA conditions at khalal and rutab stages (El-Rayes, 2009) and in Libyan 'Hurra' cultivar (Fennir and Morgham, 2016). Also, significant colour changes were reported in 'Burhi' and 'Majhool' cultivars under MA conditions (Alsmairat et al., 2013). It is worth mentioning that darkness in dates is a quite normal change and it increases with storage time advances mainly due to fruit composition.

3. Sensory analysis.

Sensory analysis was made after 13 weeks of storage under MA conditions. For 'Taboni', no significant differences due to ripening method were recorded at 0.05 level on sweetness, taste, acceptance, colour and hardness. ANOVA results were ($F_{(1,16)}$=0.569, p=0.462), ($F_{(1,16)}$=0.09825, p=0.758), ($F_{(1,16)}$=0.084, p=0.776), ($F_{(1,16)}$=6.376, p=0.23), ($F_{(1,16)}$=0.766, p=0.395) for the five tested attributes, respectively. Similarities between natural an induced ripening were also recorded. Similarly, MA treatments for the five tested attributes also were not significant, their ANOVA results were ($F_{(1,16)}$=0.569, p=0.462), ($F_{(1,16)}$=1.736, p=0.206), ($F_{(1,16)}$=0.598, p=0.451), ($F_{(1,16)}$=0.417, p=0.527), and ($F_{(1,16)}$=0.358, p=0.558) for sweetness, taste, acceptance, colour and hardness attributes, respectively. 'Bronsi' fruits sensory attributes namely, sweetness, taste, acceptance, colour, and hardness were also tested at 0.05, level, ANOVA results were ($F_{(1,16)}$=0.108, p=0.747), ($F_{(1,16)}$=0.018, p=0.896), ($F_{(1,16)}$=0.026, p=0.874), ($F_{(1,16)}$=4,125, p=0.059), ($F_{(1,16)}$=0.113, p=0.742) for the five tested attributes, respectively. MA treatments showed also insignificant effects of the MA treatment on the five tested colour attributes. ANOVA results at 0.05 level were ($F_{(1,16)}$=0.404, p=0.534), ($F_{(1,16)}$=1.138, p=0.302), ($F_{(1,16)}$=0.00, p=0.986), ($F_{(1,16)}$=1.318, p=0.268), and ($F_{(1,16)}$=0.113, p=0.742) for sweetness, taste, acceptance, colour, hardness sensory attributes, respectively.

Similar findings were recorded for 'Bronsi'. ANOVA results at 0.05 level showed no significant differences due to ripening treatment. For the two cultivars, ripening method and MA treatments did not show significant effect on all tested sensory attributes. It is worth mentioning that sensory test was made for MA treatments only; since RA treatment was discarded after 4 weeks due to severe deterioration and fungal infections. Such results confirm that whether fruits ripened naturally or be induced they had the same taste properties. Nonetheless, MA treatments of 5 and 10% CO_2 combined with 5% O_2 led to preventing microbial infections, maintaining acceptable level of quality attributes, such as taste, acceptance, colour and hardness.

'Deglet' cultivar

Sorting results of a 25-kg capacity field box is presented in Figure 2. It is quite evident that harvesting practices in Houn use freeze storage rather than cooled storage. As it can be observed 48.50% of the box capacity were fully ripe at moisture content about 25%, partially ripe were either at balah stage or spotted represented 20%, moisture content was determined at 35%, while dry dates represented 28% and its moisture content was less than 15%. Based on sorting, the constituents were stored for 24 weeks, at the end of the study colour analysis and sensory evaluations were made.

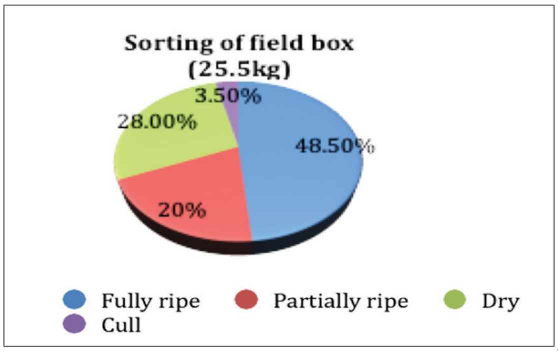


Figure 2. Constituent of field box of 'Deglet'.

1. Colour.

Analysis of variance was made by comparing colour attributes (L), (a) and (b) measured at the beginning of the storage and values were compared with measurements made after 12 and 24 weeks of the three ripening stages ripe, partially ripe (unripe) and dry. ANOVA results for (L) colour attribute were ($F_{(3,96)}$=11.31, p<0.001) for storage conditions (time zero, freezing, CA and RA), ($F_{(2,96)}$=7.53, p=0.001) for ripening stage (fully ripe, unripe and dry and ($F_{(1,96)}$=9.75, p<0.02) and for storage duration (after 12 and 24 weeks). The three treatments significantly affected (L) colour attribute. Similarly, for the (a) colour attribute, for storage conditions results were ($F_{(3,96)}$=4.36, p<0.006), for ripening stage ($F_{(2,96)}$=63.03, p<0.001) and for storage duration ($F_{(1,96)}$=24.18, p<0.001). However, (b) colour attribute results were ($F_{(3,96)}$=10.25, p<0.001) for storage conditions, ($F_{(2,96)}$=5.24, p=0.007) for ripening stage and ($F_{(1,96)}$=22.20, p<0.001) for storage duration. Interactions among the three treatments were also recorded storage conditions vs. ripening stage was at ($F_{(6,96)}$=4.9, p<0.001), while storage treatment vs. storage duration was ($F_{(3,96)}$=4.89, p=0.003) and no interaction between storage conditions and measuring time ($F_{(3,96)}$=1.99, p=0.142). Table 2 shows pairwise comparisons among the three tested treatments (ripening stage, MA and storage duration on the three colour attributes (L, a and b). As it can be seen that RA storage treatment gave lowest colour values of (L), (a) and (b), indicating that the RA condition led to darker colour. Also, MA led to comparable values with time zero and freezing. In general MA leads to preserving colour of stored fruits and vegetable reported by several authors (El-Rayes, 2009; Al-Redhaiman, 2004). The fully ripe and dry treatments resulted in good colour compared with dates stored in regular air.

Table 2. Pairwise comparisons of the effects of tested factors on colour of 'Deglet'.

Sour	L	a	b
	Ripening stage		
Ripe	6.98[a]	3.42[a]	25.94[a]
Partially ripe	7.00[a]	4.20[b]	24.30[a]
Dry	8.44[b]	6.04[c]	25.94[a]
	Storage treatment		
Time zero	7.81[b]	4.19[a]	24.65[a]
RA	5.86[a]	4.25[a]	23.81[a]
CA	7.94[b]	4.63[ab]	25.76[b]
Freezing	8.78[c]	5.10[b]	27.35[b]
	Storage duration		
12 weeks	6.93[b]	4.07[a]	24.27[b]
24 weeks	8.02[b]	5.04[b]	26.52[c]

Means with the same letter in the same column are insignificant at $p<0.05$ level (Tukey-Kramer test).

2. Sensory and visual evaluation.

After 24 weeks, treatments were subjected to sensory evaluation, and unripe treatments were evaluated in their ripening. In this regard, ripening progressed through the storage duration and full ripening was reached after about 8 weeks. During storage, unripe treatment exhibited high respiration rate, whereas air content adjustment was made at shorter rate (data not presented). Nonetheless, the other two ripening stages respired much less and hence, their air content adjustment was made at 7-10 days. Generally, treatments maintained good appearance until the end of the study. The sensory evaluation was made after 24 weeks, no differences were recorded among the treatments and average evaluation of very good was recorded. Also, CA treatment showed comparable results with freezing in appearance and taste.

CONCLUSIONS

Response of soft dates to MA storage was investigated, ('Hellawi', 'Hurra' at balah), ('Bronsi' and 'Taboni' at rutab), and ('Deglet' at fully ripe, partially ripe and dry) were stored at several MA storage conditions at 1±0.5°C. First, 'Hurra' and 'Hellawi' at balah stage were kept for 12 weeks at 10, 15 and 20% CO_2 each with 5% O_2 plus RA as control. MA treatments led to maintaining firmness and TSS compared with RA treatment which lasted way less. However, significant colour change was recorded; fruit skin colour changed to light brown. Second, 'Bronsi' and 'Taboni' soft dates naturally ripe and ripened by inducing method were kept at 5 and 10% CO_2 and 15% each with O_2 and compared with RA. MA conditions maintained fruit firmness, colour and good sensory attributes for 12 weeks, exhibiting insignificant difference among MA treatments and ripening methods on firmness, colour and sensory. Third, 'Deglet' dates at three ripening stages, fully ripe, partially ripe and dry were stored at 1±0.5°C and MA treatment of 5% CO_2 and O_2, and at RA and freezing at -18°C. Colour analysis and sensory evaluations were made after 12 and 24 weeks of storage. Results showed comparable effect of RA to freezing in colour and sensory, but RA showed less effects. Storage conditions tested led to ripening of unripe dates and acceptable sensory evaluations were also recorded. The study demonstrated the potential of using MA conditions of CO_2 and O_2 preferably (5% CO_2 and O_2) for successful storage and packaging of soft dates and their use combined with near zero temperature for long-term storage of soft dates.

ACKNOWLEDGEMENTS

The authors acknowledge the Libyan Authority for Scientific Research for funding the project.

Literature cited

Akasha, I.A.M. (2014). Extraction and characterization of protein fraction from date palm (*Phoenix dactylifera* L.) seeds. Ph.D. thesis (UK: Heriot-Watt University), pp.255.

Al-Redhaiman, K.N. (2004). Modified atmosphere improves storage ability, controls decay, and maintains quality and antioxidant contents of Barhi date fruits. J. Food Agric. Environ. *2* (*2*), 25–32.

Al-Redhaiman, K.N. (2005). Chemical changes during storage of 'Barhi' dates under controlled atmosphere conditions. HortScience *40* (*5*), 1413–1415 https://doi.org/10.21273/HORTSCI.40.5.1413.

Aleid, S.M., and Al-Saikhan, M.S. (2017). Effect of permeable modified atmosphere packaging on quality and shelf life of fresh 'Khenaizy' dates stored at low temperature. J. Food Nutr. Res. *5* (*7*), 503–507.

Alhamdan, A., Hassan, B., Alkahtani, H., Abdelkarim, D., and Younis, M. (2018). Freezing of fresh *Barhi* dates for quality preservation during frozen storage. Saudi J Biol Sci *25* (*8*), 1552–1561 https://doi.org/10.1016/j.sjbs.2016.02.003. PubMed

Alsmairat, N.G., El Assi, N.M., Al Abdalla, A.M., and Mehyar, G.F. (2013). Enhancement of edibility of Barhi and Madjool date palm cultivars at balah and mature stage. Int. J. Bot. *9* (*3*), 123–132 https://doi.org/10.3923/ijb.2013.123.132.

Alturki, S. (2013). Utilization of modified atmosphere packaging to extend the shelf-life of fresh figs. Biotech. *12* (*2*), 81–86 https://doi.org/10.3923/biotech.2013.81.86.

Colelli, G., Mitchell, F.G., and Kader, A.A. (1991). Extension of post harvest life of 'Mission' figs by CO_2-enriched atmospheres. HortScience *26* (*9*), 1193–1195 https://doi.org/10.21273/HORTSCI.26.9.1193.

Dehghan-Shoar, Z., Hamidi-Esfahani, Z., and Abbasi, S. (2010). Effect of temperature and modified atmosphere on quality preservation of Sayer date fruits (*Phoenix dactylifera* L.). J. Food Process. Preserv. *34* (*2*), 323–334 https://doi.org/10.1111/j.1745-4549.2008.00349.x.

Deltsidis, A.I., Eleni, D.P., and Brecht, J.K. (2011). Establishing CO_2 tolerance of pink tomatoes in modified atmosphere packaging at elevated handling temperatures. Proc. Florida State Horticultural Society *124*, 241–245.

El-Rayes, D.A. (2009). Effect of carbon dioxide-enriched atmosphere during cold storage on limiting antioxidant losses and maintaining quality of 'Barhy' date fruits. Meteorology. Environment and Arid Land Agriculture Sciences *20* (*1*), 3–22 https://doi.org/10.4197/met.20-1.1.

FAO. (2019). Food and Agriculture Organization of the United Nations. http://faostat.fao.org (accessed September 13, 2021).

Fennir, M.A., and Morgham, M.T. (2016). Effects of controlled atmosphere conditions on storability of Libyan 'Hurra' soft date cultivar. J. Adv. Agric. Technol. *3* (*3*), 202–206 https://doi.org/10.18178/joaat.3.3.202-206.

Fennir, M.A., and Morgham, M.T. (2019). Response of two Libyan soft date cultivars to induced ripening under controlled atmosphere conditions. Sch. J. Agric. Sci. *1* (*2*), 1–9 https://doi.org/10.21608/sjas.2019.20029.1016.

Gariepy, Y., Raghavan, G.S.V., Plasse, R., Phan, C.T., and Theriault, R. (1985). Long term storage of cabbage, celery and leeks inder controlled atmosphere. Acta Hortic. *157*, 193–202 https://doi.org/10.17660/ActaHortic.1985.157.26.

Gorris, L.G.M., and Peppelenbos, H.W. (2007). Modified-Atmosphere packaging of produce. In Handbook of Food Preservation, 2nd edn, M. Shafiur Rahman, ed. (CRC Press Taylor & Francis Group), p.315–333 https://doi.org/10.1201/9781420017373.pt3.

Harker, F.R., Elgar, H.J., Watkins, C.B., Jackson, P.J., and Hallett, I.C. (2000). Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. Postharvest Biol. Technol. *19* (*2*), 139–146 https://doi.org/10.1016/S0925-5214(00)00090-9.

Johnson, F.T., and Zhu, Y. (2015). Transcriptome changes in apple peel tissues during CO_2 injury symptom development under controlled atmosphere storage regimens. Hortic Res *2* (*1*), 15061 https://doi.org/10.1038/hortres.2015.61. PubMed

Kader, A.A. (1992). Modified atmosphere during transport and storage. In Postharvest Technology of Horticultural Crops, 2nd edn, A.A. Kader, ed., Div. Publication 3311 (University of California), p.85–92.

Kader, A.A., and Hussein, A.M. (2009). Harvesting and Postharvest Handling of Dates (Aleppo, Syria: ICARDA), pp.15.

Kudachikar, V.B., Kulkarni, S.G., and Prakash, M.N. (2011). Effect of modified atmosphere packaging on quality and shelf life of 'Robusta' banana (*Musa* sp.) stored at low temperature. J Food Sci Technol *48* (*3*), 319–324 https://doi.org/10.1007/s13197-011-0238-y. PubMed

Matar, C., Salou, T., Helias, A., Penicaud, C., Gaucel, S., Gontard, N., Guilbert, S., and Guillard, V. (2021). Benefit of modified atmosphere packaging on the overall environmental impact of packed strawberries. Postharvest Biol. Technol. *177*, 111521 https://doi.org/10.1016/j.postharvbio.2021.111521.

Racchi, M.L., Bove, A., Turchi, A., Bashir, G., Battaglia, M., and Camussi, A. (2014). Genetic characterization of Libyan date palm resources by microsatellite markers. 3 Biotech *4*, 21–32.

Salomon-Torres, R., Valdez-Salas, B., and Norzagaray-Plasencia, S. (2021). Date palm: source of food, sweets and beverage. In The Date Palm Genome, Vol. 2, J.M. Al-Khayri, J.S. Mohan, and D.V. Johnson, eds. (Switzerland: Springer Natural), p.3–26 https://doi.org/10.1007/978-3-030- 73750–4_1.

Saquet, A.A., and Streif, J. (2017). Respiration rate and ethylene metabolism of 'Jonagold' apple and 'Conference' pear under regular air and controlled atmosphere. Bragantia (Campinas) *76* (*2*), 335–344 https://doi.org/10.1590/1678-4499.189.

Sarraf, M., Jemni, M., Kahramanoğlu, I., Artés, F., Shahkoomahally, S., Namsi, A., Ihtisham, M., Brestic, M., Mohammadi, M., and Rastogi, A. (2021). Commercial techniques for preserving date palm (*Phoenix dactylifera*) fruit quality and safety: A review. Saudi J Biol Sci *28* (*8*), 4408–4420 https://doi.org/10.1016/j.sjbs.2021.04.035. PubMed

Serrano, M., Pretel, M.T., Botella, M.A., and Amoros, A. (2001). Physiochemical changes during date ripening related to ethylene production. Food Sci. Technol. Int. *7* (*1*), 31–36 https://doi.org/10.1106/Y6MD-JJDH-LT0P-Y9AE.

Sitton, J.W., and Patterson, M.E. (1992). Effect of high carbon dioxide and low oxygen controlled atmosphere on post harvest decay of apples. Plant Dis. *76* (*10*), 992–995 https://doi.org/10.1094/PD-76-0992.

Smith, R.B., and Skog, L.J. (1992). Post harvest carbon dioxide treatment enhances firmness of several cultivars of strawberry. HortScience *27* (*5*), 420–421 https://doi.org/10.21273/HORTSCI.27.5.420.

Thompson, A.K. (2003). Fruit and Vegetables: Harvesting, Handling and Storage, 2nd edn (Blackwell publishing), p.211–212.

Cultivar ‘Assiane’ parthenocarpy in Figuig oasis, Morocco

E. Hanoune[a], O. Laout and M.A. Elhoumaizi[b]

Laboratory of Agricultural Production Improvement, Biotechnology and Environment (LAPABE), Faculty of Sciences, University Mohammed First, 717 Oujda 60000, Morocco.

Abstract

The economy of the oasis system in Morocco is based mainly on the cultivation of date palm (*Phoenix dactylifera* L.). In Figuig oasis located in the southeastern region near the Algerian borders, the estimated number of date palm trees is 190,000 representing 2.8% of total Moroccan date palm. The average annual production is estimated to about 3600 t coming from a diversified cultivar profile from which ‘Aziza Bouzid’ is the best cultivar. The cultivar ‘Assiane’ is the most widely planted date palm cultivar in Figuig oasis which represents more than 51% of the total date palm, followed by the cultivar ‘Boufegous’ (14%), ‘Khalt’ (14%), ‘Aziza Bouzid’ (6%), ‘Boufegous-Gharas’ (5%) and ‘Mejhoul’ (2%). Pollination and fruit setting constitute serious problems for the date palm cultivar ‘Assiane’. It was found that the problem of parthenocarpy has lowered the yields and total production. As a result, farmers tend to substitute the cultivar ‘Assiane’ by other more productive cultivars such as ‘Mejhoul’, ‘Boufegous’ and ‘Najda’. In this study we discuss the future of the cultivar ‘Assiane’ and possibilities of processing parthenocarpic fruits toward other more profitable processed products. The cultivar ‘Assiane’ was planted in a traditional oasis. The new plantation adopts modern cultural and postharvest techniques which increased the yield and produced dates of better morphological and organoleptic quality. The result of the microbiological analyses also showed that the hygienic state of the dates in the new plantation is better than that in the traditional oasis. Resolving the parthenocarpy and the development of the know-how of the farmers in the oasis will allow the cultivar ‘Assiane’ to keep its economic position in Figuig oasis.

Keyswords: morphological, organoleptic, microbiological, parthenocarpy

INTRODUCTION

The economic activity generating income in Figuig oasis is based mainly on agriculture, given its geographical location and its climate characteristic of the palm grove, the agricultural activity is dominated by the *Phoenix* culture with 80% of the number of fruit trees.

The *Phoenix* cultural heritage of the oasis includes more than ten cultivars of which ‘Aziza Bouzid’ is the most commercialized cultivar in the palm grove and ‘Assiane’ the most produced and consumed by the oasis population and many other cultivars such as ‘Boufegous Gharas’, ‘Boufegous’, ‘Khalt’,’Mejhoul’, etc.

Following the climatic changes that have marked agriculture in recent years (appearance of inflorescence and early flowering on different tree species), due to the lack of success of pollination, more than 50% of the production of the ‘Assiane’ cultivar has developed into parthenocarpic fruit that cannot be consumed as fruit.

In our study we will study the impact of the constraints faced by the ‘Assiane’ cultivar on the yield of production, on the morphological, organoleptic and microbiological quality of the date both in the old palm grove and in the new plantation. We will also discuss the perspectives of development and preservation of ‘Assiane’ cultivar in Figuig oasis.

[a]E-mail: elbatoulhanoune@gmail.com
[b]E-mail: elhoumaizi@yahoo.fr

MATERIALS AND METHODS

Study area

This study was carried out in the eastern region of Morocco, Figuig oasis is located at the south-east, surrounded on three sides by Algeria, and located about 400 km south of the Mediterranean (UNESCO, 2011) (Figure 1).

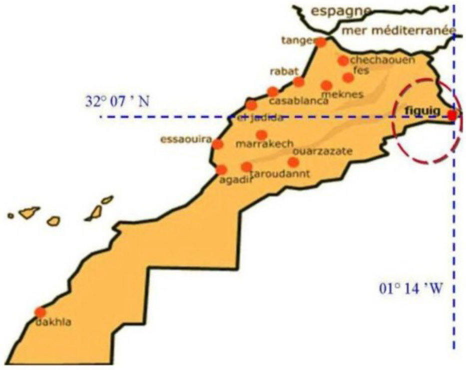


Figure 1. Location of Figuig oasis in Morocco.

The oasis extends over an area of 200 km^2, It is characterized by arid climate, hot dry summers, and cold winters with an average rainfall of 128 mm (annual average) (Commune Teritorrial de Figuig, 2014).

The palm groves are made up of small, cramped and very dense irrigated plots covering an area of about 700 ha. *Phoenix dactylifera* L. represent 75% of all fruit trees (Hakkou and Bouakka, 2004), their population is currently estimated at 190,000 trees. They are divided into about ten cultivars including the cultivar 'Assiane', the most planted date palm cultivar, which represents more than 51% of the total population, followed by the cultivar 'Boufegous' (14%), 'Khalt' (14%), 'Aziza Bouzid' (6%), 'Boufegous-Gharas' (5%) and 'Mejhoul' (2%).

Plantation densities vary according to the farms. Indeed, the average density is estimated at 225 plants ha^{-1} in the old date palm grove, while the average density in the new extension areas is estimated at 180 plants ha^{-1} (Commune Teritorrial de Figuig, 2014).

Plant material

We compared the morphological, organoleptic and microbiological characteristics of the cultivar 'Assiane' with reference to important cultivar in Figuig oasis 'Aziza Bouzid', both in the traditional palm grove of the oasis and in the new plantation called "extension".

Samples: ten females plants for each cultivar in the two palm groves were used for study (Table 1).

Table 1. Sampling of the plant material.

Cultivars	Traditional palm grove	Extension
Assiane	10	10
Aziza Bouzid	10	10
Plant age (years)	20-22	15-20

The plants of the two cultivars from the same plot and almost similar in age, have experienced the same growing conditions (temperature, irrigation, fertilization, etc.), and have all been pollinated using the traditional method and with the same pollen cultivar.

The date palm can produce about 15 bunches, but it is followed by a poor harvest and a weakening of the palm trees. For this reason, it is advisable to limit the number of bunches per palm (Benziouche and Chehat, 2014). The operations of limitation and thinning of the bunches by chiselling were carried out 4 weeks after pollination (end of May). The number of bunches retained for both samples was limited to 8 bunches palm^{-1}, which means one bunch for 8 to 9 functional green palms.

MATERIALS AND METHODS

The yield of cultivars

From the setting to maturity, the date undergoes morphological and organoleptic changes to give the characteristic fruit of each cultivar, our study was carried out on the final stage or ripe dates called "tamer".

We collected all the dates of our samples during September 2021 for 'Assiane' and October 2021 for 'Aziza Bouzid'.

To calculate the fruit yield of the two cultivars, the harvested fruits were weighed with a scale and then sorted by eliminating parthenocarpic fruits, injured or crushed fruits, and all impure fruits.

The sorting differences were weighed separately to obtain the quantity of good commercial quality fruits.

Morphological and organoleptic characteristics of fruits at tamer stage

The morphological and organoleptic characteristics of the 'Assiane' cultivar dates both in the traditional palm grove and in the new plantation were compared based on the quality criteria cited on the recognition of the geographical indication 'Assiane dates from Figuig' and the approval of the related specifications (Ministre de l'Agriculture, de la Pêche Maritime, du Développement Rural et des Eaux et Forêts, 2019).

The morphological and organoleptic analysis was carried out on two samples of 20 fruits for each one.

The morphological measurements were based on: a) dimensions: length and diameter, which were made using a caliper; and b) the mass of the whole date was measured with an analytical balance.

The organoleptic characteristics of dates used to compare the two samples were: shape, color, taste and texture. In order to evaluate the taste, we conducted a statistical analysis based on the opinion of a tasting panel consisting of 20 members, 10 farmers, and 10 consumers.

Microbiological characteristics

The microbiological analyses were carried out based on ISO standards. To assess the hygienic quality of dates we proceeded to the enumeration of total aerobic mesophilic flora TAMF according to the method of reference (ISO 4833.2013) by the technique of surface plating, and for the enumeration of CFU of yeast and mold, we based on the method (ISO 7954:1987) technique by counting colonies at 25°C.

For the sanitary safety, we have examined for *Salmonella* and *E. coli* according to standards EN/ISO 6579 and ISO 16649-1. Microbiological analysis was performed on samples of fresh dates after 30 days of their harvest. The sampling plan is detailed in Table 2.

Table 2. Sampling plan for microbiological analyses.

	n[a]	c[b]	m[c]	M[d]
TAMF	5	2	10^7 cfu g^{-1}	10^8 cfu g^{-1}
Yeast and mold	5	-	-	-
E. coli	5	2	100 cfu g^{-1}	1000 cfu g^{-1}
Salmonella	5	0	Absence in 25 g	

[a]Number of units in the sample.
[b]Number of sample units giving values between m and M.
[c]All results equal or lower are considered satisfactory.
[d]Threshold of acceptability, beyond which the results are no longer considered satisfactory, without the product being considered toxic.

RESULTS AND DISCUSSION

Yield of cultivars in the traditional palm grove and in the new plantation

The yield of fruit of good commercial quality for each sample is calculated by the following formula: % Average yield = (total quantity of good quality fruit/total fruit harvested)×100.

As well as the percentage of sorting deviations is = 100-(% average yield), the results of the evaluation parameters, weighing and sorting dates are detailed in Tables 3-6.

Table 3. Results of weighing and sorting dates in the traditional palm grove.

	Harvest quantity (kg)		Partenocarpic fruits (kg)		Injured or crushed fruits (kg)		Impure fruit (kg)		Total sorting differences (kg)		Good quality fruits (kg)	
	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid
Total (kg)	579.2	409.2	174.52	18.4	112.66	32.23	29.7	14.85	316.88	65.48	262.32	343.72

Table 4. The results of the evaluation parameters in the traditional palm grove.

	Average yield (%)	The percentage of sorting deviations
Aziza Bouzid	84.00	16.00
Assiane	45.29	54.71

Table 5. Results of weighing and sorting dates in the extension.

	Harvest quantity (kg)		Parthenocarpic fruits (kg)		Injured or crushed fruits (kg)		Impure fruit (kg)		Total sorting differences (kg)		Good quality fruits (kg)	
	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid	Assiane	Aziza Bouzid
Total (kg)	498.1	551.25	122.5	0	118.7	31.54	34.01	14.85	275.21	46.39	222.89	504.86

Table 6. The results of the evaluation parameters in the extension.

	% Average yield (%)	Percentage of sorting deviations
Aziza Bouzid	91.58	8.42
Assiane	44.75	55.25

The average yield of the cultivar ‘Assiane’ in both sites was very low compared to the average yield of the reference cultivar ‘Aziza Bouzid’. This was believed due to: mainly to the parthenocarpy (bad polinization of the flower) which increased the rate of sorting gaps, a very big problem that the farmer has to face every year, which represents more than 55% of the total sorting deviations in the traditional palm grove and more than 44% in the new plantation.

The cultivar ‘Assiane’ is the most date palm cultivar widely planted in Figuig oasis and the most consumed and appreciated cultivar by the local population, but the drop in production yields due to parthenocarpy has affected its economic position and has caused significant loss to the farmers. As a result they tend to substitute it with other cultivars of high commercial value such as ‘Aziza Bouzid’, ‘Boufegous-Gharas’ and ‘Mejhoul’.

The uprooting of the ‘Assiane’ cultivar date palms which does not exist anywhere and its substitution by other cultivars threatens its existence in Figuig oasis and unfortunately weakens its position in the local economy.

The other reasons we believed is the traditional harvesting techniques used during the harvesting have also contributed to the increase on the rate of crushed and injured fruits, which explains even the sorting deviation of the reference cultivar.

When we compare the yields of the cultivar 'Assiane' in the two palm groves, there was no remarkable difference, the values are very close either in the fruits of good commercial quality or in the sorting differences, therefore within the oasis of Figuig the cultivar 'Assiane' can be planted in the two palm groves and with the same proportions.

The sorting deviations of the cultivar exceeds half of the total quantity produced by *Phoenix* cultural campaign. It was found unfortunately used only as animal feed, which represents an economic loss and a loss of biomass resources. Hence the importance of conducting scientific research to guide and encourage the development of date production techniques and the development of sorting waste, including parthenocarpy, through the processing of date derivatives such as vinegar, date powder and date juice concentrate.

The results of the scientific research will contribute to the improvement of the know-how of the professionals, and thus save the socio-economic system of the oasis based mainly on *Phoenix* culture.

Quality characteristics of the 'Assiane' cultivar

1. Morphological characteristics.

The results of the evaluation of the morphological criteria of our samples showed that our fruits respect the norms of labilization prescribed by Morocco agriculture standards, including the length, the diameter and the mass of dates (The Minister of Agriculture, Maritime Fisheries, Rural Development and Water and Forests, 2019). But when we compare the results obtained in the two palm groves, we will notice that the dates cultivated in the extension, are the best in the three parameters, especially the mass of the dates where there is in average a difference of 1.01 g more (Table 7).

Table 7. Morphological characteristics of the cultivar 'Assiane'.

	Length (cm)	Diameter (cm)	Mass (g)
New plantation	3.44	2.02	7.06
Traditional oasis	3	1.70	6.05

The analysis of the know-how of the oasis population has shown that the modern cultural techniques adopted in the new plantation have made it possible to produce dates of better morpholigical quality. It was a successful experience that farmers must adopt even in the traditional palm grove.

2. Organoleptic characteristics.

The fruits of both samples have almost identical organoleptic characteristics which was recorded as: a) the shape is generally oval to oblong; b) the color goes from golden yellow to brown; and c) the texture is semi-soft.

Only taste was the focus of controversy. Table 8 represents the results of statistical analysis.

Fruits of cultivar 'Assiane' collected from the traditional palm grove was highly appreciated (80%) by the tasting panel members. It had a sweeter taste and a pronounced characteristic flavour.

Table 8. Statistical analysis of the panel to evaluate the dates taste.

	'Assiane' produced in traditional palm grove	'Assiane' produced in the new extension
Positive appreciation	80	20

The micro-climate in the traditional palm grove allows to produce dates of better organoleptic quality. Nevertheless, the surface of establishment does not allow any more afforestation.

The interpretation of the microbiological analyses results was based on the international regulation (Codex Alimentarius N° 2073/2005,2005) and the Moroccan decree n°293-19 (Ministre de l'Agriculture, de la Pêche Maritime, du Développement Rural et des Eaux et Forêts, 2019) fixing the list and the limits of the microbiological criteria authorized in the primary products and the food products (Table 9).

Table 9. Microbiological results of dates from the traditional oasis and from the new date palm plantation.

	TAMF				Average		Yeast and mold					Average	*E. coli*	*Salmonella*
	The traditional palm grove													
Assiane	9000	7900	6500	8100	8.5×10^3	8×10^3	2900	2600	1900	2400	2700	25×10^2	0	Absence in 25 g
Aziza Bouzid	6000	7200	5800	5900	6600	63×10^2	4900	4200	4400	3500	3000	40×10^2	0	Absence in 25 g
	The new plantation													
Assiane	260	270	240	180	300	250	140	160	150	135	115	140	0	Absence in 25 g
Aziza Bouzid	100	110	98	105	102	103	101	110	100	89	90	98	0	Absence in 25 g

The results are in accordance with the thresholds set by the regulations, whether Moroccan or international. But when we compare the results of the analyses of FMAT, yeasts and molds we notice that the hygienic quality of the dates in the new plantation is better than that of the traditional palm grove. This contrast is the result of modern techniques adopted during the harvest and postharvest treatments that respect good hygiene practices in the new plantation, contrary to the traditional palm grove where the farms are small family plots and the farmers still use traditional techniques during and post harvesting.

CONCLUSIONS

Despite all the constraints facing the production of the 'Assiane' cultivar in Figuig especially parthenocarpy, it remains the characteristic cultivar of the oasis and the dominant tree in the palm groves.

Although agriculture in Figuig records low yields, these activities remain vital for the preservation of the oasis, including the social and cultural balance of the population. Indeed, the interest of agricultural activity mainly *Phoenix* culture is not only measured in terms of monetary income, but especially in terms of social and cultural return.

Literature cited

Arrêté conjoint du ministre de l'agriculture, de la pêche maritime, du développement rural et des eaux et forêts et du ministre de la santé n°293-19 du 9 joumada II 1440 (15 février 2019); fixant la liste et les limites des critères microbiologiques autorisées dans les produits primaires et les produits alimentaires. Bulletin Officiel n°6796 du18/07/2019, p.1686.

Benziouche, S.E., and Chehat, F. (2010). La conduite du palmier dattier dans les palmeraies des Zibans (Algérie) quelques éléments d'analyse. Eur. J. Sci. Res. *42* (*4*), 644–660.

Codex alimentarius, Règlement (CE) No 2073/2005 de la Commission du 15 novembre 2005 concernant les critères microbiologiques applicables aux denrées alimentaires, pp.30.

Commune Teritorrial de Figuig. (2014). Activités Économiques, www.ville-figuig.info.

EN/ISO 6579 Microbiologie de la chaîne alimentaire – Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella – Partie 1: Recherche des *Salmonella* spp.

Hakkou, A. and Bouakka, M. (2004). Oasis de Figuig: état actuel de la palmeraie et incidence de la fusariose vasculaire. Science et Changements Planétaires / Sécheresse *15* (*2*), 147–158.

ISO 16649-1 Microbiologie de la chaîne alimentaire - Méthode horizontale pour le dénombrement des *Escherichia coli* bêta-glucuronidase positive – Partie 1: Technique de comptage des colonies à 44°C au moyen de membranes et de 5-bromo-4-chloro-3-indolyl bêta-D glucuronide.

ISO 4833-2:2013 Microbiologie de la chaîne alimentaire – Méthode horizontale pour le dénombrement des micro-organismes – Partie 2: Comptage des colonies à 30°C par la technique d'ensemencement en surface.

ISO 7954:1987Microbiologie – Directives générales pour le dénombrement des levures et moisissures – Technique par comptage des colonies à 25°C.

Ministre de l'Agriculture, de la Pêche Maritime, du Développement Rural et des Eaux et Forêts; Arreté N°1452–19 du 27 chaabane1440 (3 Mai 2019) portant reconnaissance de l'indication geographique "dattes Assiane de Figuig" et homologation du cahier des charges y afferent ; Bulletin Officiel N°6810-5 moharrem 1441(5-9-2019).

UNESCO. (2011). Oasis de Figuig. www.unesco.org/fr.

Potential of modified atmosphere packaging for storage of 'Dhakki' dates

S.A. Shahzada[1,a], A.S. Ambreen[2], M. Arshad Farooq[3], T.Z. Chohan[3] and N. Latif[4]

[1]Food Technology Section, Agriculture Research Institute, Dera Ismail Khan, Pakistan; [2]National Physical and Standards Laboratory (NPSL), PCSIR, Islamabad, Pakistan; [3]Pakistan Agriculture Research Council (PARC), Islamabad, Pakistan; [4]PARC Arid Zone Research Center, Dera Ismail Khan-29050, Pakistan.

Abstract

The date palm (*Phoenix dactylifera* L.) imparts close and everlasting association with mankind, and is a legend for the Arabic world. Since date palm is an important tree of arid and tropical regions a large area of Pakistan is under date cultivation ranking the country at 5th position world over. The 'Dhakki' cultivar of Dera Ismail Khan (KPK, Pakistan) has extra-large size (5-6×3-4 cm^2), very high pulp (96%) to small pit ratio and delicious taste, and hence it is considered one of the best cultivars worldwide. However, coincidence of ripening season with monsoon and adoption of traditional technology are a few major factors responsible for excessive wastage and quality degradation, and are the basis for low return. The 'tamar' dates intended for marketing and storage for being nutritious are prone to deterioration micro-biologically and biochemically. The use of modified atmosphere packaging (MAP) is currently underway extending shelf-life of fresh and processed food commodities. The atmosphere surrounding the product is desirably modified by passive MAP technique developing naturally a desired atmosphere through selective film packaging. The MAP is responsible for reduced respiration and ethylene production, and quality preservation. The present study was undertaken to investigate the response of MAP to 'Dhakki' date on storage at 40°C. The cured 'Dhakki' date adjusted to 0.52, 0.62 and 0.75 water activity was packed under cellophane, polyethylene and laminated film pouches and changes in physico-chemical and organoleptic quality parameters were evaluated. The quality deterioration appeared a function of both water activity and type of gaseous atmosphere produced under the package. Samples stored at 0.75 aW under cellophane rapidly deteriorated largely by slime formation, and that with 0.62 aW under laminated film maintained natural characteristic color, flavour and freshness of samples during storage. In order to extend shelf life of high quality product 'Dhakki' dates adjusted to 0.62 aW require proper MAP packaging for storage.

Keywords: 'Dhakki' date, laminated films, modified atmosphere, packaging, water activity

INTRODUCTION

Pakistan is considered the 5th largest date producing country in the world, and the date is our important cash crop and a good source of foreign exchange earnings. The total cultivated area of all type of dates in Pakistan exceeds 98.1 thousand ha with its estimated annual production over 650,000 t, which constitutes about 11% of total world production (Anonymous, 2019a, b). Pakistan is exporting mostly dried dates worth Rs. 1.786 billion annually (Anonymous, 2019a, b). Cultivation of date palm in Khyber Pakhtunkhwa (KPK) province exceeds 1500 ha with 39,000 t production out of which more than 60% is furnished from Dera Ismail Khan area. Most of the plantations in Dera Ismail Khan are concentrated in Panyala, Paharpur, Chowdhwan and Dhakki, where summer is hot, a climate responsible for early ripening of the date fruits. The temperature during June August normally ranges between 38 and 48°C rising sometimes above 50°C with about 30 mm rainfall. Among the local cultivars 'Dhakki' is the most promising cultivar with commercial importance. The date is quite popular for its extra-large size (4-5 cm long and 2-3 cm thick) of small stone and heavy

[a]E-mail: sasdikpk@gmail.com

in weights (16-20 g fruit^{-1}). It has fine texture, relish taste (Baloch, 1999) and fetches a high price in the market. However, concurrence of monsoon season with date ripening period, the crop receives heavy damages by rainstorm and insect bites. The losses are even greater in case of 'Dhakki' date, which is a late maturing cultivar and very susceptible at mature/ripened stage to hot humid climate. Moreover, during peak production period a large quantity of the fresh fruit is left over, and gluts the local market. Due to lack of appropriate processing and storage facilities the surplus produce is wasted.

Modified atmosphere packaging (MAP) is now being used increasingly for extending the shelf-life and reducing the wastage of a wide range of fruits and vegetables. MAP is a technique in which the air surrounding fruit in a package is flushed out and replaced with a carefully formulated gas mixture such as carbon dioxide, oxygen, and nitrogen. Modified atmospheres (MA), i.e., elevated concentrations of carbon dioxide and reduced levels of oxygen and ethylene inside the package. MA benefits include reduced respiration, ethylene production and sensitivity to ethylene, retarded softening, compositional changes and reduced decay (Kader, 2004; Aleid et al., 2012; Mortazavi et al., 2007). A rapid darkening is also common in dates in storage at the prevalent elevated summer temperature with high humidity, which causes a very annoying situation for the date industry and calls for attention. Oxidation of phenolic compounds and involvement of sugar are the dominant factors causing darkening at elevated temperatures (Vandercook et al., 1979). Mechanism for the browning in model systems (Hodge, 1953), and in fruits and vegetables (McWeeny et al., 1974; Wedzicha, 1987) had been reviewed thoroughly. Packing under vacuum or inert gases (Rygg, 1977; Mohsen et al., 2003) or applying sulphite treatment (McWeeny et al., 1974) had also been suggested for storage to prolong shelf life of high moisture dates and other moist foods. Previously we have reported that 'Dhakki' dates have about 0.62 water activity lying at the segment covering intermediate moisture levels (Saleem et al., 1997). The information regarding the effect of storage atmosphere at elevated temperature as well as water activity on stability of dates in general and the 'Dhakki' dates in particular are lacking. The objective of the present investigation is to explore the potential of modified atmosphere packaging and optimize water activity close to freshly cured 'Dhakki' dates in order to enhance storage stability at the elevated temperature of 40°C.

MATERIAL AND METHODS

Sample preparation

'Dhakki' dates at khalaal stage having 200-250 mm Hg cm^{-2} hardness index (Baloch et al., 2003) were procured from the local market. Well-developed fruits having good appearance were taken while the unwanted were discarded. To retain normal color and flavour of the dates during curing/drying the fruits taken in a wire-mesh basket were dipped (1 kg L^{-1}) for 1 min in potassium metabi sulphite (0.5 g 100 mL^{-1}) solution at 70°C. The treated samples were allowed to drain, taken on to stainless steel trays with single layer loading of 6 kg m^2, kept in a Pak made thermostatically controlled dehydrator equipped with hot air overflow system and then cured and dried at 40°C for 10 h until reaching about 24% moisture contents. The dates were thoroughly mixed to ensure sample uniformity. Samples required for sorption studies were made into pulp after removing seeds, whereas whole cured fruits were used for further storage studies.

Studies were carried out to observe the effect of different packaging films on physiochemical properties of dates during storage. Dates were packed at three different pouches made from polyethylene, cellophane and laminated materials. The general specification of these films is shown in Table 1. In order to find out shelf life of the 'Dhakki' dates the cured dates were adjusted to 0.62 aW as reported previously (Saleem et al., 1997). The sample was divided into four equal portions each for a specific packaging material (cellophane, polyethylene, laminate) to pack under different pouches. A control sample was also run side by side to compare the effectiveness of the treatments. The packed/sealed samples were then incubated in an oven for 4 months at 30 and 40°C. The packaging materials were investigated for permeability to moisture and atmospheric gases at both temp 30 and

40°C. The samples were periodically taken out after one-month interval, and were subjected to analysis for moisture contents, browning, acidity, pH and total soluble solids. Organoleptic evaluations were also performed. Samples were taken out from each packaging after every month and analyzed for darkening, pH and titratable acidity. The dates after removing the pits were cut into small pieces, and ground into a uniform mash. The mash was extracted with distilled water or dilutes acetic acid (2 g 100 mL^{-1}) for the measurement of pH and titratable acidity, and for darkening evaluation, respectively. The experiment was conducted simultaneously and the data analyzed statistically.

Table 1. General specifications of films.

Type of film	Water vapor transmission	O_2 permeability	N_2 permeability	CO_2 permeability	Water absorption
Laminated	1.3	550	180	2900	Low
Polyethylene	0.7	240	60	800	Low
Cellophane	0.3	1	1	13	High

g loss 24 h^{-1} 100 in^{-2}, 2, 3, 4 mL 24 h^{-1} 100 in^{-2}.

RESULTS AND DISCUSSION

Sorption isotherm

The date samples stored at water activity of 0.58 aW or below started losing weight, whereas gain in weight was observed in samples kept under water activity of 0.75 aW and above. The loss or gain in weight was rapid during initial equilibration periods, which levelled off after 5 days of equilibration. The equilibrium moisture content (EMC) increased from 10.6 to 95.4% with respective increases in water activity level from 0.12 to 0.97 aW (Table 2). A plot between EMC (%) and corresponding water activity values represents moisture sorption isotherms (Figure 1). The figure depicts a typical sorption isotherm not segmented distinctly as frequently reported in theoretical representations. Heiss (1968) also reported a number of similar isotherms pertaining to fruits. The shape of the current isotherm indicates an overlap of moisture layers from one sorption region to the other. First segment of the isotherm extends to 0.25 aw with relatively high rate of water uptake per unit change in water activity. The 2nd portion is larger in size approaching up to about 0.60aw and appears to be almost flat in shape. This portion most probably carries a moisture level for mono-layer coverage. The last portion is enlarged one with highest slope for water uptake denoted for the region of vapor and capillary water. The isotherm depicts how the water activity interacts with food components and to its moisture, and thus helps in predicting stability of the dates during storage at various water activity levels. Water activity of the sample at zero weight change, calculated from the point of intersection from the plot between water activity and loss or gain in weight (%), was found to occur at about 0.61-0.62 aW, and supposed to be the water activity of the Dhakki dates. This water activity is within the reported range for dehydrated semi moist fruits (Davies et al., 1976). The water activity of 0.61-0.62 aW corresponded to 24-25% equilibrium moisture contents.

The chosen limit of water activity covers the water activity of the dates (≈0.62 aW), extends to the range of semi-dried and moist foods stretching out to the sorption segment possibly intended for storage of freshly cured dates. Further, a temperature of 40°C was selected for storage studies so as to collect information at the elevated temperature. It is pertinent to note that the selected temperature lies within the range of most prevalent summer temperature corresponding with the high production season as well as rapid deterioration period of the freshly ripened dates.

Table 2. Saturated salt solutions of required water activity at 40°C. Source: Troller and Christian (1978).

S. no.	Name of salt	Formula	A_W
1	Lithium chloride	$LiCl_2$	0.12
2	Potassium acetate	KCH_3COO	0.23
3	Magnesium chloride	$MgCl_2$	0.33
4	Potassium carbonate	K_2CO_3	0.44
5	Magnesium nitrate	$Mg(NO_3)_2$	0.52
6	Sodium bromide	NaBr	0.58
7	Sodium chloride	NaCl	0.75
8	Ammonium sulfate	$(NH_4)_2SO_4$	0.79
9	Potassium chloride	KCl	0.83
10	Potassium chromate	K_2CrO_4	0.88
11	Potassium nitrite	KNO_2	0.94
12	Potassium sulfate	K_2SO_4	0.97

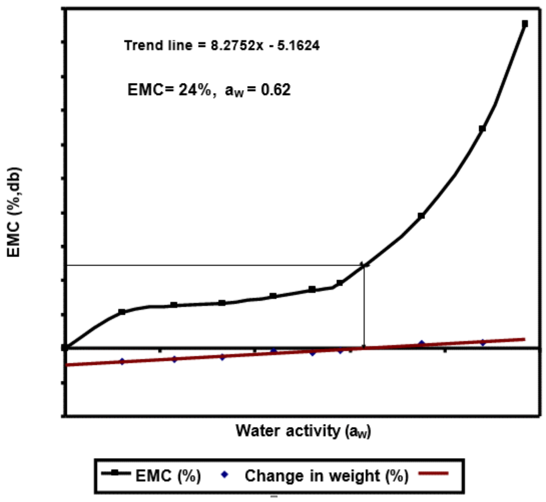


Figure 1. Sorption isotherm and water activity of 'Dhakki' dates at 40°C.

Permeability studies of packaging materials

The packaging materials were investigated for permeability to moisture and atmospheric gases at both temperatures of 30 and 40°C. The cellophane pouches showed resistance to permeability to oxygen and nitrogen whereas polyethylene pouches facilitated the gases but controlled moisture vapors to pass through. The laminated pouches showed least permeability to both water vapors and storage gases. The quality deterioration in samples stored under polyethylene and cellophane pouches continued at a rapid rate, whereas the changes were minimal under the laminated environment. The laminated material for its better structural design resisted to the passage of oxygen and moisture vapors hence the quality of the products was maintained without appreciable changes. Nevertheless all of packaging films reduced changes to a certain degree during storage for four month period. The laminated pouches proved to be superior barrier against contamination, moisture and atmospheric gases at both temperatures of 40 and 30°C, and hence protected quality of the dates to a maximal value.

Storage studies under different packaging materials

1. Darkening/browning.

The browning showed increasing trend for all the samples during the 4-month storage at both temperatures of 30 and 40°C, however, the changes took place with slower rate at 30°C (Figure 2). The samples under cellophane pack exhibited maximum browning increasing the initial absorbance of 0.04 to 0.079 nm and 0.089 whereas it was a minimum of 0.051 nm and 0.061 under the laminated at the adjusted water activity at both temperatures of 30 and 40°C, respectively. The rate of darkening was more than twice greater in cellophane than that in laminated at both temperatures. The laminated samples looked normal, whereas those under cellophane appeared dark brown. A higher intensification of browning on storage of the samples kept under cellophane as well as under polyethylene indicates that the darkening process followed both oxidative and non-oxidative routes at the elevated storage temperatures. Since the cured dates possess high amount of sugar carbohydrates together with small amount of amino acids, as well as contain a noticeable amount of tannin poly-phenolic compounds (Sawaya et al., 1982), the operating of aerobic and anaerobic deteriorative mechanisms seems acceptable. Maier and Schiller (1960) reported that the darkening at 49°C was caused primarily by non-oxidative and non-enzymatic reactions. They further reported that enzyme browning of polyphenols proceeds at observable rates at room temperature, whereas at temperature above 38°C sugar browning predominates. However, Maier and Schiller (1961a, b) made both oxidative and non-oxidative deteriorative reactions responsible for the darkening in 'Deglet Noor' at 38°C. They further reported that the former caused 20% greater darkening, which was inhibited by storage under inert gas. Maier and Metzler (1965) opined those insoluble tannins were responsible for non-enzymatic oxidative browning in dates. Mohsen et al. (2003) suggested vacuum packaging as a useful technique for reducing darkening of the date for long-term storage. The present results are consistent in principle to those reported earlier, as the rates of browning were much lower in dates stored under laminated pouches which resisted to oxygen and vapor passage.

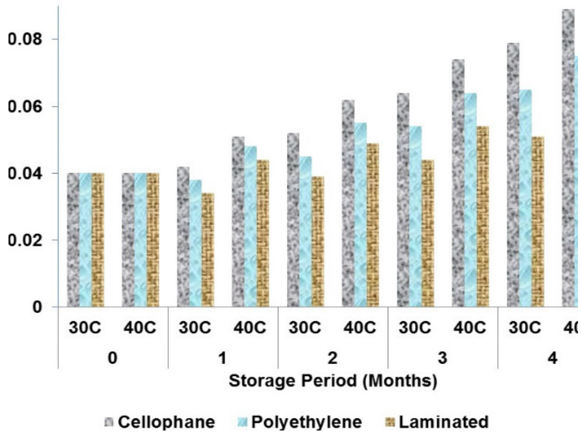


Figure 2. Effect of packaging materials on darkening of 'Dhakki' dates during storage at 30 and 40°C.

2. pH changes.

Irrespective of the packaging material under which the samples were incubated at 30 and 40°C there appeared a gradual and linear decline in pH throughout the storage (Figure 3). The pH decreased from 6.3 to as low as 3.68 and 3.58 at both storage temperature of 30 and 40°C, respectively. Maximum pH drop of 2.72 units occurred when the samples were stored under the cellophane pack whereas a corresponding reduction of 1.6 and 0.92 pH units took place for samples stored under polyethylene and laminated, respectively. Maier and Schiller (1961a) also reported a marked decrease in pH on storage of 'Deglet Noor'. A gradual decline in pH for all of the samples during storage advocates the involvement of both oxidative and non-oxidative mechanisms as reported above in case of NEB process, and the storage under laminated pouches emerged as most effective preposition for controlling the deteriorative reactions. Since the cellophane is responsive for about 66% of the total effect, it is very likely that the process causing pH changes follows mainly an oxidative pathway similar to that speculated for the darkening process.

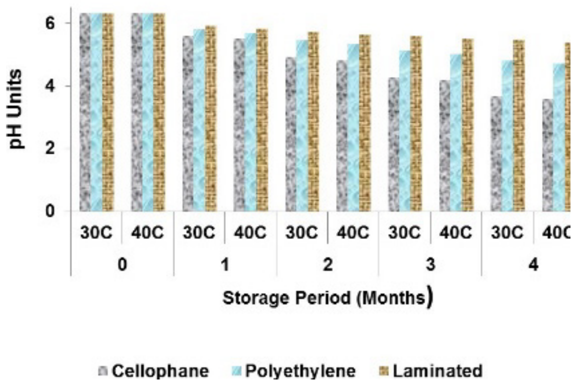


Figure 3. Effect of packaging materials on pH of 'Dhakki' dates during storage at 30 and 40°C.

3. Titratable acidity.

A consistent rise in titratable acidity was observed for all the samples during the storage at 30 and 40°C (Figure 4). However, the rates were greatly influenced by manipulations of the storage materials. Acidity content prior to storage, being 22.15×10-2% (mg g^{-1}) increased 47.45×10-2%, 64.35×10-2% to 100.55×10-2% (mg g^{-1}) and 49.75×10-2%, 66.55×10-2% to 100.95×10-2% (mg g^{-1}) after 4 month at 30 and 40°C under laminated, polyethylene and cellophane, respectively. The rate of acid formation in the samples was about 1.76 and 2.86 times higher under cellophane than for those under polyethylene or the laminated respectively (Figure 4). It is further observed that the samples, which yielded higher amount of darkening, had produced relatively greater amount of acidity as well as pH drop. Hence browning, acid formation and pH changes are related with each other and follow the similar pattern. Since there is a great concordance among the deteriorative reactions leading to darkening, acid formation and pH changes it is apprehended that all such reactions had a common pool where these are emerging. Present findings are also in line with those reported by Saleem et al. (1997) and Saddozai et al. (1998). They also reported similar increase in acidity with coexisting decrease in pH during storage of 'Dhakki' dates and milk concentrate 'Khoa', respectively.

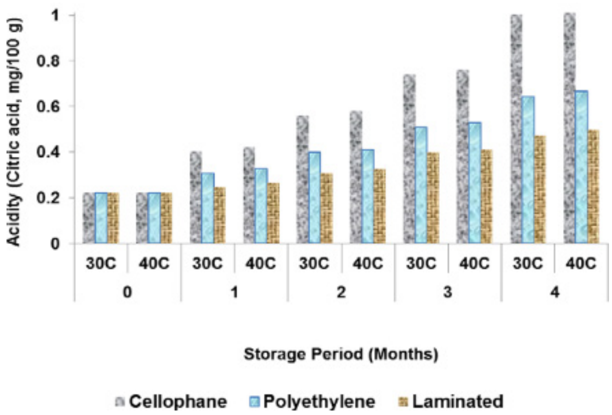


Figure 4. Effect of packaging materials on titratable acidity of 'Dhakki' dates during storage at 30 and 40°C.

CONCLUSIONS

Laminated pouches produced the least effect on moisture variations at different temperatures on account of having two types of insulating materials of lower permeability. The cellophane and polyethylene pouches for having higher permeability to moisture and to oxygen at both the temperatures could not decrease the quality changes which continued at a rapid rate, whereas the changes were minimal under the laminated environment. Nevertheless, all of packaging films reduced changes to a certain degree during storage for four month period. The laminated pouches proved to be superior barrier against contamination, and controlled variation in moisture and atmospheric gases at both temperatures of 40 and 30°C, and hence helped protecting quality of the dates. All quality parameters apparently are the function of storage atmosphere resulting from the packaging materials. The samples which received increased browning corresponded with greater overall pH drop and high acidity index. It is manifested that darkening and associated changes are mainly responsible for quality deterioration of 'Dhakki' dates. The impacts of packaging materials on quality parameter are statistically significant. Irrespective of the packaging material used changes in the quality continued slowly during four months of the storage at 30 and 40°C. The non-enzymatic browning and acidity showed increasing trend while pH and total soluble solids showed reverse trend at both of the temperatures, however, the changes took place with slower rate at 30°C. 'Dhakki' dates preferably are stored under laminated pouches free from oxygen and at moisture vapors equivalent to water activity closed to 0.62 aW so that the product maintains high quality and sufficient stability giving extended shelf life.

ACKNOWLEDGEMENTS

The author thanks the support of Pakistan Agricultural Research Council (PARC) Pakistan for providing the funds under Agricultural Linkages Programme (ALP) to Agriculture Research Institute, Dera Ismail Khan, KPK for executing of these studies.

Literature cited

Al-Eid, S.M., Barber, A.R., Rettke, M., Leo, N., Alsenaien, W.A., and Sallam, A.A. (2012). Utilization of modified atmosphere packaging to extend the shelf life of Khalas fresh dates. Int. J. Food Sci. Technol. *47* (*7*), 1518–1525 https://doi.org/10.1111/j.1365-2621.2012.03000.x.

Anonymous. (2019a) Agric. Statistics of Pakistan (Islamabad: Govt. Pakistan, Ministry Food Agric & Livestock).

Anonymous. (2019b). National Exhibition on Dates (Pakistan: Export Promotion Bureau).

Baloch, A.K. (1999) Enhancement of Post-Harvest Quality and Stability of Dhakki Dates Using Advanced Technology (Islamabad: Pakistan Science Foundation).

Baloch, A.K., Saleem, S.A., Dar, N.G., and Baloch, M.K. (2003). Influence of microwave radiation on ripening of Dhakki dates. Journal of Food Processing and Preservation *27* (*3*), 181–193.

Davies, R., Birch, G.G., and Parker, K.J. (1976). Intermediate Moisture Foods (London, UK: Applied Science Publishers).

Heiss, R. (1968). Stability and Sorption Behaviour of Low Moisture Food (Berlin, New York: Springer Verlag).

Hodge, J.E. (1953). Dehydrated foods, chemistry of browning reactions in model systems. J. Agric. Food Chem. *1* (*15*), 928–943 https://doi.org/10.1021/jf60015a004.

Kader, A.A. (2004). Controlled atmosphere storage. In The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks, Agriculture Handbook Number 66, K.C. Gross, C.Y. Wang, and M. Saltveit, eds. (Washington DC, USA: USDA, ARS.), pp4.

Maier, V.P., and Metzler, D.M. (1965). Changes in individual date phenols and their relation to browning. J. Food Sci. *30* (*5*), 747–752 https://doi.org/10.1111/j.1365-2621.1965.tb01835.x.

Maier, V.P., and Schiller, F.H. (1960). Studies on domestic dates. I. Methods for evaluating darkening. Food Technol. *14*, 139–143.

Maier, V.P., and Schiller, F.H. (1961a). Studies on domestic dates. II Some chemical changes associated with deterioration. J. Food Sci. *26* (*3*), 322–328 https://doi.org/10.1111/j.1365-2621.1961.tb01662.x.

Maier, V.P., and Schiller, F.H. (1961b). Studies on domestic dates. III Effect of temp on some chemical changes associated with deterioration. J. Sci. *26* (*5*), 529–534.

McWeeny, D.J., Knowles, M.E., and Hearne, J.F. (1974). The chemistry of non enzymic browning in foods and its control by sulfite. J. Sci. Agric. *25* (*6*), 735–746 https://doi.org/10.1002/jsfa.2740250616.

Mohsen, A., Amara, S.B., Salem, N.B., Jebali, A., and Hamdi, M. (2003). Effect of vacuum and modified atmosphere packaging on Deglet Nour date storage in Tunisia. Fruits (Paris) *58* (*4*), 205–212.

Mortazavi, S.M.H., Arzani, K., and Barzegar, M. (2007). Effect of vacuum and modified atmosphere packaging on the post harvest quality & shelf life of date fruits in khalal stage. Acta Hortic. *736*, 471–477 https://doi.org/10.17660/ActaHortic.2007.736.45.

Rygg, G.L. (1977). Date Development, Handling, and Packing in United States. Agric. Handbook No. 482 (Research Service US Department of Agriculture).

Saddozai, A.A., Baloch, W.A., Saleem, S.A., Malik, G.J., and Baloch, A.K. (1998). Characterization and storage stability of khoa (milk concentrate) as related to water activity. Pak. J. Food Sci. *8* (*1–4*), 1–7.

Saleem, S.A., Naeem, M., Baloch, W.A., and Baloch, A.K. (1997). Influence of water activity on the stability of 'Dhakki' dates. Pak. J. Food Sci. *7* (*1–2*), 1–6.

Sawaya, W.N., Khatchadourian, H.A., Khalil, J.K., Safi, W.M., and Al-Shalhat, A. (1982). Growth and compositional changes during the various development stages of some Saudi Arabian date cultivars. J. Food Sci. *47* (*5*), 1489–1492 https://doi.org/10.1111/j.1365-2621.1982.tb04967.x.

Troller, J.A., and Christian, J.H.B. (1978). Water Activity and Food (New York: Academic Press), pp.50.

Vandercook, C.E., Hasegawa, S., and Maier, V.P. (1979). Quality and Nutritive Value of Dates as Influenced by Their Chemical Composition, Vol. 54 (Date Growers' Institute).

Wedzicha, B.L. (1987). Chemistry of sulphur dioxide in vegetable dehydration. Int. J. Food Sci. Technol. *22* (*5*), 433–450 https://doi.org/10.1111/j.1365-2621.1987.tb00509.x.

Evaluation of physico-chemical parameters and bio-active compounds of date seeds of fifteen Moroccan cultivars

H. Harrak[1,a], M. Moutaoikil[1,2] and M. Guessous[1,3]

[1]Regional Centre of Agricultural Research of Marrakesh, National Institute of Agricultural Research (INRA), Rabat, Morocco; [2]Faculty of Sciences of Meknes, Moulay Ismaïl University, Morocco; [3]Faculty of Sciences and Techniques of Mohammedia, Hassan II University, Morocco.

Abstract

Date production and processing generate a huge amount of seeds that have interesting food, cosmetic, and medicinal properties. However, they are insufficiently valorised. This work aims to investigate physical and physico-chemical parameters and bio-active compounds of date seed powder (DSP) of 15 Moroccan cultivars. The studied parameters were: color parameters (L*, a*, b*, Cab*,and hab), humidity, total soluble solids, water activity (aw), pH, ash, and minerals. The evaluated bio-active compounds included total betalain, betacyanin and indicaxantin pigments, and total polyphenols. The evaluation of antioxidant activity was also carried out. The results have shown that there is a significant difference between the studied cultivars. The DSP has a high dry matter (DM) rate (89.14-91.66%) with predominance of insoluble solids. It is also characterized by a good stability according to aw values (0.41-0.56). In addition, DSP is rich in potassium and it is slightly acidic with a pH value ranging between 4.96 and 5.62. The content of indicaxanthin yellow pigments exceeds that of betacyanin red pigments. DSP also has high polyphenol contents reaching up to 6.99 g gallic acid equivalent 100 g^{-1} DM and an interesting antioxidant activity (1.95-7.63 g ascorbic acid equivalent 100 g^{-1} DM). Furthermore, the principal component analysis confirmed the divergence between the studied cultivars. 'Najda' was distinguished by the highest values of antioxidant activity, total betalains, indicaxanthins, betacyanins, ash, a* and Cab*, and the lowest values of pH, lightness L* and hue angle hab. In addition, 'Bouittob' was distinguished by low values of humidity, aw and b*. Some cultivars were characterized by potential characteristics such as 'Ademou', 'Black Bousthammi', 'Bouslikhene', 'Jihel', 'Haoua', and 'Najda'. This study highlighted that the DSP is an excellent source of nutritional and bio-active compounds, and antioxidant activity. It could therefore be appropriately used as an ingredient in functional foods and cosmetics.

Keywords: *Phoenix dactylifera* L., date seed, bio-active compound, antioxidant activity, valorisation

INTRODUCTION

The increase in the production, processing and consumption of date palm fruits (*Phoenix dactylifera* L.) leads to substantial amounts of seeds estimated to be about one million t $year^{-1}$ (Bouallegue et al., 2019). This by-product can present a risk to the environment through the accumulation of organic matter and non-biodegradable compounds. Depending on several factors such as genotype, date seeds represent 6.10 to 11.47% of the fruit weight (Habib and Ibrahim, 2009), which constitutes a massive bio-resource. In fact, date seeds are not only rich of a considerable amount of nutritional compounds (minerals, vitamins, lipids, etc.), but also of bio-active compounds represented by a wide range of phyto-chemicals especially polyphenols, carotenoids, flavonoids, and phytosterols (Al-Farsi and Lee, 2011). These biomolecules have a preventive effect against several diseases including diabetes, cardiovascular diseases, and cancer (Vayalil, 2012).

[a]E-mail: hasnaa.harrak@inra.ma

Date seed powder is widely used as an ingredient for preparing caffeine-free coffee (Habib and Ibrahim, 2009), bakery products (Platat et al., 2015), and meat products (Bouaziz et al., 2020). Date seed oil has also several applications. It can replace palm oil for cooking, frying, and seasoning (Nehdi et al., 2018). It is also used for the formulation of several cosmetic products, in particular creams, shampoos, and soaps (Devshony et al., 1992). The valorisation of date seeds can represent an important source of income for farmers and can also encourage innovation and development of new products with functional interest. The objectives of this study were the physical and physico-chemical characterization and bio-active compounds investigation of date seeds of 15 Moroccan cultivars toward their appropriate uses.

MATERIALS AND METHODS

Plant material

The date seeds of 15 Moroccan cultivars were studied: 'Ademou', 'Assiane', 'Aziza Bouzid', 'Boucerdoune', 'Boufeggous', 'Bouijjou', 'Bouittob', 'Bouskri', 'Bouslikhene', 'Black Bousthammi', 'Bouzeggar', 'Haoua', 'Jihel', 'MahElbaid', and 'Najda'. The analyses were carried out on the date seed powder (DSP) previously prepared, packaged in airtight glass jars and stored at 5°C.

Physical and physico-chemical analyses

The CIE Lab color parameters (L^*, a^* and b^*) were measured on the DSP using a spectro-densito meter (Spectro Dens, Techkon, Germany). The polar coordinates in the plane (a^*, b^*) were also calculated to determine the chromaticity (or chroma) Cab* and chrominance (or hue angle) hab according to the following equations: $Cab^* = \sqrt{(a^{*2}+b^{*2})}$ and $hab = \arctan (b^*/a^*)$.

The humidity of the DSP (%) was determined by drying a 5-g test portion in an oven (Function Line, Heraeus, Germany) at a temperature of 103±2°C during 48 h (AOAC, 1990).

The total soluble solids (TSS; %) were determined at 20°C by using a digital refractometer (RFM830,BS, France) (Harrak and Jaouan, 2010). The water activity (a_w) was measured at 25°C using an aw-meter (AWC-3, Nagy, Germany) (Harrak and Jaouan, 2010).

The pH was measured at 25°C using a pH meter (C861, Consort, Belgium) in a puree of the DSP prepared with boiled and neutralized distilled water (Harrak and Jaouan, 2010).

The ash was determined by incinerating the DSP at 600°C during 7 h (Termolyne Type 1400 Furnace, USA) (AOAC, 1990). For minerals, the ash was digested with nitric acid and diluted with de-mineralised water. The determination of potassium (K) and calcium (Ca) concentrations was carried out using a flame spectrophotometer (BMB Technology XP 2011, Germany). The results were expressed in g 100 g^{-1} of dry matter (DM).

Bio-active compounds analyses

The analysis of betalains with their two types: betacyanins and indicaxanthins (mg 100 g^{-1} DM of DSP), was carried out according to the method reported by Chougui et al. (2013).

The total polyphenol content, expressed in mg gallic acid equivalent (GAE) 100 g^{-1} DM of DSP, was determined by the Folin-Ciocalteu method (Georgé et al., 2005).

The antioxidant activity was measured in terms of radical scavenging ability using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Banerjee et al. (2005) with some modifications. It was expressed in mg ascorbic acid equivalent (AAE) 100 g^{-1} DM of DSP.

Statistical analyses

All analyses were carried out in two or three replicates except the color for which 15 measurements were performed on each DSP sample. The results are expressed as (mean ± standard deviation). To analyze the cultivar effect on studied parameters, the non-parametric Kruskal-Wallis test was used with significance levels: 0.05, 0.01, and 0.001. The Student-Newman-Keuls test allowed identifying the homogeneous groups of means. The correlation between total polyphenols, betalains, and antioxidant activity was analyzed by the Spearman test. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were also performed using all studied parameters for the 15 date cultivars.

RESULTS AND DISCUSSION

Physical and physico-chemical parameters

Color parameters (L*, a*, b*, Cab*, and hab) are significantly different between DSP cultivars (p<0.001) (Table 1). The DSP generally present a light brown color with a relatively dark lightness (L* ranging between 42.35 and 50.31 for 'Bouzeggar' and 'Jihel', respectively). Some DSP tend toward a darker brown color, such as of 'Najda' (a*=18.47) and 'Ademou' (a*=17.49), and others toward a lighter color compared to the other varieties, such as 'Bouzeggar' (b*=22.56). Chroma (Cab*) and hue angle (hab) indicate a gray color with a brown tint. These results can be explained by the nature, concentration and heterogeneity of the molecules responsible for the color such as polyphenols and flavonoids. Bouaziz et al. (2010) reported that the color of the bread enriched with dilapidated date seeds of 'Deglet Nour' and 'Allig', changed with the increase in DSP percentage.

Table 1. Color parameters of the date seed powder of 15 Moroccan cultivars.

Cultivar	L*	a*	b*	Cab*	hab
Ademou	46.14±0.74e	17.49±0.33f	21.48±0.25ef	27.71±0.34h	50.84±0.50d
Assiane	48.18±1.06fg	15.40±0.43ab	20.94±0.55de	25.99±0.65bcd	53.66±0.55 g
Aziza Bouzid	49.71±0.58hi	15.79±0.38bc	21.34±0.47ef	26.55±0.51cdf	53.50±0.70fg
Boucerdoune	47.37±0.62f	15.47±0.39ab	19.81±0.35ab	25.14±0.51a	52.02±0.29e
Boufeggous	47.49±1.01f	16.21±0.49cd	21.43±0.59ef	26.87±0.71efg	52.88±0.67f
Bouijjou	49.54±0.52hi	16.10±0.42c	20.44±0.53bd	26.02±0.62bcd	51.77±0.61e
Bouittob	43.91±0.79bc	16.72±0.55de	19.63±0.60a	25.79±0.80ad	49.57±0.37ab
Bouskri	48.05±0.64fg	14.99±0.47a	20.35±0.49bd	25.28±0.63ab	53.62±0.55 g
Bouslikhene	45.07±1.38d	16.94±0.58ef	21.47±0.79ef	27.35±0.95fh	51.72±0.55e
Black Bousthammi	43.34±0.68ab	17.29±0.33f	20.87±0.43cde	27.11±0.52efh	50.36±0.31cd
Bouzeggar	50.31±0.79i	15.75±0.53bc	22.56±0.47 g	27.52±0.67 gh	55.08±0.51h
Haoua	44.64±0.79cd	17.41±0.26f	20.07±0.29ab	26.57±0.34df	49.04±0.41a
Jihel	42.35±0.60a	16.96±0.55ef	20.21±0.52abc	26.39±0.69cde	50.01±0.66bc
Mah Lbaid	48.73±0.77 gh	14.95±0.30a	20.97±0.65de	25.76±0.65ac	54.49±0.67h
Najda	45.49±0.99de	18.47±0.51 g	21.89±0.58f	28.65±0.61i	49.84±0.95bc
p-value	p=0.00***	p=0.00***	p=0.00***	p=0.00***	p=0.00***

The means with the same letter within the column do not show significant differences according to the Student-Newman-Keuls test at p<0.05.
The p-value or Sig (two-sided) is the type 1 risk value or alpha risk according to the Kruskal-Wallis test (0 0.05 is the level of significance) and *** represent the level of significance at 0.001.

The DSP humidity showed a significant difference between varieties (p<0.001) (Table 2). The values vary between 8.34 and 10.86%, recorded for 'Ademou' and 'Aziza Bouzid', respectively. Djaoudene et al. (2019) have found higher values up to 11.4%. According to Platat et al. (2015), the addition of DSP in the traditional bread formulation has no significant effect on the moisture.

The DSP aw ranged between 0.41 and 0.56 (Table 2), recorded for 'Bouittob' and 'Bouskri', and 'Aziza Bouzid' and 'Black Bousthammi', respectively. These have a barrier effect in a product against microorganisms spoilage. Bouaziz et al. (2020) demonstrated that adding insoluble fiber extracted from DSP to turkey burgers had no significant effect regardless of rate or concentration used.

The TSS of DSP showed a significant difference between cultivars (p<0.001). The values vary from 7.00% for 'Black Bousthammi' to 17.00% for 'Najda' (Table 2). By comparison to date pulps, the latter are characterized by a higher sugar and organic acid contents, hence their TSS is higher than that of DSP and ranged around 50 and 76% (Abdul-Hamid et al., 2018).

Table 2. Physico chemical criteria of the date seed powder of 15 Moroccan cultivars.

Cultivar	Humidity (%)	Total soluble solids (%)	pH	Water activity	Ash (g 100 g^{-1} DM)	Potassium (g 100 g^{-1} DM)	Calcium (g 100 g^{-1} DM)
Ademou	8.34±0.01a	10.00±0.00e	5.278±0.004e	0.500±0.002e	8.34±0.01a	2.61±5.30abcd	0.60±0.76a
Assiane	10.11±0.01k	9.00±0.00c	5.423±0.004h	0.516±0.002f	10.11±0.01k	3.12±0.29cde	0.90±0.13ab
Aziza Bouzid	10.86±0.01o	12.00±0.00 g	5.474±0.001j	0.564±0.001h	10.86±0.01o	3.30±1.37cde	0.71±1.22ab
Boucerdoune	9.30±0.01 g	8.00±0.00b	5.444±0.009i	0.471±0.001c	9.30±0.01 g	3.09±2.57bcde	0.81±0.26ab
Boufeggous	9.94±0.01j	12.00±0.00 g	5.339±0.005f	0.517±0.001f	9.94±0.01j	2.92±1.27abcde	0.76±1.24ab
Bouijjou	8.41±0.01b	10.00±0.00d	5.343±0.001f	0.460±0.001b	8.41±0.01b	2.53±3.00abc	0.81±1.04ab
Bouittob	8.77±0.01d	8.00±0.00b	5.340±0.010f	0.415±0.001a	8.77±0.01d	2.13±0.87a	0.81±0.42ab
Bouskri	8.64±0.01c	7.00±0.00a	5.619±0.007k	0.414±0.001a	8.64±0.01c	3.53±3.37e	0.77±1.45ab
Bouslikhene	9.25±0.00f	8.00±0.00b	5.387±0.007 g	0.472±0.002c	9.25±0.00f	2.71±0.53abcde	0.66±0.47a
Black Bousthammi	10.59±0.00m	7.00±0.00a	4.957±0.001a	0.558±0.002h	10.59±0.00m	3.51±2.35e	0.82±0.00ab
Bouzeggar	9.78±0.01i	8.00±0.00b	5.149±0.011c	0.501±0.001e	9.78±0.01i	2.81±2.01abcde	0.77±1.55ab
Haoua	9.44±0.00h	11.00±0.00f	5.299±0.010e	0.506±0.000e	9.44±0.00h	2.71±0.35abcde	1.02±0.44b
Jihel	9.10±0.01e	10.00±0.00e	5.374±0.002 g	0.479±0.002d	9.10±0.01e	2.76±0.25abcde	0.86±0.04ab
Mah Lbaid	10.34±0.01l	8.00±0.00b	5.234±0.003d	0.547±0.003 g	10.34±0.01l	2.29±2.12ab	0.84±1.22ab
Najda	10.78±0.01n	17.00±0.00h	5.013±0.010b	0.522±0.001f	10.78±0.01n	3.41±0.53de	0.83±0.08ab
p-value	p=0.00***	p=0.00***	p=0.00***	p=0.00***	p=0.00***	p=0.00***	p=0.00***

The means with the same letter within the column do not show significant differences according to the Student-Newman-Keuls test at $p<0.05$.
The p-value or Sig (two-sided) is the type 1 risk value or alpha risk according to the Kruskal-Wallis test (0.05 is the level of significance) and *** represents the level of significance at 0.001.
DM: dry matter.

The pH showed a significant difference between varieties ($p<0.001$). This pH is slightly acidic ranging between 4.96 for 'Black Bousthammi' and 5.62 for 'Bouskri' (Table 2). These values are lower than that noted by El-Kholy (2018) (6.35). The use of DSP with a pH range between 5 and 6 in food products could not have a negative effect on their stability. Instead, pH adjustment could be necessary when using this powder in neutral products such as in cosmetics.

Ash contents vary between 0.84 and 1.32 g 100 g^{-1} DM recorded for 'Mah Elbaid' and 'Najda', respectively (Table 2). These contents are lower than those obtained by Najjar et al. (2022) for six UAE cultivars (2.04-6.90%).

Potassium content varies from 0.21 to 0.35 g 100 g^{-1} DM, registered for 'Bouittob' and 'Bouskri', respectively. For calcium, the content varies from 0.06 to 0.10 g 100 g^{-1} DM, recorded for 'Ademou' and 'Haoua', respectively (Table 2). Bijami et al. (2020) found close potassium content and higher calcium content for date seeds at tamar stage (0.25 and 2.73 g 100 g^{-1} DM, respectively).

Bio-active compounds and antioxidant activity

Total betalain content varies between 9.13 and 35.73 mg 100 g^{-1} DM, and shows a significant difference between varieties ($p<0.001$). The highest values are obtained for 'Najda' (35.73 mg 100 g^{-1} DM) followed by 'Haoua' (31.20 mg 100 g^{-1} DM) and 'Jihel' (28.88 mg 100 g^{-1} DM). By contrast, the lowest values are obtained for 'Bouzeggar' (9.13 mg 100 g^{-1} DM) followed by MahElbaid (10.17 mg 100 g^{-1} DM) and 'Assiane' (17.71 mg g^{-1} DM) (Table 3). Regarding the content of the two pigments betacyanins or betanins (red topurple) and indicaxanthins (yellow to orange), the content of indicaxanthins is high compared to betacyanins for the 15 cultivars. The indicaxanthins vary between 5.56 and 21.50 mg 100 g^{-1} DM, and the betacyanins are between 3.57 and 14.22 mg 100 g^{-1} DM. The methanolic extracts of some cultivars rich in betalains, in particular 'Najda' and 'Jihel', have a color tending toward orange. This can be explained by the contribution of indicaxanthins alongside other pigments to the observed orange coloration. Betalains content in seeds is generally low. For the colored quinoa seeds (*Chenopodium quinoa* Wild.), the content of these pigments varies from 0.15 to 6.10 mg 100 g^{-1} DM (Abderrahim et al., 2015).

The content of polyphenols varies from 2.35 to 6.99 g GAE 100 g^{-1} DM with a significant difference between cultivars. The highest values are obtained for 'Najda' followed by 'Aziza Bouzid' and 'Bouijjou' (6.99, 6.12 and 4.91 g GAE 100 g^{-1} DM, respectively). On the contrary, the lowest values are obtained for 'Black Bousthammi', followed by 'Boucerdoune' and 'Bouzeggar' (2.35, 2.77 and 2.80 g GAE 100 g^{-1} DM, respectively) (Table 3). These values are relatively lower than those of Metoui et al. (2018) for 12 Tunisian cultivars (5.22-9.53 g GAE 100 g^{-1} DM). Bouhlali et al. (2017) also estimated a content of phenolic compounds at 2.70 and 5.34 g GAE 100 g^{-1} DM for the Moroccan cultivars 'Bouffegous' and 'Bousthammi', respectively. The difference can be explained by various factors such as variety, maturity and storage conditions (Al-Farsi and Lee, 2011).

The antioxidant activity shows a significant difference between varieties ($p<0.001$). It is ranging from 1.95 to 7.63 g AAE 100 g^{-1} DM recorded for 'Bouittob' and 'Najda', respectively (Table 3). Chougui et al. (2013) estimated lower antioxidant activity of prickly pear seeds (*Opuntia ficus-indica*) ranging between 32.3 and 51.3 mg AAE 100 g^{-1} DM, for red and orange fruits, respectively. This can be explained by the high polyphenol content of DSP which contributes to the antioxidant activity.

Correlation analysis between total betalains, total polyphenols and antioxidant activity indicates a moderate positive correlation between antioxidant activity and indicaxanthin pigments ($r=0.624$), antioxidant activity and betacyanin pigments ($r=0.593$), antioxidant activity and total polyphenols ($r=0.632$). Besides, there is a strong and positive correlation between betacyanins and indicaxanthins ($r=0.948$), and total betalains and total polyphenols ($r=0.718$) (Table 4).

Table 3. Total polyphenols and betalains content and antioxidant activity of the date seed powder of 15 Moroccan cultivars.

Cultivar	Total polyphenols (g GAE 100 g-1 DM)	Betalains (mg 100 g-1 DM)			Antioxidant activity (g AAE 100 g-1 DM)
		Indicaxanthins	Betacyanins	Total betalains	
Ademou	4.783±0.154 gh	14.095±0.169j	9.644±0.232 g	23.739±0.396h	5.280±0.089j
Assiane	4.078±0.064e	10.578±0.099e	7.130±0.189bc	17.708±0.256d	4.498±0.078i
Aziza Bouzid	6.118±0.050i	12.397±0.613h	9.667±0.722 g	22.065±1.241 g	5.392±0.148j
Boucerdoune	2.766±0.076b	10.114±0.090d	8.012±0.256de	18.126±0.277d	2.187±0.062b
Boufeggous	4.686±0.107fg	11.457±0.134f	7.562±0.197cd	19.019±0.263e	3.363±0.085f
Bouijjou	4.910±0.068h	11.711±0.199fg	8.417±0.323ef	20.129±0.487f	3.107±0.068e
Bouittob	3.165±0.057c	11.963±0.281 g	8.135±0.395e	20.099±0.636f	1.946±0.127a
Bouskri	2.779±0.085b	8.416±0.270c	6.847±0.525b	15.262±0.787c	2.647±0.050c
Bouslikhene	3.810±0.061d	13.402±0.123i	8.757±0.231f	22.159±0.323 g	4.065±0.049h
Black Bousthammi	2.355±0.055a	10.443±0.163de	7.027±0.267b	17.470±0.375d	3.373±0.024f
Bouzeggar	2.801±0.161b	5.556±0.165a	3.570±0.122a	9.126±0.277a	3.822±0.032 g
Haoua	4.575±0.072f	18.741±0.112l	12.456±0.118i	31.197±0.163j	3.998±0.033h
Jihel	4.185±0.055e	17.272±0.140k	11.613±0.273h	28.885±0.387i	5.342±0.104j
Mah Lbaid	3.090±0.087c	6.125±0.226b	4.046±0.227a	10.171±0.421b	2.861±0.045d
Najda	6.986±0.101j	21.502±0.101m	14.225±0.215j	35.727±0.234k	7.631±0.096k
p-value	p=0.00***	p=0.00***	p=0.00***	p=0.00***	p=0.00***

The means with the same letter within the column do not show significant differences according to the Student-Newman-Keuls test at $p<0.05$.
The p-value or Sig (two-sided) is the type 1 risk value or alpha risk according to the Kruskal-Wallis test (0 0.05 is the level of significance) and *** represents the level of significance at 0.001.
DM: Dry matter; GAE: gallic acid equivalent; AAE: ascorbic acid equivalent.

Table 4. Correlation matrix showing the correlation between betalains, total polyphenols and antioxidant activity of the date seed powder of 15 Moroccan cultivars.

	Betalains	Indicaxanthins	Betacyanins	Polyphenols	Antioxidant activity
Betalains	1.000				
Indicaxanthins	0.986**	1.000			
Betacyanins	0.984**	0.948**	1.000		
Polyphenols	0.718**	0.709**	0.692**	1.000	
Antioxidant activity	0.597**	0.624**	0.593**	0.632**	1.000

**The correlation is significant at the 0.01 level (two-sided).

Principal component analysis

Correlations between the 17 studied parameters of all cultivars subjected to PCA and measured with the first two axes F_1 and F_2 (explaining 43.05 and 21.98% of the information, respectively) are presented in Figure 1. The horizontal axis 1 is formed on the positive side by all studied parameters, except pH, lightness L* and hue angle hab. The vertical axis 2 is marked by the contribution on the positive side of all studied parameters except a*, pH, calcium and betalains which they are projected on the negative side of this axis. The pH is not positively correlated to any of the studied parameters.

Figure 1 also shows differences between the 15 cultivars. 'Najda' is characterized by the highest values of biochemical parameters (antioxidant activity, total betalains, indicaxanthins, and betacyanins), ash, and color parameters (Cab* and a* tending toward red color). However, this cultivar is distinguished by low pH, lightness L* and hue angle hab. Similarly, 'Ademou' is characterized by the same interesting parameters but lower than 'Najda'. Besides, on the negative side, 'Bouzeggar' and 'MahElbaid' are characterized by a very high L* and hab. 'Haoua' and 'Jihel', characterized by high values of antioxidant activity, total betalains, betacyanins, and indicaxanthins, behave in the same way. In addition, they are distinguished by the lowest values of L* and hab. 'Bouittob' is distinguished from other cultivars by low humidity, aw and b*. ‘Bouskri’ and ‘Boucerdoune’ are characterized by high pH and low antioxidant activity, total polyphenol, and total betalain contents. ‘Aziza Bouzid’ is characterized by high humidity and aw. For the other cultivars, they are featured by average values of all studied parameters.

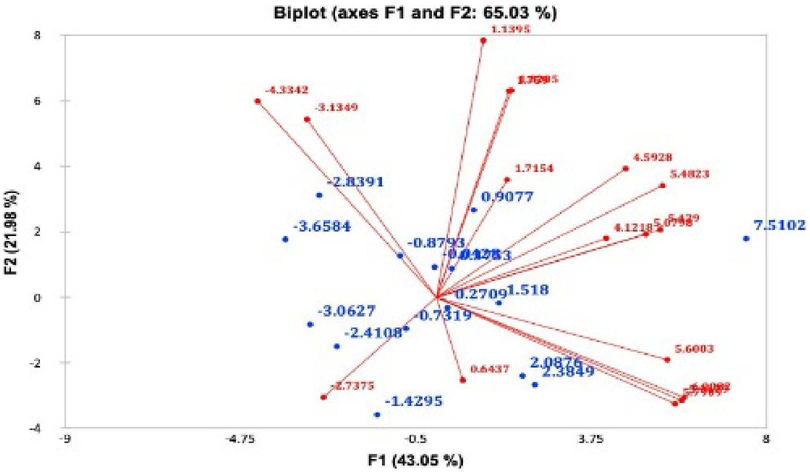


Figure 1. Principal component analysis (PCA) of the first two components (F_1 and F_2): correlation of the physical, physico chemical and biochemical criteria of date seed powders and segregation of 15 Moroccan cultivars based on these criteria.

Hierarchical classification

The studied cultivars do not behave in the same way with according to their physical and physico-chemical parameters (color parameters, pH, humidity, and water activity), and biochemical parameters (content of polyphenols, betalains, and antioxidant activity). This could be associated to many factors including genotype variability and geographical origin. The HCA also allows grouping together varieties that are close to one another into five groups: (‘Ademou’, ‘Bouslikhene’, ‘Bouijjou’, and ‘Bouittob’), (‘Bouskri’ and ‘Black Bousthammi’), (‘Assiane’, ‘Boucerdoune’, ‘Boufeggous’, and ‘Aziza Bouzid’), (‘Bouzeggar’ and ‘MahElbaid’), (‘Haoua’ and ‘Jihel’), and (‘Najda’) (Figure 2).

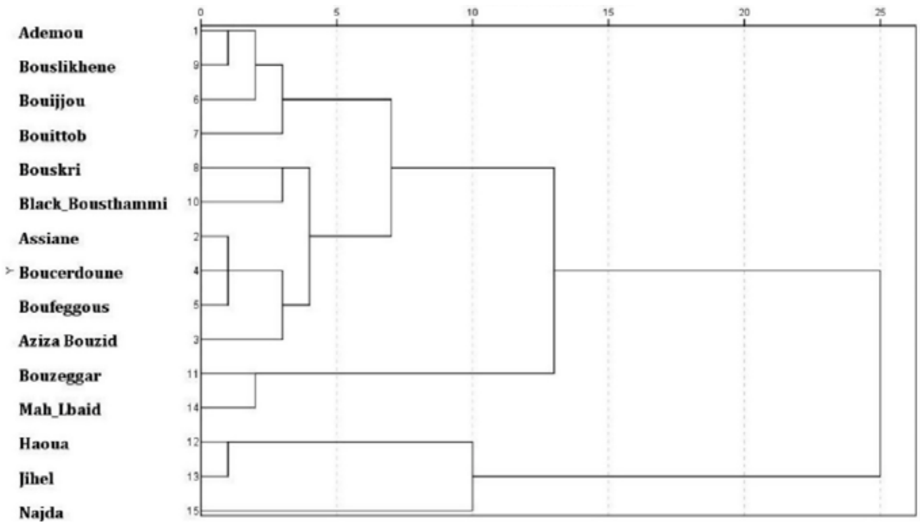


Figure 2. Hierarchical classification of 15 Moroccan cultivars based on the physical, physico-chemical and biochemical criteria of their date seed powders.

CONCLUSIONS

Date seed powder of 15 Moroccan cultivars was characterized according to some physical, physico-chemical, and biochemical parameters. Significant differences were noticed between the studied cultivars which were classified into five homogeneous groups. Furthermore, 'Ademou', 'Bouslikhene', 'Black Bousthammi', 'Haoua', 'Jihel', and 'Najda' were distinguished by interesting biochemical characteristics, including high contents of total polyphenols and total betalains and a high antioxidant activity. Thus, this study has highlighted the DSP potential to further develop innovative products for promoting sustainable progress in date palm oases.

ACKNOWLEDGEMENTS

The authors are grateful to technical staff of the Laboratory of Agri-Food Technology and Quality for providing technical support during the development of this research. They would also like to extend their thanks to the staff of INRA Experiment Station in Zagora and to the staff of oases date cooperatives for their assistance in collecting date samples.

Literature cited

Abderrahim, F., Huanatico, E., Segura, R., Arribas, S., Gonzalez, M.C., and Condezo-Hoyos, L. (2015). Physical features, phenolic compounds, betalains and total antioxidant capacity of coloured quinoa seeds (*Chenopodium quinoa* Willd.) from Peruvian Altiplano. Food Chem *183*, 83–90 https://doi.org/10.1016/j.foodchem.2015.03.029. PubMed

Abdul-Hamid, N.A., Maulidiani, M., Mediani, A., Yahya, U.I.I., Ismail, I.S., Tham, C.L., Shadid, K., and Abas, F. (2018). Physico chemical characteristics, nutritional composition, and phytochemical profiles of nine Algerian date palm fruit (*Phoenix dactylifera* L.) varieties. J. Food Biochem. *42* (*6*), e12663 https://doi.org/10.1111/jfbc.12663.

Al-Farsi, M.A., and Lee, C.Y. (2011). Usage of date (*Phoenix dactylifera* L.) seeds in human health and animal feed. In Nuts and Seeds in Health and Disease Prevention, Chapter 53, (Elsevier), p.447–452 https://doi.org/10.1016/B978-0-12-375688-6.10053-2.

AOAC (1990). Official Methods of Analysis, 15th edn (Washington, DC, USA: Association of Official Analytical Chemists).

Banerjee, A., Dasgupta, N., and De, B. (2005). In vitro study of antioxidant activity of fruit. Food Chem. *90* (*4*), 727–733 https://doi.org/10.1016/j.foodchem.2004.04.033.

Bijami, A., Rezanejad, F., Oloumi, H., and Mozafari, H. (2020). Minerals, antioxidant compounds and phenolic profile regarding date palm (*Phoenix dactylifera* L.) seed development. Sci. Hortic. (Amsterdam) *262*, 109017 https://doi.org/10.1016/j.scienta.2019.109017.

Bouallegue, A., Allaf, T., Besombes, C., Younes, R.B., and Allaf, K. (2019). Phenomenological modeling and intensification of texturing/grinding-assisted solvent oil extraction: case of date seeds (*Phoenix dactylifera* L.). Arab. J. Chem. *12* (*8*), 2398–2410 https://doi.org/10.1016/j.arabjc.2015.03.014.

Bouaziz, M.A., Amara, W.B., Attia, H., Blecker, C., and Besbes, S. (2010). Effect of the addition of defatted date seeds on wheat dough performance and bread quality. J. Texture Stud. *41* (*4*), 511–531 https://doi.org/10.1111/j.1745-4603.2010.00239.x.

Bouaziz, M.A., Bchir, B., Ben Salah, T., Mokni, A., Ben Hlima, H., Smaoui, S., Attia, H., and Besbes, S. (2020). Use of endemic date palm (*Phoenix dactylifera* L.) seeds as an insoluble dietary fiber: effect on turkey meat quality. J. Food Qual. *2020*, 8889272 https://doi.org/10.1155/2020/8889272.

Bouhlali, E.D.T., Alem, C., Ennassir, J., Benlyas, M., Mbark, A.N., and Zegzouti, Y.F. (2017). Phytochemical compositions and antioxidant capacity of three date (*Phoenix dactylifera* L.) seeds varieties grown in the South East Morocco. J. Saudi Soc. Agric. Sci. *16* (*4*), 350–357 https://doi.org/10.1016/j.jssas.2015.11.002.

Chougui, N., Tamendjari, A., Hamidj, W., Hallal, S., Barras, A., Richard, T., and Larbat, R. (2013). Oil composition and characterisation of phenolic compounds of *Opuntia ficus-indica* seeds. Food Chem *139* (*1-4*), 796–803 https://doi.org/10.1016/j.foodchem.2013.01.054. PubMed

Devshony, S., Eteshola, E., and Shani, A. (1992). Characteristics and some potential applications of date palm (*Phoenix dactylifera* L.) seeds and seed oil. J. Am. Oil Chem. Soc. *69* (*6*), 595–597 https://doi.org/10.1007/BF02636115.

Djaoudene, O., López, V., Cásedas, G., Les, F., Schisano, C., Bachir Bey, M., and Tenore, G.C. (2019). *Phoenix dactylifera* L. seeds: a by-product as a source of bioactive compounds with antioxidant and enzyme inhibitory properties. Food Funct *10* (*8*), 4953–4965 https://doi.org/10.1039/C9FO01125K. PubMed

El-Kholy, W.M. (2018). Production of probiotic yoghurt Fortified with date seeds (*Phoenix dactylifera* L.) powder as probiotic and natural stabilizer. Egypt. J. Agric. Res. *96* (*1*), 159–173 https://doi.org/10.21608/ejar.2018.132079.

Georgé, S., Brat, P., Alter, P., and Amiot, M.J. (2005). Rapid determination of polyphenols and vitamin C in plant-derived products. J Agric Food Chem *53* (*5*), 1370–1373 https://doi.org/10.1021/jf048396b. PubMed

Habib, H.M., and Ibrahim, W.H. (2009). Nutritional quality evaluation of eighteen date pit varieties. Int. J. Food Sci. *60* (*sup1*), 99-111.

Harrak, H., and Jaouan, F. (2010). Processing dates of low market value into flour: evaluation of quality and storage stability. Acta Hortic. *882*, 593–602 https://doi.org/10.17660/ActaHortic.2010.882.67.

Metoui, M., Essid, A., Bouzoumita, A., and Ferchichi, A. (2018). Chemical composition, antioxidant and antibacterial activity of Tunisian date palm seed. Pol. J. Environ. Stud. *28* (*1*), 267 274.

Najjar, Z., Alkaabi, M., Alketbi, K., Stathopoulos, C., and Ranasinghe, M. (2022). Physical chemical and textural characteristics and sensory evaluation of cookies formulated with date seed powder. Foods *11* (*3*), 305 https://doi.org/10.3390/foods11030305. PubMed

Nehdi, I.A., Sbihi, H.M., Tan, C.P., Rashid, U., and Al-Resayes, S.I. (2018). Chemical composition of date palm (*Phoenix dactylifera* L.) seed oil from six Saudi Arabian cultivars. J Food Sci *83* (*3*), 624–630 https://doi.org/10.1111/1750-3841.14033. PubMed

Platat, C., Habib, H.M., Hashim, I.B., Kamal, H., AlMaqbali, F., Souka, U., and Ibrahim, W.H. (2015). Production of functional pita bread using date seed powder. J Food Sci Technol *52* (*10*), 6375–6384 https://doi.org/10.1007/s13197-015-1728-0. PubMed

Vayalil, P.K. (2012). Date fruits (*Phoenix dactylifera* Linn): an emerging medicinal food. Crit Rev Food Sci Nutr *52* (*3*), 249–271 https://doi.org/10.1080/10408398.2010.499824. PubMed

Studies of anatomical and physical properties of date palm stem wood (*Phoenix dactylifera* L.)

F. Alla[a], K. Jdaini and M.A. Elhoumaizi[b]

Laboratory of Agricultural Production Improvement, Biotechnology and Environment (LAPABE), Faculty of Sciences, University Mohammed First, 717 Oujda 60000, Morocco.

Abstract

Palm tree belongs to the *Aceraceae* family which consists of hundreds of species. Most economically important species are date palm (*Phoenix dactylifera* L.), coconut palm (*Cocos nucifera*), and oil palm (*Elaeis guineensis*). Being monocotyledons, palm trees show distinct differences of the wood structure compared to common wood species. This study aimed to evaluate the anatomical and physical properties of date palm stem such as number, diameter, area, and percentage of vascular bundles (VBs), wood density and, moisture content in different stem zones; from the peripheral to the inner zone, and at different stem heights from the base to the top of the stem. The results indicated that the density and frequency of vascular bundles increase from the inner to the peripheral zones of the stem, thus the diameter and area of VB decrease from the first to the third zones. The density of VB in peripheral zone also decreases from the bottom to the top of the stem. In addition, it has been increased in the central and inner zones. In the peripheral and central zones, the VB are numerous and smaller in diameter. The inner zone is the broadest; the bundles reach their highest diameter. The fiber tissue percent is higher in the peripheral zone from the top of the stem (35%) than in the inner zone from the base (26%).

Keywords: vascular bundles, anatomical properties, physical properties, stem

INTRODUCTION

The quality of date palm stems as the biological raw material is determined largely by its anatomical structure. Although palm wood is a complex composite, little knowledge exists on effect of the anatomical and physical properties on biological mechanisms of the palm tree.

The anatomy of the palm stem and its variability in quantitative and characters, such as the proportion of fiber tissue, abundance and area of vascular bundles (VBs), and their distribution in different zones, can influence several biological mechanics such the rate of measured sap flow. Also, the differences in wood anatomy can cause differences in the values of the hydraulic parameters of the stem (Steppe and Lemeur, 2007).

The objective of this study was concentrated on the experimental investigation of the anatomical and physical properties of date palm stem (*Phoenix dactylifera* L.), and their effect on several biological mechanisms.

MATERIALS AND METHODS

Study area, plant material and sample preparation

Three date palm trees (*Phoenix dactylifera* L. 'Assiane' and 'Khalt') of about 15 years old, with a height of 7.2, 8 and 9.5 m, respectively, were felled at a plantation in Figuig oasis, located in the southeast of Morocco (32°10'N, 01°24'E; elevation 889 m a.s.l.). For each tree (A, B, C), three discs of 2 cm thickness and diameter of A1: 0.28 m, A2: 0.27 m, A3: 0.26 m, B1: 0.31 m, B2: 0.29 m, B3: 0.28 m, and C1: 0.32 m, C2: 0.30 m, C3: 0.29 m were cut with a chainsaw (Figure 1). The discs were then stored directly to glycerol:ethanol:water solution (2:1:1), and

[a]E-mail: fouzia.alla@gmail.com

[b]E-mail: elhoumaizi@yahoo.fr

transported in a deep freezer to the laboratory.

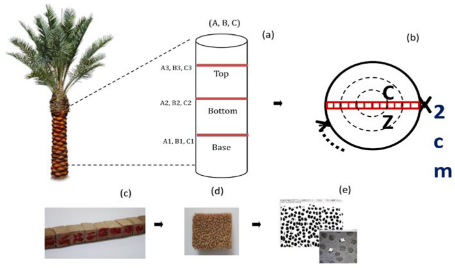


Figure 1. Schematic of cutting preparation: a) cutting positions of discs within the date palm stem, b) sketch of one disc, c) sub-sections, and d) sample of sub-section (20×20 cm^2).

Anatomical properties

In the laboratory, the discs were subsequently removed from the solution and rinsed with water, one section from each disc along the diameter of the stem (S1) was then taken and used for anatomical and physical investigation, the section was pre-cut into sub-sections of 20×20 mm^2 (Figure 1). The anatomical characteristics of date palm stem sub-sections were studied by light microscopy (LM) (Olympus, Tokyo, Japan). The images were then obtained with a digital camera (Canon Inc., USA) mounted on the microscope. Abundance of VBs, areas, and diameter of single VBs were determined using Image J software, v2 (National Institutes of Health, Bethesda, MD, USA).

Due to anatomical variations inside the stem, especially the abundance and distribution of vascular bundles over the stem height and radial section of the palm, all discs were cut from the base, the middles height and the top of the stem, also within each discs of different morphological zones: inner zone (IZ), central zone (CZ) and peripheral zone (PZ). According to geometry, each zone occupied 1/3 of the stem radius.

Physical properties

The physical properties of palm stem, including wood density (g cm^{-3}), and moisture content (water content), were investigated following the procedures described by Steppe et al. (2010).

The density is defined as the relationship of mass to volume (g cm^{-3}). The mass was recorded by submerging first the sub-sections from each height in water for at least 30 min to ensure adequate swelling, and then carefully into a beaker on a balance without touching the side walls or the bottom of the beaker. The volume of the sub-sections was estimated by applying 'Archimedes' principle. Subsequently, the dry mass was determined by drying the sub-sections in an oven at 60°C, until a constant weight is obtained. Wood density ρ"b" (g cm^{-3}) was calculated as:

$$\rho \mathrm{b} = \frac{W\mathrm{d}}{V\mathrm{f}} \tag{1}$$

where g is the oven dry mass, and, cm^3 is the volume of a freshly excised sub section of stem wood.

The moisture content (mc) was calculated as:

$$m\mathrm{c} = \frac{W\mathrm{f} - W\mathrm{d}}{W\mathrm{d}} \tag{2}$$

where g is the fresh mass.

Experimental data analysis

The experimental data were calculated and analyzed using the statistical program Excel 2010. Data results from the anatomical properties of date palm stem were interpreted according to their distribution on the different axes (axial and radial). The standard deviation (SD) was used to measure the distribution of the data around the mean value.

RESULTS AND DISCUSSION

Anatomical properties

All sub-sections from discs were observed, measured, photographed and analyzed.

The number of VBs decreases significantly from inner to peripheral of the diameter, and increases from the base to the top of the stem (Table 1). Variation in the density within the three zones was similar to the specific organization of stem: cocos-type reported by Thomas (2013) (Figure 2). The highest and the lowest percentage of fiber tissue were observed in the peripheral zone from the top of the stem (35%), and in the inner zone from the base of stem (26%).

Table 1. Description of vascular bundles.

Stem level	Number of VBs			Diameter of VBs (μm)			Percentage of fiber tissue on total area (%)		
	PZ	CZ	IZ	PZ	CZ	IZ	PZ	CZ	IZ
Top	1917±62.5	1115±55	600±58.5	510±17.33	736±11.77	827±11.33	35	34	29
Middle	1536±51.5	1023±51	569±53.5	622±14.22	706±10.88	823±10.22	32	34	28
Base	1322±63.5	957±62.5	457±45	775±12.88	846±11.11	900±11.55	34	33	26

PZ: peripheral zone, CZ: central zone, IZ: inner zone.

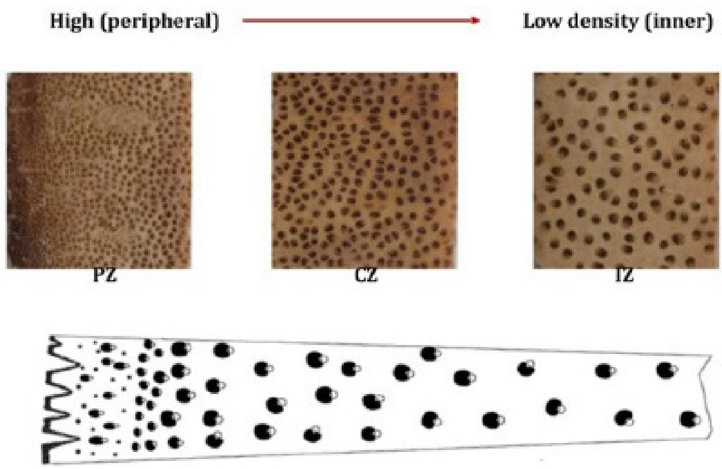


Figure 2. Specific organization of stem: cocos-type (Thomas, 2013).

In addition to the number of vessels, the diameter of VBs also remains a determining factor of homogeneity of the stem wood (Steppe et al., 2010). Area and diameter of VBs were determined in the different zones and stem height using Image J software (Table 1; Figure 3). Area values of vascular bundles varied from 0.002 to 0.011 cm^2 from the peripheral to the inner zone (Figure 3). Diameter and area of VBs were considerably larger in the inner zone, and smaller in the peripheral zone.

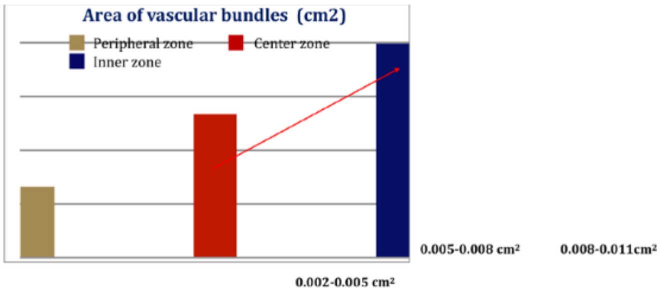


Figure 3. Area of vascular bundles.

As demonstrated in several studies (Green et al., 2003), the homogeneity of the stem is closely related with the anatomy properties of wood stem, which can influence directly the sap flux density values measured with the thermal methods . The performance of thermal methods was assumed related to the uniformity of vessel size and their distribution over the sapwood (Smith and Allen, 1996; Swanson, 1994). Then, errors in sap flow measurements were expected (Green and Clothier, 1988). From our results we described the wood of date palm as thermally inhomogeneous, which suggest the calibration of thermal method used to evaluate the sap flow densities. Studies of Sam (2016) and Fathi (2014) confirm these findings.

Physical properties

The average wood density and moisture content of date palm tree are shown in Table 2. The wood density shows a range of 0.62-0.7 g cm^{-3}. The highest mean value was recorded at the inner zone of stem on the different heights (0.7 g cm^{-3}), and the lowest value was observed in the top and middle of peripheral zone (0.62 g cm^{-3}). The highest and the lowest mean moisture content were observed in the peripheral zone of the base to the top of the stem with an average rate of 11.06±0.11 and 4.73±0.20, respectively. Values yielded from wood density measurement were not significantly different between the inner to the peripheral zone of the stem as well from the base to the top of the stem. These results show a good agreement with wood density values of date palm stem, reported by Fathi (2014).

Table 2. Wood density and moisture content within date palm stem given as mean ± standard deviation.

Stem level	Wood density (g cm^{-3})			Moisture content		
	PZ	CZ	IZ	PZ	CZ	IZ
Top	0.62±0.01	0.66±0.03	0.7±0.02	4.73±0.20	7.48±0.17	4.92±0.22
Middle	0.62±0.01	0.65±0.02	0.7±0.03	9.01±0.26	7.52±0.22	5.47±0.13
Base	0.65±0.02	0.68±0.01	0.7±0.02	11.06±0.11	6.75±0.17	5.65±0.15

PZ: peripheral zone, CZ: central zone, IZ: inner zone.

Wood density and moisture content measurements have a direct impact on physical properties of wood stem (Steppe et al., 2010). The wood density is closely related to the number and distribution of VBs (Steppe et al., 2010; Erwinsyah, 2008) along the stem height and across diameter. According to the results reported in this study, wood density and moisture content for date palm stem does not exhibit variation along the axial and radial axis, unlike to the results from coconut palm (*Cocos nucifera*), and oil palm (*Elaeis guineensis*) (Fathi, 2014).

On the other hand, physical properties of stem wood, namely wood density and moisture content, are important parameters required for the estimation/precision of transpiration and sap flow density (Steppe et al., 2010). For example, sap flow methods that use heat as a tracer, (e.g., heat pulse velocity method (Huber and Schmidt, 1937)), are very sensitive to the physical properties, namely wood density and moisture content (Green et al., 2003; Steppe et al., 2010; Sam, 2016). Results from this study, may therefore be used for the all-heat pulse techniques for measuring transpiration in date palm trees.

CONCLUSIONS

This study of the anatomical and physical properties of stem wood remain important factors in the knowledge of biological mechanisms. Anatomical and physical properties of date palm stem have a large influence on thermal measurements used for estimating tree transpiration. Date palm stem wood is described as thermally inhomogeneous.

Literature cited

Erwinsyah, E. (2008). Improvement of oil palm trunk properties using bioresin. Ph.D. dissertation (Dresden, Germany: Technische Universität).

Fathi, L. (2014). Structural and Mechanical Properties of the Wood from Coconut Palms, Oil Palms and Date Palms (Staats-und Universitätsbibliothek Hamburg Carl von Ossietzky).

Green, S., and Clothier, B. (1988). Water use of kiwifruit vines and apple trees by the heat-pulse technique. J. Exp. Bot. *39* (*1*), 115–123 https://doi.org/10.1093/jxb/39.1.115.

Green, S., Clothier, B., and Jardine, B. (2003). Theory and practical application of heat pulse to measure sap flow. J. Agron. *95* (*6*), 1371–1379 https://doi.org/10.2134/agronj2003.1371.

Huber, B., and Schmidt, E. (1937). Eine Kompensationsmethode zur thermo-elektrischen Messung langsamer Saftstrome. Ber. Dt. Bot. Ges. *55*, 514–529 https://doi.org/10.1007/978-3-0348-5325-5.

Sam, M.C. (2016). Calibration of sap flow techniques in citrus using the stem perfusion method. M.Sc. Horticulture (South Africa: University of Pretoria).

Smith, D., and Allen, S. (1996). Measurement of sap flow in plant stems. J. Exp. Bot. *47* (*12*), 1833–1844 https://doi.org/10.1093/jxb/47.12.1833.

Steppe, K., and Lemeur, R. (2007). Effects of ring-porous and diffuse-porous stem wood anatomy on the hydraulic parameters used in a water flow and storage model. Tree Physiol *27* (*1*), 43–52 https://doi.org/10.1093/treephys/27.1.43. PubMed

Steppe, K., De Pauw, D.J., Doody, T.M., and Teskey, R.O. (2010). A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods. Agric. For. Meteorol. *150* (*7–8*), 1046–1056 https://doi.org/10.1016/j.agrformet.2010.04.004.

Swanson, R.H. (1994). Significant historical developments in thermal methods for measuring sap flow in trees. Agric. For. Meteorol. *72* (*1–2*), 113–132 https://doi.org/10.1016/0168-1923(94)90094-9.

Thomas, R. (2013). Anatomy of the endemic palms of the Near and Middle East: archaeobotanical perspectives. Revue d'Ethnoécologie *4* (*4*), 2–13 https://doi.org/10.4000/ethnoecologie.1366.

The effect of preharvest factors on bio-active components of date fruits (*Phoenix dactylifera* L.)

K. Jdaini[a], F. Alla, O. Laout, H. M'hamdi and M.A. Elhoumaizi[b]

Laboratory for Agricultural Productions Improvement, Biotechnology and Environment (LAPABE), Faculty Sciences, University Mohammed First, BP-717, 60000 Oujda, Morocco.

Abstract

Date palm exposed to various agro-ecological factors causes a modification of synthesis, transport, and accumulation of certain bio-active compounds. The aim of this study is to evaluate agro-ecological factors responsible for the variation of bio-active dates. To achieve this, estimations were carried out on 12 samples of 'Aziza Bouzid' from the oasis of Figuig. We also studied preharvest practices, including irrigation, fertilizers and different bunch fruits practices. ANOVA results showed a significant variation between different samples. The high level of total content of phenols and flavonoids was recorded for dates sampled taken from Hamam Tahtani Ht (total phenolic content (TPC) = 201.79 mg gallic acid equivalent (GAE) 100 g^{-1} dry weight, total flavonoid content (TFC) = 132.73 mg quercetin equivalent (QE) 100 g^{-1} dry weight); however, the lowest values were total phenolic content (TPC) = 100.61 mg gallic acid equivalent (GAE) 100 g^{-1} dry weight and total flavonoid content (TFC) = 62.10 mg quercetin equivalent (QE) 100 g^{-1} dry weight, respectively for Znaga 2 (Zng 2). Correlation analysis of antioxidant capacities and TPC content showed a high correlation (IC$_{50}$ of DPPH scavenging activity r^2=-0.771 and FRAP activity r^2=0.752). Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of the studied fruits may be conditioned by the bunch management technique used by farmers, such as bagging thinning, which can promote light penetration into the fruit. The identification of the main factors influencing the phenolic compounds of dates would improve their medicinal and antioxidant properties.

Keywords: dates, phenol, flavonoid, antioxidant activities, agro-ecological factors

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a perennial monocotyledonous woody fruit tree of the *Arecaceae* family. It is regarded as a pivotal crop that is extensively growing in the Middle East and Africa (Chao and Krueger, 2007).

Date fruits are an excellent food since they contain a wide variety of important components that provide several possible health benefits. Carbohydrates are the main component of dates (mainly sugars: sucrose, glucose and fructose). Dates are rich in fiber and contain a variety of vitamins and minerals. Dates have also been shown to contain high bio-active content and antioxidant properties (Khan et al., 2008).

Antioxidants are biologically active molecules that can delay oxidation processes or inhibit the propagation stage of free radical reactions by quenching reactive free radicals, like hydrogen peroxide, hydroxyl radical, and super-oxide radical (Liu et al., 2018). Therefore, antioxidants are important for human health because they reduce the risk of some chronic diseases cancer, cardiovascular disease, neurological disorders, and diabetes (Barros, 2020). The antioxidant properties recorded in date fruits are due to the high level of phenolic acids, including gallic, p-coumaric, ferulic, syringic, and caffeic, and flavonoid compounds, including rutin, quercetin, quercetin, and isoquercitrin, (Biglari et al., 2008; Benmeziane-Derradji, 2019). The phenolic compounds of dates can be affected by a variety of biotic and abiotic

[a]E-mail: ajdaini91@gmail.com
[b]E-mail: Elhoumaizi@yahoo.fr

factors such as climatic conditions, soil characteristics, nutrient supply, water irrigation and tree conditions (age, formation size, light penetration, etc.). These factors lead to a modification of the different pathways of synthesis and accumulation of phenolic compounds (Jacobo-Velázquez and Cisneros-Zevallos, 2012), which provokes a variation in the biological activity, and the medicinal properties of dates.

Extensive studies have been carried out on the variation phenolic composition of date fruits; however, few studies evaluated the effect of agro-ecological factors on the bio-active compounds. Al-Turki et al. (2010) showed a significant variation in phenolic contents of date palm (225.0 to 507.0 mg GAE 100 g^{-1} FW) depending on cultivar and location. The study of Abdulaal et al. (2017) conducted in the effect of irrigation with sewage water on the antioxidant properties, revealed a high effect of irrigation water quality on these compounds. According to Madani et al. (2021), thinning and covering of date fruit during the pollination and kimri phases improve total antioxidant activity and total phenolic compounds.

Knowledge of the many elements that influence the phenolic compounds in dates allows better management and application of appropriate postharvest procedures. The objective of this study is to assess the main interactions led between agro-ecological factors and the bio-active content of date palm fruits.

MATERIAL AND METHODS

Study area

This survey was carried out from the oasis of Figuig located in south-eastern at the oriental region of Morocco, 32°07N latitudes and 1°14W longitudes, at an altitude of 900 m (Figure 1). This oasis is characterized by an arid Mediterranean climate with minimum and maximum temperatures varying, respectively, from 3 to 16°C in winter and from 24 to 42°C in summer. The average annual precipitation does not exceed 125 mm.

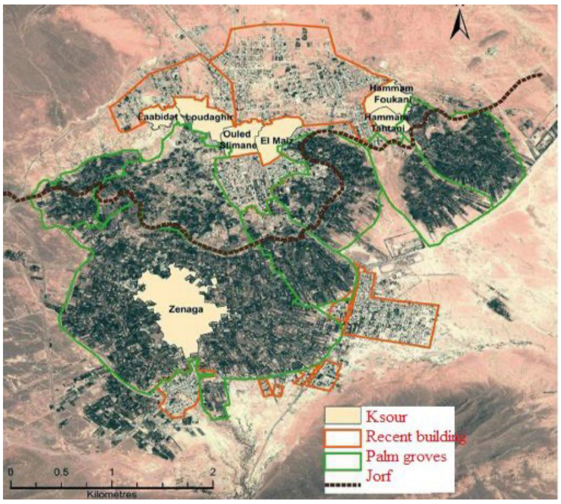


Figure 1. Figuig oasis (Sources: Google Earth, 2009. Treatment: ArcGis 10©. Production: G. Janty, 2014; UMR Ladyss, University Paris Diderot).

Fruit material

Twelve 'Aziza Bouzid' cultivar samples (one kg of each sample) were collected from different plots on Figuig oasis (Table 1). Fruits were picked at commercial maturity (tmar stage) during the 2018 harvest season (October-November). Five trees (same age and same height) were taken randomly as replicates for each sample. All dates were stored in a refrigerator at approximately -20°C until analysis.

Table 1. Study locations.

Locations	Code	Latitude	Longitude	Altitude	Irrigation technics	Fertilizer type
Aarja	Arj	N32°10'04.43	W1°13'05.01	860	Drip	Organic
CooperativeAziza	$Cop._A$	N32°09'33.97	W1°18'36.27	960	Drip	Mineral/organic
Znaga 1	Zng1	N32°05'43.86	W1°14'24.78	869	Gravity	Organic
Znaga 2	Zng2	N32°05'43.65	W1°14'26.12	838	Gravity	Organic
Znaga3	Zng3	N32°05'38.89	W1°13'47.29	869	Gravity	Organic
Znaga4	Zng4	N32°05'45.71	W1°15'31.60	863	Drip	Organic
Hamam tahtani	Ht	N32°06'27.75	W1°12'43.86	910	Drip	Organic
Laabidate	Lb	N32°06'22.55	W1°12'23.86	908	Gravity	Organic
Oulad Imaiz 1	OM1	N32°06'26.29	W1°13'17.78	937	Gravity	Organic
Oulad Imaiz 2	OM2	N32°06'25.88	W1°13'17.20	914	Gravity	Organic
Oulad slimane	O_S	N32°06'04.87	W1°13'19.93	904	Gravity	Organic
Loudaghir	Lg	N32°06'39.06	W1°14'24.98	894	Gravity	Organic

Field investigation

The survey was divided into four sections. The first one based on a plot description, included information about surface area, density, varietal profiles and presence or not of intercropping. The second one covered the age, length, and a fruit bunches number of each tree. The investigation included also; the mode, frequency, quantity, and type of different agricultural practices such as irrigation, fertilization, and pollination. In addition to maintenance practices used for bunches such as thinning, bending, and bagging.

Extraction of antioxidant compounds

The phenolic content of date fruits were extracted as described by Al-zoreky and Al-Taher (2015) with a slight modifications. 0.6 g of each date pulp was mixed with 6 mL of acetone/water (50/50, v/v). The mixture was vortexed for 2 min before being centrifuged for 10 min at 5000 g. The experiment was performed at triplicate and data were recorded as mean ± SD.

Total phenolic contents (TPC)

The total phenolic in each date extract were determined as reported by Singleton et al. (1999), using the Folin-Ciocalteu reagent and gallic acid as a reference standard. In 50 mL of each extract, 2.0 mL of diluted Folin-Ciocalteu reagent was added. After the mixtures were left to rest at room temperature for 5 min, 2.0 mL of a 10% solution of sodium carbonate (Na_2CO_3) was added. The absorbance was measured at 760°C after 60 min of incubation at room temperature. The results were expressed in equivalents of gallic acid 100 g^{-1} of dry weight (mg GAE 100 g^{-1} DW).

Total flavonoid content (TFC)

The total flavonoid content of the extract was measured using the Al-Farsi and Lee (2008) method 500 µL of extract were homogenized with 120 L of sodium nitrite 5% and 50 L of aluminum chloride 10%. After 6 min of incubation at room temperature, 2 mL sodium hydroxide 1 M and 500 L distilled water were added. Absorbance was measured at 510 nm immediately after mixing. Total flavonoid content was expressed as quercetin equivalent 100 g^{-1} of dry weight (mg QE 100 g^{-1} DW).

Free radical scavenging activity using DPPH radical

The anti-oxidant activity of dates was evaluated by DPPH free radical scavenging activity using a technique reported by Sahin et al. (2004). The DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) technique is an electron-transfer antioxidant test that results in a violet solution in methanol. 0.1 mL of each extracts was allowed to react with 1.6 mL of DPPH solution for 20 min in dark condition. The stock solution of DPPH was prepared by mixing 8 mg DPPH with

200 mL methanol which absorbance is 1.1 at 515 nm. The percentage reduction of DPPH was calculated following the formula: % Reduction of DPPH = [1-(OD sample/OD control)]×100.

The graph representing the percentage of DPPH reduction as a function of extract concentrations makes it possible to determine the IC_{50} that represents the extract concentration necessary to reduce absorption by 50%.

Ferric reducing antioxidant power assay (FRAP)

The potassium ferricyanide ferric chloride technique developed by Kasrati et al. (2015) was used to evaluate the ferric reducing power FRAP of extracts. To 0.5 mL of extract, 1.25 mL of phosphate buffer (0.2 M, pH 6.6) as well as 1.25 mL of 1% (w/v) potassium ferricyanide was added. Then 1.25 mL of trichloroacetic acid (10%) was added to the mixture to stop the reaction. 1.25 mL of the mixture was blended with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (w/v) at 0.1%. The intensity was measured at 700 nm.

Statistical analysis

Statistical analyses were performed with the SPSS 19.0 package (SPSS Inc., Chicago, USA). The analysis of variance (ANOVA) and Duncan's multiple range tests were conducted to determine significant differences between means and to separate groups of means with a probability of $p \leq 0.05$. The results were expressed as an average of four determinations ± standard deviation. The Pearson bivariate test was used to detect a linear correlation.

RESULTS AND DISCUSSION

The results of total phenolic and flavonoid content from 12 'Aziza Bouzid' samples studied are presented in Table 2. Significant differences were revealed in terms of total phenolic, total flavonoid content. Results that were obtained of TPC 'Aziza Bouzid' varied from 100.61 (Zng2) to 201.79 mg GAE 100 g^{-1} DW (Ht). The highest rate of Total flavonoid content (TFC) was recorded for Ht (132.73 mg QE 100 g^{-1} DW) and the lowest was found on dates sampled from Zng1 (62.10 mg QE 100 g^{-1} DW). Our results, especially at tmar stage, are in agreement, with those found by Hasnaoui et al. (2012) for 'Aziza Bouzid' (171.3 mg GAE 100 g^{-1} DW). The polyphenols content in this study was lower compared to the study of Bouhlali et al. (2017) for the Moroccan cultivars which varied, respectively, from 331.86 to 537.07 mg GAE 100 g^{-1} DW.

Table 2. TPC and TFC of 'Aziza Bouzid' date cultivars.

	TPC (mg GAE 100 g^{-1} DW)	TFC (mg QE 100 g^{-1} DW)
Arj	129.74±0.19^{bc}	70.29±1.19^{b}
Cop.A	159.99±3.21^{f}	87.19±3.65^{i}
Zng1	127.84±3.59^{bc}	81.25±2.64^{f}
Zng2	100.61±2.09^{a}	62.10±1.1^{a}
Zng3	127.09±3.59^{bcx}	80.85±1.92^{e}
Zng4	150.75±16.45^{f}	102.98±1.35^{j}
Ht	201.79±3.78^{g}	132.73±2.64^{k}
Lb	143.56±9.07^{de}	84.80±0.94^{h}
OM1	119.90±2.56^{b}	74.25±1.71^{d}
OM2	135.98±11.72^{cd}	72.53±4.48^{c}
OS	147.70±9.08^{e}	80.98±1.05^{e}
Lg	122.00±2.59b	83.75±0.92 g

Values are average (n=5) ± standard deviation. Significant differences in the same row are shown by different litters (a-k).

TPC: total phenolic compounds, TFP: total flavonoid compounds, DW: dry weight, GAE: equivalent gallic acid, QE: equivalent quercetin.

Significant variation ($P<0.05$) in antioxidant activities was recorded for 'Aziza Bouzid' collected from different plots in the Figuig oasis (Table 3). In this study the content of total phenolic compounds was extremely significantly correlated with the two antioxidant capacities; IC_{50} of scavenging activity DPPH r^2=-0.771 and FRAP activity r^2=0.752. IC_{50} of scavenging activity based on DPPH fluctuates from 3.55 to 7.81 g date L^{-1}. FRAP results of 'Aziza Bouzid' showed good reducing power, ranging respectively from 520.05 to 924.84 µmol 100 g^{-1} DW for Zng2 and Ht samples. These results correspond to those reported by Bouhlali et al. (2017) for Moroccan dates, DPPH and FRAP activities varied, respectively, from 2.05 to 6.25 g date L^{-1} and from 416.61 to 860.89 µmol 100 g^{-1} DW. Hatem et al. (2018) also showed that IC_{50} DPPH of date cultivars from Saudi Arabia owing from 3.42 µg mL^{-1} for 'Khenazy' to 4.89 µg mL^{-1} for 'Seqah'. As for Tunisian dates, El Arem et al. (2011) presented lower values of DPPH than those reported in this study.

Table 3. Free radical scavenging activity using DPPH radical and ferric reducing antioxidant power assay (FRAP) of 'Aziza Bouzid' date cultivar.

Locations	IC_{50} DPPH (g date^{-1} L^{-1})	FRAP (µmol 100 g^{-1} DW)
Arj	7.81±0.83^{g}	573.92±14.58^{c}
Cop.A	4.82±0.36^{b}	823.89±22.31^{d}
Zng1	7.53±1.5^{fg}	682.46±18.32^{e}
Zng2	7.69±0.72^{fg}	520.05±10.36^{a}
Zng3	4.99±0.19^{b}	561.00±6.78^{b}
Zng4	6.96±1.47^{e}	803.39±7.56^{j}
Ht	3.55±0.12^{a}	924.87±7.21^{k}
Lb	5.69±0.54^{c}	682.46±3.28^{e}
OM1	7.80±1.03 g	702.12±4.65ef
OM2	6.40±0.78d	765.45±9.32h
OS	6.03±0.35cd	780.74±1.32i
Lg	7.25±0.98ef	754.53±4.56f

Values are average (n=5) ± standard deviation.
Significant differences in the same row are shown by different litters (a-k).

Plants generate phenolic compounds as secondary metabolites (Cartea et al., 2010). The concentrations of phenolic compounds can be affected by environmental variables such as soil composition, temperature, rainfall, and UV light incidence (Kouki and Manetas, 2002; Monteiro et al., 2006). In this study water quality and soil composition were not found responsible for total phenolic and flavonoid content (no significant correlation). High quantities of polyphenols, flavonoids and antioxidant activities found in 'Aziza Bouzid' samples from Ht and Cop. A is most likely owing to the fact that these plots belong to new plantations. Farmers of Ht and Cop. A plot adopt great agricultural practices such as proper row spacing, bagging bunches with appropriate material, thinning and limiting fruits bunches. All these practices ensure a good penetration of solar light.

Spacing row trees alters the interception and distribution of solar radiation within the tree canopies (Jackson, 1980; Lauri et al., 2009) and affects the different processes including photosynthesis, flower bud formation, growth, and fruit quality. Awad and Al-Qurashi (2012) concluded that bunch craft bagging after pollination decreased total phenols. Hussain et al. (2016) stated that thinning treatments of 'Hillawi' and 'Khadrawi' increased TPC in comparison with control. According to Madani et al. (2021) thinning treatments of dates improved TPC, probably because of greater light exposure to the fruit in response to less fruit density in each bunch. Thinning dates during the kimri phase increased the TPC.

Direct exposure to sunlight has been found to control phenolic compound production by modifying the expression of genes that code for biosynthetic pathway enzymes (Azuma et al., 2012; Martínez-Lüscher et al., 2014). Light-induced flavonoid synthesis provokes a change in gene expression mediated by the photo receptors. According to Li et al. (2013) the rate of many phenolic compounds and the enzymatic activities involved in their metabolism in the sun-exposed peel of apple fruit are upregulated by high radiance. The accumulation of flavonoids and the expression of their bio-synthetic genes are greatly increased by the exposure to light during grape ripening (Cortell and Kennedy, 2006; Downey et al., 2004).

CONCLUSIONS

From the present study, we were able to determine the main factors that affect the bio-active composition of 'Aziza Bouzid' samples. The use of good preharvest practices such as the proper row spacing, bagging bunches with appropriate material, thinning, and limiting allows a better distribution of the sunlight within dates bunches. In this research light intensity and quality are considered as a major factor responsible for the bio-synthesis and accumulation of the bio-active compounds. These observations can be used to avoid any activity that may have a negative impact on the quality of dates and to control post-harvest practices in order to produce good quality dates with high medical properties.

Literature cited

Abdulaal, W.H., Zeyadi, M., Baothman, O.A.S., Zamzami, M.A., Choudhry, H., Almulaiky, Y.Q., Saleh, R.M., and Mohamed, S.A. (2017). Investigation of antioxidant and detoxifying capacities of some date cultivars (*Phoenix dactylifera* L.) irrigated with sewage water. RSC Advances *7* (*21*), 12953–12958 https://doi.org/10.1039/C6RA28760C.

Al-Farsi, M.A., and Lee, C.Y. (2008). Optimization of phenolics and dietary fibre extraction from date seeds. Food Chem *108* (*3*), 977–985 https://doi.org/10.1016/j.foodchem.2007.12.009. PubMed

Al-Turki, S., Shahba, M., and Stushnoff, C. (2010). Diversity of antioxidant properties and phenolic content of date palm (*Phoenix dactylifera* L.) fruits as affected by cultivar and location. J. Food Agric. Environ. *8* (*1*), 253–260.

Al-zoreky, N.S., and Al-Taher, A.Y. (2015). Antibacterial activity of spathe from *Phoenix dactylifera* L. against some food-borne pathogens. Ind. Crops Prod. *65*, 241–246 https://doi.org/10.1016/j.indcrop.2014.12.014.

Awad, M.A., and Al-Qurashi, A.D. (2012). Gibberellic acid spray and bunch bagging increase bunch weight and improve fruit quality of 'Barhee' date palm cultivar under hot arid conditions. Sci. Hortic. (Amsterdam) *138*, 96–100 https://doi.org/10.1016/j.scienta.2012.02.015.

Azuma, A., Yakushiji, H., Koshita, Y., and Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. Planta *236* (*4*), 1067–1080 https://doi.org/10.1007/s00425-012-1650-x. PubMed

Barros, L. (2020). Natural antioxidants and human health effects. Curr Pharm Des *26* (*16*), 1757–1758 https://doi.org/10.2174/138161282616200519085826. PubMed

Biglari, F., Alkarkhi, A.F., and Easa, A. (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera* L.) fruits from Iran. Food Chem. *107* (*4*), 1636–1641 https://doi.org/10.1016/j.foodchem.2007.10.033.

Bouhlali, E.T., Ramchoun, M., Alem, C., Ghafoor, K., Ennassir, J., and Zegzouti, Y.F. (2017). Functional composition and antioxidant activities of eight Moroccan date fruit varieties (*Phoenix dactylifera* L.). J. Saudi Soc. Agric. Sci. *16* (*3*), 257–264 https://doi.org/10.1016/j.jssas.2015.08.005.

Cartea, M.E., Francisco, M., Soengas, P., and Velasco, P. (2010). Phenolic compounds in *Brassica* vegetables. Molecules *16* (*1*), 251–280 https://doi.org/10.3390/molecules16010251. PubMed

Chao, C.T., and Krueger, R.R. (2007). The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. HortScience *42* (*5*), 1077–1082 https://doi.org/10.21273/HORTSCI.42.5.1077.

Cortell, J.M., and Kennedy, J.A. (2006). Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) pinot noir fruit and extraction in a model system. J Agric Food Chem *54* (*22*), 8510–8520 https://doi.org/10.1021/jf0616560. PubMed

Derradji, F.B. (2019). Nutritional value, phytochemical composition, and biological activities of Middle Eastern and North African date fruit: an overview. EuroMediterr. J. Environ. Integr. *4* (*1*), 39 https://doi.org/10.1007/s41207-019-0132-y.

Downey, M.O., Harvey, J.S., and Robinson, S.P. (2004). The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust. J. Grape Wine Res. *10* (*1*), 55–73 https://doi.org/10.1111/j.1755-0238.2004.tb00008.x.

El Arem, A., Guido, F., Behija, S.E., Manel, I., Nesrine, Z., Ali, F., and Helal, N.A.L. (2011). Chemical and aroma volatile compositions of date palm (*Phoenix dactylifera* L.) fruits at three maturation stages. Food Chem. *127* (*4*), 1744–

1754 https://doi.org/10.1016/j.foodchem.2011.02.051.

Hasnaoui, A., Elhoumaizi, M.A., Borchani, C., Attia, H., and Besbes, S. (2012). Physico-chemical characterization and associated antioxidant capacity of fiber concentrates from Moroccan date flesh. Indian J. Sci. Technol. *5* (*7*), 2954–2960 https://doi.org/10.17485/ijst/2012/v5i7.4.

Hatem, A., Abdel Rahman, A., Amr, F., and Mohamed, S. (2018). Effect of maturation stages on flavour profile and antioxidant activity of date palm fruits (*Phoenix dactylifera*) grown in Saudi Arabia. Int. J. Pharmacol. *14* (*3*), 407–414 https://doi.org/10.3923/ijp.2018.407.414.

Hussain, I., Ahmad, S., Amjad, M., and Ahmed, R. (2016). Execution of strands thinning improves the phyto-chemicals and sugars profiling in date palm (*Phoenix dactylifera* L.) fruits. Pak. J. Pharm. Sci. *4*, 12091215.

Jackson, J.E. (1980). Light interception and utilization by orchard systems. Hortic. Rev. (Am. Soc. Hortic. Sci.) *2*, 208–267 https://doi.org/10.1002/9781118060759.ch5.

Jacobo-Velázquez, D.A., and Cisneros-Zevallos, L. (2012). An alternative use of horticultural crops: stressed plants. Agriculture *2* (*3*), 259–271 https://doi.org/10.3390/agriculture2030259.

Kasrati, A., Alaoui Jamali, C., Bekkouche, K., Wohlmuth, H., Leach, D., and Abbad, A. (2015). Comparative evaluation of antioxidant and insecticidal properties of essential oils from five Moroccan aromatic herbs. J Food Sci Technol *52* (*4*), 2312–2319 https://doi.org/10.1007/s13197-014-1284-z. PubMed

Khan, M., Sarwar, A., Wahab, M., and Haleem, R. (2008). Physico-chemical characterization of date varieties using multivariate analysis. J. Sci. Food Agric. *88* (*6*), 1051–1059 https://doi.org/10.1002/jsfa.3187.

Kouki, M., and Manetas, Y. (2002). Resource availability affects differentially the levels of gallotannins and condensed tannins in *Ceratonia siliqua.* Biochem. Syst. Ecol. *30* (*7*), 631–639 https://doi.org/10.1016/S0305-1978(01)00142-9.

Lauri, P.É., Costes, E., Regnard, J.L., Brun, L., Simon, S., Monney, P., and Sinoquet, H. (2009). Does knowledge on fruit tree architecture and its implications for orchard management improve horticultural sustainability? An overview of recent advances in the apple. Acta Hortic. *817*, 243–250 https://doi.org/10.17660/ActaHortic.2009.817.25.

Li, P., Ma, F., and Cheng, L. (2013). Primary and secondary metabolism in the sun-exposed peel and the shaded peel of apple fruit. Physiol Plant *148* (*1*), 9–24 https://doi.org/10.1111/j.1399-3054.2012.01692.x. PubMed

Liu, Z., Ren, Z., Zhang, J., Chuang, C.C., Kandaswamy, E., Zhou, T., and Zuo, L. (2018). Role of ROS and nutritional antioxidants in human diseases. Front Physiol *9*, 477 https://doi.org/10.3389/fphys.2018.00477. PubMed

Madani, B., Dastjerdy, A.M., and Shahriyari, A. (2021). Improving ‘Piyarom’ date palm fruit quality with fruit thinning and bunch covering treatments. Adv. Hortic. Sci. *35* (*1*), 11–19 https://doi.org/10.36253/ahsc-9548.

Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I., and Gomès, E. (2014). Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profiles in grapevine berries through transcriptomic regulation. Plant Cell Physiol *55* (*11*), 1925–1936 https://doi.org/10.1093/pcp/pcu121. PubMed

Monteiro, J.M., Albuquerque, U.P., Neto, E.M.F.L., Araújo, E.L., Albuquerque, M.M., and Amorim, E.L.C. (2006). The effects of seasonal climate changes in the Caatinga on tannin levels in *Myracrodruon urundeuva* (Engl.) Fr. All. and *Anadenanthera colubrina* (Vell.) Brenan. Rev. Bras. Farmacogn. *16* (*3*), 338–344 https://doi.org/10.1590/S0102-695X2006000300010.

Sahin, F., Güllüce, M., Daferera, D., Sökmen, A., Sökmen, M., Polissiou, M., Agar, G., and Özer, H. (2004). Biological activities of the essential oils and methanol extract of *Origanum vulgare* sp. *vulgare* in the Eastern Anatolia region of Turkey. Food Control *15* (*7*), 549–557 https://doi.org/10.1016/j.foodcont.2003.08.009.

Singleton, V.L., Orthofer, R., and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. *299*, 152–178 https://doi.org/10.1016/S0076-6879(99)99017-1.

The effect of natural elicitors on quality of storability of date palm fruit at rutab stage

S.N.A. Alblooshi, N. Kaur, D. Shahwar and Z.F.R. Ahmed[a]

Integrative Agriculture Department, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain 15551, United Arab Emirates.

Abstract

Known for centuries, date palm fruit 'Khesab' has been widely consumed in the Arab region. Besides its popularity, it gets wasted many times more than the quantity that is used. Therefore, it is a challenge to preserve it after harvest. In the present study, combinations of natural elicitors were employed as preharvest treatment: chitosan (Ch) 1% alone or combined with salicylic acid (SA) 2 mM and calcium chloride (Ca) 3% was investigated on the physiological changes in 'Khesab' date fruits during 6 weeks of cold storage. Total soluble solids (TSS) were lower in Ca, Ch+SA+Ca, Ch+SA treated fruit as compared with control fruits. Similarly, Ch+SA treated fruits did not show any decay after 6 weeks of storage. The total phenolics (TPC), tannins (TTC) and flavonoids (TFC) content were the highest in the Ca treatment followed by Ch+Ca+SA and Ch by the end of the storage period. Antioxidant activities were found in all treatments, which was significantly higher in Ch+SA+Ca, Ch+SA and Ch compared to control. Our results show that the use of elicitor combinations increase the shelf life of date rutab fruit during cold storage by preserving its quality and reducing spoilage which could be used as a potential strategy.

Keywords: date palm, postharvest, quality, 'Khesab', shelf-life, elicitors

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the native crops of North African countries and the Middle East. The fruits of the date palm are considered of great agricultural and economic importance as they are highly nutritional and possess a huge amount of nutrients (Al-Mssallem et al., 2020; Bentrad and Hamida-Ferhat, 2020; Ahmed et al., 2022b). There are different cultivars of date palm grown in these areas out of which 'Khesab' is one of the most preferable consumer's choices due to its extremely sweet flavour at rutab stage (the fruit softens, changes color to light brown, begins to lose weight and accumulates more sugars (Ahmed at al., 2022a; Alyafei et al., 2022). These mature fruits are very delicious and soft in texture, but they are more prone to injuries and microbial degradation leading to a huge postharvest and market loss. Therefore, preserving their integrity is a critical challenge (Ahmed and Palta, 2010; Mohamed et al., 2014).

Various techniques have been employed in the past to reduce plant stress and increase the shelf life of fruits such as modified atmosphere packaging (MAP), controlled atmosphere storage (CA), optimization of storage temperature, and transportation procedures (Ahmed and Palta, 2011; Aleid and Saikhan, 2017; Alhamdan et al., 2015; Alsawmahi et al., 2018, Maan et al., 2021; Xylia et al., 2021; Nadeem et al., 2022). Another approach is the application of natural elicitors (Moreno-Escamilla et al., 2018; Ahmed et al., 2022a). Different eco-friendly elicitors have been used such as chitosan (Rahman et al., 2018; Abu-Shama et al., 2020; Ahmed et al., 2021b; Xylia et al., 2022), salicylic acid (Mohamed et al., 2014; Chen et al., 2020; Gomes et al., 202; Khalil et al., 2022) and calcium chloride (Sohail et al., 2015; Atia et al., 2018) to suppress the ripening, enhance the quality, and prevent the fruit decay. The mechanism by which these elicitors exert their effects involves triggering the phytochemical pathways, enhancing the production of antioxidant enzymes, and reducing ethylene production in

[a]E-mail: zienab.ahmed@uaeu.ac.ae

vegetables and fruit (Romanazzi et al., 2018; Maan et al., 2021).

To the best of our knowledge, only a few reports are available on the combinatorial effects of natural compounds on 'Khesab' (rutab) fruit, as a preharvest spray application. Hence, the aim of this study was to investigate the synergistic effects of chitosan in combination with SA and $CaCl_2$ on 'Khesab' (rutab) fruit quality at harvest and during storage time for 6 weeks, as well as to investigate the influence of these elicitors on the chemical properties and phytochemical content after harvesting and during cold storage.

MATERIAL AND METHODS

Plant material and sampling

Six date palm trees (*Phoenix dactylifera* L. 'Khesab') were selected in the experimental farm of the College of Food and Agriculture located in the Al Foah region, Al Ain, UAE. The experiment was designed as randomized complete block with 6 palms (replicate) receiving 7 different treatments (one treatment for each bunch). Each treatment was sprayed during 5 and 15 weeks from pollination, with different elicitors: chitosan, SA, and $CaCl_2$ alone and in combinations, and bunch treated with water only was used as a control (Table 1). After harvest, fruits were washed and sorted to select Rutab only. Samples of 100 fruit from each treatment were collected and stored in plastic bags (4 bags treatment^{-1} for each storage time) at 2°C and 90-92% relative humidity (RH) for 6 weeks. Analysis of samples was done every 2nd week.

Physiochemical analysis

Upon harvest, fruit dimensions were recorded according to Rastegar et al. (2012). The fruit were visually observed for decay every other week till complete spoilage of fruit occurred during storage for 6 weeks. The decay percentages were calculated by using the following equation (Kumar et al., 2013):

$$\text{Decay } (\%) = \frac{\text{Number of decay fruit}}{\text{Total number of fruits}} \times 100$$

Total soluble solids (TSS)

Hundred g cut fruits were blended with 100 mL of distilled water and filtered to get the clear juice. The TSS as a percentage value was determined in the juice using a digital refractometer (DR 6000, A. Kruss Optronic GmbH, Hamburg, Germany).

Extraction of bio-active compounds

The bio-active compounds were extracted from date fruit samples by homogenizing 2 g tissue in 20 mL of 80% methanol with water bath shaker at 150 rpm for 24 h, 45°C. Then the samples were filtered using Whatman #1 filter paper. The supernatant (date extract) was used for further analysis.

Phytochemical analysis

The total phenolic content (TPC), total tannin content (TTC), and total flavonoid content (TFC) were measured according to Ahmed et al. (2021a). For TPC, results were expressed as milligram gallic acid equivalents (GAE) per 100 g of fresh weight (mg GAE 100 g^{-1} FW) and for TTC and TFC, results were expressed as milligram of catechin equivalents per 100 g of fresh weight (mg CE 100 g^{-1} FW).

Antioxidant activities

The antioxidant activity was measured by using two different protocols as reported by Ahmed et al. (2021a). The IC_{50} values (mg mL^{-1}) were calculated for each sample by DPPH assay and Trolox equivalents (TE) per 100 g fresh weight (mg TE 100 g^{-1} FW) were calculated by ABTS assay.

Statistical analysis

The data from the completely randomized design with six replicates were subjected to statistical analysis using analysis of variance (ANOVA) in the SAS statistical software (SAS Institute Inc., 2000, Cary, NC, USA). Mean values were compared using Least significant differences (LSD) at level P≤0.05.

RESULTS AND DISCUSSION

Physical attributes of fruit at harvest

The data (Table 1) showed significant differences (p<0.05) in the physical characteristics of treated fruits. All treatments have increased the fruit size compared to control fruits. Fruits treated with SA, Ch+SA, and Ch+SA+Ca were found with the largest dimensions. SA could be a chief player that helps the cell in maintaining the volume (Kassem et al., 2010). Similar results were obtained in the study done by Mohamed et al. (2014).

Table 1. Effect of different pre-harvest treatments on the physical characteristics of 'Khesab' dates at harvest.

	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)
Control	9.51±0.08b	22.23±0.11c	32.10±0.13c
Ch	9.89±0.08b	22.97±0.09bc	32.23±0.49c
Ca	9.82±0.11b	22.53±0.12c	33.44±0.24ab
SA	11.34±0.29a	23.41±0.18b	34.09±0.40a
Ch+SA	11.82±0.22a	25.64±0.24a	32.79±0.19bc
Ch+CA	9.91±0.17b	22.91±0.38bc	33.06±0.22b
Ch+SA+Ca	11.34±0.10a	23.84±0.28b	34.19±0.51a

Values are the mean (n=25) ± SE. Means with different letters in the same column are significantly different at P>0.05 using LSD test.

Fruit decay during storage

The results of fruit decay percentage during cold storage of 6 weeks are presented in Figure 1. It was observed that the decay began after 4 weeks of cold storage. The maximum degradation was found in control fruits after 6 weeks of storage, while fruit treated with Ch and SA showed no decay (Figure 1). Additionally, the decay rate of Ca, Ch+Ca, Ch+SA+Ca, and Ch+SA treated fruit were 44, 19, 9, and 6%, respectively. In has been reported that SA and Ca, alone or in combination, increased fruit hardness and delayed softening, resulting in less decay in strawberry fruit. The present results demonstrated that using Ch in combination with Ca and SA could protect the 'Khesab' fruit from degradation during cold storage.

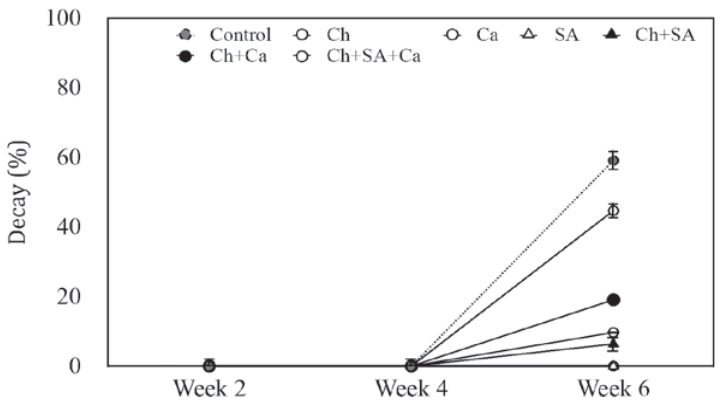


Figure 1. Effect of different pre-harvest treatments on 'Khesab' fruit decay during cold storage, values are the mean (n=25) ± SE. Means comparison were done at P<0.05 using LSD test.

Total soluble solids (TSS)

The fruit TSS level was highly modulated by all the elicitors with significant variation ($p<0.05$) among each other (Figure 2). The fruits treated with Ch+SA+Ca were found with lowest TSS after 6 weeks of cold storage. On the other hand, no significant differences were found in Ca, SA, Ch+SA, and CH+Ca, treated fruits at 4th week of storage.

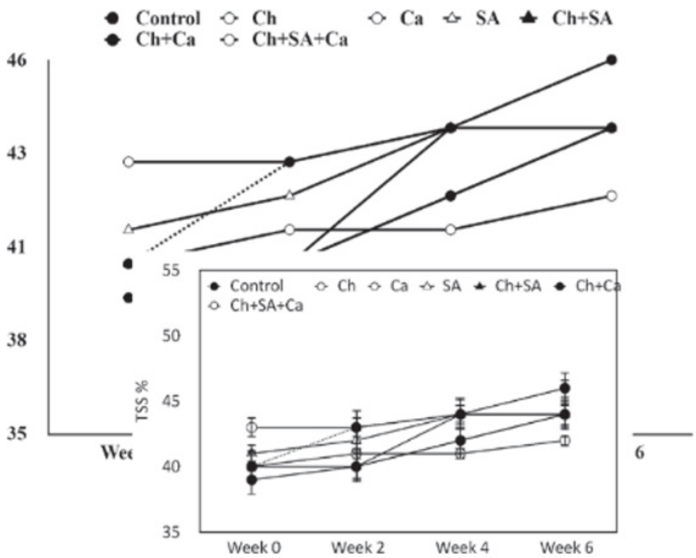


Figure 2. Effect of different pre-harvest treatments total soluble solids content of ‘Khesab’ fruit during cold storage, values are the mean (n=25) ± SE. Means comparison were done at $P<0.05$ using LSD test.

In this study, the observed TSS concentrations were found similar to those described by Kassem et al. (2010), in SA treated date fruit. However, Mohamed et al. (2014) reported no significant difference between SA treated and the control date fruit, in relation to TSS content. Generally, the increase in TSS is the result of degradation of large polysaccharide molecules into smaller sugars via enzymatic activities and loss of water during storage (Hazbavi et al., 2015). Based on our findings, Ca alone or in combination with Ch and SA reduced TSS concentrations in treated ‘Khesab’ fruit.

Total phenolic content of fruit at harvest and during storage

The total phenolic content of ‘Khesab’ fruit at harvest and during cold storage are presented in Table 2. Significant ($p<0.05$) changes were observed in the fruit TPC in all elicitor applications. The Ch (381.8-78.2 mg GAE 100 g^{-1} FW) treatment had the highest TPC in comparison to others including control from the week 0 until 6th week of storage. Whereas, SA had the lowest TPC (280.6-50.1 mg GAE 100 g^{-1} FW) from week 0 to week 6 of cold storage. However, variations in the concentration of TPC among different date cultivars have been reported by several studies (50-400 mg GAE 100 g^{-1} FW) (Al-Qurashi and Awad, 2011; Awad et al., 2011a; Mohamed et al., 2014), which was believed to be due to cultivar variations and/or environmental factors. SA has ability to induce antioxidant systems during cold temperature (Wang et al., 2006). The application of elicitors such as chitosan, SA and Ca has been reported to improve the levels of polyphenols in fruit and therefore enhances their quality (Ruiz-García and Gómez-Plaza, 2013).

Table 2. Effect of different preharvest treatments on total phenolics content on 'Khesab' dates at harvest and during cold storage.

Treatment	Week 0	Week 2	Week 4	Week 6
Control	319.2±2.98d	76.3±1.23d	67.8±2.99c	64.8±1.23b
Ch	381.8±4.20a	92.1±2.06b	86.4±1.32a	78.2±1.28a
Ca	315.2±5.78d	94.8±2.39ab	86.1±1.54a	65.7±1.51b
SA	280.6±2.40e	82.7±1.44c	77.9±1.25b	50.1±1.88d
Ch+SA	338.0±5.40cd	82.1±1.20c	79.6±1.76b	63.4±1.96bc
Ch+Ca	372.2±3.23b	98.0±2.30a	64.6±2.12c	61.0±1.72c
Ch+SA+Ca	349.9±4.30c	76.7±1.06d	64.9±1.96c	63.0±1.41bc

Values are the mean (*n*=25) ± SE. Means with different letters in the same column are significantly different at P>0.05 using LSD test.

Total tannin, flavonoids, and antioxidants concentrations at harvest

The effect of different preharvest treatments on fruit tannins (TTC), flavonoids (TFC), and antioxidants were determined upon harvest and the results are tabulated in Table 3. All the elicitors have highly impacted the bio-active compounds with significant variation ($p<0.05$) among different treatments. In comparison to all treatments, Ch treated fruits had the highest TTC 51.99 mg CE 100 g^{-1} FW followed by Ch+Ca, Ca, SA, Ch+Sa, Ch+SA+Ca, control with 49.09, 46.25, 38.71, 36.77, 26.13, 20.41 mg CE 100 g^{-1} FW, respectively. Ch+Ca and Ch treated fruits were found with highest flavonoid content with 59.8 and 55 mg CE 100 g^{-1} FW, respectively whereas no significant difference was found between SA and Ch+Ca+SA. The antioxidants activity was higher in Ch treated fruit as measured by ABTS (346.4 mg TE 100 g^{-1} FW) and DPPH (IC_{50}=1.90 mg mL^{-1}) radical scavenging activities (Table 3). The smallest the IC_{50} in DPPH, highest the radical scavenging activity. The results are consistent with 0% decay observed with the same treatment (Table 3; Figure 1) highlighting the key role of phenolic compounds in providing defense support to the fruits. A similar relationship was found in other fruits (Awad et al., 2011a, b; Rahman et al., 2018). According to the above findings, preharvest application of Ch alone and in combination with Ca and SA could improve the levels of antioxidant compounds in date fruit.

Table 3. Effect of different preharvest treatments on phyto-chemicals content and antioxidant activity of 'Khesab' fruit.

Treatments	Tannin (mg CE 100 g^{-1} FW)	Flavonoids (mg CE 100 g^{-1} FW)	ABTS (mg TE 100 g^{-1} FW)	IC_{50} (mg mL^{-1})
Control	20.41±1.83c	45.1±1.21e	274.9±1.57d	2.97±0.93a
Ch	51.99±1.61b	55.0±1.37b	346.4±2.83a	1.90±0.61c
Ca	46.25±3.25a	50.7±1.18c	177.8±2.74b	2.37±0.35b
SA	38.71±1.78b	51.7±1.20d	220.5±2.24d	3.42±0.32b
Ch+SA	36.77±1.51c	45.8±1.15e	154.2±1.56d	2.49±0.34ab
Ch+Ca	49.09±1.61c	59.8±1.10e	225.1±1.91c	2.39±0.75a
Ch+SA+Ca	26.13±3.35a	51.7±1.44a	145.4±1.42c	2.24±0.24c

Values are the mean (*n*=3) ± SE. Means with different letters in the same column are significantly different at P<0.05 using LSD test.

CONCLUSIONS

The following conclusion can be drawn from the study: SA and Ch, have significantly prevented the microbial growth on fruits with 0% decay after 6 weeks of storage. SA alone and in combination with chitosan have maintained the fruit quality with least weight loss. Ch alone and in combination with Ca increased the TPC content. Antioxidant's activity was also found highest in Ch and Ch+SA+Ca treatments. Combinations of elicitors used in this study can be suggested for a wide scale use. Also, they can be tested on different date cultivars to compare the effects. This method can be a more efficient, cost-effective, and a natural way for improving date fruit quality and protection against a wide range of decaying microbes.

ACKNOWLEDGEMENTS

The authors appreciate the UAEU for financial support and the College of Agriculture and Veterinary Medicine, Integrative Agriculture Department, Horticulture lab, Food Science lab for assisting the sample analysis.

Literature cited

Abu-Shama, H.S., Abou-Zaid, F.O.F., and El-Sayed, E.Z. (2020). Effect of using edible coatings on fruit quality of Barhi date cultivar. Sci. Hortic. (Amsterdam) *265*, 109262 https://doi.org/10.1016/j.scienta.2020.109262.

Ahmed, Z.F.R., and Palta, J.P. (2010). Lysophosphatidylethanolamine, a natural phospholipid, may retard senescence and improve the shelf life of banana fruit. HortScience *45*, S66–S66.

Ahmed, Z.F.R., and Palta, J.P. (2011). Hormone-like effect of a natural lipid, lysophosphatidylethanolamine, can mitigate calcium deficiency injury in potato shoot cultures. HortScience *46*, S196–S197.

Ahmed, Z.F.R., Alblooshi, S.S.N.A., Kaur, N., Maqsood, S., and Schmeda-Hirschmann, G. (2021a). Synergistic effect of preharvest spray application of natural elicitors on storage life and bio-active compounds of date palm (*Phoenix dactylifera* L., cv. 'Khesab'). Horticulturae *7* (*6*), 145 https://doi.org/10.3390/horticulturae7060145.

Ahmed, Z.F.R., Al Shaibani, F.Y., Kaur, N., Maqsood, S., and Schmeda-Hirschmann, G. (2021b). Improving fruit quality, bio-active compounds, and storage life of date palm (*Phoenix dactylifera* L., cv. Barhi) using natural elicitors. Horticulturae *7* (*9*), 293 https://doi.org/10.3390/horticulturae7090293.

Ahmed, Z.F.R., Kaur, N., Maqsood, S., and Schmeda-Hirschmann, G. (2022a). Preharvest applications of chitosan, salicylic acid, and calcium chloride have a synergistic effect on quality and storability of date palm fruit (*Phoenix dactylifera* L.). HortScience *57* (*3*), 422–430 https://doi.org/10.21273/HORTSCI16416-21.

Ahmed, F.R.A., Kaur, N., and Hassan, F.E. (2022b). Ornamental date palm and sidr trees: fruit elements composition and concerns regarding consumption. Int. J. Fruit Sci. *22* (*1*), 17–34 https://doi.org/10.1080/15538362.2021.1995570.

Al-Mssallem, M.Q., Alqurashi, R.M., and Al-Khayri, J.M. (2020). Bio-active compounds of date palm (*Phoenix dactylifera* L.). In Bio-Active Compounds in Underutilized Fruit and Nuts, H.N. Murthy, and V.A. Bapat, eds. (Cham: Springer International Publishing), p.91–105.

Al-Qurashi, A.D., and Awad, M.A. (2011). Quality characteristics of Bisr 'Barhee' dates during cold storage as affected by postharvest dipping in gibberellic acid, naphthaleneacetic acid and benzyladenine. Fruit. *66* (*5*), 343–352 https://doi.org/10.1051/fruits/2011048.

Aleid, S., and Saikhan, M.S.A.L. (2017). Effect of permeable modified atmosphere packaging on quality and shelf life of fresh Khenaizy dates stored at low temperature. Food Nutr. Res. *5*, 503–509.

Alhamdan, A.M., Elkhair, D.O., and Ehmed, K.A. (2015). Modeling of respiration rate of fresh date fruit (Barhi cultivar) under aerobic conditions. J. Advanced Agric. Technol. *2* (*2*), 120–124 https://doi.org/10.12720/joaat.2.2.120-124.

Alsawmahi, O.N., Al-Juhaimi, F., Alhamdan, A.M., Ghafoor, K., Adiamo, O.Q., Mohamed Ahmed, I.A., Hassan, B.H., Ehmed, K.A., Babiker, E.E., Abdelkarim, D., et al. (2018). Phenolic, tannin, antioxidant, color, and sensory attributes of Barhi date (*Phoenix dactylifera*) fruit stored in modified atmosphere packages. J. Food Biochem. *42* (*5*), e12576 https://doi.org/10.1111/jfbc.12576.

Alyafei, M.A.S., Al Dakheel, A., Almoosa, M., and Ahmed, Z.F.R. (2022). Artificial pollination of date palm (*Phoenix dactylifera* L.) using a platform mounted robotic pollination system (drone). HortScience *57*, 1298–1305 https://doi.org/10.21273/HORTSCI16739-22.

Atia, A., Abdelkarim, D., Younis, M., and Alhamdan, A. (2018). Effects of calcium chloride and salicylic acid postharvest treatments on the quality of Khalal Barhi dates at different ripening levels during cold storage. J. Food Meas. Charact. *12* (*2*), 1156–1166 https://doi.org/10.1007/s11694-018-9729-0.

Awad, M.A., Al-Qurashi, A.D., and Mohamed, S.A. (2011a). Antioxidant capacity, antioxidant compounds and antioxidant enzyme activities in five date cultivars during development and ripening. Sci. Hortic. (Amsterdam) *129* (*4*), 688–693 https://doi.org/10.1016/j.scienta.2011.05.019.

Awad, M.A., Al-Qurashi, A.D., and Mohamed, S.A. (2011b). Biochemical changes in fruit of an early and a late date palm cultivar during development and ripening. Int. J. Fruit Sci. *11* (*2*), 167–183 https://doi.org/10.1080/15538362.2011.578520.

Bentrad, N., and Hamida-Ferhat, A. (2020). Date palm fruit (*Phoenix dactylifera*): nutritional values and potential benefits on health. In The Mediterranean Diet, 2nd edn, V.R. Preedy, and R.R. Watson, eds. (Academic Press), p.239–255.

Chen, Y., Sun, J., Lin, H., Lin, M., Lin, Y., Wang, H., and Hung, Y.C. (2020). Salicylic acid treatment suppresses *Phomopsis longanae* Chi-induced disease development of postharvest longan fruit by modulating membrane lipid metabolism. Postharvest Biol. Technol. *164*, 111168 https://doi.org/10.1016/j.postharvbio.2020.111168.

Hazbavi, I., Khoshtaghaza, M.H., Mostaan, A., and Banakar, A. (2015). Effect of postharvest hot-water and heat treatment on quality of date palm (cv. Stamaran). J. Saudi Soc. Agric. Sci. *14* (*2*), 153–159 https://doi.org/10.1016/j.jssas.2013.10.003.

Kassem, H., Alobeed, R., and Ahmed, M. (2010). Extending harvest season, improving fruit quality and shelf life of 'Barhee' date palm by preharvest sprays. Acta Hortic. *882*, 147–154 https://doi.org/10.17660/ActaHortic.2010.882.16.

Khalil, H.A., El-Ansary, D., and Ahmed, Z.F.R. (2022). Salicylic acid mitigates the adverse effects of salinity stress on pomegranate (*Punica granatum* L. cv. Wonderful) by activating the antioxidant enzymatic mechanism, protecting morphological structure, and regulating lipid peroxidation level. Horticulturea *8*, 375 https://doi.org/10.3390/horticulturae8050375.

Kumar, D., Mishra, D.S., Chakraborty, B., and Kumar, P. (2013). Pericarp browning and quality management of litchi fruit by antioxidants and salicylic acid during ambient storage. J Food Sci Technol *50* (*4*), 797–802 https://doi.org/10.1007/s13197-011-0384-2. PubMed

Maan, A.A., Reiad Ahmed, Z.F., Khan, M.K.I., Riaz, A., and Nazir, A. (2021). *Aloe vera* gel, an excellent base material for edible films and coatings. Trends Food Sci. Technol. *116*, 329–341 https://doi.org/10.1016/j.tifs.2021.07.035.

Mohamed, S.A., Awad, M.A., and Al-Qurashi, A.D. (2014). Antioxidant activity, antioxidant compounds, antioxidant and hydrolytic enzymes activities of 'Barhee' dates at harvest and during storage as affected by pre-harvest spray of some growth regulators. Sci. Hortic. (Amsterdam) *167*, 91–99 https://doi.org/10.1016/j.scienta.2014.01.003.

Moreno-Escamilla, J.O., Alvarez-Parrilla, E., de la Rosa, L.A., Núñez-Gastélum, J.A., González-Aguilar, G.A., and Rodrigo-García, J. (2018). Effect of elicitors in the nutritional and sensorial quality of fruit and vegetables. In Preharvest Modulation of Postharvest Fruit and Vegetable Quality, M.W. Siddiqui, ed. (Academic Press), p.71–91.

Nadeem, A., Ahmed, Z.F.R., Hussain, S.B., Omar, A.E.-D.K., Amin, M., Javed, S., Ali, A., Ullah, S., Razzaq, K., Rajwana, I.A., et al. (2022). On-tree fruit bagging and cold storage maintain the postharvest quality of mango fruit. Horticulturae *8* (*9*), 814 https://doi.org/10.3390/horticulturae8090814.

Rahman, M., Mukta, J.A., Sabir, A.A., Gupta, D.R., Mohi-Ud-Din, M., Hasanuzzaman, M., Miah, M.G., Rahman, M., and Islam, M.T. (2018). Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. PLoS One *13* (*9*), e0203769 https://doi.org/10.1371/journal.pone.0203769. PubMed

Rastegar, S., Rahemi, M., Baghizadeh, A., and Gholami, M. (2012). Enzyme activity and biochemical changes of three date palm cultivars with different softening pattern during ripening. Food Chem *134* (*3*), 1279–1286 https://doi.org/10.1016/j.foodchem.2012.02.208. PubMed

Romanazzi, G., Feliziani, E., and Sivakumar, D. (2018). Chitosan, a biopolymer with triple action on postharvest decay of fruit and vegetables: eliciting, antimicrobial and film-forming properties. Front Microbiol *9*, 2745–2745 https://doi.org/10.3389/fmicb.2018.02745. PubMed

Ruiz-García, Y., and Gómez-Plaza, E. (2013). Elicitors: a tool for improving fruit phenolic content. Agric. *3* (*1*), 33–52 https://doi.org/10.3390/agriculture3010033.

Sohail, M., Ayub, M., Khalil, S.A., Zeb, A., Ullah, F., Afridi, S.R., and Ullah, R. (2015). Effect of calcium chloride treatment on postharvest quality of peach fruit during cold storage. Int. Food Res. J. *22*, 2225–2229.

Wang, L., Chen, S., Kong, W., Li, S., and Archbold, D.D. (2006). Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. Postharvest Biol. Technol. *41* (*3*), 244–251 https://doi.org/10.1016/j.postharvbio.2006.04.010.

Xylia, P., Chrysargyris, A., Ahmed, Z.F.R., and Tzortzakis, N. (2021). Application of rosemary and eucalyptus essential oils and their main component on the preservation of apple and pear fruits. Horticulturae *7* (*11*), 479 https://doi.org/10.3390/horticulturae7110479.

Xylia, P., Chrysargyris, A., Shahwar, D., Ahmed, Z.F.R., and Tzortzakis, N. (2022). Application of rosemary and eucalyptus essential oils on the preservation of cucumber Fruit. Horticulturae *8* (*9*), 774 https://doi.org/10.3390/horticulturae8090774.