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

Bio-Stimulants for sustainable agriculture in oasis ecosystem towards improving date palm tolerance to biotic and abiotic stress.

Author:

Prof. Abdelilah Meddich

Cadi Ayyad University, Faculty of Science Semlalia,
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Coordination and Supervision:

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Bio-Stimulants for sustainable agriculture in oasis ecosystem
Towards improving date palm tolerance to biotic and abiotic stress.

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Author/Prof. Abdelilah Meddich
Cadi Ayyad University, Faculty of Science Semlalia,
Laboratory of Agro-Foods, Biotechnologies,
and Valorisation of Plant Bioressources

Coordination and supervision/Prof. Abdelouahhab Zaid
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Preface

Bayoud disease, drought, salinity, lack of soil organic matter and mineral content, and spread of many pests are the major causes of date palm (*Phoenix dactylifera*) plantations losses during the last decades. These problems can be tackled by exploiting the use of biofertilizers in sustainable crop production.

The book presents research within a relevant practical framework to improve date palm-appropriately called “the tree of life”- growth, tolerance to biotic (vascular *Fusarium* wilt and pests) and abiotic (drought and salinity) constraints. Further, it is intended to cater to the needs of creating inexpensive, energy neutral, and eco-friendly methods to improve nutrient supply, conserve the field management, and increase the yield of the date palm in the arid and semi-arid ecosystems and its underlying crops.

There has been a great demand for a book dealing with the potential application of biofertilizers, date palm growth, and productivity, and improved tolerance to the harmful effects of stresses with a special emphasis on mechanisms triggering these changes.

Seven chapters have been elaborated to outline an innovative approach aiming in developing sustainable agriculture in fragile ecosystems such as palm grove oases. In this regard, setting up efficient biological protocols integrating organic amendments such as compost and natural microflora of the soil which constitutes all groups of useful bacteria and fungi including the arbuscular mycorrhiza fungi (AMF) and plant growth-promoting rhizobacteria (PGPR). Besides, these biofertilizers also contribute to the development and production of young tolerant palms able to cope with the aforementioned stresses and they serve in the reinvigoration/rejuvenation tree-planting programs in the oasis ecosystems.

Herein, we also specify the processes and mechanisms developed by date palm plants treated with selected exotic and native bacteria and fungi, under controlled and field conditions, to understand the agro-physiological and biochemical bases of biofertilizers towards sustainable agriculture in reducing problems associated with the use of chemicals fertilizers and large-scale and global environmental changes.

This book will certainly provide useful information on various strategies adopted by using agriculturally useful microbial populations and compost, interactions, and positive effects in their implementation to date palm under climate change-driven biotic and/or abiotic stresses. It should serve as a reference and a source of information for extension specialists, date growers, agricultural/environmental scientists, environmentalists, regulators and policymakers, students, and, above all, anyone interested in the biofertilizers and date palm status.

Prof. Abdelilah Meddich

The Award's General Secretariat's word

Since its establishment in 2007, the General Secretariat of Khalifa International Award for Date Palm and Agricultural Innovation, has been keen to work according to a clear strategic plan, through which it seeks to achieve its objectives for which it was established, and implement the UAE's leaders' wise vision, in supporting and developing the date palm cultivation sector, and promoting agricultural innovation at the national, regional and international levels.

This success achieved by the United Arab Emirates in supporting and developing the infrastructure of the date palm cultivation sector at the regional and international levels, and the significant footprint achieved by the Award during its fourteen years journey, made us feel proud. These achievements would not have been without the support and care of H.H. Sheikh Khalifa Bin Zayed Al Nahyan, President of the UAE, and the Award's patron, "May God protect him", where the Award is honored to be named after His Highness. The Award is also honored to gain the blessings of H.H. Sheikh Mohammed Bin Zayed Al Nahyan, Crown Prince of Abu Dhabi, Deputy Supreme Commander of the UAE Armed Forces, and the support of H.H. Sheikh Mansour Bin Zayed Al Nahyan, Deputy Prime Minister, Minister of Presidential Affairs, and the continuous follow-up of H.E. Sheikh Nahayan Mabarak Al Nahayan, Minister of Tolerance and Coexistence, Chairman of the Award's Board of Trustees, confirms the leadership's interest in shaping a better future to the date palm cultivation and agricultural innovation sectors, which is a fundamental component of the food security equation to achieve sustainable development.

In appreciation of this role, the Award's General Secretariat seeks continuously to strengthen and empower its target groups, by issuing specialized scientific books on various topics related to date palm cultivation, and agricultural innovation.

Prof. Abdelouahhab Zaid

Book summary

The date palm oasis ecosystem, which coexists with deserts in arid regions, plays a major role in balancing and safeguarding the global environment and the settlement of human populations and their socio-economic wealth (Hadagha et al., 2018). Accordingly, the stability of the oasis ecosystem directly relates to sustainable development in arid and semi-arid regions. Nevertheless, this ecosystem has been largely impacted by natural and/or anthropogenic constraints (e.g. spread of bayoud disease and pests, drought, salinity, erosion, desertification, soil depletion, over-exploitation of water resources), thereby leading to the environmental degradation and desertification (Ait-El-Mokhtar et al., 2019a; Boutaj et al., 2019a; Dihazi et al., 2012; Meddich et al., 2015a,b). These latter have become the main obstacle to the sustainable development of date palm oasis ecosystem. Therefore, several past and ongoing studies on innovative prospects to overcome the impacts of these issues and preserve the oasis agriculture are indispensable for the sustainable development in arid regions (Antoniou et al., 2017; Boutasknit et al., 2020a; Diacono et al., 2019; El Maaloum et al., 2020; Meddich et al., 2019).

Plant fitness is intimately influenced by the rhizosphere, soil layer surrounding plant roots, microbiome. Telluric microorganisms such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) provide fitness benefits to their respective hosts and improve biomass and tolerance against biotic and abiotic constraints. The microbial inoculants have paramount significance in integrated nutrient management systems, nutrient use efficiency of fertilizers, and protection against pathogens to sustain agricultural productivity and healthy environment (Santhanam et al., 2015; van der Heijden et al., 2016). Besides, the dynamics of microbe-microbe interactions have recently emerged as an important feature of the phyllosphere (Agler et al., 2016). The efficiency of the health-promoting soil microbiomes strategy can be promoted by the assistance of organic amendments to the soil such as green compost. The valorization of organic wastes by the composting process and their application in agriculture as an organic amendment has been given lastly a great deal of attention owing to their economic and ecological strategies and the need for strategies to recycle such waste. Composting facilitates the conversion of organic waste into new usable organic matter that helps restore the depleted soils. Besides, composts represent a sustainable way to suppress diseases and improve plant growth. Each of these biofertilizers has been identified as having the potential to the enrichment of soil environment in micro- and macro-nutrients *via* N fixation, P and K solubilization or mineralization, the release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha et al., 2010). Through the rhizosphere-enriched strategies, microorganisms and composts have great potential for future exploitation and management to facilitate a variety of sustainability programs in oasis ecosystem, conservation and restoration, considerations that are relevant during this time of environmental disasters, global change, and widespread degradation of natural resources.

This book will bring together an exciting body of practical research toward (i) creating environmentally-friendly and efficient biological protocols integrating indigenous microorganisms and organic fertilizers originated from a vegetal, animal, and agro-industrial wastes, (ii) improving the biomass and yield of the date palm tree and its underlying crops (wheat, maize, alfalfa, tomato, lettuce, garlic, and leek), (iii) assessing the potential roles of rhizosphere-enriched microorganisms and composts in plant defense, protection and fitness improving, (iv) exploring relationships and significance of microbes and composts in oasis ecosystem stability and development, and (v) understanding the underlying regulation mechanisms from whole plant to cell the dual and multiple combinations of compost and/or microorganisms confer to the host plant toward the stable use of biofertilizers in agriculture and to have knowledge of their potential effects.

During the last decade, several experiments have been carried out by our team under controlled and open-field systems to provide evidence that rhizosphere-enriched microbes and composts can be an efficient strategy for the construction and sustainability of the oasis ecosystem. For this purpose, soils collected from one of the major palm groves (Tafilalet) in Morocco and the leading producer of dates in the region. These soils are poor in N- and P- availability and showed a significant mycorrhizal potential, particularly with the Aoufous mycorrhizal consortium, which is characterized by a highe rmycorrhization capacity (Chapter 1). To remedy the poverty of these soils, several organic amendments were prepared by an efficient and low-cost composting technology: grass scraps alone (GW), dead grass-sheets (WGDL), olive cakes olive mill wastewater and garbage (OCOMWWG), and phosphate-grass sludge (GWSP) (Chapter 2). In consequence, herein we proposed a methodology to select the most suitable amendments, best dose, and combination with AMF effects. Also, owing to its low content of potentially health-threatening contaminants and its highest quality, composts proved to be the most suitable amendment to boost the growth and yield of leguminous (alfalfa), cereals (wheat, maize), and horticultural (tomato, lettuce, leek, garlic) plants.

In Chapters 3 and 4, we used controlled and open-field assays to test the combination of microbial (AMF and organic biofertilizers) and/or PGPR on date palm performance. This would be particularly relevant to address the ecological role of microorganisms mutualists and soil conditioners addition, particularly in the context of desertification. Therefore, it is worth considering in future strategies of fertilization, species management, and restoration of native plants.

Subsequently, Chapter 5 covers the application of a native mycorrhizal consortium as a potential biofertilizer triggering date palm tolerance to the increasing salinity in the oasis ecosystem. The beneficial microorganisms, through activating mechanisms, improve date palm tolerance to salinity contamination. This chapter may have a significant impact from an agronomic point of view, since the application of native fungi can produce a significant increase in the growth of this important plant and this could be a first step towards encouraging farmers to autonomously produce their AMF inocula, starting from native soils.

Thereafter, the role of biofertilizers such as AMF and other biological treatments on the tolerance of date palm under abiotic (drought) and/or biotic (Bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa) and *Potosia opaca* larvae attack) constraints have been addressed in the Chapters 6 and 7. The application of selective native and exogenous AMF showed that alterations of plant-soil biotic interactions could occur under climate change and the mortality rates in palm plants caused by Foa decreased significantly in plants inoculated with beneficial microorganisms. Further, soil beneficial organisms potentially mediate date palm fitness and growth. The Chapter 6 also deciphers several key players involved in the modulation of the root colonization process when both beneficial and pathogenic microorganisms or drought are jointly applied.

Finally, since the climate change is affecting the biology, distribution, and outbreak potential of pests in a vast range of crops, including date palm and oasis landscape, Chapter 7 gives an overview of the efficient application of an olive mill wastewaters-based bioinsecticide as a biological agent against the new pest *Potosia opaca*, which is associated with palm tree mortality.

Altogether, this book is of particular importance for oasis systems and may provide promising management options to mitigate the negative impacts of climate change.

Keywords: Drought, climate change, compost, environmental driver, *Fusarium oxysporum* f. sp. *albedinis,* mycorrhizae, natural-based solutions, oasis ecosystem, PGPR, *Potosia Opaca*, salinity, soil degradation, sustainability, tolerance, waste.

Background

Date Palm Oases are ecosystems in arid and semi-arid areas and are known for their complexity and fragility. For centuries, these ecosystems have played important roles in the socio-economic development of local populations and in ecological security. Date palm, *Phoenix dactylifera* L., is considered the mainstay of this ecosystem and one of the most economically important perennial plants in arid and semi-arid regions (Arias et al., 2016). The geographical distribution of date palm in the world clearly shows that 150 million date palms are distributed in around 40 countries including more than 100 million in the Arab countries (Figure 1).

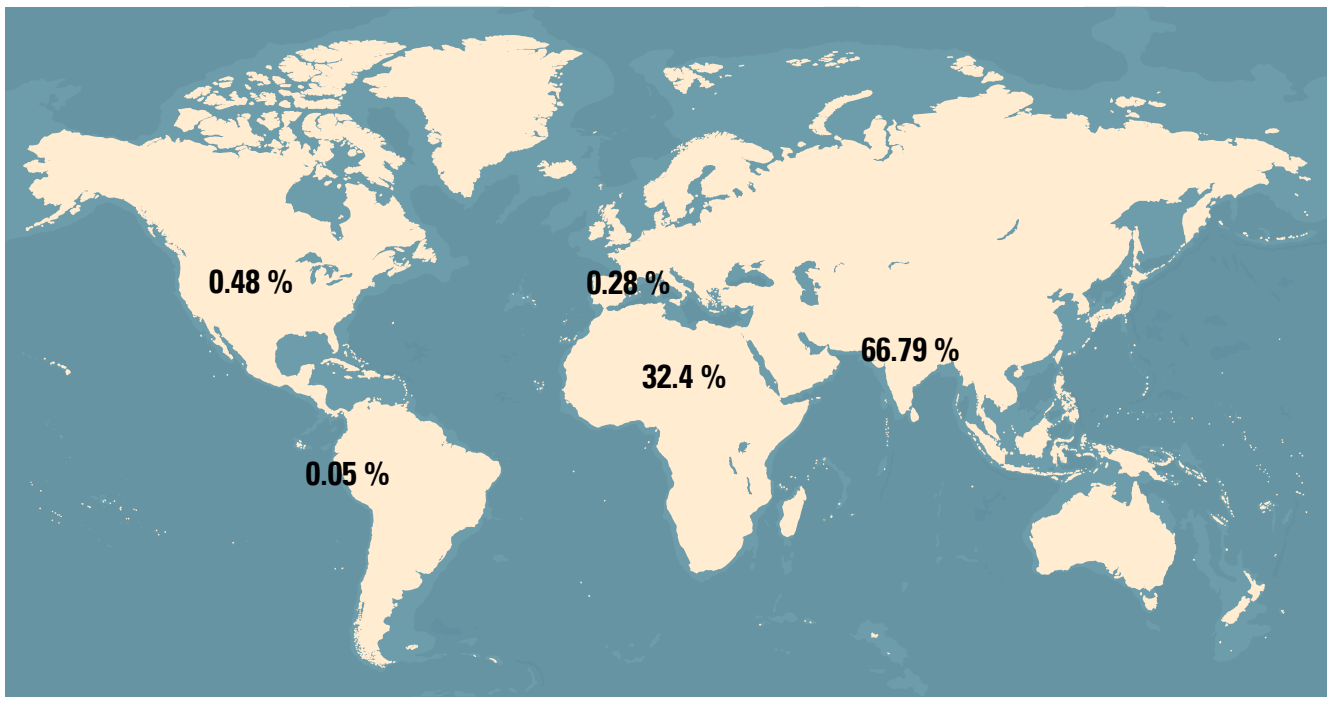

Figure 1: Geographical distribution of date palm in the world (Source: blank map http://onlinemaps.blogspot.com/2011_11_01_archive.html).

Dates are a good source of essential nutrients and form an important part of the daily human diet (Arias et al., 2016). Dates demand for international consumption has increased over the years in comparison to the available supply. This has prompted several countries to import dates (Oihabi 2020). Total world date production was 8.5 million tons in 2018 (FAO, 2020) with an increase of 27% from 6.44 million tons in 2000 (Figure 2).

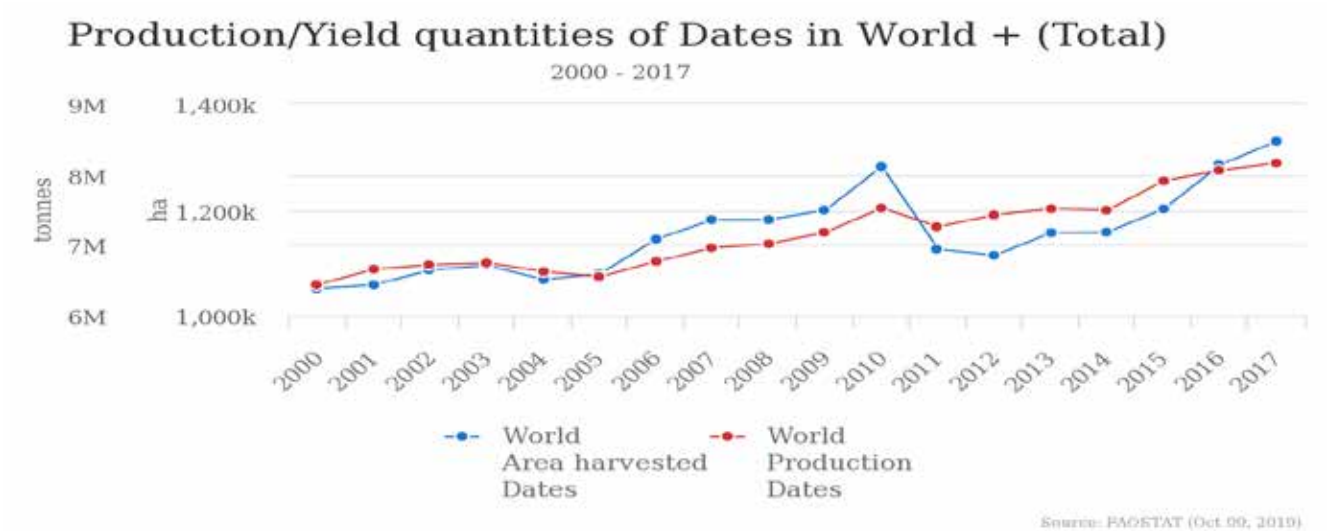

Figure 2: Evolution of the harvested area and world date production between 2000 and 2017 (FAO, 2020)

Table 1 presents the values of date production in the main producing countries in 2018 (FAO, 2020).

Table 1. Ranking of the top 14 date-producing countries (FAO, 2020).

Rank	Country	Production (tons)	Rank	Country	Production (tons)
1	Egypt	1,562,171	8	Oman	368,808
2	Saudi Arabia	1,302,859	9	United Arab Emirates	345,119
3	Iran	1,204,158	10	Tunisia	335,000
4	Algeria	1,094,700	11	Libya	176,229
5	Iraq	614,584	12	China	158,294
6	Pakistan	471,670	13	Morocco	111,701
7	Sudan	440,871	14	Kuwait	96,656

Date palm in Morocco

In Morocco, the palm grove is a unique heritage, key to the economic future of the oasis regions. The culture of date palm has been present in the country since ancient times, mainly in the south of the Atlas Mountains. Date palms are mainly cultivated in the valleys bordering the great rivers of the region such as the Ziz, the Draa, the Gheris and the Guir. Oases have also flourished around the water tables of the Baniou and Saghro regions. Furthermore, it is also established, further north in the east of the Kingdom, precisely in Figuig where it draws its water from local springs along the wadi Zousfana. Very demanding, the palm tree needs intense light and heat as well as a dry climate to thrive and bud. Morocco is therefore ideal for this plant which is present in almost a third of its territory (471,000 km2). Four large regions with a phoenicultural vocation for several thousand years welcome this plant with multiple uses: The Oriental, the Draa-Tafilalet, the Souss Massa and finally the region of Guelmim Oued Noun. The date palm helped shape the people and landscapes of Morocco since time immemorial. Its multiple uses, food for people and livestock, raw material for everyday objects but also the ease of conservation of the date makes it a unique product. In addition, the palm grove supplies the necessary raw material for various activities such as traditional construction or the production of handmade objects. Moreover, it is a vital energy source in a region that is poor in fuel resources. It is not surprising that it has imposed itself in the Saharan areas where it had once thrived the trans-Saharan caravan trade and where today it is the pillar of the local international agrarian economy (MAFRDF, 2018). For these reasons, the Kingdom of Morocco has adopted numerous programs in collaboration with international organizations and various stakeholders to develop oasis agricultural systems and increase the sector's resilience to climate change. The programs that are most relevant are presented below:

The Green Morocco Plan (GMP) was officially launched in 2008 by the Ministry of Agriculture, Fisheries, Rural Development and Forestry (MFRDF). The GMP is the government's national strategy to develop the sustainibility of the agricultural sector. It aims to improve the agricultural revenues of people living in rural areas by supporting investments, exportation and aggregation between production and the commercial and industrial phases. The GMP implementation is supported by two pillars and various cross-cutting programs. The first pillar is related to high-productivity and intensive production agriculture with a direct relationship to the market. The second pillar is focused on consolidating small farmers by increasing crop production intensification where required, and the use of crops that are considered more suitable in relation to environmental conditions and market demand. The GMP consists of 1500 projects requiring more than US$10 billion to be implemented until 2020 (Badraoui 2014).

The date production sector, linked to the oasis area, is one of the GMP's concerns. The GMP has considered this sector as very important for the strategy of Moroccan agricultural development. The sector is important because national demand is high and date production is potentially ecologically sustainable and economically competitive, even for export. Actually, the biggest project in Morocco, which is part of the GMP, is the program to accelerate the restoration of the palm groves of Oriental, Souss-Massa, Guelmim-Oued Noun and Draa-Tafilalet, with the following objectives (Pegna et al., 2019):

- Creation of multiple new date retrieval and storage units in Souss-Massa (capacity of 3,400 tons), Meknes Tafilalt (capacity of 2,600 tons), Guelmim Es-smara (capacity of 1,000 tons), eastern region (capacity of 100 tons).
- Creation of a National Laboratory for the date palm tissue culture in Errachidia to increase the national production to 50,000 budding strains per year.
- Planting 3 million date palms by 2020 with high-quality and tolerant varieties, using the laboratories for the vitroplants production.
- Reaching a production of 160,000 tons by 2020.

The sustainable oasis ecosystem restoration in Morocco is the key goals of others development programs carried out in the last decade. A list of these programs is presented in table 2.

Table 2. Rehabilitation programs launched by the Moroccan government with different organizations in the last decade (Pegna et al., 2019).

Programs	Description
Tafilalet Oasis Program (TOP)	TOP concerns Tafilalet and was carried out by the Ministry of Energy, Mines, Water and Environment Management in collaboration with Directorate of Spatial Planning between 2006 and 2015.
Draa Oasis Program (DOP)	A program to mitigate desertification, fight poverty, and protect and enhance the traditional oasis of Draa production (2006-2015).
ANDZOA Program	The National Agency for the Development of the Oasian and Argan Zones (ANDZOA) founded in 2010, and designed to maximize national and international resources in order to protect and rehabilitate the traditional oasis ecosystem threatened by desertification.
Globally Important Agricultural Heritage Systems (GIAHS) program	A program that protects the oases of Imilchil-Amellago, Ait Mansour, Akka, Assa and Figuig since 2011 and is aimed at fighting against the increasing degradation of the oasis area. It was launched by FAO, in partnership with the Ministry of the Environment, the Ministry of Agriculture, ANDZOA, Development of the Moroccan Southern Provinces (DPS), the National Institute of Agronomic Research (INRA) and the Global Environment Facility (GEF).
OASIL	A project, sponsored by FAO in cooperation with ANDZOA and GEF (2017-2021). The project objectives are: - to reinforce oasis based agro-ecosystems in the Draa-Tafilalet zone to improve their productivity, suitability and fitness, and to support and make more resilient the livelihoods of local communities. - to support the knowledge dissemination regarding oases, to strengthen policy discussions and support the adoption of national and regional strategies as well as plans for the oases management sustainability. - to encourage the use of an integrated management approach for the oasis agro-ecosystem, with deep participation of all stakeholders in the decision-making process.
Oriental Integrated Local Development (DeLIO) program	DeLIO is a program which is focused on the oasis of Figuig, among others (2008-2011). It's interested in the development of 5 local products (dates and the most relevant handcrafted products), by promoting and supporting their chain of production.
Mohammed VI Foundation for the Protection of the Environment	The project initiated in 2006 and has benefited the palm grove of Marrakech and has allowed the planting of 581,000 date palm trees and the maintenance of 81,000 adults date palm trees.

Morocco ranks 13th in the world in the production of dates in 2018, with 111,701 tons (FAO, 2020). In Morocco, dates production areas are mainly located along the Ziz and Draa valleys, with nearly 7 million date palm trees, of which nearly 41% are productive. The sector contributes to the formation of agricultural income up to 50% for 2 million inhabitants. The varietal composition is characterized by the existence of a multitude of varieties: Mejhoul, Boufeggous, Bouskri, Jihel but with predominance of khalts (unidentified varieties). The main pillar of the oasis economy is the phoeniculture activity. It generated an average annual revenue of 1.965 billion dirhams over the period 2015-2018 (MAFRDF, 2018). It provides farmers annually with an average added value estimated at 1,423 billion dirhams over the same period. The phoenicultural sector supports the local purchasing power since it provides 3.6 million working hours per year to produce an average production of 117,000 tons of dates each year. It therefore greatly improves the living conditions of hundreds of thousands families who see an improvement in their own socio-economic situation (MAFRDF, 2018).

As for the market, some varieties are specific to certain production areas:

- 50% of the production is marketed.
- 30% of the production is for self-consumption.
- 20% remaining is allocated to cattle feed.

Table 3 below shows the importance of the main production areas for date palms in Morocco. The Draa Tafilalet region is well distinguished by its surface areas and date productivity.

Table 3. Cultivated area of date palm and date production in the main phoenicultural areas in Morocco (2017/2018) (MAFRDF, 2018).

Region	Cultivated area (ha)	Production (tons)	Production (%)
Draa Tafilalet	48,453	94,000	86.21
Souss Massa	8,000	8,041	7.37
Figuig	1,787	4,000	3.67
Guelmim Oued Noun	1,400	3,000	2.75
Total	59,640	109,041	100.00

During the last decades, the date palm cultivation area has undergone intense degradation under various biotic and abiotic stresses (Ait-El-Mokhtar et al., 2020a; Arias et al., 2016; Botes et Zaid, 2002; Meddich and Boumezzough, 2017; Oihabi, 1991; Sedra 2015). These stresses are considered the most important and damaging agricultural and eco-environmental issues limiting the growth and development of date palm (Al-Nadabi et al., 2020). Nowadays, the use of chemical fertilizers has increased the degradation of soil quality and their impoverishment. Among the main abiotic and biotic constraints with which the date palm is permanently exposed, we can cite the following:

Abiotic constraints

In arid lands and under climate change, the amount of available water to crops restricts plants' average growth and development. About 40% of the earth surface is occupied by drylands (Vallejo et al., 2012). Globally, around 8% of total land and 20% of cultivated land is affected by salinity (FAO-IPTRID 2006) and more than 50% of arable land would be affected by this constraint by 2050 (FAO, 2020). Full-grown plants may tolerate water deficit and salt stress through their growth flexibility, as well as morphological and physiological adjustments; however, new plantations are highly sensitive to water and salt stress; this is the case until their root system is established well in the soil (Meddich et al., 2018; Vallejo et al., 2012). Plants establishment is a challenging problem in dryland conditions, especially under water and salt stress conditions. The mortality rate can be high and posing a serious challenge in the process of establishment. It is necessary to discover techniques that can improve plant growth with little irrigation in reclamation plans in arid environments (Vallejo et al. 2012), especially in climate change scenarios.

Thus, drought, salinity, high temperatures and soil poverty represent recently a serious worldwide problem and play a particularly destructive role in the agricultural sector in countries with a low annual rainfall. These constraints cause a significant loss in yield and quality in date palm (*Phoenix dactylifera* L.), despite the fact that the date palm can survive a wide range of extreme abiotic stresses such as drought and soil salinity (Ait-El-Mokhtar et al., 2019; Al Kharusi et al., 2019; Meddich et al., 2018).

Biotic constraints

Main insect pests: red weevil, white mealybug and *Potosia opaca*

The red weevil (*Rhynchophorus ferrugineus*) is a serious pest of date palm and causes significant ecological and economic damage to farmers. The adult can remain inside the trunk or can disperse and spread infestations to other palms (Al-Dosary et al., 2016). In the other hand, the white mealybug (*Parlatoria blanchardi targ*), is one of the main pests of the date palm because it affects the green parts of the tree, preventing and disrupting photosynthesis, respiration and transpiration of the tree. Among the damage caused by *P. Blanchardi* is the drying out, reduction and dieback of the leaves of the palm tree. In the case of adult trees precisely in the leaves, this pest gives a yellowish or salt-green appearance and also decreases the quality and quantity of date production (Babaousmail et al., 2016). Otherwise, *Potosia opaca* is an insect beetle that affects date palms and Canary palms. The larvae also develop at the base of the leaves of palms and the weakened rachis (Meddich and Boumzough, 2017).

Main fungus diseases: Bayoud and inflorescence rot

Bayoud disease, caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), has been responsible for the destruction of Moroccan and Algerian palm plantations (more than 10 million plants) causing considerable socio-economic and ecological damage (Botes et Zaid, 2002; Oihabi 1991; Sedra 2012, 2015). Inflorescence rot or Khamedj is a disease caused by a fungus of the order *Mauginiella scaettae*. It shows a brownish-brown color on the still closed husks of the spats (El Bouhssini 2018; Sedra 2012).

Beside *F. oxysporum* f. sp. *albedinis*, several studies reported the association of different Fusarium species with date palm diseases. These include *Alternaria* species, which caused leaf spot in Oman (Al-Nadabi et al., 2020), *F. fujikuroi*, the cause of date palm pollen rot in Iraq (Abedalred et al., 2019), *F. solani* associated with sudden decline of date palms in Pakistan (Ali Maitlo et al., 2013), *F. solani and F. proliferatum,* associated with chlorosis and other date palm diseases in Saudi Arabia (Saleh et al., 2017) and *F. proliferatum* associated with date bunch fading in Iran (Mansoori 2012). *Thielaviopsis paradoxa* in Iran and *T. punctulata* in United Arab Emirates have been recorded as causal agents of black scorch or neck bending on date palm (Mirzaee et al., 2014; Saeed et al., 2016).

Biological approach as an alternative

Despite the numerous rehabilitation programs launched to address the critical situation of palm groves (Meddich et al., 2018; Pegna et al., 2019), these initiatives remain incomplete since any ecosystem restoration program must incorporate components from different levels of the ecosystem, in particular soil microorganisms that are closely related to plants. Symbiotic microorganisms have often improved the growth and tolerance of date palm to biotic and abiotic stresses (Ait-El-Mokhtar et al., 2020a; Anli et al., 2020a; Baslam et al., 2014; Meddich et al., 2019, 2018; 2015a, Naser et al., 2016).

The use of arbuscular mycorrhizal fungi (AMF), PGPR bacteria and organic soil amendment through compost is one of the agrobiological processes to mitigate biotic and abiotic stresses in date palms (Ait-El-Mokhtar et al., 2020b; Anli et al., 2020a, 2020b, 2020c; Meddich et al., 2015b; Naser et al., 2016; Toubali et al., 2020). The application of these biofertilizers reduces biotic and abiotic stresses by improving soil rhizospheric characteristics, nutrient acquisition, water absorption, photosynthetic activity and the antioxidant system (Ait-El-Mokhtar et al., 2020b; Al Kharusi et al., 2019; Toubali et al., 2020).

General concepts

1. Key features

Date palm constitutes the corner stone of oasis agroecosystems, the main landscape of food security and ecosystem services in arid areas. The date palm has a large genetic stock and promotes the creation of a favorable microclimate for the development of underlying crops (e.g. arboriculture, cereal crops, forage crops, and horticultural species). Date palm plantation contributes primarily to the stabilization and sustainability of people's lives and food security in arid and semi-arid regions, where the natural resources are limited and the living conditions are harsh (Ehsine et al., 2014). However, less attention has been given to identifying the trends and driving forces affecting the agricultural oasis stability and their impact on the oasis landscape.

North-African oases, especially in Morocco, are currently facing the additional challenge of climate change and constraints related to urbanization, desertification, erosion, water scarcity, salt increasing, diseases such as Bayoud caused by *Fusarium oxysporum* f. sp. *albidinis* and pest attacks, soil depletion, and tree aging (Awad 2006; Botes et Zaid, 2002; Jaiti et al., 2008; Meddich et al., 2015b; Meddich and Boumezzough, 2017; Oihabi 1991; Saaidi 1992; Ziouti 1998). All these problems have resulted in rural-urban migration, thereby leading to farmland abandonment, degradation, and regression of these landscapes. Besides, the date palm has greatly contributed to the creation, maintenance, and development of the economy in the oases. At the end of the 19th century, Morocco ranked the 3rd largest producer of dates in the world (Oihabi 1991) with 15 million date palm trees. At the beginning of the 20th century, this number decreased to 4.25 million palm trees. Multiple, simultaneous environmental changes, in climatic/abiotic factors and direct human influences, are affecting date palm populations and thus oasis landscape (FAO, 2012).

Although, some of these changes may be more impactful than others. For instance, the non-adapted farming practices, such as the increased inputs of chemical fertilizers, weakened the soil quality and ecological stability of the oasis ecosystem by decreasing their content of organic and inorganic matters (Choudhary et al., 2018). The repeated overuse of chemical fertilizers can have negative effects on soil health and soil microbial community structure, crop production, and the environment (Solgi et al., 2018). Therefore, an appropriate and efficient strategy is required to overcome these constraints and to ensure the protection and restoration of oases. In the last two decades, scientists carried out several investigations dealing with the beneficial association of plants with symbiotic soil micro-organisms such as AMF and PGPR bacteria. The use of these symbiotic microorganisms can improve crop yields, soil fertility, and reinstate the fertility of degraded soils. This contention was supported by findings from Al-Yahya'ei et al. (2011), Ait-El-Mokhtar et al. (2020c), Meddich et al. (2015b) and Oihabi (1991) suggesting that date palms roots are receptive to the AMF and are capable to grow in harsh environments. Similarly, other studies have shown the positive effects of mycorrhizal symbiosis on the growth and health of date palms (Al-Karaki, 2013 and Meddich et al., 2015b). Taken together, such studies revealed that (i) AMF promoted the growth of date palm seedlings in nursery conditions (Shabbir *et al.*, 2011) compared to controls treated with chemical fertilizers (Symanczik et al., 2014) (ii) increasing availability of nutrients in soil cultures (Al-Karaki et al., 2007), and (iii) improving the absorption of water and nutrients in saline conditions (Bearden and Peterson, 2000), thereby protecting date palm

seedlings against the increasing salinity (Ait-El-Mokhtar et al., 2019b). Other studies emphasized the essential role of AMF in improving the drought and salt stress tolerance in other crops such as lettuce (Baslam and Goicoechea, 2012; Ruiz-lozanoet al., 1995; Vicente-Sánchez et al., 2014), sorghum (Augé et al., 1995; Symanczik et al., 2018), corn (Subramanian and Charest, 1997), clover (Meddich et al., 2000; Oihabi and Meddich, 1996), barley (Meddich 2001; Tao et al., 2014) and carob (Boutasknit et al., 2020b). The mutually beneficial mycorrhizal interactions can improve plant resistance/tolerance to biotic stresses (Azcon-Aguilar and Barea, 1996; Jung et al., 2012; Linderman 1994; Meddich et al., 2015b; Thygesen et al., 2004; Xiao et al., 2014).

Plant growth-promoting rhizobacteria represent a heterogeneous group of easily accessible beneficial bacteria that colonize plant roots. PGPR are capable of improving plant growth, yield, and protection against biotic and abiotic stresses (Dimkpa et al., 2009; Glick, 2012; Grover et al., 2011). They improve plant growth through enhanced nutrient uptake from soil and a wide variety of mechanisms such as biological N fixation, P (Abdi et al., 2017) and K (Ana et al., 2009) solubilization, antifungal activity, systemic resistance induction through modulation of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase expression, phytohormones (e.g., abscisic acid, gibberellic acid, auxin, and cytokinins), and siderophores (Etesami and Maheshwari, 2018) production. They can control plant pathogens by different mechanisms including the generation of extracellular enzymes hydrolyzing the fungal cell wall, competition for nutrients (niches) within the rhizosphere, induction of systemic resistance, and the production of antibiotics and siderophores (Compant et al., 2005; Shirinbayan et al., 2019; Defez et al., 2019; Naseem et al., 2018). Such mechanisms make PGPR potentially usable biofertilizers and biopesticides, which make them an effective organic alternative that could help to sustain environmental health and soil productivity.

The combination of socio-economic development and population growth increased large quantities of solid and liquidwastes generated mainly by households, industry, and health facilities (Laarousi et al., 2006). For instance, Morocco produces significant quantities of green waste annually from the maintenance of green spaces. The couch grass and palm leaves from green waste as organic waste have been receiving increasing attention owing to their high abundance in the gardens of the city of Marrakesh (18,000 tones/year) and their improving effect of the mixture structure ensuring a carbon source for microbial growth. Besides, the pomace olives -produced in very high quantities estimated at 180,000 tons/year- and olive mill wastewater -estimated at 400,000 m^3/year- have been also considered as other sources of carbon required for microbes (CFC/IOC, 2008). Besides, owing to that Morocco is the world's largest producer of phosphate, and contains about 75% of the world's estimated reserves, the sludge phosphates (21.64% P_2O_5) do not undergo any recycling or adequate treatment. Their production is estimated at 2 million tons/year. Currently, the production of sludge from water treatment in Marrakesh is estimated at 64,800 tons/year. Finally, the manure and bedding produced from poultry units (590,000 tons/year) and horse manure are generated in large quantities without any use. The quantity (volume or mass) of these manures and its nutrient content are the most critical factors that govern its use as a nutrient source.

Technologies for sustainable wastes and manure management are needed. Composting these wastes can be a valuable economic and ecological solution, since it will allow the return of organic matter to the soil and thus its reintegration to the vital ecological cycles (Francou 2003). The amendment of soils with compost improves their physicochemical characteristics and stimulates plant growth (Tejada et al., 2009). The compost upgrades the organic matter with organic molecules, diversified degradation products, and humus substances in soils and their structure by interacting with minerals and aggregation of clay particles. This interaction increases soil stability by producing micro-aggregates (Clapp et al., 2001; Seul et al., 2009; Stevenson 1994). This organic matter decreases the soil density and promotes root growth and penetration by improving nutrition, photosynthesis, and plant biomass (Boutasknit et al., 2020a; Nardi et al., 1996; Rauthan and Schnitzer, 1981; Schnitzer and Poapest, 1967). Similarly, the compost increases the cation exchange capacity and soil water retention by ensuring a good flow of water and limiting of leaching (Giusquiani et al., 1995; Somerville et al., 2019; Takeda et al., 2009). Compost stimulates the activity of microorganisms and accelerates the cycle of elements and mineral alteration. The gradual decomposition requires large amounts of macro- and micronutrients necessary for plant nutrition (Clapp et al., 2001). The compost has become one of the most promising strategies for controlling and inhibiting the development of phytopathogenic agents (Chilosi et al., 2018; Tautorus and Townsley, 1983; Vassilev et al., 2009). Nevertheless, the effects of composts in the oasis ecosystem as a strategy to combat climate change, and improve the date palm, and its underlying crops, growth, and fitness remain poorly studied and underestimated (Barje et al., 2016; Meddich et al., 2015c).

The present book is the first of its kind on the effects of combined application of indigenous AMF and/or PGPR and/or organic amendments on improving the tolerance of date palm to biotic and abiotic stresses. Integrating biofertilizers for the development of date palm might be considered as an appropriate strategy to reverse land degradation trends and encourage sustainable patterns for the remediation of soils in arid and semi-arid areas, especially in fragile ecosystems such as palm grove oases. Furthermore, we describe an approach to improve the growth and tolerance of date palm under climate change scenario through the use of indigenous and/or exogenous mycorrhizal strains, indigenous PGPR, and composts produced from different waste types under controlled and open-field systems.

2. Research theme expectations

The recent progress in microbiology focused in optimizing bio-fertilizer application in the combination of appropriate agricultural practices, this book will offer opportunities to improve resource management, ecosystem services, and will generate socio-economic benefits and policies aiming to improve farmer income, poverty alleviation, and rural development. Herein we describe an innovative technology by implementing integrated organic biofertilizers combining modern and high-tech methods and agricultural practices for date palm production and cultivation in oasis landscape. This series of research aimed to boost the growth and development of date palms and its underlying crops by the adoption of innovative practices to improve soil fertility, preserving water resources, environmentally-friendly, and ensuring the development of sustainable organic agriculture. The overall objective is to establish an efficient biological protocol integrating biological and organic fertilizers to promote the growth, yield, fitness of oasis crops. As such, the book offers practices that maximize biomass, mineral nutrition, and plant tolerance to environmental constraints together with methods of soil microorganisms' evaluation.

Other interesting prospects included in this book:

- Early integration of biofertilizers during invitro plant propagation to improve the survival and establishment of plants during acclimation and reduction of input rates of mineral fertilizers and pesticides.
- Organic bio-fertilizer (OBF) technology to reduce the time needed for the propagation of date palms via tissue cultures.
- Application of on-farm biological and organic biofertilizers to improve growth, nutrition, productivity, and disease-resistance of in vitro plants in a greenhouse hardening. Also, offshoot cultivation in open-field of young date palm plantations for asubstantial economic benefit for smallholder farmers.
- Selection of indigenous AMF and PGPR and composts production and their exploitation in oasis agroecosystems.
- Implementation of an integrated OBF technology to increase soil fertility leading to ecosystem services improvement, natural resources conservation, and rural stability and sustainability.

This is the first book to encompass the effects of selected AMF, PGPR, and composts on improving oasis crops such as date palm and its underlying crops and the impact of climate change stressors. The book covers the latest research on beneficial fungal, bacterial, and recycling of biodegradable waste. Also, the book provides overview knowledge about different mechanisms from the whole plant to cell underlying the role of biofertilizers in triggering date palm fitness and tolerance under the climate change scenario.

Research topics

Chapter 1. Assessment of Mycorrhizogenic Potentials of Moroccan Palm Groves as a Key of Efficient Ecological Restoration.

Chapter 2. Composting of Green, Animal, and Agro-industrial Wastes: Organic Biofertilizer Production and Crop Performance.

Chapter 3. Effectiveness of Arbuscular Mycorrhizal Fungus inoculation and Compost Amendments to improve growth and physiological parameters of *Phoenix dactylifera.*

Chapter 4. Soil Inoculation with Symbiotic Microorganisms (Mycorrhizas and Rhizobium) and Compost Promote Date Palm Performance under Drought condition: From controlled-condition to open-field system.

Chapter 5. Arbuscular Mycorrhizal Fungi in Saline Soil: Alleviating Date Palm under High-Salt Stress.

Chapter 6. Optimizing growth and tolerance of date palm (*Phoenix dactylifera* L.) to drought and vascular *fusarium-induce*d wilt (*Fusarium oxysporum*) by application of Arbuscular Mycorrhizal Fungi (AMF).

Chapter 7. Olive Mill Wastewaters as Bio-insecticide Agent to Control the New Pest (*Potosia Opaca*) in Date Palm cultivation: Potential of Biopesticides for Integrated Crop and Pest Management.

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II. Research topics

Chapter 1

Assessment of Mycorrhizogenic Potentials of Moroccan Palm Groves as a Key of Efficient Ecological Restoration

Mohamed Ait-El-Mokhtar[1], Abdessamad Fakhech[2], Mohamed Anli[1,2], Raja Ben-Laouane[1], Abderrahim Boutasknit[1], Youssef Ait-Rahou[1], Marouane Baslam[3], Said Wahbi[1], Abdallah Oihabi[4] and Abdelilah Meddich[1*]

* Corresponding author: a.meddich@uca.ma

[1]Laboratoire d'Agro-Alimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Faculté des Sciences Semlalia, Université Cadi Ayyad, BP 2390 Marrakech, Maroc.
[2]Laboratoire de Biotechnologies Microbiennes, Agrosciences et Environnement (BioMAgE), Faculté des Sciences Semlalia, Université Cadi Ayyad, BP511 Marrakech, Maroc.
[3]Laboratory of Biochemistry, Faculty of Agriculture, Niigata University, Niigata, Japan.
[4]X Full University Professor and Independent International Consultant.

Abstract

Oases in arid and semi-arid areas figure among the most affected and degraded ecosystems. An efficient restoration of these ecosystems requires involvement of their different components like arbuscular mycorrhizal fungi (AMF), which play a pivotal role in plant growth and resistance to biotic and abiotic stresses, soil structure and fertility improvement. To date, indigenous AMF remain poorly unexplored and underutilized but could be a potential resource for successful restoration programs. In this study, we evaluated the infectivity of AMF of five degraded oasis soils in Morocco (Marrakesh, Tafilalet, Draa, Tata-Guelmim and Figuig palm groves). Our data show that mycorrhizal status (AMF spore number, mycorrhizal infectious potential (MIP) and AMF infection), in the arid palm groves soils (Tafilalet, Draa, Tata-Guelmim and Figuig palm groves) was higher than in semi-arid ones (Marrakesh palm grove). Strong positive correlations ($r \geq 0.702$, $p<0.001$) were recorded between the different mycorrhizal parameters. Principal component analysis (PCA) showed that the percentage of sand in the soil had a positive impact on AMF parameters ($r=0.260$, $p<0.01$), while soil available phosphorus, total nitrogen, electrical conductivity, organic matter, CaCO3 content, C/N ratio and clay percentage had a negative impact on these parameters ($r \geq -0.176$, $p<0.05$). Results of the present study demonstrate that the mycorrhizal status of these palm groves soils is closely linked to the edaphic parameters and suggest that autochthonous AMF can be used to restore the ecological function of degraded oases in arid and semi-arid area.

Keywords: Arbuscular mycorrhizal fungi, ecological restoration, palm groves, arid, semi-arid, edaphic parameters.

Introduction

The oasis ecosystem constitutes a natural and cultural heritage in many arid and semi-arid areas. It plays important socio-economic and environmental roles. Like many North African oases, Moroccan palm groves (PGs) were subjected to different environmental constraints in the last decades such as soil poverty, drought, salinity, desertification, intensive exploitation, and lack of restoration initiatives, thus causing considerable economic, ecological and social damages (Meddich et al., 2018). Marrakesh, Tafilalet, Draa, Figuig and Tata-Guelmim oases are the main important PGs in Morocco but also the most affected, disorganized and fragile ecosystems. This plight has been the reason for lunching many rehabilitation programs. One of those programs was initiated by Mohammed VI Foundation for Environmental Protection for Marrakesh PG with the plantation of more than 500,000 palm trees (Meddich et al., 2017).

During the last years, ecologists are becoming more aware that any restoration and reestablishment programs of fragile and degraded ecosystems should incorporate components from different levels of the ecosystem specially soil microorganisms which are closely linked to plants (Asmelash et al., 2016). Arbuscular mycorrhizal fungi (AMF) are well known to be a main component of the natural ecosystem in the world (soil component). AMF form mutualistic associations with the roots of more than 80% of the terrestrial plants and are beneficial to plant survival in harsh environments and soil fertility (Smith and Read, 2008). They are known to increase plant growth and nutrient uptake (Ait-El-Mokhtar et al., 2019; Fakhech et al., 2019a), their tolerance to biotic and abiotic stress (Meddich et al., 2018; Ben-Laouane et al., 2019; Fakhech et al., 2019b; Boutasknit et al., 2020). Allied to this, AMF contribute to edaphic

stability by promoting soil aggregation and water retention through the mycelial network and glomalin production (Rillig and Mummey, 2006). The ecological impact of AMF is particularly relevant under the extreme conditions of desert ecosystems. Today, they are considered as an important component in ecosystem functioning restoration and rehabilitation of vegetation in fragile or degraded ecosystems (Asmelash et al., 2016). However, Duponnois et al. (2013) showed that these microorganisms are little used in agricultural fields, mainly because of the compatibility lack of introduced strains with local edaphic characteristics. Thus, it is important to point out that native AMF strains may be best adapted to actual soil and climatic conditions (El Faiz et al., 2015).

In this context, this study is conducted to evaluate the mycorrhizal fungi infectivity of the PGs soil in Morocco in relation with edaphic conditions, with a view to a possible valorization of their AMF resources. Therefore, it may be interesting to select indigenous strains adapted to Moroccan oases harsh environment to use in restoration programs.

Material and methods

Study sites and sampling

The study was carried out in 42 sites in Moroccan PGs. Two sites in Marrakesh and Figuig PGs; eleven sites in Tata-Guelmim PG; twelve sites in Draa PG and fifteen sites in Tafilalet PG (Figure 1). Marrakesh PG is characterized by a semi-arid climate, whereas the other PGs are characterized by arid climate. Samples of rhizospheric soils (4 Kg) were obtained, between September and December 2017, from each site at 1 m from the stipes of palm trees and in a depth between 10 and 40 cm, an area rich in mycorrhized roots.

Soil physico-chemical analyses

Prospected soils texture determination was performed using the hydrometer method described by Klute (1986). Soil pH and electrical conductivity (EC) were estimated using the electrometric method (1:5 water soil ratio). Available phosphorus (AP) assessment was carried out according to Olsen method (Olsen et al., 1954). For the total soil organic carbon (TOC), we adopted the potassium dichromate oxidation method (Pansu and Gautheyrou, 2007) and the organic matter (OM) values were calculated using the Wasksman coefficient (1.72). The total nitrogen (TN) was extracted by Kjeldahl method (Egli 2008) and total limestone ($CaCO_3$) content was determined using the Bernard calcimeter method (Vatan 1967).

AMF spore extraction

AMF spores were extracted from 100 g of each soil sample using the wet-sieving method (Gerdemann and Nicolson, 1963), followed by sucrose gradient centrifugation method (Daniels and Skipper, 1982). After centrifugation, the supernatant was poured through 800 µm and 50 µm meshes and quickly rinsed with tap water. Isolated spores were counted using a Petri dish under a stereoscopic microscope (Figure 2).

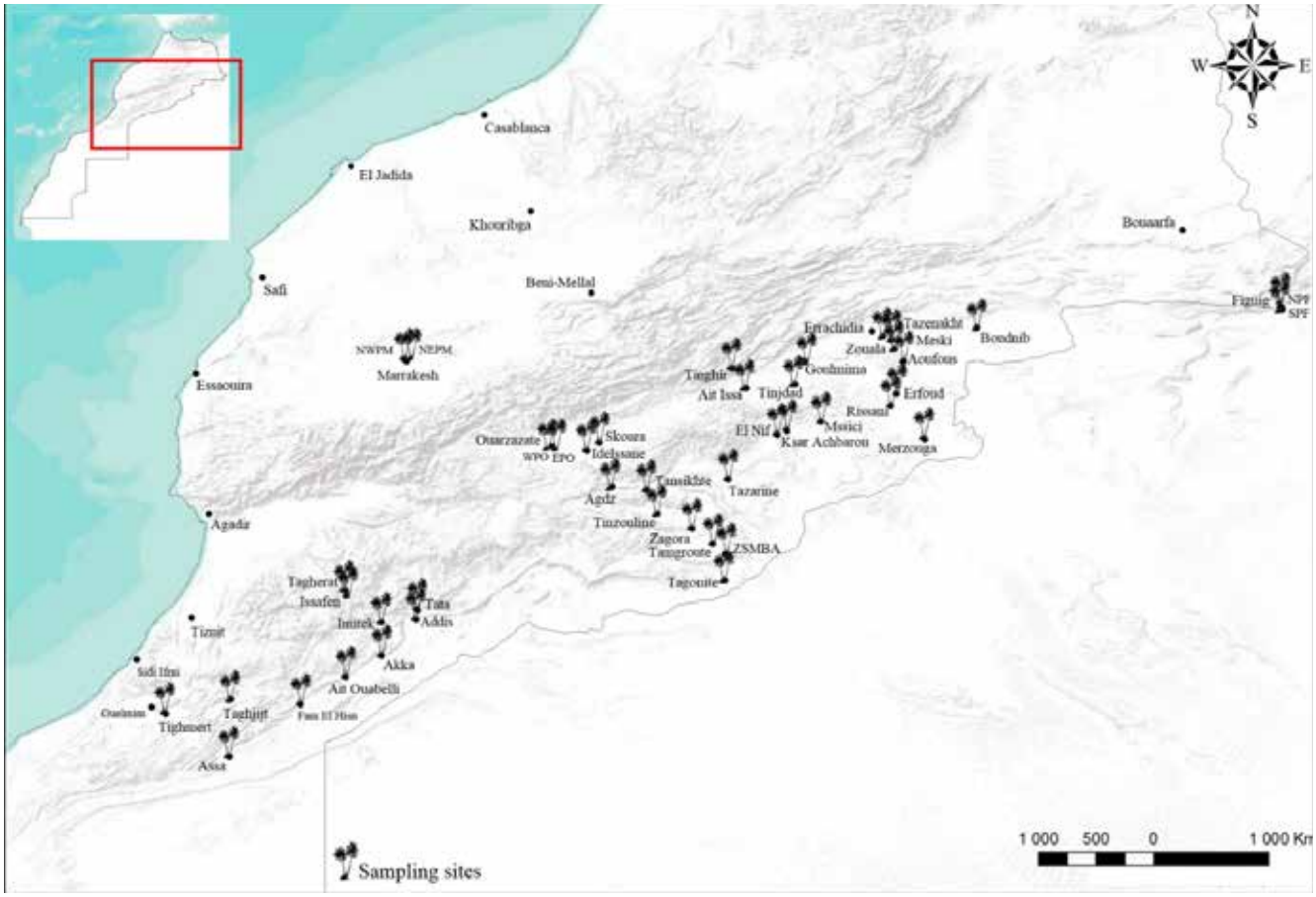

Figure 1. Study area: sampling sites are marked with palm tree signs. NPF: North PG of Figuig ; SPF: South PG of Figuig ; NEPM: North-east PG of Marrakesh ; NWPM: North-west PG of Marrakesh ; EPO: East PG of Ouarzazate ; WPO: West PG of Ouarzazate ; ZSMBA: Zaouiat Sidi El Mokhtar Ben Ali. (the symbols are the same as in Tables 1 and 2 and Figures 3, 4, 5, 6A and 7).

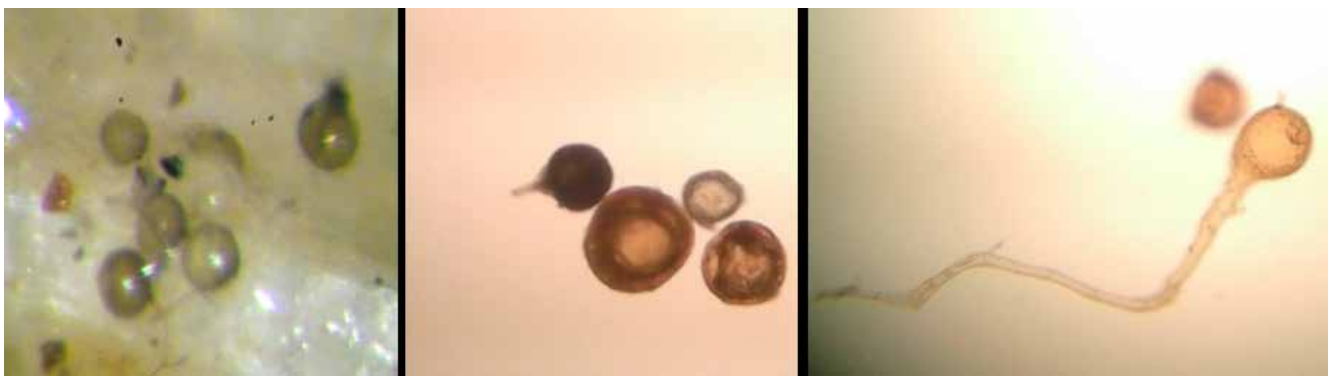

Figure 2. Spores of Genus Glomus isolated from soils of Aoufous (Tafilalet PG) Gx100.

Most probable number (MPN) assay for MIP estimation

MPN assays of each of the 42 soil samples were carried out under natural light in a greenhouse (with 25/18 °C day/night and 70-75% relative humidity) at the Faculty of Science Semlalia, Cadi Ayyad University Marrakesh, Morocco. Soil was passed through a 2 mm sieve and mixed with sterilized sand to make six successive dilutions (4^0 – 4^{-5}) of five replicates each and placed in 200 mL pots according to Sieverding protocol (1991). Three seeds of maize (*Zea mays* L.) were sown in each pot and one corn plant was left per pot after seedling emergence. No chemical amendments were applied, and watering was done according to need. The plants were harvested 30 days later by cutting roots from the center of each pot. The roots are cleared with 10% of KOH at 90 °C water bath for 15 min, then rinsed with 1% HCl for 5 min, then repeatedly with distilled water and were stained with Trypan blue 5 ‰ at 90 °C water bath for 15 min (Phillips and Hayman, 1970). Root were cut into 1 cm segments and mounted on microscope glass slides and examined under a light microscope. All the root system was examined. AMF typical structures occurrencewas recorded as "+" or "-" for presence or absence, respectively. Most probable number of infective propagules (MPN-IP) for each soil sample was calculated with the method described by Porter (1979).

AMF infection percentages

Fine roots of 1 cm in length, issues from the first dilution (4^0) of MPN assay, were examined under a Zeiss Axioskop 40 microscope. The number of root segments forming arbuscular mycorrhizal (AM) symbiosis was counted with criss cross lacing method and used to calculate AMF colonization (McGonigle et al., 1990). AMF infection frequency and intensity were calculated using the following equations:

$$\text{AMF infection frequency (Fa) (\%)} = \left(\frac{\text{Infected root segments}}{\text{Total root segments}}\right) \times 100$$

$$\text{AMF infection intensity (Ma) (\%)} = \frac{(95n5+70n4+30n3+5n2+n1)}{\text{Total root segments}}$$

*n5, n4, n3, n2, n1 = number of fragments denoted 5, 4, 3, 2 and 1, respectively. Class 5: more than 91%, Class 4: between 51% and 90%, Class 3: between 11% and 50%, Class 2: less than 10%, Class 1: trace and Class 0: no mycorrhization.

Statistical analyses

Statistical analysis was performed using Microsoft Excel 2013 software for Windows with XLSTAT 2016. Statistically significant differences between means were analyzed using analysis of variance (ANOVA) and Duncan's Multiple RangeTest (DMRT). Differences were considered significant at $p<0.05$. Correlations between measured parameters were evaluated by determining Pearson correlation. Data were analyzed by principal components analysis (PCA) to explore the different interactions among soils and AMF parameters of the prospected soils. The PCA were followed by performing hierarchical cluster analysis (HCA), using Ward's method, in order to organize all the sites from the study area in homogeneous groups according to their AMF status and edaphic parameters.

Results

Soil analysis

The physical and chemical analyses of the samples are presented in Table 1. Regarding the soil texture, 29 from 42 samples from the PGs were sandy-loamy (11 samples from Draa PG, 9 samples from Tata-Guelmim PG, seven samples from Tafilalet PG, NEPM sample from Marrakesh PG and SPF sample from Figuig PG), ten samples were loamy-sandy (eight samples from Tafilalet PG, Tazarine sample from Draa PG and NPF sample from Figuig PG), two samples were sandy (ZSMBA sample from Draa PG and Ait Ouabelli sample from Tata-Guelmim PG) and one sample was loamy (NWPM sample from Marrakesh PG). The pH of the soil samples showed a very slight variance (pH 7.43–8.76). The EC of the samples ranged from 0.36 to 8.88mS.cm^{-1} with a high average in Draa PG samples (4 mS.cm^{-1}). The mean AP concentrations of the samples collected from Draa PG (11 g.Kg^{-1}) is the lowest compared to that of samples from Marrakesh PG which recorded the highest value (57 g.Kg^{-1}). TOC, OM and TN presented high contents in Marrakesh PG soils than in the soils from the other PGs. Considering the C/N ratio average, Tafilalet PG samples had the highest ratio (40) compared to Marrakesh PG which recorded the lowest ratio (9). The total $CaCO_3$ content ranged from 6 to 365 g.Kg^{-1} with a low content in NWPM (Marrakesh PG) and Tinzouline (Draa PG) samples.

Table 1. Prospected soils physical and chemical analyses

Palme grove	Site	pH	EC (mS.cm^{-1})	AP (mg.kg^{-1})	TOC (g.kg^{-1})	OM (g.kg^{-1})	TN (g.kg^{-1})	C/N	$CaCO_3$ (g.kg^{-1})	Texture
Marrakesh	NEPM	7.78±0.01^{ij}	1.2±0.04^{ij}	46.71±12.17^{b}	11.94±1.32^{cd}	20.59±2.27^{cd}	1.29±0.20^{b}	9.25±0.40^{j-p}	186.21±6.08^{u}	Sandy-loamy
	NWPM	7.54±0.02^{n-q}	1.86±0.01^{h}	64.68±2.27^{a}	21.07±1.27^{a}	36.33±2.19^{a}	2.47±0.09^{a}	8.51±0.40^{k-p}	58.24±5.31^{v}	Loamy
Draa	Agdz	7.85±0.01^{hi}	1.14±0.02^{ij}	11.86±1.82^{klm}	8.13±1.43^{c-g}	14.02±2.47^{efg}	0.94±0.09^{c}	8.59±0.78^{k-p}	118.65±18.17^{uv}	Sandy-loamy
	Tamegroute	7.78±0.10^{ij}	0.71±0.01^{k}	19.76±1.39^{fgh}	5.26±1.44^{j-l}	9.07±2.47^{i-l}	0.90±0.09b^{c}	5.75±1.06^{nop}	197.59±13.87^{u}	Sandy-loamy
	Tinzouline	7.99±0.02^{g}	0.36±0.01^{k}	19.17±1.15^{pqr}	12.52±2.17^{k-n}	21.58±3.75^{k-n}	0.74±0.16^{efg}	17.12±1.63^{k-p}	65.10±5.56^{v}	Sandy-loamy
	Skoura	8.00±0.18^{g}	7.15±1.06^{c}	8.44±0.51^{n-q}	14.59±0.35^{b}	25.15±0.60^{b}	0.25±0.03^{hij}	61.69±3.80^{c}	84.44±5.14^{pq}	Sandy-loamy
	Tagonit	7.73±0.03^{j-l}	8.88±0.27^{a}	7.78±0.71^{n-q}	9.50±0.50^{e}	16.37±0.86^{e}	0.14±0.04^{ij}	68.96±15.14^{b}	77.78±7.14^{r}	Sandy-loamy
	Zagora	7.58±0.01^{m-p}	0.46±0.02^{k}	10.40±0.14^{lmn}	1.35±0.34^{o-r}	2.32±0.58^{o-r}	0.14±0.04^{ij}	10.32±0.06^{j-p}	104.04±1.43^{n}	Sandy-loamy
	Tansikht	7.47±0.01^{pq}	2.96±0.13^{f}	9.39±0.43^{m-p}	0.29±0.01^{r}	0.50±0.01^{r}	0.12±0.01^{ij}	2.49±0.14^{p}	93.94±4.28^{o}	Sandy-loamy
	Tazarine	7.72±0.06^{jkl}	0.47±0.01^{k}	5.86±0.57^{qrs}	6.26±0.13^{hij}	10.78±0.23^{hij}	0.14±0.04^{ij}	46.09±11.88^{d}	58.58±5.71^{s}	Loamy- sandy
	ZSMBA	7.81±0.01^{hij}	1.30±0.03^{ij}	9.39±0.14^{m-p}	0.57±0.01^{qr}	0.99±0.01^{qr}	0.08±0.04^{j}	7.61±3.49^{l-p}	93.94±1.42^{o}	Sandy
	Idelsane	7.77±0.06^{ijk}	7.57±0.18^{b}	18.69±1.28^{h}	2.59±0.50^{m-q}	4.46±0.86^{m-q}	0.17±0.01^{hij}	15.49±2.84^{g-o}	186.87±12.86^{h}	Sandy-loamy
	EPO	7.64±0.11^{k-n}	2.99±0.13^{a}	9.5±0.58^{mno}	5.76±0.99^{h-k}	9.92±1.72^{h-k}	0.08±0.04^{j}	69.39±18.17^{b}	149.49±5.71^{op}	Sandy-loamy
	WPO	7.43±0.05^{q}	8.69±0.66^{f}	14.95±0.57^{ij}	3.45±0.49^{l-o}	5.95±0.86^{l-o}	0.16±0.01^{hij}	20.84±2.47^{f-k}	91.00±0.13^{j}	Sandy-loamy

Palme grove	Site	pH	EC ($mS.cm^{-1}$)	AP ($mg.kg^{-1}$)	TOC ($g.kg^{-1}$)	OM ($g.kg^{-1}$)	TN ($g.kg^{-1}$)	C/N	$CaCO_3$ ($g.kg^{-1}$)	Texture
Tafilalet	Aoufous	7.92±0.01^{gh}	0.46±0.00^{k}	5.92±1.94^{rs}	8.82±1.29^{ef}	15.21±2.23^{ef}	0.48±0.15^{def}	17.92±5.36^{f-m}	363.98±1.33^{t}	Sandy-loamy
	Zouala	7.99±0.02^{g}	0.36±0.01^{k}	19.17±1.15^{gh}	12.52±2.17^{cd}	21.58±3.75^{cd}	0.74±0.16c^{d}	17.12±1.63^{f-o}	347.13±46.94^{t}	Sandy-loamy
	Guelmima	8.4±0.01^{cd}	1.5±0.14^{i}	31.61±1.29^{d}	11.60±1.52^{d}	20.01±2.62^{d}	0.20±0.04^{hij}	59.64±4.28^{c}	316.16±12.86^{c}	Sandy-loamy
	Erfoud	8.61±0.03^{b}	1.11±0.01^{ij}	22.33±0.13^{f}	10.86±0.54^{d}	19.38±0.93^{d}	0.14±0.04^{ij}	106.10±19.13^{a}	223.33±1.29^{f}	Sandy-loamy
	Rissani	8.44±0.01^{c}	0.61±0.02^{k}	26.97±0.71^{e}	14.47±1.52^{b}	24.96±2.62^{b}	0.22±0.01^{hij}	60.33±3.80^{bc}	269.70±7.14^{e}	Sandy-loamy
	Tinjdad	8.18±0.05^{f}	1.45±0.21^{ij}	13.66±0.39^{ijk}	13.40±1.01^{bc}	23.09±1.75^{bc}	0.28±0.01^{hi}	39.49±0.96^{d}	136.66±3.86^{kl}	Loamy- sandy
	Tinghir	7.62±0.03^{lmn}	0.58±0.01^{k}	32.83±0.14^{d}	4.58±0.05^{j-m}	7.88±0.09^{j-m}	0.20±0.04^{hij}	23.72±4.47^{e-i}	328.28±1.43^{b}	Loamy- sandy
	Merzouga	7.43±0.06^{q}	3.65±0.03^{e}	11.55±0.51^{klm}	3.63±0.19^{lmn}	6.26±0.32^{lmn}	0.20±0.04^{hij}	18.52±2.57^{f-n}	115.55±5.14^{m}	Loamy- sandy
	Elnif	7.45±0.23^{q}	0.52±0.01^{k}	4.14±0.14^{rs}	5.76±0.48^{h-k}	9.92±0.86^{h-k}	0.20±0.04^{hij}	28.48±5.75^{ef}	41.41±1.43^{t}	Sandy-loamy
	Taznakht	7.65±0.01^{k-n}	1.35±0.01^{ij}	20.70±0.14^{fgh}	2.83±0.07^{m-p}	4.88±0.12^{m-p}	0.11±0.01^{ij}	25.27±0.61^{e-i}	207.07±1.43^{g}	Loamy- sandy
	Mcisi	8.18±0.08^{f}	0.35±0.01^{k}	5.96±0.14^{qrs}	2.84±0.05^{m-p}	4.91±0.09^{m-p}	0.17±0.01^{hij}	16.87±0.29^{f-o}	59.60±1.42^{s}	Loamy- sandy
	Meski	7.6±0.04^{l-o}	0.60±0.02^{k}	32.02±.0.99^{d}	1.24±0.16^{pqr}	2.15±0.29^{pqr}	0.08±0.04^{j}	15.42±7.27^{g-o}	320.20±9.99^{c}	Loamy- sandy
	Boudnib	7.48±0.08^{opq}	0.62±0.03^{k}	16.06±0.14^{ij}	4.32±0.45^{j-n}	7.44±0.86^{j-n}	0.20±0.04^{hij}	21.42±1.21^{f-j}	160.60±1.42^{i}	Loamy- sandy
	Ait Issa	7.70±0.11^{j-m}	1.23±0.03^{ij}	14.95±0.86^{ij}	4.58±0.05^{j-m}	7.88±0.09^{j-m}	0.20±0.04^{hij}	23.72±4.47^{e-i}	149.49±8.57^{j}	Sandy-loamy
	Ksar Achbaro	7.49±0.01^{opq}	1.09±0.02^{j}	3.53±0.14^{s}	2.30±0.49^{n-q}	3.40±0.86^{n-q}	0.20±0.04^{hij}	10.49±2.12^{i-p}	35.35±1.43^{t}	Loamy- sandy
Tata- Guelmim	Tagherat	8.39±0.11^{cd}	0.38±0.01^{k}	21.01±0.28^{fgh}	2.83±0.06^{m-p}	4.87±2.82^{m-p}	0.11±0.00^{ij}	25.27±0.61^{e-h}	210.10±2.86^{g}	Sandy-loamy
	Issafen	8.32±0.01^{de}	0.71±0.03^{k}	16.46±0.43^{i}	8.05±1.49^{efg}	13.89±2.58^{efg}	0.34±0.06^{gh}	23.98±2.23^{e-i}	164.65±4.28^{i}	Sandy-loamy
	Imitek	8.18±0.01^{f}	1.19±0.07^{ij}	15.96±1.71^{ij}	7.48±0.50^{fgh}	12.90±0.86^{fgh}	0.39±0.06^{fg}	19.25±1.88^{f-m}	159.60±17.14^{i}	Sandy-loamy
	Tata	8.24±0.04^{ef}	0.7±0.04^{k}	36.46±0.14^{c}	7.48±0.50^{fgh}	12.90±0.86^{fgh}	0.58±0.06^{e}	12.56±0.49^{h-p}	364.65±1.43^{a}	Sandy-loamy
	Addis	8.41±0.02^{cd}	0.64±0.01^{k}	13.94±0.57^{ijk}	2.55±0.43^{m-q}	4.40±0.79^{m-q}	0.48±0.03^{ef}	5.34±0.52^{op}	139.39±5.71^{kl}	Sandy-loamy
	Akka	8.23±0.03^{ef}	1.32±0.06^{ij}	20.40±0.57^{fgh}	2.30±0.23^{n-q}	3.96±0.86^{n-q}	0.52±0.03^{ef}	4.37±0.66^{op}	204.04±5.71^{g}	Sandy-loamy
	Ait Ouabelli	7.99±0.09^{g}	4.53±0.10^{d}	6.97±0.14^{o-r}	2.59±0.38^{m-q}	4.47±0.86^{m-q}	0.13±0.03^{ij}	20.27±4.72^{f-l}	69.70±1.42^{r}	Sandy
	Fam El Hisn	8.00±0.11^{g}	1.47±0.06^{ij}	13.13±0.28^{jkl}	1.29±0.24^{pqr}	2.23±0.42^{pqr}	0.19±0.03^{hij}	6.91±0.10^{m-p}	131.31±2.86^{l}	Sandy-loamy
	Assa	8.13±0.08^{f}	0.72±0.03^{k}	11.82±0.14^{klm}	4.31±0.41^{j-n}	7.44±0.86^{j-n}	0.22±0.00^{hij}	19.27±2.23^{f-m}	118.18±1.43^{m}	Sandy-loamy
Tata- Guelmim	Taghjijt	7.87±0.04^{hi}	3.54±0.16^{e}	28.38±0.43^{e}	6.04±0.49^{hij}	10.42±0.86^{hij}	0.26±0.03^{hij}	23.21±1.04^{e-i}	283.84±4.28^{d}	Sandy-loamy
	Tighmert	8.11±0.01^{f}	2.43±0.03^{g}	14.04±0.14^{ijk}	6.62±0.45^{ghi}	11.41±0.86^{ghi}	0.24±0.03^{hij}	27.41±1.48^{efg}	140.40±1.43^{k}	Sandy-loamy
Figuig	NPF	8.76±0.11^{a}	0.68±0.01^{k}	21.72±1.57^{fg}	2.74±0.24^{m-p}	4.73±0.41^{m-p}	0.08±0.04^{j}	34.89±12.99^{e}	217.17±15.71^{f}	Loamy- sandy
	SPF	7.7±0.10^{j-n}	0.47±0.01^{k}	22.53±0.14^{f}	1.14±0.02^{pqr}	1.96±0.04^{pqr}	0.08±0.04^{j}	15.08±6.78^{g-p}	225.25±1.42^{f}	Sandy-loamy

Values represent means (±SE) (n=6). Same letters mean no significant difference according to DMRT ($p<0.05$). AP: Soil available Phosphorus; TN: Soil total Nitrogen; $CaCO_3$: Soil $CaCO_3$; EC: Soil electrical conductivity; TOC: Soil total organic Carbone; OM: Soil organic matter; C/N: C/N ratio (the symbols are the same as in Table 3 and Figure 7B).

AMF statustraits

AMF spore number (AMF-SN) in the rhizospheric soils is shown in Figure 3. The studied soils AMF spore counts ranged from 257 to 2234 spores /100g of dry soil. Aoufous soil sample, from Tafilalet PG, presented the highest AMF-SN while Tamegroute soil from Draa PG showed the lowest. By considering averages, Tafilalet PG samples had higher AMF-SN (1044 spores /100 g dry soil) while the lowest average was recorded in Marrakesh PG samples (407 spores /100 g dry soil).

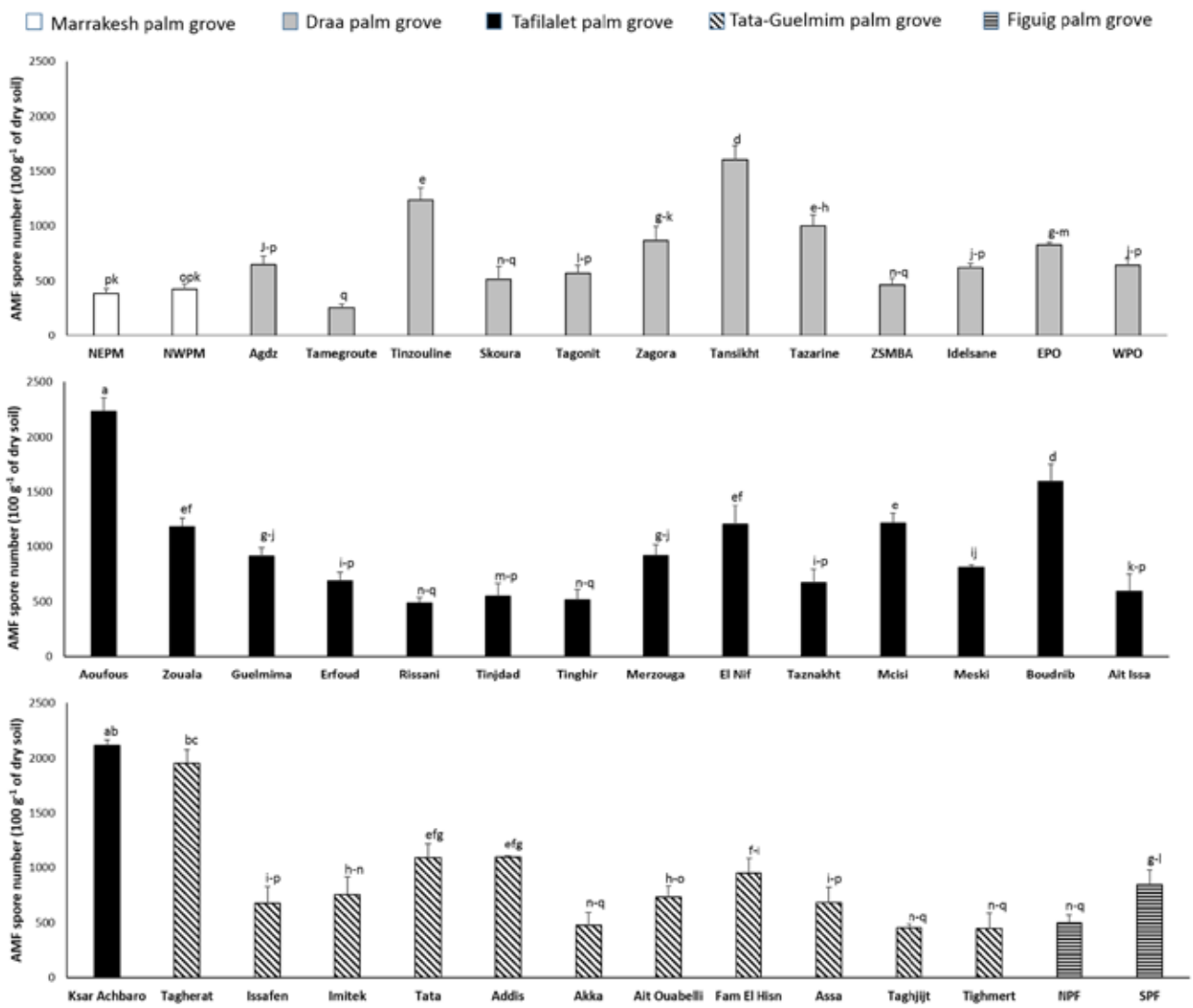

Figure 3. Prospected soils AMF spore number. Same letters mean no significant difference according to DMRT ($p<0.05$).

The most probable number of AMF infective propagules (MPN-IP) quantified by trap-culture assay ranged from 131 propagules /100 g of dry soil to 1149 (Table 2). Among the prospected soils, Aoufous, Zouala, El Nif, Mcisi, Boudnib, Ksar Achbarou from Tafilalet PG, Tinzouline, Tansikht and Tazarine from Draa PG and Tagherat, Tata and Addis from Tata-Guelmim PG exhibited the highest MPN-IP. In contrast, Tamegroute (Draa PG) and NEPM (Marrakesh PG) samples showed the lowest MPN-IP with 243 and 131 MPN counts /100 g of dry soil respectively.

Table 2. Most probable number of AMF infective propagules in the prospected soils.

Palme grove	Site	Most probable number of infective propagules /100 g of dry soil
Marrakesh	NEPM	131.54
	NWPM	464.21
Draa	Agdz	660.88
	Tamegroute	242.60
	Tinzouline	1149.11
	Skoura	660.88
	Tagonit	660.88
	Zagora	992.05
	Tansikht	1149.11
	Tazarine	1149.11
	ZSMBA	464.21
	Idelsane	660.88
	EPO	870.86
	WPO	660.88
Tafilalet	Aoufous	1149.11
	Zouala	1149.11
	Guelmima	870.86
	Erfoud	660.88
	Rissani	464.21
	Tinjdad	660.88
	Tinghir	660.88
	Merzouga	870.86
	Elnif	1149.11
	Taznakht	660.88
	Mcisi	1149.11
	Meski	1149.11
	Boudnib	1149.11
Tata - Guelmim	Tagherat	1149.11
	Issafen	660.88
	Imitek	870.86
	Tata	1149.11
	Addis	1149.11
	Akka	464.21

Palme grove	Site	Most probable number of infective propagules /100 g of dry soil
Tata - Guelmim	Ait Ouabelli	660.88
	Fam El Hisn	992.05
	Assa	660.88
	Taghjijt	464.21
	Tighmert	464.21
Figuig	NPF	464.21
	SPF	870.86

All samples showed a very important AMF infection frequency (Fa) (>50%), except for Akka, Tighmert, Taghjijt (Tata-Guelmim PG), NPF (Figuig PG), ZSMBA (Draa PG), Rissani (Tafilalet PG) and NEPM (Marrakesh PG) samples (Figure 4). The highest Fa (100%) was recorded in two sites, Aoufous from Tafilalet PG and Tinzouline from Draa PG, while NEPM site had the lowest Fa (34%).

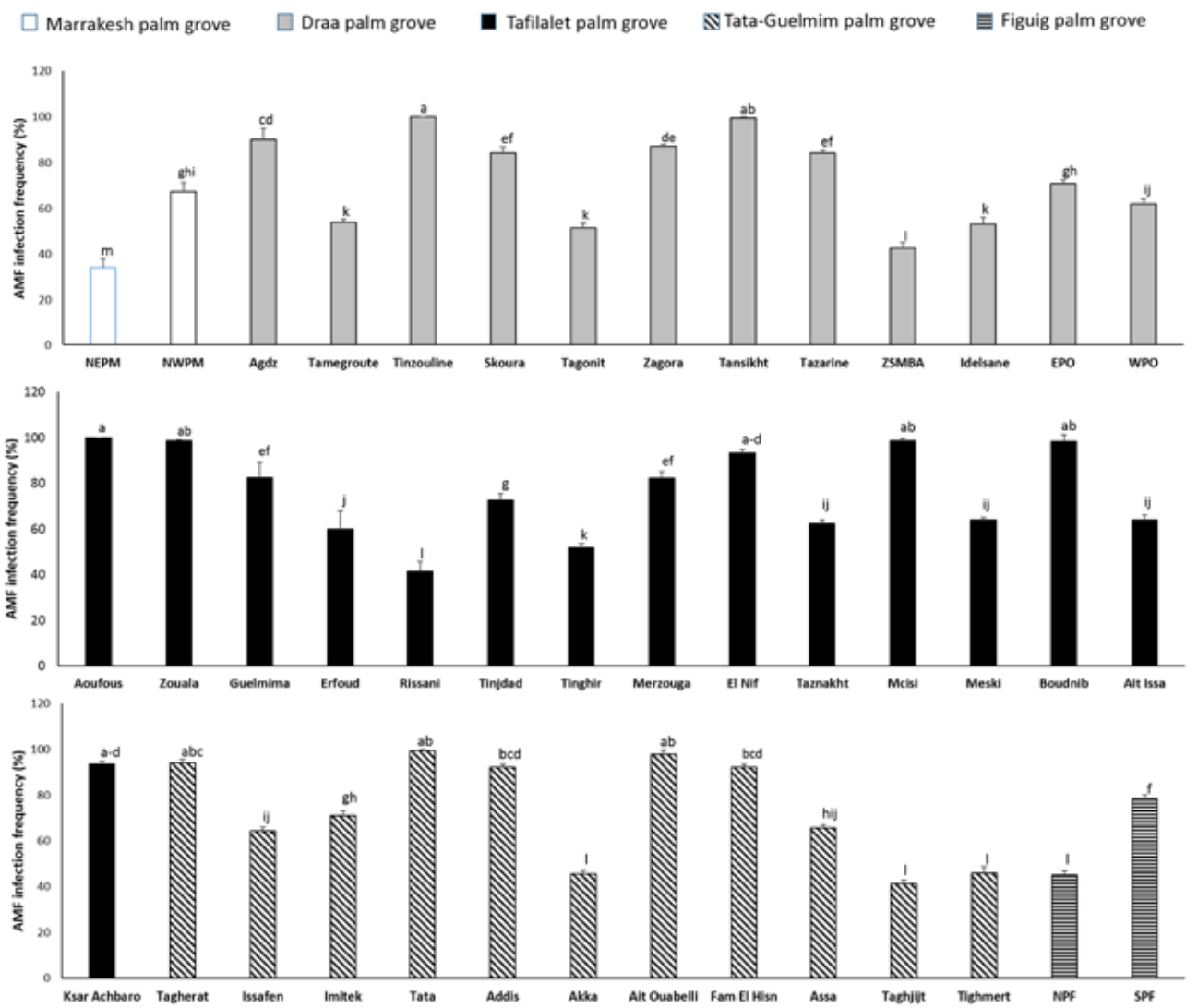

Figure 4. Prospected soils AMF infection frequency. Same letters mean no significant difference according to DMRT ($p<0.05$).

When measured by AMF infection intensity (Ma), only three sites exceeded 50% mycorrhization intensity, namely, Aoufous and Ksar Achbarou from Tafilalet PG and Tagherat from Tata-Guelmim PG (Figure 5). Averages comparison of Ma showed that Tafilalet PG had the highest percentage (30%) followed by Tata-Guelmim PG (24%), Draa PG (22%) and Figuig PG (18%), while Marrakesh PG recorded the lowest Ma (6%).

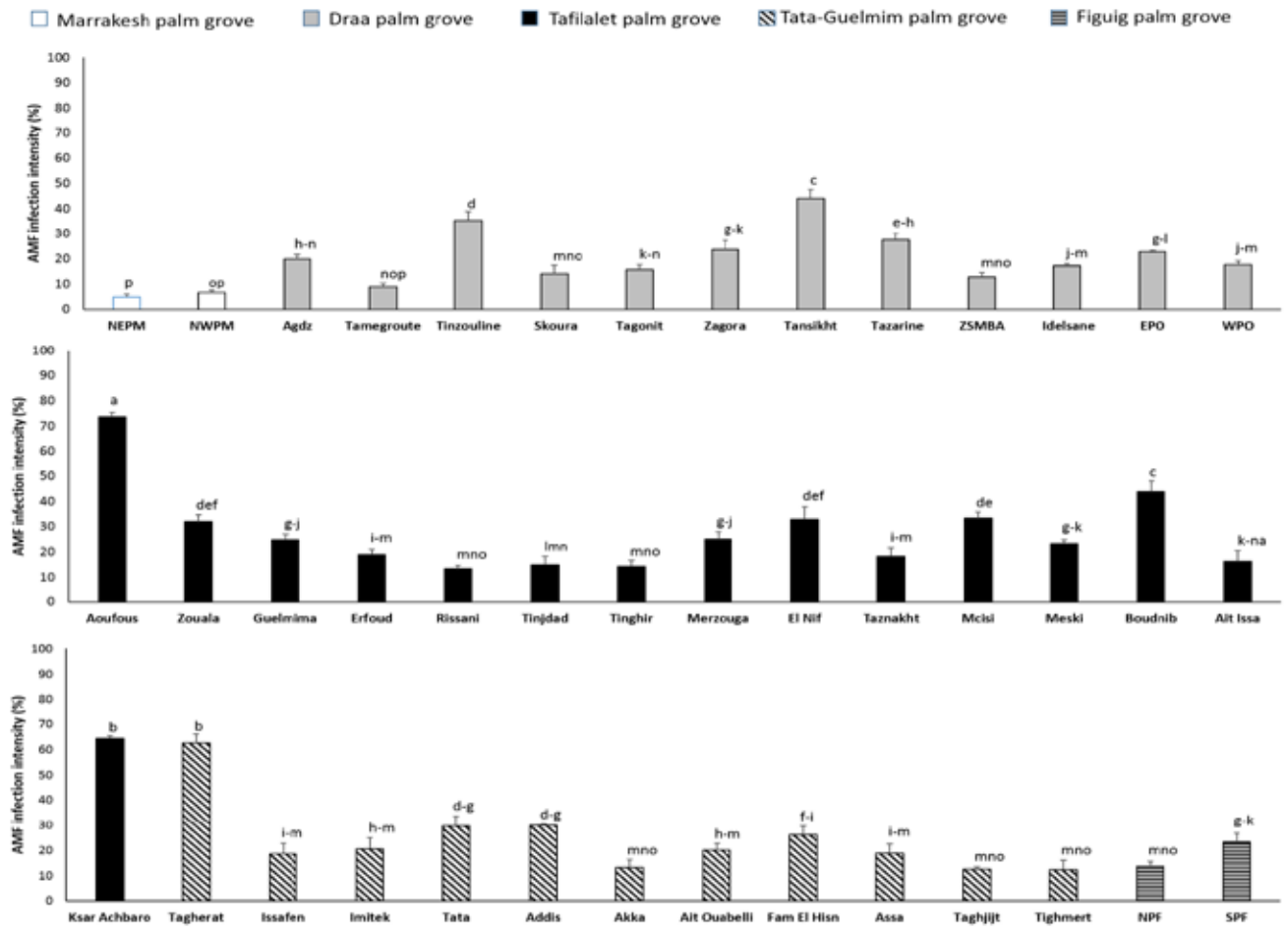

Figure 5. Prospected soils AMF infection intensity. Same letters mean no significant difference according to DMRT (p<0.05).

According to our findings, the different mycorrhizal parameters are more important in the arid PGs samples (Tafilalet, Draa, Figuig and Tata-Guelmim oases) compared to semi-arid ones (Marrakesh oases).

The relationship between edaphic parameters and AMF status

The correlation matrix obtained from the observed variables using Pearson's coefficient (Table 3) showed a very significant and strong positive correlation between mycorrhizal status parameters viz. AMF-SN, MPN-IP, Ma and Fa (r≥0.702,*p*<0.001). The only mycorrhizal parameter that showed a significant positive correlation with soil physico-chemical parameters was MPN-IP, which was positively correlated with the percentage of sand in the soil (r=0.260, *p*<0.01). However, all mycorrhizal parameters were negatively correlated to AP (r≥-0.365, *p*<0.001). Three of the mycorrhizal parameters (AMF-SN, MPN-IP and Ma) are negatively correlated to TN (r≥-0.192, *p*<0.05), EC (r≥-0.182, *p*<0.05), OM (r≥-0.243, *p*<0.01), and to the percentage of clay in the soil (r≥-0.176, *p*<0.05). Furthermore, CaCO3 contents and C/N ratio had a significant negative correlation with Fa ([r=-0.286, *p*<0.001] and [r=-0.196, *p*<0.05] respectively).

Principal components analysis (PCA) of the prospected soil variables showed that first and second components had a cumulative variance of 51.23% (32.54% and 18.69% respectively) (Figure 6). According to the score plot (Figure 6A), Ksar Achbarou, Tansikht, Aoufous, and Boudnib sites showed the highest positive scores along the first axis (PC1), while NWPM, NEPM and Rissani sites showed the highest negative scores along the same axis. The second component (PC2) showed a high positive correlation with NWPM and Aoufous sites while it showed the highest negative correlation with ZSMBA site.

Table 3. Correlation matrix (Pearson) of soil properties and mycorrhizal status parameters.

Variables														
AMF-SN	AMF-SN													
MPN-IP	**0.817**^{c}	MPN-IP												
Fa	**0.733**^{c}	**0.872**^{c}	Fa											
Ma	**0.988**^{c}	**0.784**^{c}	**0.702**^{c}	Ma										
AP	**-0.365**^{c}	**-0.457**^{c}	**-0.397**^{c}	**-0.400**^{c}	AP									
TN	**-0.192**^{a}	**-0.290**^{c}	-0.069	**-0.225**^{a}	**0.654**^{c}	TN								
$CaCO_3$	-0.168	-0.101	**-0.286**^{c}	-0.150	**0.364**^{c}	**-0.373**^{c}	$CaCO_3$							
EC	**-0.232**^{b}	**-0.182**^{a}	-0.171	**-0.227**^{b}	-0.171	-0.141	-0.110	EC						
pH	-0.119	-0.101	-0.117	-0.084	0.072	-0.086	**0.327**^{c}	**-0.224**^{a}	pH					
TOC	**-0.243**^{b}	**-0.265**^{b}	-0.132	**-0.258**^{b}	**0.470**^{c}	**0.611**^{c}	-0.131	0.093	**0.201**^{a}	TOC				
OM	**-0.243**^{b}	**-0.265**^{b}	-0.132	**-0.258**^{b}	**0.470**^{c}	**0.611**^{c}	-0.131	0.093	**0.201**^{a}	1	OM			
C/N	-0.173	-0.094	**-0.196**^{a}	-0.155	-0.083	**-0.312**^{c}	**0.209**^{a}	**0.408**^{c}	**0.288**^{b}	**0.439**^{c}	**0.439**^{c}	C/N		
Sand	0.138	**0.260**^{b}	0.151	0.143	**-0.450**^{c}	**-0.633**^{c}	**0.187**^{a}	-0.024	-0.016	**-0.491**^{c}	**-0.491**^{c}	-0.029	Sand	
Silt	0.026	-0.137	-0.068	0.041	**0.244**^{b}	**0.257**^{b}	-0.013	**-0.199**^{a}	**0.055**^{c}	**0.219**^{a}	**0.219**^{a}	0.010	**-0.643**^{c}	Silt
Clay	**-0.200**^{a}	**-0.176**^{a}	-0.123	**-0.221**^{a}	**0.297**^{c}	**0.502**^{c}	**-0.206**^{a}	**0.224**^{a}	0.002	**0.375**^{c}	**0.375**^{c}	0.033	**-0.552**^{c}	**0.268**^{b} Clay

[a]correlation is significant at p<0.05, bcorrelation is significant at p<0.01, ccorrelation is significant at p<0.001. AMF-SN: AMF spore number; MPN-IP: AMF infective propagules; Ma: AMF infection intensity; Fa: AMF infection frequency (the symbols are the same as in Figure 7B).

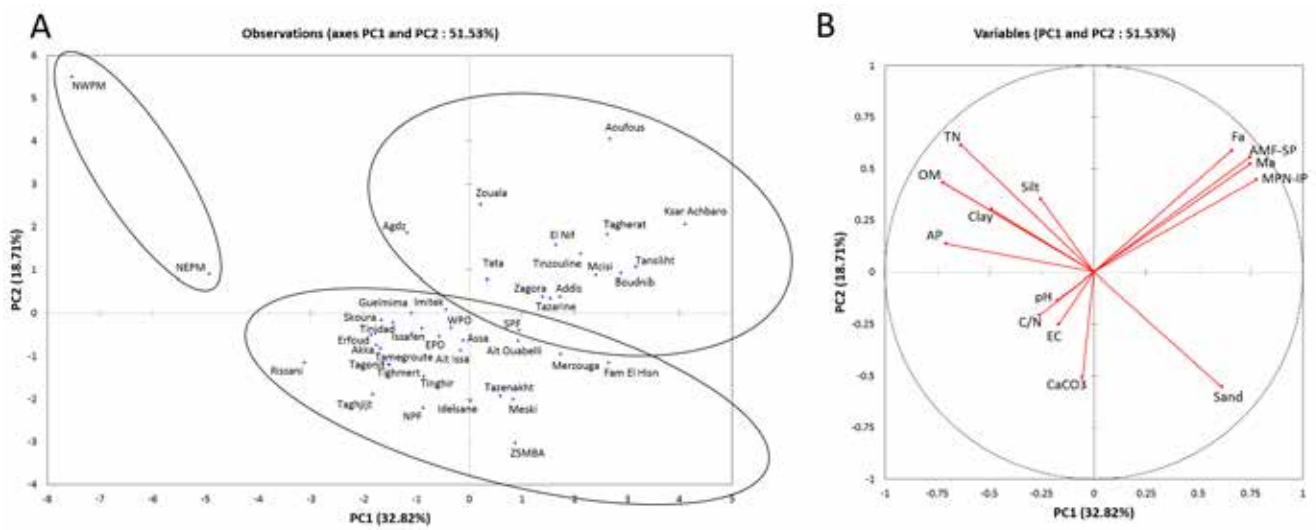

Figure 6. Scoring plot of samples (A) and loading plot of variables (B) ordinated in PCA.

The results of the score plot showed that AMF rich soils (Aoufous, Ksar Achbarou, Tagherat, El Nif, Tansikht, Tinzouline, Mcisi and Boudnib) were located towards the right end of the first PCA axis of the scoring plot (Figure 6A), where AMF-SN, MPN-IP, Ma and Fa in the corresponding loading plot were located (Figure 6B). In contrast, AMF poor soils (NWPM, NEPM, NPF, Taghjijt and Rissani) were positioned towards the left end point of the axis (Figure 6A) in which OM, AP, TN, EC, CaCO3 and clay percentage were located in the same position in the loading plot (Figure 6B).

Three different groups were obtained by hierarchical cluster analysis (Figures 6A and 7). The first group is characterized by an important mycorrhizal status and consists of 15 sites, namely Aoufous, Zouala, El Nif, Mcisi, Boudnib, Ksar Achbarou (Tafilalet PG), Agdz, Tinzouline, Zagora, Tansikht, Tazarine (Draa PG), Tagherat, Tata, Addis (Tata-Guelmim PG) and SPF (Figuig PG). The second group is represented by the samples from NWPM and NEPM sites (Marrakesh PG) and showed a very low mycorrhizal status. The third group consists of soil samples from the remaining 15 sites: Guelmima, Erfoud, Rissani, Tinjdad, Tinghir, Merzouga, Tazenakht, Meski, Ait Issa (Tafilalet PG), Idelsane, EPO, WPO, Issafen, Imitek, Akka, Ait Ouabelli, Fam El Hisn, Assa, Taghjijt, Tighmert (Tata-Guelmim PG), Tamegroute, Skoura, Tagonit, ZSMBA (Draa PG) and NPF (Figuig PG). This group presents an intermediate mycorrhizal status between the two previously mentioned groups.

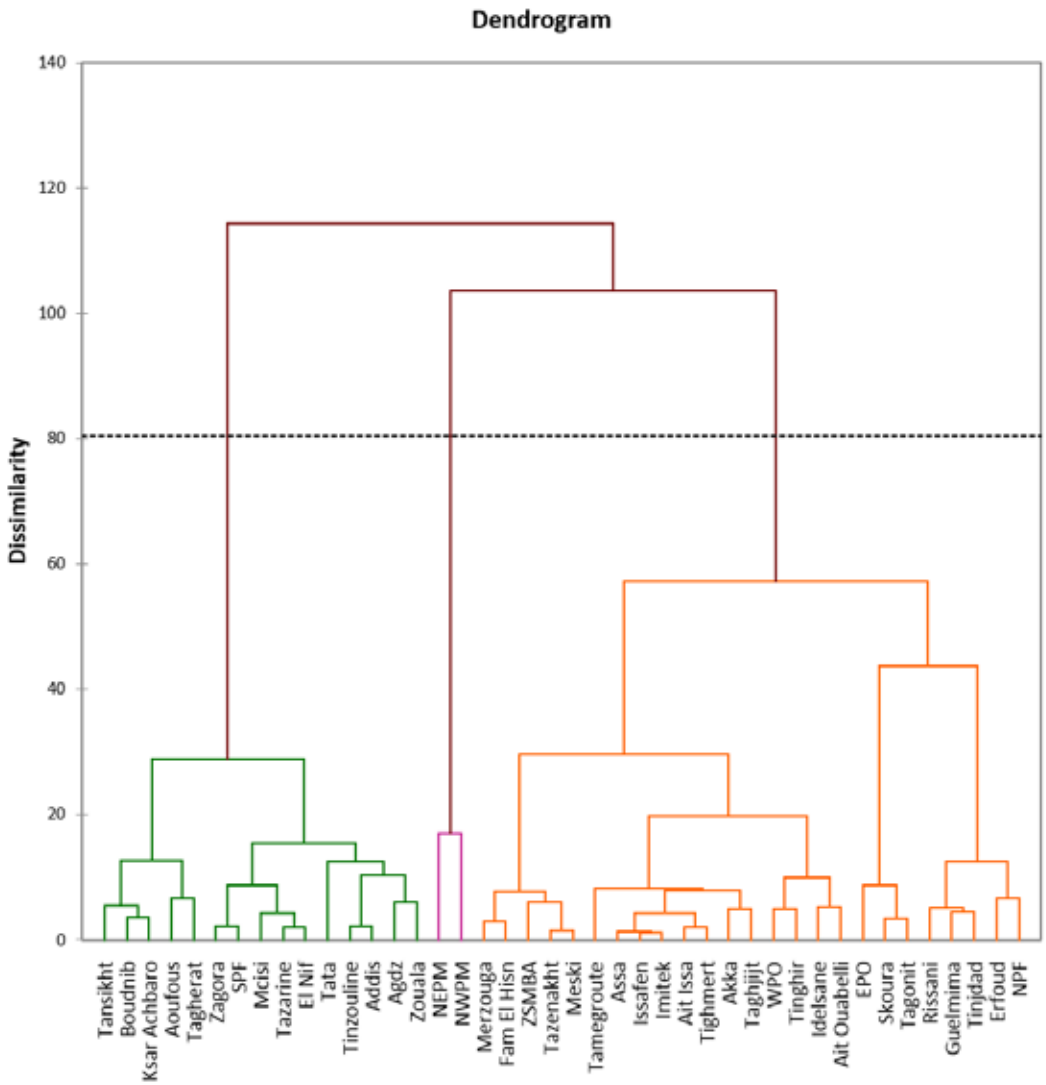

Figure 7. Dendrogram of the ascending hierarchical classification (AHC) of prospected soils.

Discussion

The spore counts recorded in this study were important and close to those recorded in some arid and semi-arid ecosystems where AMF abundance ranged from 200 to 3500 spores per 100 g of soil (Yang et al., 2008; de Souza et al., 2016). MPN-IP estimates were in line with previous reports carried out in semi-arid ecosystem of southern Spain (Alguacil et al., 2009) showing that MPN-IP varied between 5 to 623 infective propagules per 100 g of soil. According to our findings, the different mycorrhizal parameters were more important in the arid PGs compared to the semi-arid ones which can be explained by the hot and arid environmental conditions. It is known that high temperature and high light intensity could increase AMF sporulation (Cardoso et al., 2003). In addition, da Silva et al. (2014) showed that dry environment may enhance AMF spore production and promote AMF establishment. Through our investigation, PCA analysis and Person correlation matrix showed that Fa, Ma, MPN-IP and AMF-SN had a very significant strong positive correlation ($r \geq 0.702$, $p<0.001$), which sounds logical, when the number of AMF propagules in soil is high the colonization percentages will be high and vice versa.

Soil environment can significantly influence life and activity of rhizosphere microorganisms. Previous studies reported the preference of AMF for a near neutral or alkaline soil pH (Helgason and Fitter, 2009). In the present investigation, no significant correlation was observed for soil pH with different mycorrhizal parameters, possibly due to the slight variation of this parameter (7.43–8.76) in the prospected soils. Otherwise, soil texture can influence plant growth as well as mycorrhizal effectiveness in many different ways, such as through drainage, aeration, and limitations in nutrients (Joshi and Singh, 1995). Person's correlation revealed that mycorrhizal parameters are negatively correlated with clay content ($r \geq -0.176$, $p<0.05$), while a significant positive correlation ($r=0.260$, $p<0.01$) was recorded between the percentage of sand and the MPN-IP. Carrenho et al. (2007) suggested that clay soil inhibited the development of mycorrhizal association while sand soil stimulated it. It has been shown that high soil AP content inhibits AMF growth, development, and AM formation as well as their functions (Cai et al., 2005). Our findings confirm those results since, we have noticed a very significant negative correlation ($r \geq -0.365$, $p<0.001$) between mycorrhizal parameters and soil AP. AMF-SN, MPN-IP and Ma are negatively and weakly correlated to TN and OM ($r \geq -0.192$, $p<0.05$), which is also supported by C/N ratio having a significant negative yet weak correlation with Fa ($r=-0.196$, $p<0.05$). The negative correlation is consistent with the widely accepted idea that mycorrhizae is disadvantaged in soils rich in OM and is due to the high availability of macro- and micronutrients added to the soil through OM decomposition (Watts-Williams and Cavagnaro, 2014). AMF-SN, MPN-IP and Ma also showed a significant weak negative correlation ($r \geq -0.182$, $p<0.05$) with electrical conductivity (soil salt content). Giri et al. (2007) reported that high salt content of the soil can prevent AMF colonization capacity and spore germination. Furthermore, $CaCO_3$ content was significantly and negatively correlated ($r=-0.286$, $p<0.001$) with Fa. This correlation is confirmed by numerous

data showing inhibition of AMF spore germination and hyphae elongation in the presence of high $CaCO_3$ content in the soil (Moradi et al., 2015). PCA analysis had grouped Aoufous, Ksar Achbarou, Tagherat, El Nif, Tansikht, Tinzouline, Mcisi and Boudnib due to their soil richness in AMF (Figure 7A), while NWPM, NEPM, NPF, Taghjijt and Rissani were grouped in the same side due to their AMF poor soils. AMF-SN, MPN-IP, Ma and Fa were logically grouped together because of their close development (Figure 6B). Previous studies have highlighted the use of the Aoufous mycorrhizal consortium of the Tafilalet PG in the restoration of oases area by improving the tolerance of crops in this ecosystem to various biotic and abiotic stresses (Baslam et al., 2014, Meddich et al., 2015; Ait-El-Mokhtar et al., 2019; Ben-Laouane et al., 2019). Indeed, Meddich et al. (2015) demonstrated the effect of AMF from this consortium in increasing the tolerance of date palm to Bayoud disease and water stress. In the same crop, Ait-El-Mokhtar et al. (2019) highlighted the role of this consortium in mitigating the negative effects of saline stress on growth, physiology and mineral nutrition. Ben-Laouane et al. (2019) demonstrated the effectiveness of the application of AMF from the Aoufous consortium in improving the tolerance of alfalfa to salt stress. This support the effectiveness of native AMF in the rehabilitation of these ecosystems.

Conclusion

The mycorrhizal status varied significantly among the prospected soil samples from Moroccan PGs with high mycorrhizal parameters in soils of arid oases (Tafilalet, Draa, Figuig and Tata-Guelmim PGs) compared to semi-arid ones (Marrakesh PG). The parameters characterizing this status namely, AMF spore number, MPN of infective propagules, AMF infection frequency and AMF infection intensity were positively correlated. The soil parameters acted differently on AMF mycorrhizal status, since soil available phosphorus, total nitrogen, organic matter, electrical conductivity, $CaCO_3$ content, clay percentage and C/N ratio had negative effects on their status. In the opposite, the percentage of sand in the soil had a positive effect on mycorrhizal infection. It clearly appeared that AMF of oasis ecosystems, especially in arid ones, could be an important asset in selecting these microorganisms and achieving successful restoration programs in these areas based on the similarity of mycorrhizal profile and soil physico-chemical parameters between the oases in question.

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Chapter 2

Composting of Green, Animal, and Agro-industrial Wastes: Organic Biofertilizer Production and Crop Performance

Meddich Abdelilah[1*], Elouaqoudi Fatima-zohra[2], Khadra Ahmed[2] and Bourzik Widad[3]

[1]Laboratoire d'Agro-Alimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Faculté des Sciences-Semlalia, Université Cadi Ayyad, BP2390 Marrakech, Maroc.
[2]Laboratoire de Biotechnologies Microbiennes, Agrosciences et Environnement (BioMAgE), Faculté des Sciences Semlalia, Université Cadi Ayyad, BP511 Marrakech, Maroc.
[3]Service de l'Environnement, Wilaya de la Région Marrakech-Safi, Maroc.

***Corresponding author: a.meddich@uca.ma**

Abstract

Highquality and quantity food production have influenced agricultural practices around the world. In fact, in order to satisfy and respond to the increasing needs of the population intensive use of chemical fertilizers has led to the degradation and depletion of cultivated soils. Moreover, in Morocco, the combination of socio-economic development and population growth has been accompanied by an increase in waste such as liquid effluents and solid (olive-pomace) from the olive sector, phosphogypsum and sludge from phosphate wastes and green waste from maintenance of green spaces. These huge amounts of different types of accumulated wastes remain without any treatment and no valorization method adapted to the Moroccan context has been committed. The implementation of landfills for wastes elimination could be considered as wastage of raw materials and may cause serious damage to the environment. However, recycling organic matter and biodegradable compounds has become an important environmental act for the restoration of the soil ecosystem and for the improvement of crop yields. The objective of our study is to investigate the possibility to treat various agro-industrial wastes by aerobic processes for further agronomic valorization. This process of composting has double interest by mitigating the environmental impact of wastes and recycling these deposits of organic matter in major natural cycles. In order to assess the biodegradation of wastes at different stages of composting, we monitored the main physico-chemical parameters including temperature, pH, organic carbon, nitrogen and ash content. Mixtures of OCOMWWG (olive cakes olive mill waste water and Garbage), GW (grass scrap alone) and GWSP (grass -phosphate sludge) were found to reach maturity and stability after 3 months of composting with a C / N ratio <19. On the other hand, date palm substrates (DD) and DDW required more composting time to achieve a good level of stability. During composting and for almost all used wastes, pH undergoes a slight increase. Similarly, the rate of ash increases with higher values in the case of the DD and DDW than for other tested mixtures (GW, GWDL and OCOMWWG). On the other hand, the ratio NH_4^+/NO_3^- has decreased to low values (< 1) after 3 months of composting for GW, GWDL, GWSP and OCOMWWG mixtures. The decrease in this ratio indicates a significant oxidation of organic matter and a good maturity of composts. The use of mature compost in low doses has clearly stimulated the growth and the yield of tested crops such as alfalfa, wheat, maize, tomato, lettuce, leek and garlic.

Keywords: Soil degradation, waste, composting, agronomic development, crops, growth, yield enhancement.

Introduction

Depletion of organic matter in soils is a common characteristic of Mediterranean soils, particularly in the arid and semi-arid soil and climate context (Mallard et al., 2006; Abouelwafa 2009). The excessive supply of mineral fertilizers to improve crop yields continues to accelerate and worsen the deterioration of soil quality by decreasing their organic matter contents.

Morocco is one of the Mediterranean countries threatened by the most serious environmental problems that are waste and their inadequate management. The combination of socio-economic development and population growth in our country has been accompanied by an increase in the amount of solid waste produced mainly by households, industries, health institutions and livestock units (Laarousi et al., 2006). Solid waste production is around 6,832,000 tons / year in Morocco (Atlas Waste, 2014). These wastes are usually deposited in anarchic dumps without any treatment or control (PNDM, 2010). This has serious consequences for both public health and the environment (Makan et al., 2014). The process of washing, flotation and decantation of natural phosphates generates large quantities of phosphates and phophogypse sludge waste estimated at 2 million tons / year in the case of phosphate sludge (OCP communication). Furthermore, Morocco produces significant quantities of green waste annually from the maintenance of green spaces and which are evacuated to the dump. The palm grove and gardens of Marrakech produce annually a significant amount of green waste estimated at 38% of global waste of the city. Adding to this, other types of waste are generated continuously and in large quantities by industrialists that are mainly liquid effluents (olive oil mill wastewater) (400,000 m3 / year) and solid (Olive pomace) (180,000 T / year)) of the olive sector (CFC / IOC, 2008), and manure from livestock units. Waste treatment has become a mandatory necessity for the environmental protection (Oueslati et al., 1995). All waste is recoverable according to its mechanical and physicochemical properties (Jung 2013; Zhang and Sun, 2016). Organic recovery of biodegradable waste is one of the priorities of the waste management policy in many countries and that of maintaining the organic matter content in certain soils (Mallard et al., 2006; Zhang and Sun, 2016; Castán et al., 2016). In our case, the sustainable reuse of the waste in agriculture and nurseries in the form of organic fertilizers will help to reduce the peat's import bill that the government pays in currency as well as reducing the significant amounts of waste and its negative effects on the environment. There are several procedures for the treatment and management of waste among which are techniques for energy or agricultural recovery. In fact the biomethanation and composting are green technologies that transform waste into high value-added products while minimizing the risk of pollution (Prevot 2000). The different studies carried out in this field have often confirmed the beneficial role of the treatment of different types of waste in order to produce organic fertilizers that are pathogens free (Youssef and Eissa, 2014; Ge et al., 2016). In this context, the objective of this research theme is the treatment of the different waste mixtures of the abovementioned raw materials by aerobic aerial bioprocesses and their agronomic valorization. This technique of transformation of biodegradable solid waste in an aerobic environment is economically more profitable (Francou 2003; Ruggieri et al., 2009). It is an appropriate environmental alternative for the transformation of organic matter into high-quality organic fertilizers (Shak et al., 2014). It has the dual interest of mitigating the environmental impacts of waste and recycling these deposits of organic matter into their natural cycles.

In this perspective, the present study aims to understand the scientific mechanisms involved in the composting process, especially the evolution of organic matter and the different physicochemical parameters (pH, humidity, total organic carbon, nitrogen, C / N ratio, decomposition rate and available phosphorus...) and the production of valuable composts in agriculture. We will also aim to study the impact and the effect of the organic fertilizers produced on the growth and development of the date palm and the underlying crops including barley, wheat and alfalfa.

Materials and experimental procedure

Raw materials used

The raw materials used for the conduct of our tests are:

- Date palm wastes (PW)
- Grass waste only (GW)
- Waste of green areas especially leaves
- Waste of cactus plants
- Waste from livestock units: Poultry and horse manure
- Sludge generated from the wastewater treatment of Marrakech
- Sludge from natural phosphates and phosphogypsum
- Olive pomace and olive mill waste water
- Waste from sugar beet industry
- Waste of domestic refuse.

Among the mixtures of waste produced:
Date palm wastes alone (DD)

- Grass waste only (GW)
- Grass waste and dead leaves (GWDL)
- Olive cake, olive oil mill wastewater, and Garbage (OCOMWWG)
- Grass waste and sludge of washed phosphate (GWSP).

Compost preparation and environmental conditions

All tests were conducted in a composting platform which consists of a metal frame of 2400 m, at the municipal nursery of city Marrakech (average external temperature of 15.68° C, average relative humidity of 47.17% and light of 295 µmol m^{-2} s^{-1}).

Mixtures moistened to 60-70% are deposited on plastic sheeting to prevent runoff and leaching phenomena during wetting and mixtures are covered by other similar pierced sheeting to reduce the evaporation and the drying windrows and heat loss.

For ventilation of windrows during the composting process, regular and manual mixing are provided with pitchforks and shovels until the end of composting.

After each brewing, samples were taken at 10 different levels windrows (deep, surface, side, center), according to the principle of quartering method (AFNOR, 1999).

Measured parameters

We have measured the physico-chemical parameters of the mixtures of waste used and the composts produced. The agronomic aspect of the composts obtained was evaluated by measuring the biomass of plants widely cultivated in Morocco that are mainly date palm, barley, alfalfa and wheat. The pH is measured by an electrometric method using a pH meter on a suspension of 10 g of fresh sample in 20 ml of distilled water.

The variation of the temperature is recorded daily by measuring this parameter at different levels windrows using a metal temperature probe. The temperature of the windrow is the average of six measurements in each side of the windrow and at different depths (100 cm, 70 cm and 30 cm). The ash content is determined by calcining a sample previously dried in a muffle furnace at a temperature of 600° C for 06 hours. The increase in temperature must be achieved by heat bearing (105° C [1 hour], 200° C [1 hour], 600° C [6:00]), to avoid the sudden destruction of organic matter resulting in material losses in the oven. The total organic carbon content is determined by the oxidation method of organic material in cold condition with an excess of potassium dichromate $K_2Cr_2O_7$ in the presence of concentrated sulfuric acid according to the protocol described by Aubert (1978). The measurement of the total nitrogen is based on the transformation of organic nitrogen into ammonium nitrogen. The sample undergoes a mineralization by concentrated sulfuric acid and in the presence of Kjeldahl catalyst, and then the formed ammonia is displaced by NaOH (40%). Then, the entrained ammonia by the water vapor is fixed by the boric acid and titrated with sulfuric acid. NKT content is determined by the distillation unit Velp-UDK132 according to the protocol described by Rodier (1984).

Ammonium levels are determined according to the Kjeldahl method (AFNOR, 1975) from fresh sample (2g) by distillation in an alkaline medium with 10 ml of sodium hydroxide 40%.

Nitrates are reduced through a granulated cadmium column in nitrites which are assayed colorimetrically. After 30 minutes of reaction with the diazotation reagent, the nitrate is measured in a spectrophotometer at 537nm. Available phosphorus is determined by Olsen method. The produced composts were used as growing medium for examining their effect, on the growth of underlying crops with a short development cycle under greenhouse (controlled conditions). The response of treated plants with or without compost, was evaluated by determining the shoots dry mass (SDM) and root dry mass (RDM), a reliable indicator of biomass, measured after drying in the oven at 105 ° C for 24 hours. In addition, the yield was measured in the case of crops grown in the field in two successive campaigns.

Statistical analysis

All results were analyzed statistically with the CO-STAT software (Statistical Software, New Style Anova). The study includes an analysis of variance followed by the Newman and Keuls test at the 5% threshold.

Principle results

Physico-chemical characteristics of waste raw materials and substrates for composting

The physico-chemical characteristics of the raw materials used in the composting process are presented in Table 1 and Pictures 1, 2 and 3. Analysis of the results shows that palm leaf waste has very low moisture levels not exceeding 11% compared to other waste used. The pH of the majority of the waste is slightly acidic to neutral, favorable to the development of different types of microorganisms. The dandelions, phosphate sludge, sludge of wastewater treatment, waste of sugar beet and poultry and horse manure have an alkaline pH (~8). Sludge of wastewater treatment and poultry manure are rich in total nitrogen (> 3%). Dandelions and waste of domestic refusetested were rich in organic matter and contain moderate amounts of total nitrogen. The C / N ratio of the majority of waste is favorable for good microbiological activity and for an adequate composting process.

Table 1. Physical and chemical characteristics of the waste before composting.

Rawmaterials	Humidity (%)	pH	Total organic carbon (%)	Total Kjeldahl nitrogen (%)	Ratio C/N
Dandelions	69.00	8.01	58.10	1.68	34,58
Date palm leaves	8.14	5.50	54.70	1.10	50.00
Date palm stipes	11.00	6.60	55.00	0.80	70.00
Phosphate sludge	59.00	8.29	2.00	0.073	27.39
Leaves of green waste	58.00	6.64	55.40	1.37	40.44
Olive pomace and liquid effluents	54.00	5.77	46.52	1.34	34.72
Sludge of wastewater treatment	41.00	8.00	37.60	3.64	10.33
Waste of sugar beet industry	2.70	8.60	3.70	0.27	14.00
Waste of domestic refuse	84.40	5.20	60.80	2.41	25.23
Poultry manure	39.00	8.00	47.30	3.41	13.87
Horse manure	31.00	8.60	44.50	1.67	26.65
Phosphogypsum	2.87	5.49	1.69	0.054	31.29

***Picture 1**. Preparation of raw material waste and composting platform.*

Phosphate sludge ***Date palm leaves***

Green waste

Picture 2. *Start the composting process.*




Dandelions

Picture 3*. Maintenance and monitoring of the composting process.*




Mixtures of waste during composting

In Picture 4, the different phases of composting are illustrated. The evolution of the temperature during the composting of different substrates and made mixtures is shown in Figure 1. The biodegradation can be assessed by the temperature of the compost which shows a gradual increase during the mesophilic phase, reaching maximum values during the thermophilic phase. Then it gradually decreases to reach values around the temperature of the air during the maturation phase. The temperature curves' profile looks the same except in the case of waste constituted by palm only (DD) where the temperature increased slowly during the first month and then showed a more important increase from the second month to reach a maximum temperature of 43° C in the third month. For other waste including DDW, the temperature increased rapidly in the first days of composting to reach maximum values of 56° C to 69° C. Thereafter, the temperature gradually decreases to values between 30° C and 40° C.

Picture 4. *Illustration composting phases.*

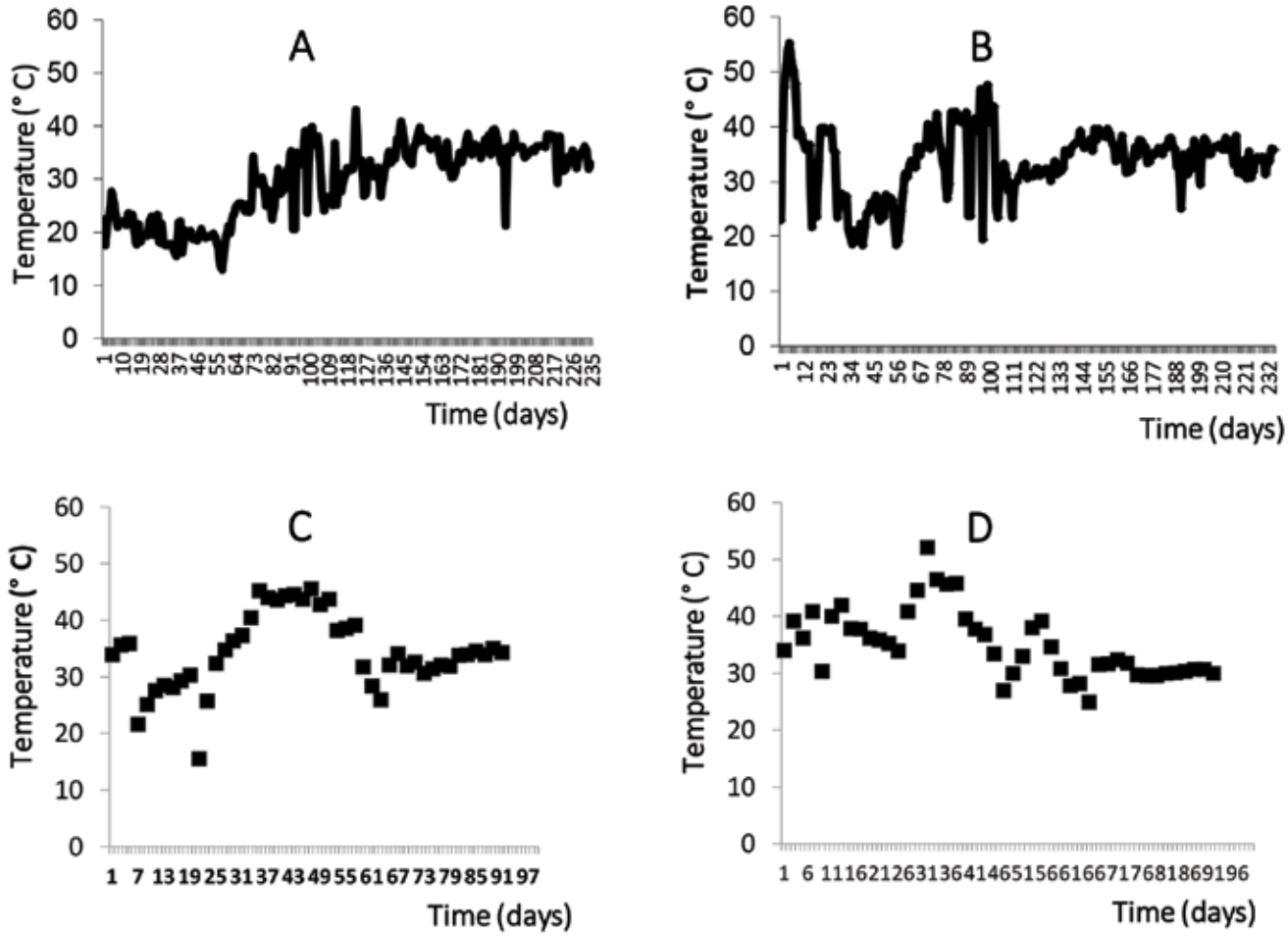
Phase Decomposition Mesophilic

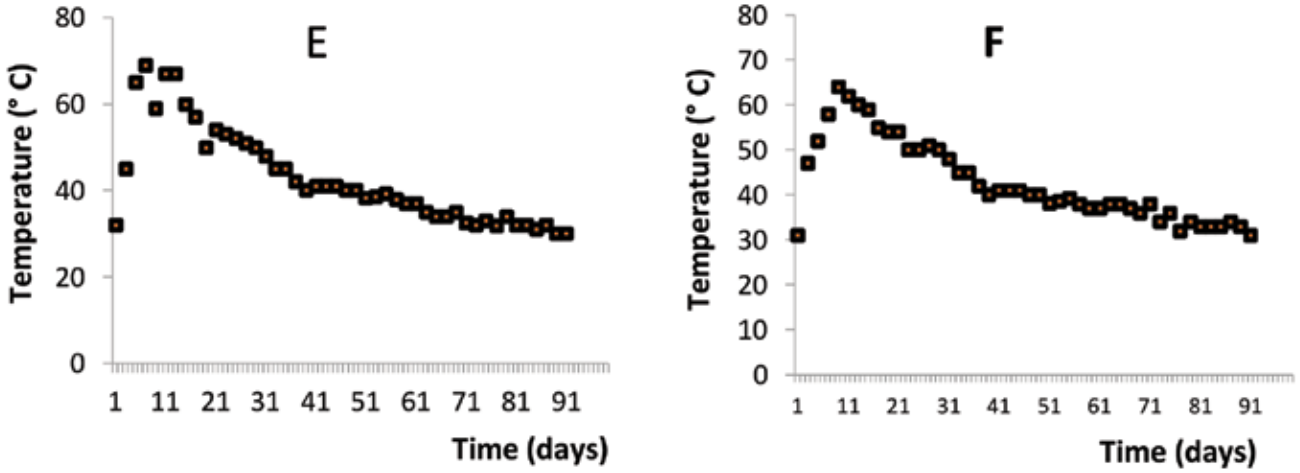
Phase Decomposition Thermophilic

Phase Decomposition Cooling

Phase Maturation - Stability compost-

Figure 1. Temperature evolution during composting of different types of waste.





DD (date palm waste) (A) ; mixture DDW (date palm-grass waste) (B) ; DW (Grass waste only) (C) ; GWSP (Grass waste and sludge of washed phosphate) (D) ; GWDL (Grass waste and dead leaves) (E) and OCOMWWG (Olive cake, olive oil mill wastewater and Garbage) (F).

A slight increase in pH was recorded from 6 to 7; 6.4 to 7.6; 7.23 to 8.4 and 5.49 to 6.06 for DD, DDW, GWDL and OCOMWWG respectively (Table 2). For Grass waste alone or associated with waste sludge phosphate, the pH is slightly alkaline and remained without significant change during composting. The evolution of the C / N ratio in mixtures DW, GWDL, GWSP and OCOMWWG shows a rapid decrease respectively from 34 to 14, 33 to 11, 64 to 12 and 32 to 19 after the third month (Table 2). As opposed to the C / N ratio of DD and DDW which slowly decreased from 56 to 39 and from 55 to 33, respectively, in the sixth month. The ash content varies from compost to another; their increase is due to the mineralization of organic matter and concentration of mineral elements (NH_4+, NO_3-, P...) in compost products. Higher ash content was observed in the case of DD and DDW than for the other mixtures used. Composts obtained from palm waste alone or mixed with grass presented very low levels of available phosphorus, which do not exceed 9 ppm. The values of this element remained high in the case of compost of grass alone or combined with phosphate sludge. The measurement of the ammonium nitrogen N-NH_4+ and nitrate nitrogen N-NO_3- shows a simultaneous increase of these two forms of nitrogen throughout the process for both DD and DDW with significant values of NH_4+, as a result NH_4+/NO_3- ratios values were very high even after 6 months of composting. By cons, for mixtures DW, GWDL, GWSP and OCOMWWG a decrease in the ratio NH_4+ / NO_3- was recorded (<1) after only 3 months of composting.

Table 2. Physicochemical parameters during composting.

(DD: date palm waste, DW: Grass waste only, DDW: date palm-grass waste, GWDL: Grass waste and dead leaves, GWSP: Grass waste and sludge of washed phosphate and OCOMWWG: Olive cake, olive oil mill wastewater and Garbage).

Stade de compostage	pH	TOC (%)	TKN (%)	C/N Ratio	Ashes (%)	NH_4^+ (mg/g)	NO_3^- (mg/g)	P avai. (mg/g)*	NH_4^+/ NO_3^-
DDi	6.00	49.30	1.20	56.50	15.20	724.00	0.16	0.006	$4.52.10^3$
DD6	7.00	40.80	1.06	38.60	29.80	738.00	0.70	0.009	$1.05.10^3$
DWi	8.013	58.071	1.680	34.566	40.00	0.066	0.046	0.141	1.45
DW3	7.863	30.654	2.190	14.00	49.00	0.031	0.069	0.270	0.44

Stade de compostage	pH	TOC (%)	TKN (%)	C/N Ratio	Ashes (%)	NH_4^+ (mg/g)	NO_3^- (mg/g)	P avai. (mg/g)*	NH_4^+/NO_3^-
DDWi	6.40	50.00	0.90	55.30	13.80	555.00	0.36	0.009	$1.51.10^3$
DDW6	7.60	34.80	1.07	32.60	40.00	770.00	4.00	0.008	$0.19.10^3$
DWDLi	7.23	41.60	1.25	33.28	19.00	11.13	2.07	-	5.38
DWDL3	8.40	18.40	1.60	11.20	31.54	5.75	5.96	-	0.96
DWSPi	7.863	25.00	0.392	63.77	50.00	0.013	0.018	0.167	0.68
DWSP3	7.77	7.637	0.616	12.39	82.00	0.0036	0.032	0.300	0.11
OCOMWWGi	5.49	50.50	1.57	32.16	3.90	5.44	1.89	-	2.88
OCOMWWG3	6.06	37.30	1.98	18.84	5.60	2.82	7.60	-	0.37

(I: initial, 3 months and 6 months); * P avai. : Available phosphorus.

Characteristics of compost used at maturity stage

Composts GWSP, GW, GWDL are characterized respectively by an alkaline pH values equal to 7.77, 7.86 and 8.40 respectively. While the pH of the compost OCOMWWG remained slightly acidic and near neutral in the order of 6.06.

Composts OCOMWWG and GW have a very high total level of organic carbons reaching 37.30% and 30.65% respectively. These latter levels were higher than those obtained with composts GWDL and GWSP with rates of 18.40 % and 7.63% respectively. Moreover, the tested composts GWDL, GWSP, GW and OCOMWW presented weak C / N ratios whose values are, respectively, 11.20, 12.39, 14.00 and 18.84.

The analysis of nitrogen ammonia N-NH_4+/ nitrogen nitrate N-NO_3- ratio showed values below one, (NH_4+ / N-NO_3- <1). It was found that the composts used GWSP, OCOMWWG, GW and GWDL had NH_4+ / N-NO_3- ratio of 0.11, 0.37, 0.44 and 0.96, respectively.

Determination of the agronomic value of the composts produced

Impacts of composts on plants grown in greenhouses under controlled conditions

In order to test the effectiveness of composts produced, we have chosen crops with a short development cycle and economic interest in Morocco. The weight of the dry matter is the criterion that gives the best estimate of the growth. The agricultural soil used is the same for the different crops. It is characterized by a lack of organic and mineral matter with sandy loam texture.

The application of compost from the couch grass and dead leaves (GWDL) with doses of 5% and 20% has a beneficial effect on improving the production of shoot dry matter (SDM) and root dry matter (RDM) of **alfalfa** (*Medicag sativa*, variety Demnate) compared to the control, after 2 months of culture (Picture 5, Table 3). In the absence of any nutrient intake, the dose of 20% GWDL compost induced a significant increase in the production of SDM and RDM compared to the control plants or compared to the treatment with 5% compost.

Picture 5. Effect of MCA and compost GWDL on alfalfa growth.

Coontrol **5%C** **20%C**

The application of compost from waste pomace olive and Garbage (OCOMWWG) at a dose 10% caused a slight increase (aboveground biomass) of SDM of **wheat** compared to the control plants (Table 3). On the other hand, the same treatment showed a significant increase (4.5 times higher than the level obtained with the control) in the dry matter production of wheat roots (RDM).

Moreover, the same compost OCOMWWG applied at the same dose 10% induced an increase (1.5 times higher) in the SDM of **maize** compared to the control plants (Table 3). For RDM, applying compost OCOMWWG showed no effect on this root biomass.

Based on the evaluation of SDM, the production of shoot dry weight of **tomato** was doubled by compost treatment at a dose of 10% from the dandelions and phosphates washing sludge (GWSP) (Table 3).

Table 3. Impacts of composts tested on crop growth under controlled conditions.

Type of crop		Crops under controlled conditions	Measured parameters	
		Treatment	Shoot dry matter (SDM)	Root dry matter (RDM)
Leguminous	Alfalfa	Control plants	0.006193±0.00405	0.005767±0.003911
		GWDL compost 5%	0.378833±0.007962	0.233833±0.029583
		GWDL compost 20%	0.770033±0.056556	0.476567±0.067596
Cereals	Wheat	Control plants	0.068083±0.009034	0.004017±0.00084
		OCOMWWG Compost 10%	0.081583±0.010027	0.01820±0.001641
	Maize	Control plants	0.43855±0.032032	0.13950±0.012960
		OCOMWWG compost 10%	0.648267±0.062857	0.13705±0.035880
Vegetable crops	Tomato	Control plants	0.06±0.007073	0.02±0.00278
		GWSP Compost 10%	0.13±0.010548	0.03±0.002948

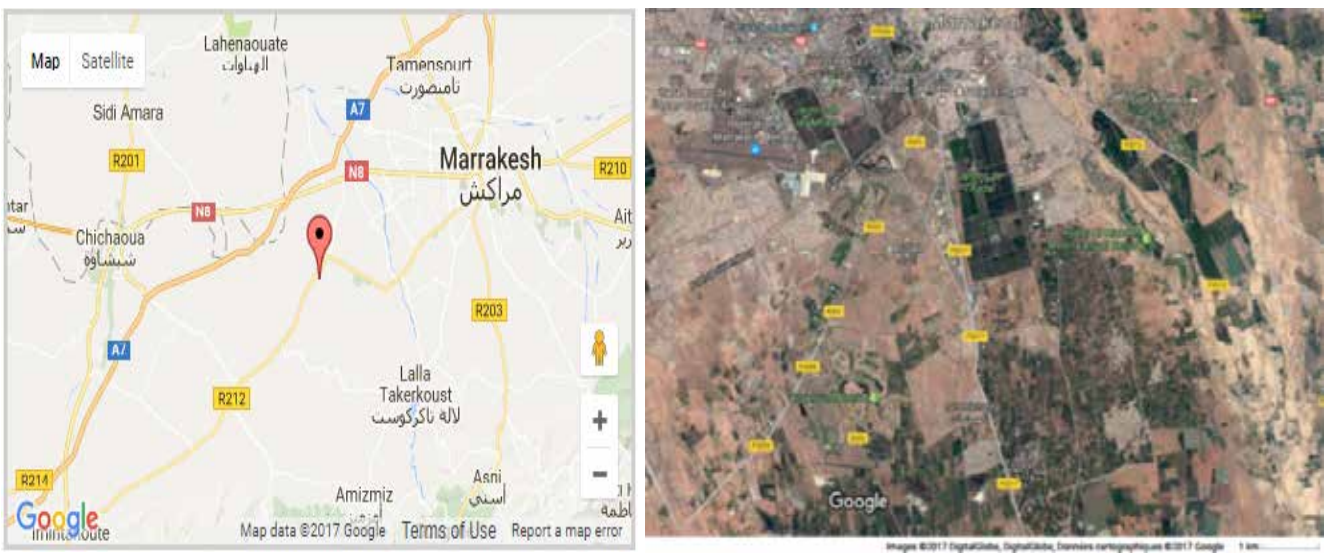
Impacts of composts on plants yield in field

The experiments were carried out in the field on an area of 6,000 m2 in the municipality of Tamesloht of Marrakech, in Morocco (N 31 54 176; W 008 02 087; Elevation 531m) (Picture 6). The regional climate of the experimental site is typically Mediterranean, with an annual average of temperature of about 20.5°C and 281 mm per year for precipitation. Two crop campaigns were carried out on the same plot during the years 2017-2018 and 2018-2019. No herbicides and no chemical fertilizers were applied in previous growing seasons. For plant material, we chose vegetable crops. The selected species (Green Lettuce, Red Lettuce, Garlic and Leek) are known for their importance, interest and widespread use by farmers in the region. The selected biological seeds were disinfected and then washed successively with sterile distilled water. They were germinated in alveoli in the laboratory. Then, the seedlings were transferred to the field on agricultural soil with the following characteristics: sandy loam texture (sand: 74.75%, silt: 13.55% and clay: 11.69%); pH: 8.12; electrical conductivity: 138.3 µs / cm; phosphorus available: 57.42 ppm; organic matter: 0.87% and limestone content: 5.04%.

The seedlings were treated by the various biological fertilizers such as composts of different origins and produced locally by Meddich et al. (2016).

An uninoculated control for each crop was carried out under the same conditions without any biological or organic amendment. The plots of land carried out are each subdivided into several basic blocks of 1.5 m × 0.8 m each, ie 1.2 m^2 (Picture 7). The plots and their blocks are equipped with a drop-by-drop system and are arranged in a random manner. A device of 12 blocks repeated for the same treatment and the same culture was used to evaluate the impact of compost on the cultures tested. Other fallow blocks were used. The yield evaluation of the crops studied was determined by measuring the fresh weight of fruit produced.

Picture 6. Geographical location of the study area (Google maps, 2017).



Picture 7. Development of blocks and plots in the field equipped with drip.

Each crop was randomly arranged in the different blocks of plots realized (Picture 8). The majority of applied composts improved the yield of vegetable crops (lettucess, garlic and leek) (Table 4).
The different composts applied at low doses 10% have increased the yield of green lettuce with a clear improvement in the case of amendment with the composts from green waste associated with phosphate sludge or phosphogypsum.

Composts of green waste, whether or not associated with poultry manure, have considerably increased the yield of red lettuce and leek. The yield was increased 4 times more in the case of leek amended with compost 10% compared to the control.

The green waste composts associated with animal oragro-industrial waste have increased the yield of garlic, especially in the case of green waste mixed with phosphogypsum, olive cakes and horse manure (Table 4, Picture 9).

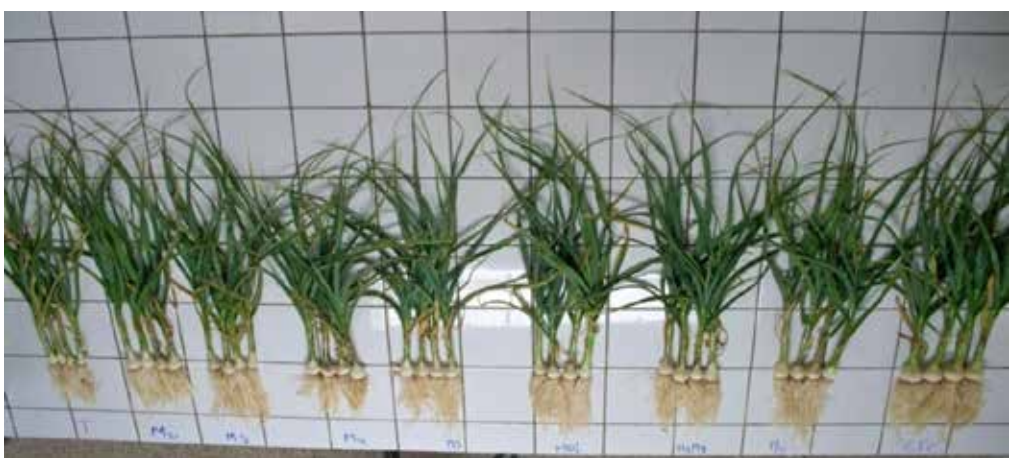
Picture 8. Implementation and randomization of plantations of leek and red lettuces subjected to different fertilizers.

Table 4. Impacts of composts tested on crop yields in the field.

Type of crop		Field crops	Measured parameters
		Treatment	Yield (g / plant) (Average of 2 campaigns)
Vegetablecrops	Green Lettuce	Control plants	357.10 ±43.28
		Compost of horse manure and green waste 10%	404.25 ±40.78
		Compost of poultry manure and green waste 10%	337.37 ±54.89
		Compost of olive cakes and green waste 10%	336.93 ±39.33
		Compost of phosphogypsum and green waste 10%	464.75 ±65.44
		Compost of grass waste and phosphate Sludge 10%	500.75 ±76.88
	Red Lettuce	Control plants	465.60 ±32.95
		Compost of poultry manure and green waste 10%	685.15 ±59.68
		Compost of green waste 10%	710.47 ±52.10
	Leek	Control plants	5.27 ±1.53
		Compost of poultry manure and green waste 10%	23.00 ±5.49
		Compost of green waste 10%	20.90 ±4.12
	Garlic	Control plants	8.78 ±0.74
		Compost of horse manure and green waste 10%	13.43 ±0.77
		Compost of poultry manure and green waste 10%	9.45 ±1.34
		Compost of olive cakes and green waste 10%	13.92 ±0.71
		Compost of phosphogypsum and green waste 10%	15.57 ±1.14
		Compost of grass waste and phosphate Sludge 10%	11.20 ±0.50

Individual mean of 12 repetitions belonging to each of the 12 blocks repeated for the same treatment and the same culture (Neumans and keuls test $P < 0.05$).

Picture 9. Positive impact of compost on the growth of Garlic, after 3 months of culture.



T: Control; M 2: Compost of horse manure and green waste; M3: Compost of poultry manure and green waste; M4: Compost of olive cakes and green waste; M5: Compost of phosphogypsum and green waste; MD2': Compost of grass waste and phosphate Sludge.

Discussion

The choice of wastes and mixtures for recycling is justified by their abundance and their high content in minerals or organic matter. At the start of the composting of the waste used, the gradual rise in temperature (below 42° C) during the first mesophilic phase, results from the heat released during the degradation of the compounds easily assimilated by mesophilic microorganisms, especially bacteria and fungi which degrade simple molecules such as sugars and amino acids as well as part of the complex molecules such as lipids and proteins (Mustin1987). The increase in heat released during this phase depends on the nature of the composted waste and the type of isolation from the outside environment (Ahn et al., 2009). The increase in temperature inside the compost mass allows the installation of thermotolerant microorganisms during the thermophilic phase (Viel 1989). During this phase the temperature can reach 60° C to 75° C, which stops the activity of microorganisms which do not resist high temperatures like fungi, on the other hand it promotes the development of thermophilic bacteria and neutralizes pathogenic organisms (Francou 2003; Hachicha et al., 2009). During the cooling phase which follows the thermophilic phase, the gradual decrease in temperature are linked to the decrease in the quantity of easily degradable organic matter, which causes a slow down in microbial activity (Mustin 1987; Petiot et Guardia, 2004). Finally, the temperature gradually decreases to reach values around ambient temperature generally below 40° C during the maturation phase. During this last phase, the humification process predominates, the rate of humic substances improves by polymerization and condensation of the substances released during the decomposition of organic matter (Mustin 1987). The slow degradation of resistant compounds such as cellulose, lignin and tannins does not allow the high temperature to be maintained and leads to a dark brown to black coloration of the compost which becomes finer and more homogeneous (Hsu and Lo, 1999). The microbiological activity is weak during this phase and the mature compost will serve as nutrients for the plants.

For the waste used in this study, we have noted the following four phases of temperature evolution: mesophilic, thermophilic, cooling and maturation. The evolution of the temperature for compost obtained from palm waste (DD) is related to the lignocellulosic nature of the substrate which is little fermentable. Melillo et al. (1982) reported that the amount of lignin in cells can affect biodegradation through its action as a barrier to decomposition. Thus, cellulose and hemicellulose degradation is slower for lignocellulosic plant substrates (Huang et al., 2010). As a result, the DD compost will degrade more slowly than the other composts tested.

During composting, the pH of the organic mass changed. At the beginning of the degradation process, an acidogenic phase occurs resulting from the production of organic acids and CO2 by the acidogenic bacteria by lowering the initial pH. Then, this parameter increases during the alkalinization phase before stabilizing at the end of the composting towards neutral pH depending on the nature of the substrate (Tiquia and Tam, 2000). This second phase corresponds to the bacterial hydrolysis of nitrogen with the production of ammonia (NH_3-) associated with the degradation of proteins and the breakdown of organic acids (Haug 1993; Mustin 1987). In general, the increase in pH can be explained by an accumulation of ammonia and / or a loss of short chain fatty acids and volatiles that result from microbial activity (Lim et al., 2014 and Shak et al., 2014). Overall, the compost with a pH value range between 6 and 8.5 is compatible for most plants (Hachicha et al., 2009).

The C/N ratio is used to assess the maturity of the compost. Changes in this ratio during composting reflect the decomposition and stabilization of organic matter. The C/N ratio decreases during the composting to stabilize towards the end of the process. For our composts produced, the rapid decrease in C/N ratio at the third month of composting could be explained by significant reduction in easily metabolizable organic carbon related to biodegradation of organic matter, and that is mainly obtained in the case of quackgrass waste, leaves green waste, olive cake and olive oil mill wastewater. Palm waste, however, is degraded at a slow rate due to its lignocellulosic nature (Solano et al., 2001; El ouaqoudi 2015). Compost with a C/N ratio below 20 is considered mature and can be used without any restrictions (Jimenez and Garcia, 1989; Bernal et al., 1998; Koivula et al., 2000; Som et al., 2009). A C / N ratio value range of 8-15 is often considered as an index of humic material formation and stability of composts produced (Rynk 1992; Lim et al., 2014). This ratio depends on the composition of the substrates to be composted. The substrates can be rich in carbon with high C/N ratio and resistant to decomposition by microorganisms (Straw, lignocellulosic products), or substrates with low C/N ratio which are rich in nitrogen and very fermentable (food waste, residual sludge ...). As a result, depending on C/N ratio values, DD and DDW composts would require more composting time to achieve a high level of stability.

Ash rates remained higher in the case of compost from DWSP than in the case of DW compost alone. The evolution of the rate of organic matter is also considered a good criterion for the maturity of the compost (Som 2006). It decreases significantly during the composting process with 35 to 50% loss of the organic matter (Abouelwafa 2009).

The $N\text{-}NH_4^+$ / $N\text{-}NO_3^-$ ratio is also used as an indicator of the compost maturity. A decrease in this ratio indicates that the compost contains high nitrate levels and it also indicates that the compost is mature (Morale et al., 2005; Raviv et al., 2005). A value of the NH_4^+ / NO_3^- ratio lower or equal to 1 indicates significant oxidation of organic matter, degradation of the substrate and maturity of the compost (Barje et al, 2012; El Fels et al, 2014). The recorded values of $N\text{-}NH_4^+$ after 6 months of composting of DD and DDW show that their maturity is not yet complete. Zucconi and Bertoldi (1987) suggested a value of 400 mg kg-1 of $N\text{-}NH_4^+$ as its maximum level in mature compost. However, the slow biodegradation of palm substrates can be improved by adding another more easily biodegradable substrate and / or by finer grinding of the starting material in order to reduce the composting time.

Our study was carried out to elucidate the impact of mature composts on the growth and yield of plants grown in the greenhouse and in the field and which are of major economic importance especially in the oases, where they are highly appreciated. The agricultural soil used in our study is characterized by low contents of organic matter and available phosphorus. This is in favor of the formation, development and proper functioning of symbiosis between plants and microorganisms such as PGPR and AMF (Meddich et al., 2015a et 2017). In this context, we used low doses of compost to promote plant growth without inhibiting the beneficial microorganisms often associated with roots in the rhizosphere of crops.

Overall, the low doses 5%, 10% and 20% of the used composts and mature have beneficial effects on improving the growth of alfalfa, wheat, maize and tomato within the greenhouse.

The production of shoot dry weight of tomato was increased by using compost treatment 10% from the dandelions and phosphates washing sludge (GWSP). Morocco has the largest phosphate reserves in the world and is also one of the largest producers of phosphate fertilizers. But this activity also produces phosphate washing sludge reaching 28.1 million tonnes in 2010. A rate which is expected to increase due to mining activity linked to the enrichment of natural phosphates which generates a large amount of sludge. In Morocco little is done to capture value from phosphate washing sludge waste. However, it is rather stored in large basins which require the acquisition and immobilization of land and storage monitoring at high cost. The phosphorus contained within these sludges is an essential nutrient required for plant growth. This sludge could therefore be incorporated into and during the composting of biodegradable waste. The compost obtained can then be incorporated into soils poor in organic matter and phosphorus.

Mature composts increased the yield of lettuce, leek and garlic grown in the field. Similar results that emphasize the utility of composts were also noted for lettuce and maize (Mrabet et al., 2011). Green waste compost applied with poultry manure significantly increased the yield of leek and red lettuce. Our results are comparable to those obtained by Koulibaly et al. (2015) who tested doses of compost obtained from cotton and significantly improved cotton yield by 65% compared to the control. Copetta et al. (2011) showed that the use of compost from green waste considerably improves the yield and quality of the tomato fruit. Composts improve the different physicochemical and biological properties of soils (Toumpeli et al., 2013, Killi et Kavdir, 2013; Mehta et al., 2014) and consequently increase the yield of plants (Akram Quasi et al., 2009; Motta and Maggiore, 2013). They are able to improve the mineral and water status of the plant (Gharib et al., 2008; Meddich et al., 2015b). The application of the compost can be an advantage in improving the fertility of agricultural soils. These results are due to the enrichment of the soil by the organic matter and the microorganisms brought by this amendment. Organic matter provides many biological functions. It makes available and store nutrients for plants, it promotes biological activity as a source of energy for microorganisms, and it has a positive effect on structuring, physicochemical properties and aeration of the soil. In addition to all the benefits already mentioned about the use of composts, it also prevents sandy soils and colloidal particles from being washed. The compost avoids the erosion phenomena by playing a role in the retention of the particles set in motion by the rain and by effectively absorbing the drops (Bodet and Carioli, 2001; Roca-Pérez et al., 2009). Compost can provide agricultural soils in addition to organic matter, major nutrients (N, P, K), secondary (Ca, Mg, Na, S ...) and essential trace elements (Fe, Cu, Co, Ni , Zn,...) necessary for the intensification of agriculture (El Ouaqoudi 2015). The high content of organic matter in composts improves the biological and enzymatic activity of substrates and the bioavailability of nutrients after mineralization of organic matter (Pascual et al., 1999; Gustafsson et al., 2000; Gregorich et al., 2003; Hofman and Dušek, 2003).

Conclusion

The mixtures of waste studied OCOMWWG (Olive cake, olive oil mill wastewater and Garbage), DW (Grass waste only) and GWSP (Grass waste and sludge of washed phosphate) reached their maturity and stability after 3 months of composting with a C / N ratio lower than 19. By against DD and DDW date palm substrates require more composting time to reach a good level of stability.

The use of mature composts significantly boosted the growth of the tested crops. Low doses of compost are effective in improving the growth and yield parameters of the crops used.
The use of such organic fertilizers from aerobic bioprocess could constitute an efficient biotechnological means. It could reduce the impact of the waste generated in large quantities on one hand and improve the growth of plants under constrained pedoclimatic conditions of the environmenton the other hand.

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Chapter 3

Effectiveness of Arbuscular Mycorrhizal Fungus Inoculation and Compost Amendments to improve Growth and Physiological Parameters of *Phoenix dactylifera*

Mohamed Anli[1,5], Sarah Symanczik[2], Abdelilah El abbassi[1], Mohamed Ait-el-mokhtar[1], Abderrahim Boutasknit[1], Raja Ben-laouane[1], Marouane Baslam[3,4], Paul Mäder[2], Mohamed Hafidi[5] and Abdelilah Meddich[1*]

[1]Laboratoire Agro-Alimentaire, Biotechnologies et Valorisation des Bioressources Végétales, Department of Biology, Faculty of Sciences – Semlalia, Cadi Ayyad University, Marrakech 400001, Morocco.
[2]Soil Science Department, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, 5070 Frick, Switzerland.
[3]Laboratory of Biochemistry, Faculty of Agriculture, Niigata University, Niigata, Japan.
[4]Department of Life and Food Science, Graduate School of Science and Technology, Niigata University, Niigata, Japan.
[5]Laboratory of Microbial Biotechnologies, Agrosciences, and Environment (BioMAgE), Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakesh, Morocco.

[*]**Corresponding author: a.meddich@uca.ma**

Abstract

Date palm (*Phoenix dactylifera*) is an important crop in arid zones and plays significant ecological and socio-economic roles. During the last decades, date palm groves were subjected to degradation due to extensive soil exploitation and low soil fertility. The use of biological techniques is essential to improve date palm development. The present work aims at assessing the effect of two different doses of compost (5% and 20%) and inoculation with the arbuscular mycorrhizal fungus (AMF) *Rhizoglomus irregulare* on growth and development of date palm seedlings. The treatments comprised control and application of 5 and 20% of compost with *R. irregulare* individually or in combination. Growth, physiological, histological, and mycorrhization traits were assessed. Obtained results showed that compost applied at a low dose (5%) alone or in combination with the AMF stimulated root length, leaf area, root dry weight, nitrogen, phosphorus and potassium contents, stomatal conductance and chlorophyll fluorescence and increased the number of sclerenchyma fibers, the number of vascular bundles, root diameter, and lignification of the endodermis of date palm seedlings compared to control. Compost at low doses combined or not with AMF can successfully be applied as biofertilizers for improving the growth and development of date palm.

Keywords: Date palm, arbuscular mycorrhizal fungi, compost, growth, physiological parameters, histological changes.

Introduction

Soil quality decline and abiotic and biotic stresses represent significant constraints on agriculture productivity and sustainability in the world (Al-Karaki 2013; Agegnehu et al., 2017). However, harmful quantities of chemical fertilizers are applied annually in agricultural soils (Baligar et al., 2001). Furthermore, the application of inorganic fertilizers (Baligar et al., 2001; Subhashini 2016) is not a sustainable solution for maintaining maximum yields and improving soil fertility (Rivera-Becerril et al., 2017). To minimize chemical fertilizer inputs and improve soil quality, plant productivity as well as the tolerance of plants to environmental stresses, several agroecological practices were investigated. Among these practices, biofertilizers such as organic fertilizers and arbuscular mycorrhizal fungi (AMF) are receiving much attention and should be exploited to improve agricultural production (Padilla et al., 2017; Meddich et al., 2018), reduce the use of pesticides and chemical fertilizers and protect crops and soil quality (Shen et al., 2013; Kumar et al., 2016; Júnior et al., 2018).

Several studies have shown that AMF can promote growth (El-Amri et al., 2013; Asghari and Cavagnaro 2014), mineral nutrition of plants (Kumar et al., 2015), and to enhance tolerance against biotic (Meddich et al., 2018) and abiotic stresses (Baslam et al., 2014; Symanczik et al., 2018; Ait-El-Mokhtar et al., 2019; Ben-Laouane et al., 2019). To substitute mineral fertilizers, compost represents a valuable alternative providing plants with assimilable nutrients and improving soil properties such as water retention capacity (Agegnehu et al., 2017), soil suppressiveness (Mehta et al., 2014), and soil organic matter contents (Ning et al., 2017). However, compost application is known as a beneficial organic fertilizer that promotes not only available nutrients and soil properties but also the growth, performance, physiology, yield, and fruit quality of crop plants (Dada et al., 2017; Padilla et al., 2017). Shen et al. (2013) and Liu et al. (2018) suggested that the application of compost in the soil increased the supply of organic carbon and nitrogen for the microbial community and improved soil health and crop productivity.

Date palm (*Phoenix dactylifera* L.) is a common crop adapted to arid regions (Oihabi 1991) and plays significant ecological and socio-economic roles in many countries. Indeed, many farmer families depend on date palms as a single financial source not only through its fruits, the dates, for their food and feed, but also by its wood and its young palms used especially in the artisanal sector (Arias et al., 2016). Besides, date palms with their large leaves form a microclimate that is essential for the cultivation of underlying crops such as fruit trees, vegetables, and forage species (Meddich et al., 2015b). However, agricultural soils of date palm groves are subjected to biotic and abiotic constraints as well as low soil fertility (Meddich and Boumezzough, 2017) that cause intense destruction of date palm groves and deterioration of oasis ecosystems, limiting agricultural production in these extreme environments that are in a fragile balance (El Modafar 2010; Arias et al., 2016).

Few studies reported the effect of AMF and/or compost on date palm growth and development (Souna et al., 2010; Baslam et al., 2014, Meddich et al., 2015a). To our knowledge, no data are available about the application of local compost, prepared from green waste alone and/or in combination with *R. irregular* (formerly *Glomus irregulare*) to improve growth and development traits of date palm seedlings. In this context, this study aims at assessing the effects of locally made compost in combination with the AMF *R. irregular* on the performance of the date palm. The following specific questions were asked: 1) does the application of compost and AMF improve the growth and development of date palm seedlings in a nutrient-poor substrate? and 2) does the application of compost positively affect the mycorrhization of date palm roots?

Material and methods

Preparation and application of biofertilizers

The fungal material used was *R. irregulare*, a pure AMF strain (DAOM 197198) provided by the Plant Biotechnology Institute of Montreal (Canada). *R. irregular* was enriched in propagules by co-cultivation with corn. Corn roots containing hyphae, vesicles, and spores were harvested and cut to small pieces and used as inoculum. The mycorrhization of date palm seedlings was performed by adding 4.8 g (fresh weight) of mycorrhizal corn roots near the root system (the roots of second and third orders) of each palm plant, according to Ait-El-Mokhtar et al. (2019).

The compost was prepared from grass waste as described by Meddich et al. (2016). The physicochemical properties of the compost are pH: 7.9; Olsen available phosphorus: 0.3 mg/g; total organic carbon: 30.6%; total nitrogen: 2.2%; C/N: 14 and NH_4^+/NO_3^- : 0.4.

Experimental design

Seeds of *Phoenix dactylifera* 'Boufgouss' were disinfected with sodium hypochlorite: water (1:2; v:v) and rinsed three times with sterile distilled water before germination in plastic basins containing a sandy substrate moistened with sterile distilled water. Seeds had been subsequently incubated in the oven at 38 °C for three weeks. With the age of two months (one-leaf stage), seedlings were transplanted into plastic bags containing 2.2 kg of river sand collected from "Oued Lahjar" limiting the Northeastern palm grove of Marrakesh (phosphorus (P): 0.001%, potassium (K): 0.001%, magnesium (Mg): 0.006%, Iron (Fe): 0.012%, calcium (Ca): 0.01%, sodium (Na): 0.002%, silicon (Si): 0.002%, aluminum (Al): 0.01%,

electrical conductivity (EC): 0.29 dS cm−1, and pH: 9.31) previously sterilized during 3 hours at 180°C, alone or mixed with compost at two doses of 5% or 20%. Half of the plants were amended with AMF-inoculated corn roots (mycorrhizal plants) and the other half were kept as control treatment amended with sterilized AMF-inoculated corn roots. Plants were watered and maintained at 75% field capacity. The experiment was carried out in a transparent plastic greenhouse (average temperature 24°C, average relative humidity 69%, and light intensity of 330 µm-2/s) at the Faculty of Sciences - Semlalia, Cadi Ayyad University of Marrakesh (Morocco).

The experiment consisted of six treatments: (1) control treatment not receiving any amendment (control), (2) AMF: treatment receiving only AMF, (3) C5: treatment receiving compost alone at 5%, (4) C20: treatments receiving compost alone at 20%, (5) C5+AMF: treatment receiving AMF and compost at 5%, and (6) C20+AMF: treatments receiving AMF and compost at 20%. The experiment was set up in a fully randomized design with five replicates per treatment. During the experimentation, no chemical input was applied to maintain a nutrient-deficient system and to promote the effect of compost and/or the infectivity of the applied mycorrhizal strain.

Plant growth traits and mineral analysis of plant tissues

Growth performance of date palms was assessed by measuring the length of shoots and roots, the number of leaves, leaf area, and biomass production (shoot and root dry matters). Briefly, shoots were cut shortly above the soil surface and weighed to determine to shoot fresh weight. Roots were carefully washed to remove the remaining substrate, gently dried with a soft paper towel to remove any free surface moisture, and immediately weighed to determine the root fresh weight. Two grams of fine roots and thin root sections were taken for mycorrhizal and histological assessments, respectively, as described below. Shoot and root dry weights were assessed after drying the shoots and roots in an oven-dried at 80°C till constant weight.

Shoots were excised, dried, and ground for analysis of mineral content. The total content of nitrogen (N) in plants was carried out according to the method described by Rodier (1984). Shoot powder (250 mg) was incinerated in a muffle furnace before acid extraction and the P concentration was determined using the Olsen method (Olsen and Sommers, 1982). The contents of Na, K, and Ca in shoots were measured by flame photometry (JENWAY, PFP7) as described by Wolf (1982).

Stomatal conductance measurement and chlorophyll fluorescence analysis

Four months after the experiment was started, stomatal conductance expressed in $mmol.m^{-2}.s^{-1}$ was measured on the leaves of the second rank using a portable porometer (Decagon Device, Inc. DECAGON DEVICES, version, 2012) between 11:00 am and 14:00.

During the harvest period, chlorophyll fluorescence was measured by a fluorometer (OPTI-SCIENCE, OS30p). Dark adaptation was made on the upper side of the second fully developed leaf by obscuring it for 20 min. This parameter was measured by transmission at 650 nm on a leaf area of 12.5 mm^2. The fluorescence signal was recorded for a second at an acquisition speed of 10 µs (Strasser 1995).

Effects of AMF and/or compost on histological traits

Histological cross-sections were prepared at the primary root 5 cm below the stem roots from the lateral root system of the same order. Root sections were placed in sodium hypochlorite (12% active chlorine) for 15 minutes to destroy cell contents. Sections were rinsed for 1 min to remove the excess of sodium hypochlorite and were placed in diluted acetic acid (1%) for 5 min. The double staining method with carmine green was applied to color the lignified tissue in green (suberin, lignin, and cutin) and the cellulosic cell walls in red. Excess stain was blotted away and the collected tissue was quickly washed in distilled water, dehydrated, and mounted on glass slides in water. Root sections were observed under an optical microscope (Mondolot et al., 2001).

Estimation of arbuscular mycorrhizal fungus colonization

Root samples were cleared with 10% KOH at 90 °C for 30 min, following by washing with distilled water, acidified with 2% HCl for 10 min, and stained with Trypan blue at 90°C for 20 min to identify and count AMF structures inside the root according to Phillips and Hayman (1970). Microscopic estimation of mycorrhizal root colonization rates was performed according to the method of Trouvelot et al. (1986), which provides information concerning the frequency and intensity of root colonization by AMF. Fine roots of 1 cm in length were examined under a Zeiss Axioskop 40 microscope at 40–100× magnification. A minimum of 50 segments for each replicate sample was observed to assess the structural colonization of AMF (hyphae and vesicles) associated with roots. The evaluated mycorrhizal traits were the frequency (F%) and the intensity (M%) of root AM colonization and were determined according to the following two formulas :

Mycorrhizal frequency (F%): F% = Nf x 100/N

*Nf: number of mycorrhizal fragments, N: number of fragments observed

Mycorrhizal intensity (M%): $M\% = \frac{(95n5 + 70n4 + 30n3 + 5n2 + n1)}{N}$

*n5, n4, n3, n2, n1 = number of fragments rated 5, 4; 3, 2 and 1, respectively. Class 5: more than 91%, class 4: between 51% and 90%, class 3: between 11% and 50%, class 2: less than 10%, class 1: trace and class 0: no mycorrhization.

Statistical analysis

Data were analyzed by ANOVA method, followed by Tukey's Honestly Significant Difference (HSD) test using a significance level of 5% ($p \leq 0.05$). Normality of residuals was tested using the Shapiro-Wilk test. Mycorrhizal root colonization rates were arcsin-square root transformed to fit the assumption of normal distribution. Principal Component Analysis (PCA) was performed using XLSTAT v. 2014. To obtain an effective data analysis with PCA, only the dependent variables that showed significant differences between the treatments were selected for these analyses. Analyses were performed using CO-STAT Statistical Software (CoStat 6.4).

Results

AMF and/or compost effects on date palm growth traits, mineral nutrition, and physiology

Effects on date palm growth, mineral nutrition, and physiology

Root length was significantly increased in treatments amended with 5% compost alone (C5) or in combination with AMF (C5+AMF) compared to the control (Table 1). Leaf area was increased in date palms inoculated with AMF alone (AMF) and those amended with 5% compost (C5) compared to the control (Table 1). No significant differences were observed in shoot height and number of leaves among treatments (Table 1). Moreover, shoot and root dry weights were significantly increased only in treatments C5+AMF and C5, respectively, as compared to the control (Table 1).

Table 1. Effects of compost application and inoculation with arbuscular mycorrhizal fungus (AMF) on growth (shoot height, root length, number of leaves, shoot dry weight, and root dry weight) of date palm (*Phoenix dactylifera* L.).

Treatments	Shoot height (cm)	Root length (cm)	Number of leaves (-)	Leaf area (cm^2)	Shoot dry weight (g)	Root dry weight (g)
Control	22.12±0.57^{a}	22.12±0.78^{c}	2.40±0.24^{a}	18.00±0.93^{c}	0.76±0.03^{b}	0.53±0.04^{b}
AMF	24.98±0.49^{a}	22.42±1.43^{bc}	2.60±0.24^{a}	28.47±2.02^{a}	1.02±0.05^{ab}	0.63±0.01^{b}
C5	27.22±0.77^{a}	28.44±1.29^{ab}	3.00±0.00^{a}	25.39±2.07^{ab}	1.01±0.08^{ab}	0.90±0.09^{a}
C5+ AMF	26.68±1.26^{a}	32.00±1.56^{a}	3.00±0.00^{a}	18.83±0.51^{bc}	1.08±0.06^{a}	0.62±0.05^{b}
C20	25.54±0.69^{a}	26.12±1.54^{abc}	2.60±0.24^{a}	22.09±1.82^{abc}	0.91±0.07^{ab}	0.63±0.06^{b}
C20+ AMF	26.56±1.11^{a}	23.14±1.59^{bc}	2.80±0.20^{a}	20.83±1.66^{bc}	0.96±0.07^{ab}	0.70±0.05^{ab}
F_{ANOVA}	2.262	7.993	1.6	6.196	3.298	5.268
p-Value	Ns	***	Ns	***	*	**

C5 and C20: compost amended at a dosage of 5% and 20%, respectively; AMF: inoculation with Rhizoglomus irregulare ns: not significant, *; ** and ***: significant at p≤0.05; p≤0.01 and p≤0.001. Values with the same letter are not significantly different (p≤0.05). Data represent means ± SE (n = 5).

All treatments significantly improved shoot N content compared to control (Table 2). The P content was significantly enhanced in treatments C5, AMF, and C20+AMFcompared to control seedlings (Table 2). K and Na contents were significantly increased in treatments amended with C5 or in combination with AMF compared to their respective control plants (Table 2). Ca content showed no significant differences among treatments.

Stomatal conductance was significantly affected by compost and AMF, C5+AMF, and C20 showing significantly higher values compared to the other treatments (Table 2). Compared to control plants, the chlorophyll fluorescence measured by quantum photochemistry (Fv/Fm) performance was significantly higher in all the amended treatments (Table 2).

Table 2. Effects of compost and inoculation with arbuscular mycorrhizal fungus AMF on mineral content (N, P, K, and Ca in mg/plant) and physiological parameters (stomatal conductance and chlorophyll fluorescence) of date palm (*Phoenix dactylifera* L.) seedlings.

Treatments	N	P	K	Ca	Na	gs (mmol m^{-2} s^{-1})	(Fv/Fm)
Control	0.13±0.01^{d}	0.41±0.05^{b}	3.97±0.53^{c}	3.09±0.33^{a}	2.83±0.26^{b}	28.20±2.02^{c}	0.756±0.003^{b}
AMF	0.27±0.02^{c}	0.65±0.06^{a}	6.32±0.35^{bc}	4.74±0.44^{a}	5.37±0.46^{a}	33.74±4.07^{bc}	0.788±0.004^{a}
C5	0.48±0.03^{b}	0.66±0.04^{a}	13.14±1.58^{a}	4.63±0.94^{a}	5.46±0.77^{a}	63.08±4.34^{a}	0.800±0.003^{a}
C5+ AMF	0.54±0.02^{ab}	0.60±0.07^{ab}	8.80±1.36^{ab}	3.92±0.70^{a}	5.33±0.74^{a}	59.42±4.19^{a}	0.799±0.004^{a}
C20	0.60±0.04^{a}	0.52±0.03^{ab}	8.10±0.95^{bc}	2.52±0.25^{a}	4.00±0.38^{ab}	48.40±4.61^{ab}	0.789±0.004^{a}
C20+ AMF	0.63±0.04^{a}	0.72±0.03^{a}	7.01±0.81^{bc}	2.82±0.34b^{a}	2.98±0.22^{b}	35.36±4.63^{bc}	0.789±0.006^{a}
F_{ANOVA}	53.923	5.157	8.957	2.897	5.664	12.602	15.309
p-Value	***	**	***	ns	**	***	***

Ca: Calcium; Fv/Fm: Photosynthetic quantum yield; gs: Stomatal conductance; K: Potassium, Na: Sodium; N: Nitrogen; and P: Phosphorus. C5 and C20: compost amended at a dosage of 5% and 20%, respectively; AMF: inoculation with Rhizoglomus irregulare, ns: not significant, *; ** and ***: significant at p≤0.05; p≤0.01 and p≤0.001. Values with the same letter are not significantly different (p≤0.05). Data represent means ± SE (n = 5).

Effects on the histological traits of date palm

Figure 1 shows transverse histological sections of date palm roots and the cytological structures studied at the root level, namely the number of vascular bundles (xylem and phloem), number of sclerenchyma fibers, and the status of endoderm lignification after four months of compost and AMF application. Furthermore, C5 treatment significantly increased the diameter of date palm roots compared to the control (Table 3). The number of vascular bundles (phloem and xylem) was significantly increased in C5, C20 and C20+AMF treatments compared to control seedlings (Table 3). The number of sclerenchyma fibers was significantly increased in all the amended treatments compared to the control (Table 3). The lignification of the endoderm showed the highest values in date palm seedlings amended with C5+AMF, AMF, C5, and C20 seedlings and lowest values in seedlings amended with C20+AMF (Table 3).

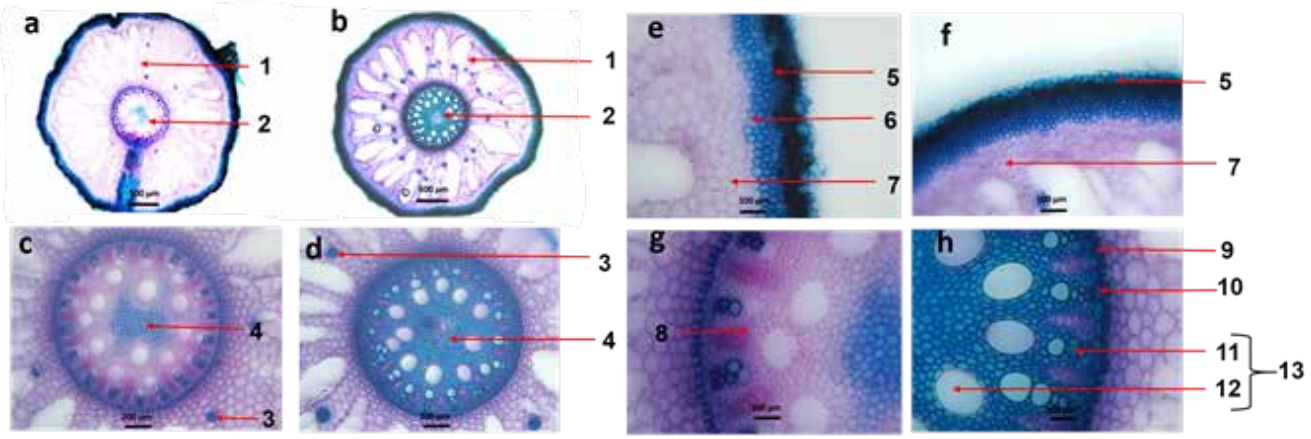

Figure 1. Histological cross sections of *Phoenix dactylifera* roots after four months of growth in the greenhouse (a and b: magnification x40, c and d: x100, and e, f, g, and h: magnification Gx200): a: entire section of control plant; b: the whole section of *Phoenix dactylifera* amended with C5+*Rhizoglomus irregulare*; c: central cylinder and part of the control bark; d: central cylinder and part of the bark of *Phoenix dactylifera* amended with C5+*Rhizoglomus irregulare*; e: part of the bark of control; f: part of the bark of *Phoenix dactylifera* amended with C5+*Rhizoglomus irregulare*; g: part of the central cylinder and part of the bark of control and h: part of the central cylinder and part of the bark of *Phoenix dactylifera* amended with C5+*Rhizoglomus irregulare* (Bark: 1: Central cylinder; 2: Sclerenchyma fiber; 3: Sclerenchyma; 4: Ectoderm; 5: Suberoid; 6: cortical parenchyma; 7: Phloem; 8: Endoderm; 9: Pericycle; 10: Protoxylem; 11: Metaxylem: 12 and Xylem: 13).

Table 3. Effects of compost application and inoculation with arbuscular mycorrhizal fungus (AMF) on the histological parameters (root diameter, number of vascular bundles, number of sclerenchyma fibers, and Endoderm lignification) of date palm (*Phoenix dactylifera* L.) roots.

Treatments	Root diameter (mm)	Number of vascular bundles	Number of sclerenchyma fibers	Endoderm lignifications
Control	2.83±0.09^{bc}	18.04±0.45^{cd}	4.24±0.25^{d}	endoderm lignified
AMF	2.63±0.10^{c}	16.24±0.63^{d}	10.76±0.31^{c}	endoderm lignified
C5	3.73±0.05^{a}	25.04±0.42^{a}	16.28±0.50^{b}	endoderm lignified
C5+AMF	2.96±0.03^{b}	19.72±0.22^{bc}	20.16±0.79^{a}	endoderm more lignified
C20	3.09±0.06^{b}	20.76±0.66^{b}	20.16±0.23^{a}	endoderm lignified
C20+AMF	2.80±0.08^{bc}	20.92±00.56^{b}	20.08±0.24^{a}	endoderm lignified
F_{ANOVA}	27.007	34.213	225.401	
p-Value	***	***	***	

C5 and C20: compost amended at a dosage of 5% and 20%, respectively; AMF: inoculation with Rhizoglomus irregulare and ***: significant at p≤0.001. Values with the same letter are not significantly different (p≤0.05). Data represent means ± SE (n = 5).

Effect of compost on the colonization of date palm roots by AMF

Mycorrhization frequency (Figure 2a) and intensity (Figure 2b) estimated in date palm roots was significantly decreased by compost application in seedlings amended with compost at 20% compared to those at 5% compost or no-compost. The treatments receiving only sterilized AMF did not show any mycorrhizal structures.

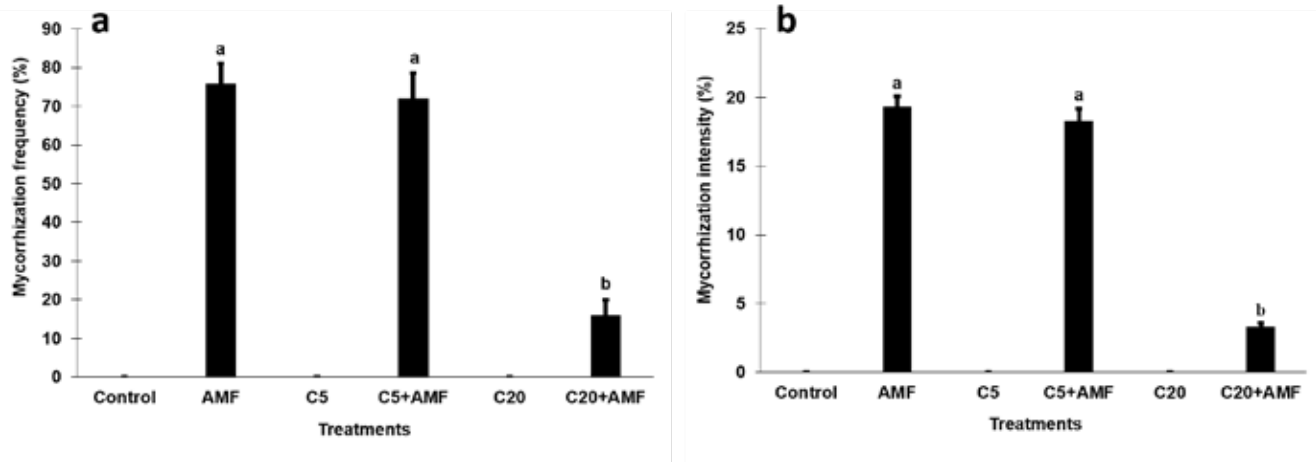

Figure 2. (a) Frequency and (b) intensity of mycorrhization of *Phoenix dactylifera* seedlings after four months of plant growth. C5 and C20: compost amended at a dosage of 5% and 20%, respectively; AMF: inoculation with *Rhizoglomus irregulare*. Values with the same letter are not significantly different (p≤0.05). Data represent means ± SE (n = 5)

Principal component analysis (PCA)

Principal components analysis was performed to obtain a smaller number (15) of linear combinations of all parameters and only those which significantly differed among treatments were analyzed by PCA. Two main components were extracted which accounted for ca. 76% of the variability observed in the data (Figure 3). The first and second components of the PCA accounted for 51% and 25%, respectively, of the total variation of the traits investigated. The eigenvectors satisfactorily characterized the six different treatments (Figure 3a), which were grouped into three main sectors of the score plot: control versus C5, C5+AMF, C20, C20+AMF and AMF treatments (separated by the first principal component) and the C5+AMF and AMF treatments versus C5, C20, and C20+AMF treatments (separated by the second principal component). The parameters stomatal conductance, chlorophyll fluorescence, shoot and root dry weights, root length, P and K contents, and the number of sclerenchyma fibers was positively correlated (at the highest level) with the first component (PC1), while mycorrhizal parameters (frequency and intensity of mycorrhization) were positively correlated with the second component (PC2). Root diameter and the number of vascular bundles were positively correlated with PC1 and negatively with PC2 (Figure 3b).

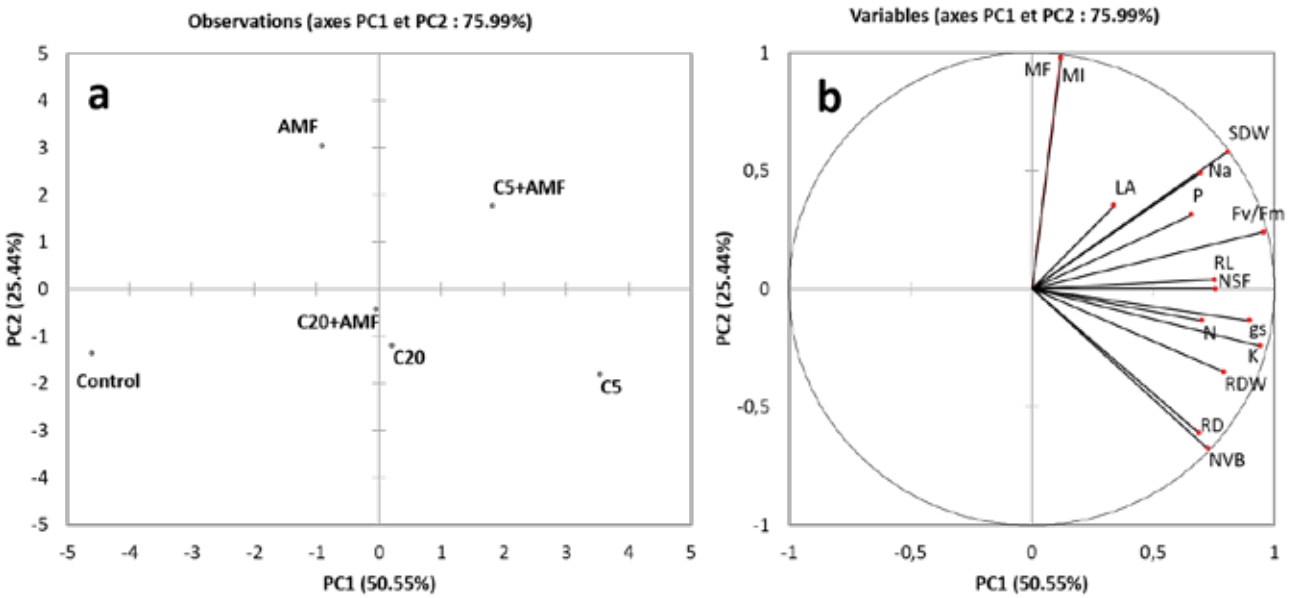

Figure 3. Principal components analysis (PCA) of (a) treatments and (b) studied parameters of *Phoenix dactylifera* seedlings after four months of growth. C5 and C20: compost amended at a dosage of 5% and 20%, respectively; **AMF:** inoculation with *Rhizoglomus irregulare;* **Fv/Fm:** photosynthetic quantum yield; **gs:** stomatal conductance, **K:** potassium; **LA:** leaf area; **MF:** mycorrhization frequency; **MI:** mycorrhization intensity; **Na:** sodium; **N:** nitrogen; **NSF:** number of sclerenchyma fibers; **NVB:** number of vascular bundles; **P:** phosphorus; **RD:** root diameter; **RDW:** root dry weight; **RL:** root length; and **SDW:** shoot dry weight.

Discussion

Biofertilizers such as green waste compost and AMF are known as bio-based products that can contribute to the improvement of plant growth and performance. However, only a few studies have tested their efficacy in the context of early date palm cultivation. Thus, the first objective of this study was to evaluate the single and double effects of local compost and/or *R. irregulare* on growth and development of date palm seedlings. Our results revealed that compost applied at low dose alone (5%) or in combination with AMF can effectively improve seedlings' performance in terms of physiology (stomatal conductance), growth (shoot and root dry weights, leaf area, and root length), mineral nutrition (N, P, and K), and root histology (root diameter and the number of vascular bundles and sclerenchyma fibers).

The values of growth parameters such as leaf area, root length, and shoot and root dry weights of date palm seedlings were increased either by compost or AMF alone or in combination. Similarly, Barje et al. (2016) observed that compost produced from olive mill waste increased leaf area, root length, and shoot and root dry weights of the date palm. Also, El Kinany et al. (2018) reported the growth-promoting effects of compost when date palm was grown in sand amended with 25% compost. Besides, previous studies testing the effect of AMF on date palm growth showed that the inoculation with a consortium of *Glomus* sp. pl., *G. intraradices* or with a native AMF consortium increased the growth of seedlings particularly leaf area, root length, and shoot and root weights under nursery and greenhouse conditions (Zougari-Elwedi et al., 2012; Baslam et al., 2014; Meddich et al., 2018). Furthermore, Souna et al. (2010) demonstrated a positive effect of the dual use of compost and *G. intraradices* on the growth of date palm, especially shoot dry weight.

The observed beneficial effects of compost alone or in combination with *R. irregulare* on date palm growth may result from the improved uptake of mineral nutrients as reflected by enhanced N, P, K, and Na contents in date palm shoots. This is consistent with other studies reporting an increment of shoot nutrient contents after compost amendment and inoculation with AMF. Meddich et al. (2015a) observed an increase in shoot N, P, and K contents after inoculation with three individual AMF species and with an AMF complex grown for nine months in the greenhouse. El Kinany et al. (2018) have shown that compost amendment significantly increased K content in date palm shoots grown for twelve months under greenhouse conditions. Similar effects of compost and AMF were observed in studies on carob trees (Manaut et al., 2015) and olive plants (Bati et al., 2015). Additionally, we observed an increase in shoot Na content when biofertilizers were applied alone or in combination. Subbarao et al. (2003) considered Na+ among the elements that promote plant growth, thereby suggesting its classification as a functional nutrient. Previous studies have shown a significant increase in Na content in mycorrhizal date palm (Ait-El-Mokhtar et al., 2019) and woody plants amended with compost (Marosz 2012). This increase of Na and other nutrients such as N, P, and K either results from their direct uptake from the compost via the plant roots or their delivery *via* the AMF mycelium.

In the present study, stomatal conductance and chlorophyll fluorescence were increased in date palm seedlings amended with compost (C5, C20) alone or combined with AMF (C5+AMF), which could improve CO_2 assimilation and photosynthetic activity. Recent studies have reported that the application of the compost alone and/or combined with AMF improved the geranium and sorghum photosynthetic activity through enhanced chlorophyll contents, carotenoids, as well as leaf water contents (Akhter et al., 2015; Abd El-Mageed et al., 2018). Other studies demonstrated that inoculation with *R. irregulare* improved chlorophyll concentration, water status along with an increase in gas exchange in ginseng and poplar plants (Cho et al., 2009; Liu et al., 2015). This result could be explained by the increase in shoot K contents as it is an essential element playing an important role in photosynthetic activities by triggering the opening or closing of stomata (Cakmak 2005).

In addition to the agro-physiological and mineral nutrition changes observed in date palm, the root architecture in terms of root diameter, the number of vascular bundles (xylem and phloem), and sclerenchyma fibers, and endoderm lignification were improved by compost amendment and/or AMF inoculation. The increase of root thickness and phloem and xylem bundles is an indicator of improved root growth, which is supported by the increase in root dry weight of date palms amended with a low dose of compost. Higher numbers of phloem and xylem bundles can promote a better exchange between roots and shoots for water/nutrients and photosynthetic products (Konrad et al., 2018; El Amerany et al., 2019) and might have resulted into the observed increase in shoot P and K contents. Additionally, it has been observed that compost and

AMF alone or in combination increased the number of sclerenchyma fibers as well as endoderm lignification, thus increasing the rigidity of the roots which can ameliorate the impermeability of the root system to water and increase the defense of plants against pathogens and herbivores (Benjelloun et al., 2014). Furthermore, Chen et al. (2013) and Hashem et al. (2016) reported that cucumber and acacia plants inoculated with AMF increased the lignin content in mycorrhizal plants. Other studies showed that application of compost or inoculation with AMF increased the production of the antioxidant enzymes such as peroxidase (POX) and polyphenol oxidase (PPO) in date palm, cucumber, and pot marigold plants (Chen et al., 2013; Baslam et al., 2014; Meddich et al., 2015a; Ait-El-Mokhtar et al., 2019; Khosravi Shakib et al., 2019), which mediates the lignin accumulation in plants (Whetten et al.,1998). Similarly, the lignification of endoderm and sclerenchyma fibers of date palm under the combined treatment (C5+AMF) might also have resulted from the activation of antioxidant enzymes biosynthesis.

The results of mycorrhization frequency and intensity showed the ability of *R. irregulare* alone or combined with compost at a low dose, to extensively colonize the roots of the date palm. This is following other studies investigating date palm root colonization by AMF. Indeed, Meddich et al. (2018) and Baslam et al. (2014) found the roots of date palm grown under greenhouse conditions to be colonized between 50% and 100% depending on the inoculated AMF species. Similar root colonization frequencies were observed when date palms were grown under field conditions (Bouamri et al., 2006; Zougari-Elwedi et al., 2016). In contrast, it was observed that compost amended at high doses inhibited date palm root colonization by *R. irregulare*. This could have resulted from the high nutrient input provided by the higher compost dosages, which could allow the plant to be less dependent on the AMF symbiosis to acquire sufficient amounts of nutrients. Similar results have been observed for tomato and soursop plants, showing hence reduced colonization rates following 30% and 50% compost application (Copetta et al., 2011; Júnior et al., 2018).

Figure 3b illustrates the relationship between the studied parameters. The increase in shoot mineral nutrients (P and K) showed the same tendency as the biomass production (shoot and root dry weights), the physiological (stomatal conductance and chlorophyll fluorescence), and histological parameters (root diameter, number of vascular bundles and sclerenchyma fibers), which indicates the high affinity among these parameters.

Conclusions

Altogether, it can be suggested that the application of compost at low doses alone and in combination with AMF could play an important role in promoting date palm growth and development, especially via increased nutrient uptake. Thus, the input of chemical fertilizers can be reduced or even completely replaced by adding organic matter to the systems when transplanting date palms into field soils generally characterized by low soil organic matter contents and fertility.

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Chapter 4

Soil Inoculation with Symbiotic Microorganisms (Mycorrhizas and Rhizobium) and Compost Promote Date Palm Performance under Drought condition: From controlled-condition to open-field system

Mohamed Anli[1], Marouane Baslam[2,3], Abderrahim Boutasknit[1], Mohamed Ait-El-Mokhtar[1], Raja Ben-Laouane[1], Youssef Ait Rahou[1], Mohamed Hafidi[4] and Abdelilah Meddich[1*]

[1]Laboratoire d'Agro-Alimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Facultédes Sciences Semlalia, Université Cadi Ayyad, BP2390 Marrakech, Maroc.
[2]Laboratory of Biochemistry, Faculty of Agriculture, Niigata University, Niigata, Japan.
[3]Department of Life and Food Science, Graduate School of Science and Technology, Niigata University, Niigata, Japan.
[4]Laboratoire de Biotechnologies Microbiennes, Agrosciences et Environnement (BioMAgE), Faculté des Sciences Semlalia, Université Cadi Ayyad, BP511 Marrakech, Maroc.

Corresponding author: a.meddich@uca.ma

Abstract

Drought stress is one of the main constraints threatening crop production. Plant root associations with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) can improve plants' nutritional status and health and mitigate the negative effects of drought stress. In the current study, we implemented an adapted management program to improve date palm development and its tolerance to water deficit by using single or multiple combinations of exotic and native AMF, and/or selected consortia of PGPR, and/or composts (C) from grasses and green waste in greenhouse and field conditions. We analyzed the potential for physiological functioning (photosynthesis, water status, osmolytes, mineral nutrition) to evolve in response to drought since this will be a key indicator of plant resilience in future environments. As result, the dual-inoculation of AMF/PGPR amended with composts alone or in combination boosted the biomass accumulation under water deficit conditions to a greater extent than in non-inoculated and/or non-amended plants. Both single and dual biofertilizers improved physiological parameters byelevating stomatal conductance, photosynthetic pigments (chlorophyll and carotenoids content), and photosynthetic efficiency. The dual inoculation and compost significantly enhanced, especially under drought stress, the concentrations of sugar and protein content, and antioxidant enzymes (polyphenoloxidase and peroxidase) activities as a defense strategy as compared with controls in greenhouse and field conditions. Inoculation of date palms with AMF alone or in combination with PGPR and composts mitigated the stress as reflected by plant growth enhancement, phosphorus and nitrogen uptake and plant water relationship improvement under drought stress. We summarize the extent to which the dual and multiple combinations of microorganisms can overcome challenges related to drought by enhancing plant physiological responses.

Keywords: Arbuscular mycorrhizal fungi, compost, PGPR, water stress, physiological responses, tolerance, date palm vitroplants, greenhouse and open field conditions.

Introduction

Drought damaging effects to crops represents an environmental severe stress for agriculture, particularly in the arid and semi-arid regions of the world (Maia et al., 2018). Due to the recent and increasing impacts of climate change, the adverse impacts of drought stress on various crops will further intensify a serious threat to global food security demands (Lamaoui et al., 2018; Ait-El-Mokhtar et al., 2019a). In that context, soil moisture required for plant production is strongly limited, and the availabilities and transport of nutrients to plants are reduced due to water shortage, when the soil is exposed to extensive dry periods (Vurukonda et al., 2016; Kanwal et al., 2017; Hao et al., 2019a). In addition, drought stress can induce changes in plants' physiological, biochemical as well as molecular functions, and ultimately affects plants productivity (Augé et al., 2014; Symanczik et al., 2018; Baraldi et al., 2019).

On a higher scale, when plants are exposed to drought, the antioxidative defense system is altered as a consequence of cell dehydration leading to an accumulation of reactive oxygen species (ROS) in cells such as hydrogen peroxide (Duc et al., 2018; Begum et al., 2019; Li et al., 2019). The ROS production interferes with the plants' photosynthesis machinery and subsequently impairs with the photosynthetic activity and decreases chlorophyll concentrations (Becklin et al., 2016; Duo et al., 2018; Li et al., 2019). Under such circumstances, improvement in crop productivity remains an important challenge to meet food security demands of an ever-increasing population and therefore requires a better understanding of plants adaptation strategies to mitigate drought stress, especially in date palm trees.

The date palm (*Phoenix dactylifera* L.) is a dioecious perennial long-lived monocotyledonous tree of significant ecological and socio-economic importance in most arid and semi-arid regions where its cultivation mainly dominates (Chao and Krueger, 2007). Date fruit production is ranked after cereal production, the second most important staple food crop produced and consumed worldwide (Arias et al., 2016; Puch-Hau et al., 2016). The fruit is valued due to its high nutritious values, and rich carbohydrates, proteins, fibers, fats, various vitamins, and other minerals (Arias et al., 2016; Al-Shwyeh 2019). Other environmental benefits of date palm trees are linked to the abilities to use their leaves as mulch and shelter for ground crops and contribute to the development of a microclimate enabling the establishment of other associated crops (Meddich et al., 2020). However, under their growing environmental conditions, high temperatures, water stress, and salinity are adverse factors that negatively contribute to poor plant establishment, growth and consequently to a decrease in fruit production (Arias et al., 2016; Meddich and Boumezzough, 2017; Whitman 2019). Various diseases and pests are also reported to affect date fruit production and threaten the sustainability of the crop with significant negative economic return (Hadrami et al., 2011; Yaish and Kumar, 2015).

To cope with the drastic environmental conditions, especially drought stress, plants initiate both rapid and long-term tolerance strategies grouped into escaping, avoiding, and tolerating mechanisms (Ait-El-Mokhtar et al., 2019b; Begum et al., 2019; Ben-Laouane et al., 2019). Alternatively, plants' roots associated with beneficial soil microbe' groups such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) to mitigate the stress and improve their health and productivity (Raklami et al., 2019; Anli et al., 2020b, 2020a; Boutasknit et al., 2020; El Amerany et al., 2020). AMFs colonized plants are capable of increasing the synthesis of biomolecules, in particular low weight plant metabolites (i.e., proline and sugar) that act as osmolytes during the osmotic stress and contribute to the drought and/or water stress mitigation (Baslam et al., 2014; Zhang et al., 2018; Boutasknit et al., 2020). PGPRs contribute to nutrient recycling, solubilization of nutrients such as phosphorus (P) and potassium (K), degradation of organic matter in the soil, phytohormone and antibiotics production, improvement of the soil structure and aggregation, and confer plant tolerance's to environmental stresses (Dimkpa et al., 2009; Bompadre et al., 2014). Furthermore, the integration of management practices exploring the use of composts can represent an alternative towards the improvement of date palm adaptation to stressful environmental conditions (Meddich et al., 2015; Ortuño et al., 2018; Anli et al., 2020b). The use of local composts representsan eco-friendly alternative for plant growth, mineral nutrition, plant resistance to different environmental stresses increasing the photosynthetic activity (Tartoura 2010; Hirich and Jacobsen, 2014; Abd El-Mageed et al., 2019; Khosravi Shakib et al., 2019).

In the current research, we explored the agro-physiological and biochemical responses involved in drought adaptation in date palms, and the functionality of the single and dual-use of selected strains of PGPR and native and exotic AMF with or without the addition of two composts. The objective of this study was: i) to evaluate the morpho-physiological basis of drought responses in date palms under implementation of eco-friendly cultivation practices, ii) to assess the combined application of PGPR, AMF and compost on tissues cultured date palm growth and tolerance under well-watered and drought conditions, and iii) to evaluate the behavior of date palm plants treated with the same biofertilizers in the open field subjected to severe water stress. The results obtained here will provide a deeper understanding of the mechanisms of date palm tolerance to long-term drought stress as well as paving the way for identification of the best factors that led to successful outcomes in the biofertilization experiments for other crops.

Materials and methods

Biological materials

Young plants of two leaves growth stage of date palm generated from in vitro vegetative multiplication were obtained from the commercial laboratory of "Domaine Agricole El Bassatine", Meknes, Morocco. The vitro plants were propagated from the elite variety "Boufgouss". Two types of AMF inoculants were used in our experiment: (i) an exogenous AMF strain (*Rhizophagus irregularis*, DAOM 197198) was obtained from the Plant Biotechnology Institute of Montreal, Canada and (ii) an indigenous consortium of AMF selected from the Tafilalet palm grove located 500 Km southeast of Marrakesh and containing a mixture of native species: (i) *Glomus* sp. (15 spores/g of substrate), (ii) *Sclerocystis* sp. (9 spores/g of substrate), and (iii) *Acaulospora* sp. (1 spore/g of substrate) (Meddich et al., 2015). The inoculum was propagated using maize (*Zea mays* L.) as a host plant and the inoculum was prepared using spores enriched with infective root segment of maize plants.

The PGPR inoculum contains two consortia of four PGPR strains (Z1, Z2, Z4, and ER21) originally isolated from date palm rhizosphere in the Tafilalet region (31°47'20.8"N and 4°14'59.3" W and elevation: 1,046 m). The inoculum was prepared by propagating the two PGPR consortia in Tryptic Soy Broth (TSB) liquid medium at 28 °C. Viable cell concentrations were determined at 600 nm using a UV visible spectrophotometer (UV-3100PC spectrophotometer, VWR). The quantification in vitro of plant growth-promoting traits of the strains used was examined by standard protocols: phosphate solubilization was performed by the production of halo on agar medium as described by Alikhani et al. (2006) and the tolerance to water deficiency was tested by the resistance to polyethylene glycol. A confrontation assay was carried out to confirm the absence of inhibition between the four strains. The PGPR characteristics of the four strains are listed in Table1.

The composts used were prepared from grass waste (C1) and a mixture of green waste (C2). The composts were produced from organic wastes collected from municipality gardens in Marrakesh city as described by Meddich et al. (2016). Briefly, organic parts of the wastes were piled in the composting platform and allowed to the organic part to decompose. The composting process was conducted for three months and no additional inorganic fertilizers products were added. The physicochemical properties of the mature compost are illustrated in Table 2.

Table1. Phosphate solubilization and resistance to polyethylene glycol (tolerance to water deficiency) of the four tested PGPR strains (Z1, Z2, Z4, and ER21).

Activity	Z1	Z2	Z4	ER21
Phosphate solubilization	+	+	+	+
Resistance to polyethylene glycol 6000	+	+	-	-

Table 2. Physico-chemical properties of the composts used in this study.

Composts	Ph	EC (mS/cm)	COT (%)	NTK (%)	C/N	P (mg/g)
Compost (C1)	7.86	7.10	30.65	2.19	14.00	0.270
Compost (C2)	7.80	8.50	27.24	1.32	20.64	0.266

EC: electrical conductivity, TOC: total organic carbon, TKN: total Kjeldahl-nitrogen, C/N: carbon-to-nitrogen ratio, P: phosphorous.

Acclimatisation of date palm vitro plants, AMF and PGPR inoculation procedure, and supplementation of compost

The vitro plants were acclimatized for 3 months under greenhouse condition with day/night temperature of 25.5/17°C, fluorescent lighting (500 µmol.m^{-2}.s^{-1}), 16/8 h light/dark photoperiod and relative humidity of 68.5% as previously described by Anli et al. (2020b). The soil used to growth the vitro plants were collected in the Urban area of the Commune of Marrakesh (31°39'07.3"N 8°04'04.3"W). The soils were passed through 4 mm sieve and autoclaved (at 180°C for 3h and repeated 3 times consecutively). The soil had the following chemical properties: available phosphate, 11 mg kg-1; total nitrogen, 1%; organic matter, 1%; total organic carbon, 0.58%; electrical conductivity (EC), 0.19 mS/cm; and pH, 8.6. Each plantlet was transferred to a 2.4 L plastic pot filled with 2.6 kg of the sterile soil. For the pots to which compost was supplemented, 2.25 kg of soil was mixed with 0.130 kg of compost (soil/compost ratio of 1:5, w:w) and homogenized following the procedure described by Meddich et al. (2015). Date palm plants were randomly arranged in the greenhouse and maintained at 75% field capacity by daily watering.

The inoculation of date palm was performed by adding 40g of the inoculums (roots and substrate containing spores) to the date palm root system. Non-mycorrhizal (NM) treatments received an equal quantity of filtered inoculum in an attempt to restore other soil free-living microorganisms accompanying the AMF. The filtrate for each pot was obtained by passing the mycorrhizal inoculum in 20mL of distilled water through a layer of 15- to 20- µm filter papers (Whatman, GE Healthcare, Buckinghamshire, UK).

The PGPRs inoculation was done twice at 15 days inoculation intervals by injecting 4 mL of the bacterial suspension in contact with the roots at a concentration of 5 x 109 CFU/mL. The date palm plants were grown under controlled conditions for an additional three months under well-watered conditions to ensure successful acclimatization. Afterwards, the drought stress was imposed by withholding irrigation in drought-imposed plants during six months for greenhouse experiment and nine months for plants destined to field experiment. The positions of plants in the greenhouse were regularly re-arranged at monthly intervals to ensure homogeneity in received light and solar radiation during the experiment.

Experimental design and greenhouse experiment

After three months from application of biofertilizers, two water regimes were imposed 75% and 25% of field capacity (FC). Then, after 6 months of greenhouse cultivation under these 2 water regimes (9 months after biofertilizer application), we proceeded to harvest the palm trees and to measure the following parameters: Growth (number of leaves, aerial height, root elongation, leaf area, total dry weight), mineral (total phosphorus and total nitrogen of the leaves), physiological (stomatal conductance, leaf water potential, chlorophyll fluorescence, chlorophyll a, b and total, and carotenoids), biochemical (sugars,

proteins, polyphenol oxidase (PPO), peroxidase (POX), Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) in the leaves) traits. The experiment was carried out in a fully randomized design with 6 biological replicates for each treatment (in a total of 54 treatments) and all plants were randomly placed in the greenhouse (Table 3).

Table 3. Different treatments (and their nomenclature) applied in greenhouse.

Treatments	Water regime		
Control	75% FC	25% FC	Plants non-amended with compost and no-inoculated with AMF/PGPR
B1			Plants non-amended with compost, no-inoculated with AMF, and inoculated with PGPR consortia B1 (Z1+Z2)
B2			Plants non-amended with compost, no-inoculated with AMF, and inoculated with PGPR consortia B2 (Z1+Z2+Z4+ER21)
C1			Plants amended with compost C1 (grass waste), no-inoculated with AMF/PGPR
C1 + B1			Plants amended with compost C1, no-inoculated with AMF, and inoculated with PGPR consortia B1
C1 + B2			Plants amended with compost C1. no-inoculated with AMF, and inoculated with PGPR consortia B2
C2			Plants amended with compost C2 (mixture of green waste) and no-inoculated with AMF/PGPR
C2 + B1			Plants amended with compost C2, no-inoculated with AMF, and inoculated with PGPR consortia B1
C2 + B2			Plants amended with compost C2. no-inoculated with AMF and inoculated with PGPR consortia B2
AMF1			Plants inoculated with AMF1 (exogenous *R. irregular*), non-amended with compost, and no-inoculated with PGPR
AMF1 + B1			Plants inoculated with AMF1, inoculated with PGPR consortia B1, and non-amended with compost
AMF1 + B2			Plants inoculated with AMF1, inoculated with PGPR consortia B2, and non-amended with compost
AMF1 + C1			Plants inoculated with AMF1, amended with C1, and no-inoculated with PGPR
AMF1 + C1 + B1			Plants inoculated with AMF1, inoculated with PGPR consortia B1, and amended with C1
AMF1 + C1 + B2			Plants inoculated with AMF1, inoculated with PGPR consortia B2, and amended with C1
AMF1 + C2			Plants inoculated with AMF1, amended with C2, and no-inoculated with PGPR
AMF1 + C2 + B1			Plants inoculated with AMF1, inoculated with PGPR consortia B1, and amended with C2
AMF1 + C2 + B2			Plants inoculated with AMF1, inoculated with PGPR consortia B2, and amended with C2

Treatments	Water regime
AMF2	Plants inoculated with AMF2 (indigenous consortium of AMF), non-amended with compost, and no-inoculated with PGPR
AMF2 + B1	Plants inoculated with AMF2, inoculated with PGPR consortia B1, and non-amended with compost
AMF2 + B2	Plants inoculated with AMF2, inoculated with PGPR consortia B2, and non-amended with compost
AMF2 + C1	Plants inoculated with AMF2, amended with C1, and no-inoculated with PGPR
AMF2 + C1 + B1	Plants inoculated with AMF2, inoculated with PGPR consortia B1, and amended with C1
AMF2 + C1 + B2	Plants inoculated with AMF2, inoculated with PGPR consortia B2, and amended with C1
AMF2 + C2	Plants inoculated with AMF2, amended with C2, and no-inoculated with PGPR
AMF2 + C2 + B1	Plants inoculated with AMF2, inoculated with PGPR consortia B1, and amended with C2
AMF2 + C2 + B2	Plants inoculated with AMF2, inoculated with PGPR consortia B2, and amended with C2

Experimental design and field transplantation of date palms

After 12 months of treatment with the same biofertilizers in a controlled greenhouse and facing the same water levels (75 and 25% FC for nine months), a lot of vitro-plants from the remaining 6 replicates per treatment was randomly transplanted in the open field, in an area of 10,000 m². The field experiment was established at Saada (31°37'39.9'' N and 08°07'46.7'' W) around Marrakesh region in Morocco. The climate is characterized as semi-arid with a maximum temperature of 36.2 °C, minimum temperature of 12.8 °C and the average annual temperature of 19.5 °C. Average annual rainfall recorded during the lifespan of the field experiment was 248 mm. The analyzed physical characteristics of the field site soils were sand, 52%; clay, 24% and loam, 24%. The chemical properties of field soils were available soil phosphorus, 31 mg kg-1; total nitrogen, 0.15%; organic matter, 1.3%; total organic carbon, 0.80%; electrical conductivity (EC), 1.7 mS/cm; and pH, 7.9.

The design used to establish the field experiment was a factorial arrangement with two main factors; factor 1: water regime (W) testing two levels, well-watered (WW) and drought (D) conditions and factor 2: biofertilization treatments with 8 levels: (1) control (without inoculation and compost supplementation), (2) *R. irregularis* inoculated (AMF1); (3) PGPR consortium inoculated (B1), (4) compost supplemented (C1), (5) AMF1 + B1, (6) AMF1 + C1, (7) B1 + C1 , and (8) AMF1 + B1 + C1 (Table 4). Six replicates for each treatment were established. The date palms were transplanted at 5 × 5 m distance between lines and rows. Under field conditions, the volume of water during irrigation was adjusted to 32 L per hour and 16 L per hour for the WW and D conditions, respectively. Plants were kept under these two water regimes and daily watered until the harvest. Morphological, biochemical, and physiological parameters of the date palm were measured on sampled leaves and roots at the harvest.

Table 4. Different treatments (and their nomenclature) applied in field experiment.

Treatments	Water regimes		
Control	WW	D	Plants non-amended with compost (C1) and no inoculated with *R. irregularis* (AMF1)/PGPR (B1)
B1			Plants non-amended with compost C1, no inoculated with AMF1, and inoculated with PGPR consortium B1 (Z1+Z2)
C1			Plants amended with compost C1, no inoculated with AMF1/PGPR (B1)
C1+B1			Plants amended with compost C1, no inoculated with AMF1, and inoculated with PGPR consortium B1
AMF1			Plants inoculated with AMF1, non-amended with compost C1, and no inoculated with PGPR consortium B1
AMF1+B1			Plants inoculated with AMF1, inoculated with PGPR consortium B1, and non-amended with compost C1
AMF1+C1			Plants inoculated with AMF1, amended with C1, and no inoculated with PGPR consortium B1
AMF1+C1+B1			Plants inoculated with AMF1, inoculated with PGPR consortium B1, and amended with compost C1

WW: Well-Watered and D: Drought.

Data collection and analyses

Stomatal conductance, quantum yield of photosystem II and leaf water potential

Stomatal conductance (g_s) was measured at the two youngest fully mature date palm leaves between 10:00 AM and 01:00 PM using a porometer (CI-340, Handheld Photosynthesis System, WA USA) following the procedure described by Harley et al. (1992). Chlorophyll fluorescence (PSII (F_v/F_m) was measured by a fluorometer (OPTI-SCIENCE, OS30p). Dark adaptation was made on the upper side of the second fully developed leaf by obscuring them for 20 minutes. This parameter was measured by transmission at 650 nm on a leaf area of 12.5 mm2. The fluorescence signal was recorded for a second at an acquisition speed of 10 µs (Strasser, 1995). The water potential (Ψ_w) of mature leaves (i.e. positioned in the third from the apex) was measured between 06:00 AM and 08:00 AM using a pressure chamber (Model 600-EXP Super Pressure Chamber, PMS instrument, Albany, USA). The leaf water potential was measured immediately after the gas exchange measurement and values are expressed in MPa.

Photosynthetic pigments quantification

The chlorophyll pigment (chlorophyll a, b, total chlorophyll, and carotenoids) contents were measured as described by Arnon (1949). The different pigments were extracted in homogenized leaves tissues in 80% acetone (v/v) and centrifuged at 3000 × g for 10 minutes. The absorbance of supernatant extracts was measured at 480, 645, and 663 nm, respectively using a UV visible spectrophotometer (UV-3100PC spectrophotometer, VWR).

Growth performance

The growth performance of date palm was assessed by measuring the leaf number, leaf area, shoot height, root length, and the shoot and root dry weights. The dry weight of shoots and roots were recorded from oven-dried samples at 80 °C for 48 h. Also, the shoot and root P concentrations were measured. Briefly, 0.5 g of samples finely ground through 1 mm mesh, was digested in concentrated H_2SO_4 at 500°C. The P concentration in the extracts was measured using a spectrophotometer (UV-3100PC spectrophotometer, VWR, China), at 620 nm as described by Murphy and Riley (1962). The concentrations of N in the shoot and root extracts were measured according to the method described by Novozamsky et al. (1983).

Total soluble sugars content of date palm

For measurement of the total soluble sugar (TSS), the method of Dubois et al. (1956) was followed. Fresh shoot samples of 0.1g were homogenized in 80% ethanol solution (v/v) and ground in a cold mortar. Each extract of 0.2 mL was supplemented with 0.2 mL of phenol and 1 mL of concentrated sulfuric acid (v:v) and allowed to cool at room temperature for 15 minutes. The TSS content was measured at 485 nm using a UV visible spectrophotometer (UV-3100PC spectrophotometer, VWR, China).

Antioxidant enzymatic, peroxidase (POX), polyphenol oxidase (PPO) activities, and total soluble protein contents of date palm

For enzymatic activity, 0.1g of a fresh sample of the shoot and root tissues was homogenized in a cold mortar with 4 mL of 0.1 M phosphate buffer (pH 7.8) containing 5% polyvinylpolypyrrolidone. The homogenized substrate was centrifuged at 15,000 ×g (Model MIKRO 220|R centrifuge, Regenstein Centrifuge Co., New York, NY, USA) for 15 min at 4 °C and the antioxidant enzymatic activity measured in 2 mL supernatant following the method of Tejera García et al. (2004). The POX and PPO activities were measured as described following Hori et al. (1997). In brief, 0.1 mL extract was used in 3 mL reaction mixture containing 100 mM phosphate buffer (pH 7.8), 20 mM guaiacol and 40 mM H_2O_2 to initiate the chemical reaction for 3 min. The POX activity was read at 470 nm after 3 min using a UV visible spectrophotometer (UV-3100PC spectrophotometer, VWR, China). The PPO was measured in 20mM catechol in 0.1M phosphate buffer (pH 7) solution to which 100 µL of the extract was added. The absorbance was read at 420nm after 3 min after cooling at room temperature. A modified procedure from Bradford (1976) was used to measure the total soluble protein contents of the different samples.

Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) contents of date palm

The H_2O_2 concentration in leaf and root samples were measured using the procedure described by Velikova et al. (2000). Frozen samples of 0.25 g were homogenized with 5 ml 10% (w/v) trichloroacetic acid and centrifuged at 15,000 ×g (Model T centrifuge, Regenste in Centrifuge Co., New York, NY, USA) for 15 min at 4°C. 0.5 mL of extract was recovered with 0.5 ml potassium phosphate buffer (10 mM, pH 7) and 1 mL of iodic potassium (1 M) was added in the final solution. H_2O_2 concentrations were measured at 390 nm absorbance after one hour of incubation at room temperature using a UV visible spectrophotometer (UV-3100PC spectrophotometer, VWR, China). Malondialdehyde (MDA) content in leaves was estimated by homogenizingthe frozen leaf powder subsamples (0.25g) in 10 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuging at 18,000xg for 10 min as described by Madhava Rao and Sresty

(2000). Two milliliters of supernatant were mixed with 2 mL of 20% TCA containing 0.5% Thiobarbituric acid (TBA). The mixture was then heated in a water bath at 100 °C for 30 min and immediately cooled in an ice bath. The absorbance was read at 532 nm. The nonspecific turbidity was corrected by subtracting A600 from A532, and the MDA content was calculated as follows: [MDA] = 6.45 (A_{532}–A_{600}) - 0.56A_{450}.

Microscopic observation of mycorrhizal structures

Root samples were stained to observe the AMF structures inside the root using the method of Phillips and Hayman (1970). Microscopic assessment of mycorrhizal root colonization rates was performed according to the method of Trouvelot et al. (1986). The mycorrhizal colonization frequency (MCF) was determined using the following formula:

MCF = [(number of colonized root fragments/ number of total root fragments] × 100

The mycorrhization intensity (MI) was determined using the following formula:

MI = [(mycorrhization index × number of total root fragments)/number of colonized root fragments)×100

The mycorrhizal index was assigned from 0 to 5, where 0 represents no colonization, 1: traces of colonization, 2: colonization rate less than 10%, 3: colonization between 11 to 50%, 4: colonization rate from 51 to 90%, and 5: colonization rate from 91 to 100 %, respectively.

Statistical analysis

Statistical analysis of data was performed using the CO-STAT (CoStat 6.4) and SPSS 10.0 software's. A two-way analysis of variance (ANOVA) was performed to test the water regimes and biofertilization treatments effects and their interaction on the measured parameters. When the ANOVA test denoted a significant Fischer's (F) value at $p< 0.05$, a Tukey's honestly significant difference test was used to compare treatment means. Data presented are means ± standard error (SE). Data from mycorrhizal frequency and intensity were arcsin-square root transformed to fit data to a normal distribution. The Principal Components Analysis (PCA) was performed to fit data into patterns to be easily interpreted and visualized under the WW and D conditions. The PCA was conducted on a correlation matrix to detect variables which could distinguish clusters of experimental cases. The PCA was performed using XLSTAT v. 2014 and the biplots of the first principal components (PC1 and PC2) are presented.

Results of greenhouse experiment

Mycorrhization parameters

Our results showed that no mycorrhizal structure was observed in the roots of non-treatment controls. The frequency and intensity of AMF in date palm roots were significantly affected by water regime, biofertilization treatments and their interaction (Table 5). The plants inoculated with AMF, especially for AMF2 and AMF1, without compost and PGPR showed the higher root colonization intensity compared to plants treated with compost and PGPR (Figures 1A and 1B). AMF infection frequency and intensity showed no significant difference between date palm inoculated with AMF alone orcombined with PGPR and/or composts (bi- and tripartite combinations) under drought stress conditions (Figures 1A and 1B).

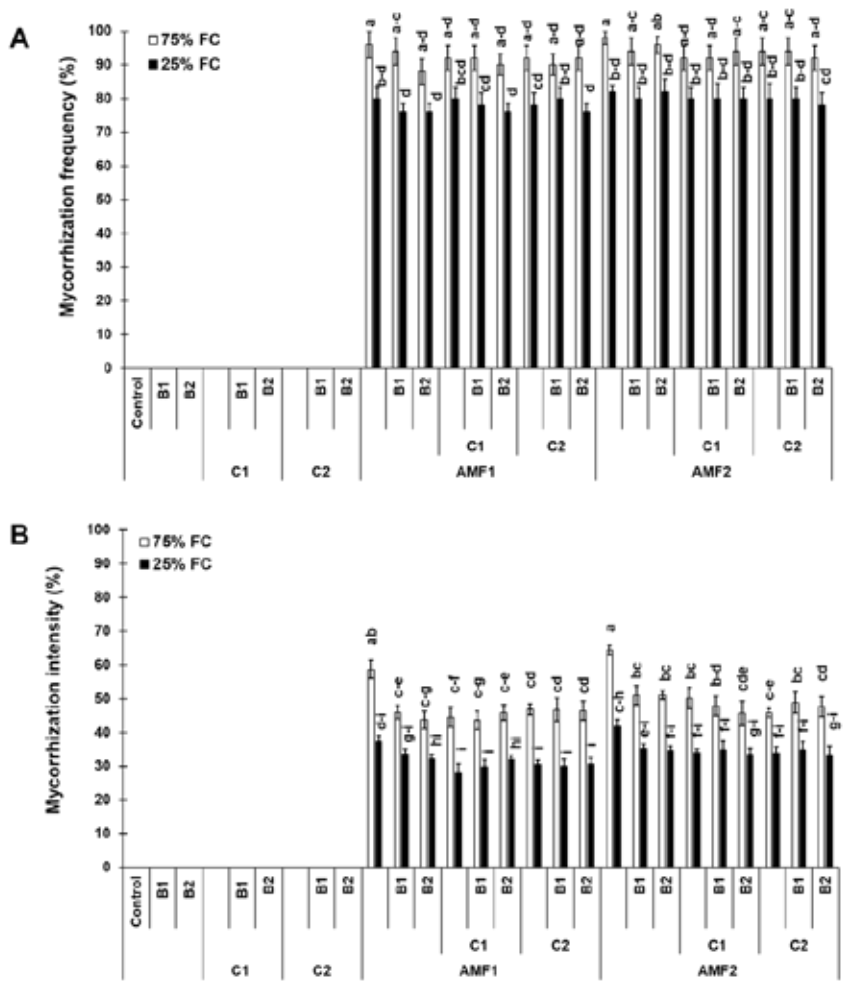

Figure 1. Influence of different water regimes (75% field capacity (FC); open bars and 25% FC; filled bars) on (A) mycorrhization frequency and (B) intensity in control plants (non-amended, non-inoculated), and plants amended with composts (C1 or C2) and/or inoculated with arbuscular mycorrhizal fungi (AMF, exogenous AMF1 or native AMF2) or plant growth promoting rhizobacteria (PGPR) strains (B1 or B2). Data are mean ± SE of six biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

Table 5. Result of 2-way ANOVA test for independent variables including testing the water stress (2 levels, well-watered and drought), inoculation and compost supplementation treatments (T), and the water stress x biofertilization treatments on the measured date palm parameters. Where: ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

Parameters	Water regime (W)	Biofertilization treatments(T)	W x T
Mycorrhizal frequency (%)	136.5 (***)	212.8 (***)	3.0 (***)
Mycorrhizal intensity (%)	408.6 (***)	617.5 (***)	8.7 (***)
Number of leaves	205.3 (***)	6.0 (***)	1.1 (ns)
Shoot height (cm)	259.2 (***)	8.2 (***)	0.8 (ns)
Root length (cm)	336.7 (***)	12.7 (***)	1.0 (ns)
Leaf area (cm^2)	773.4 (***)	12.0 (***)	1.0 (ns)
Biomass dry weight (shoot+ root) (g $plant^{-1}$)	864.2 (***)	21.8 (***)	6.7 (***)

Parameters	Water regime (W)	Biofertilization treatments(T)	W x T
Shoot phosphorus content (mg P plant $^{-1}$)	1540.4 (***)	55.6 (***)	21.2 (***)
Shoot N content (mg N plant $^{-1}$)	853.1 (***)	30.6 (***)	5.0 (***)
Leaf water potential (MPa)	965.4 (***)	20.0 (***)	3.2 (***)
Stomatal conductance (mmol m^{-2} s^{-1})	783.7 (***)	7.0 (***)	0.6 (ns)
Chlorophyll fluorescence Fv/Fm	726.6 (***)	3.1 (***)	0.8 (ns)
Chlorophyll a (mg. g^{-1} FW)	1537.4 (***)	14.6 (***)	9.3 (***)
Chlorophyll b (mg. g^{-1} FW)	573.2 (***)	17.6 (***)	15.2 (***)
Total Chlorophyll (mg. g^{-1} FW)	1635.1 (***)	26.6 (***)	19.0 (***)
Carotenoid (mg. g^{-1} FW)	551.8 (***)	19.4 (***)	6.0 (***)
Shoot total sugar content (mg. g^{-1} FW)	944.1 (***)	31.7 (***)	8.5 (***)
Shoot total soluble proteins (mg. g^{-1} FW)	1213.6 (***)	66.7 (***)	14.0 (***)
Shoot peroxidase activity (**µmol mg**$^{-1}$ protein min^{-1})	2138.0 (***)	19.0 (***)	7.0 (***)
Shoot polyphenol oxidase activity (**µmol mg**$^{-1}$ protein min^{-1})	1930.6 (***)	30.3 (***)	14.3 (***)
Shoot H_2O_2 content (nmol g-^{1} FW)	5762.0 (***)	56.0 (***)	6.6 (***)
Shoot MDA content (nmol g-^{1} FW)	2583.0 (***)	16.1 (***)	7.0 (***)

Growth assessment and mineral nutrition

Drought, biofertilization treatments, and their respective interaction significantly affected the total dry matter, P, and N contents of date palm (Table 5). Our results showed that the un-inoculated and un-amended control performed very weak response in all the growth parameters compared to the treated plants under both well-watered and drought stress conditions (Figure 2 and Table 6). Under drought stress, however, the application of bi-and tripartite combinations of biofertilizers (AMF1+C1, AMF1+C1+B1, AMF2+C2+B1, and AMF2+C1+B2) showed positive effects by promoting date palm shoot height and root length to a greater extent than in non-inoculated and non-amended plants. Moreover, the compost alone, bi and tripartite combinations (AMF1+C2+B1, AMF2+C2, C2) increased the number of leaves as compared to non-inoculated and non-amended date palm plants under well-watered and water deficit conditions. The plants treated with AMF1+C1, AMF2+B2, and AMF2+C2 improved the leaf area compared to non-inoculated and non-amended date palm plants under water deficit. A positive effect on the total dry weight of date plants subjected to water stress was recorded after application of biofertilizers (Figure 2). Indeed, the AMF and compost alone, bi and tripartite combinations (5g in AMF2+C2 and C2, 4.8g in AMF1+C1, and 4,6g in AMF2, AMF1+B1, and AMF2+C1+B1) showed the highest values of this parameter under water deficit in comparison with non-inoculated and non-amended plants (ca. 2.4g).

Table 6. Influence of different water regimes on growth parameters of non-amended and non-inoculated plants (control), and plants amended and inoculated date palm plants with composts (C1 or C2) and/or arbuscular mycorrhizal fungi (AMF, exogenous AMF1 and native AMF2), and/or plant growth promoting rhizobacteria (PGPR) (B1 or B2). Means followed by the same letters are not significantly different $P < 0.05$ (Tukey's HSD).

Parameters	Leaf number		Shoot height (cm)		Root length (cm)		Leaf area (cm²)	
Treatments	75% FC	25% FC	75% FC	25% FC	75% FC	25% FC	75% FC	25% FC
Control	4.6 ± 0.2 fg	3.8 ± 0.2 g	23.6 ± 0.8 lm	21.1 ± 0.6 m	21.9 ± 0.6 nop	18.5 ± 0.9 p	29.8 ± 1.4 g-j	21.7 ± 0.9 j
B1	5.2 ± 0.2 c-f	4.6 ± 0.2 fg	25.7 ± 0.5 c-m	23.4 ± 0.7 lm	25.2 ± 0.7 d-n	23.2 ± 1.6 i-o	35.0 ± 1.8 c-h	22.7 ± 1.4 ij
B2	6.4 ± 0.2 abc	5.4 ± 0.2 b-f	27.2 ± 0.4 b-l	23.0 ± 0.7 lm	26.3 ± 1.1 a-m	22.4 ± 1.4 m-p	34.0 ± 1.8 d-h	26.3 ± 1.6 hij
C1	6.4 ± 0.2 abc	5.4 ± 0.2 b-f	28.8 ± 1.2 a-j	24.1 ± 0.6 j-m	26.1 ± 0.8 a-m	22.5 ± 1.0 l-p	39.8 ± 1.9 a-f	28.3 ± 1.0 g-j
C1+B1	6.2 ± 0.2 a-d	4.6 ± 0.2 fg	27.5 ± 0.9 b-l	25.0 ± 0.8 f-m	26.7 ± 1.1 a-k	21.9 ± 0.9 nop	42.5 ± 0.9 a-d	32.7 ± 0.9 fgh
C1+B2	6.0 ± 0.3 a-e	5.0 ± 0.3 d-g	29.8 ± 0.8 a-f	26.6 ± 1.0 b-l	26.6 ± 0.5 a-l	23.4 ± 1.3 i-o	43.3 ± 2.1 abc	29.3 ± 1.3 g-j
C2	6.8 ± 0.2 a	5.6 ± 0.2 a-f	30.1 ± 0.6 a-d	25.7 ± 0.5 c-m	27.1 ± 0.5 a-j	22.8 ± 0.8 k-o	45.3 ± 1.5 a	34.5 ± 0.8 c-h
C2+B1	6.2 ± 0.2 a-d	5.4 ± 0.2 b-f	27.6 ± 0.9 a-l	25.1 ± 0.9 e-m	24.5 ± 0.2 g-o	21.0 ± 0.4 op	42.0 ± 1.2 a-e	32.0 ± 0.7 fgh
C2+B2	5.8 ± 0.2 a-f	5.4 ± 0.2 b-f	31.0 ± 1.1 ab	26.7 ± 0.9 b-l	28.7 ± 0.9 a-f	23.4 ± 0.5 i-o	48.5 ± 1.7 a	33.0 ± 1.6 fgh
AMF1	6.4 ± 0.2 abc	5.4 ± 0.2 b-f	30.0 ± 1.3 a-d	27.2 ± 0.5 b-l	27.9 ± 1.3 a-h	23.1 ± 0.4 j-o	43.3 ± 1.7 abc	31.5 ± 0.7 f-i
AMF1+B1	6.2 ± 0.3 a-d	5.2 ± 0.2 c-f	30.2 ± 0.7 abc	25.6 ± 0.9 c-m	29.3 ± 0.8 a-d	25.3 ± 0.5 d-n	44.0 ± 1.6 ab	33.0 ± 1.3 fgh
AMF1+B2	6.6 ± 0.2 ab	5.4 ± 0.2 b-f	29.6 ± 0.9 a-g	26.4 ± 0.6 b-l	29.2 ± 1.3 a-d	24.4 ± 0.5 g-o	43.0 ± 1.1 abc	34.0 ± 1.1 d-h
AMF1+C1	5.6 ± 0.2 a-f	4.8 ± 0.2 efg	29.9 ± 1.0 a-e	27.6 ± 0.7 a-l	30.0 ± 1.1 a	25.0 ± 0.5 e-o	46.0 ± 1.9 a	34.7 ± 1.5 c-h
AMF1+C1+B1	6.8 ± 0.2 a	5.2 ± 0.2 c-f	30.4 ± 1.3 abc	27.5 ± 1.1 a-l	29.5 ± 0.5 abc	27.1 ± 0.5 a-j	45.0 ± 2.2 a	31.3 ± 0.9 f-i
AMF1+C1+B2	5.8 ± 0.2 a-f	5.2 ± 0.2 c-f	29.5 ± 0.5 a-h	26.8 ± 0.9 b-l	30.0 ± 0.8 a	24.9 ± 0.6 e-o	42.0 ± 2.0 a-e	33.0 ± 1.3 fgh
AMF1+C2	6.4 ± 0.2 abc	5.4 ± 0.2 b-f	28.6 ± 1.2 a-j	24.7 ± 0.7 h-m	28.2 ± 1.2 a-g	26.2 ± 0.6 a-m	45.3 ± 1.7 a	31.7 ± 1.1 fgh
AMF1+C2+B1	5.8 ± 0.2 a-f	5.8 ± 0.2 a-f	27.2 ± 0.8 b-l	24.4 ± 0.7 j-m	27.3 ± 0.4 a-i	24.7 ± 0.5 f-o	44.3 ± 1.9 ab	31.7 ± 1.6 fgh
AMF1+C2+B2	6.2 ± 0.2 a-d	5.0 ± 0.3 d-g	30.2 ± 0.6 abc	24.8 ± 0.7 g-m	29.0 ± 0.7 a-e	25.6 ± 0.7 b-n	47.8 ± 1.7 a	32.6 ± 1.5 fgh
AMF2	6.2 ± 0.2 a-d	5.4 ± 0.2 b-f	28.9 ± 0.7 a-j	24.8 ± 0.6 g-m	28.8 ± 0.9 a-f	24.4 ± 0.5 g-o	47.8 ± 2.4 a	32.3 ± 1.1 fgh
AMF2+B1	6.0 ± 0.3 a-e	5.2 ± 0.2 c-f	29.5 ± 1.2 a-h	25.9 ± 0.6 c-m	27.9 ± 0.6 a-h	25.2 ± 0.3 d-n	46.3 ± 1.4 a	33.7 ± 1.7 d-h
AMF2+B2	6.2 ± 0.2 a-d	5.4 ± 0.2 b-f	28.5 ± 0.7 a-k	24.6 ± 0.6 i-m	28.8 ± 0.9 a-f	25.4 ± 0.5 c-n	47.5 ± 1.5 a	35.0 ± 0.9 c-h
AMF2+C1	5.8 ± 0.2 a-f	5.0 ± 0.0 d-g	32.3 ± 1.2 a	27.0 ± 0.7 b-l	26.0 ± 0.4 a-n	22.6 ± 0.7 k-p	43.3 ± 1.2 abc	32.8 ± 1.1 fgh
AMF2+C1+B1	6.2 ± 0.2 a-d	5.4 ± 0.2 b-f	29.4 ± 0.9 a-l	23.8 ± 0.7 klm	26.1 ± 0.5 a-m	24.0 ± 0.7 h-o	44.5 ± 2.0 ab	34.0 ± 1.4 d-h
AMF2+C1+B2	5.8 ± 0.2 a-f	5.2 ± 0.2 c-f	31.0 ± 0.6 ab	28.4 ± 0.8 a-k	29.6 ± 1.2 ab	27.1 ± 0.7 a-j	45.3 ± 2.2 a	33.5 ± 1.6 e-h
AMF2+C2	6.2 ± 0.2 a-d	5.6 ± 0.2 a-f	29.9 ± 1.3 a-e	26.8 ± 0.4 b-l	26.2 ± 0.8 a-m	23.5 ± 0.5 i-o	46.0 ± 2.1 a	36.0 ± 1.1 b-g
AMF2+C2+B1	6.0 ± 0.3 a-e	4.8 ± 0.2 efg	32.3 ± 1.1 a	27.3 ± 0.9 b-l	28.5 ± 0.4 a-g	24.8 ± 0.6 f-o	45.0 ± 1.9 a	32.3 ± 1.4 fgh
AMF2+C2+B2	6.0 ± 0.0 a-e	4.8 ± 0.0 efg	29.9 ± 0.6 a-d	25.3 ± 0.5 d-m	26.5 ± 0.6 a-m	22.7 ± 0.7 k-o	44.5 ± 0.8 ab	33.8 ± 1.6 d-h

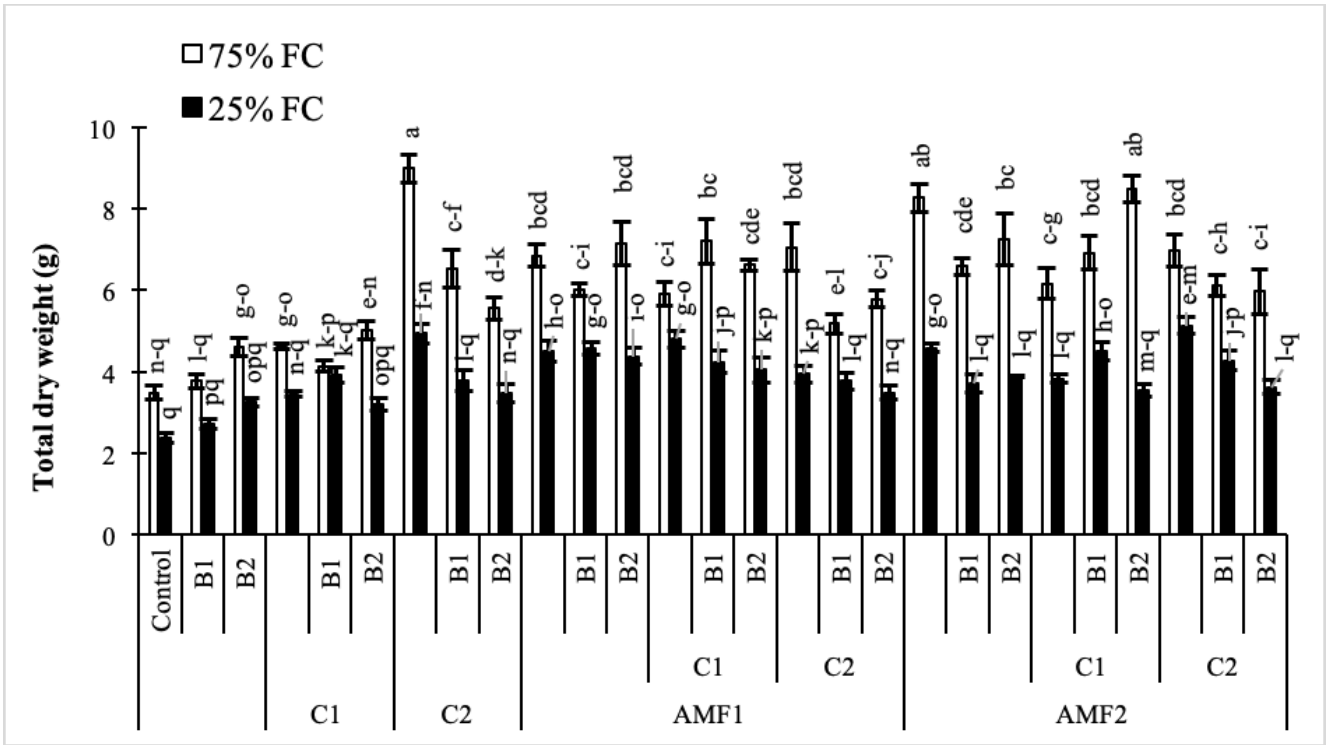

Figure 2. Influence of different water regimes (75% field capacity (FC); open bars and 25% FC; filled bars) on date palm total dry matter in control (non-amended, non-inoculated), and plants amended with composts (C1 or C2) and/or inoculated with arbuscular mycorrhizal fungi (AMF, exogenous AMF1 or native AMF2) or plant growth promoting rhizobacteria (PGPR) strains (B1 or B2) date palms. Data are mean ± SE of six biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

We assayed the P and N content in shoots of date palm plants under drought and different biofertilizers, since the degree of stress and growth depend on their uptake and translocation. Under the control condition (75% FC), shoot P was significantly increased in plants treated with AMF (Figure 3A) as compared to non-amended and non-inoculated control plants, whereas under drought stress, it was decreased. Under water deficit, shoot P content was significantly increased by C1+B1, AMF1, AMF1+B2, AMF1+C1+B1, AMF2, AMF1+B1+C1, AMF2+C1+B2, and AMF2+C2 in comparison with non-treated plants (Figure 3A). Under 75% FC, N levels in leaves of all treated plants remained significantly higher than in control conditions. Drought stress decreased N content in all treatments, and all the biofertilizer treatments were able to maintain higher content than non-amended and non-inoculated control plants (Figure 3B).

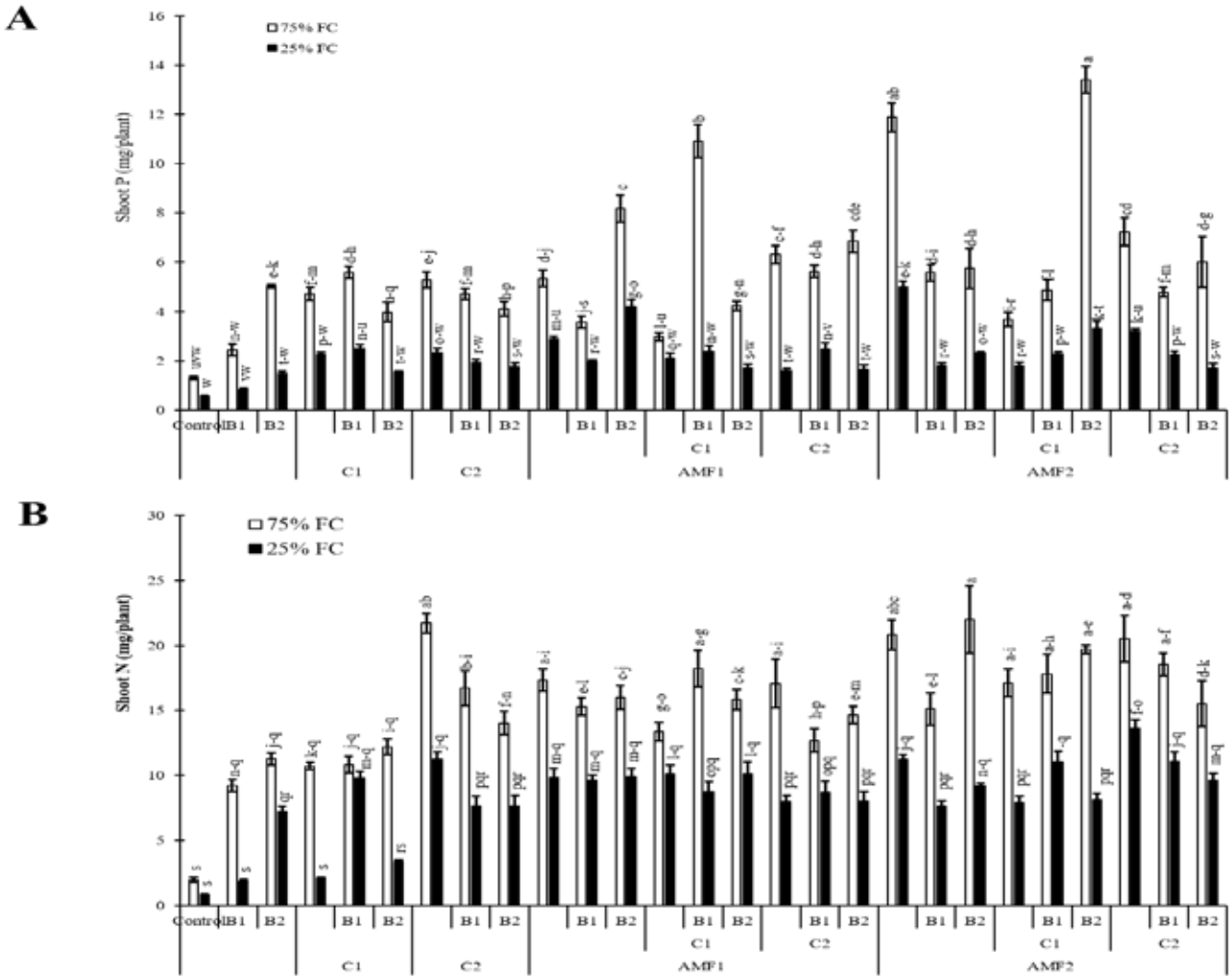

Figure 3. (A) Phosphorous (P) and (B) nitrogen (N) content in date palm shoots under two water regimes (75% field capacity (FC); open bars and 25% FC; filled bars) of the tested control (non-amended and non-inoculated) and biofertilizers treatments (composts C1 or C2, arbuscular mycorrhizal fungi (AMF, exogenous AMF1 and native AMF2), and/or plant growth promoting rhizobacteria (PGPR) (B1 or B2). Data are mean ± SE of six biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

Physiological traits

Leaf water potential, stomatal conductance, chlorophyll fluorescence (Fv/Fm), chlorophyll a and b, and carotenoid contents were all significantly affected by water regime and biofertilization treatments (Table 5). However, leaf water potential, chlorophyll a and b, and carotenoid contents were significantly affected by the interaction between water regime and biofertilization treatments (Table 5). Under water scarcity, the leaf water potential values were decreased in non-inoculated and non-amended control plants. Plant inoculation with AMF and/or PGPR amended or not with the compost yielded an improvement in leaf water potential under water deficit, especially AMF1 (-1 MPa), AMF1+C1+B1 (-1.15 MPa), and AMF2+C2+B2 (-1.20 MPa) versus non-inoculated and no-amended plants (-2.18 MPa) (Figure 4A).

Under water control condition, there were obvious stomatal conductance differences between non-amended/non-inoculated and treated plants with AMF and/or PGPR. Under water stress, stomatal

conductance values decreased in date palm plants. However, the application of biofertilizers increased stomatal conductance, with AMF1 alone, the bi-(C2+B1, AMF1+B1, AMF1+B2, AMF1+C2, and AMF2+B1) and tripartite (AMF1+C2+B1, AMF1+C2+B2, AMF2+C1+B2, and AMF2+C2+B2) combinations being the most effective in improving this parameter compared to control plants (Figure 4B).

As shown in Figure 4C, the chlorophyll fluorescence (Fv/Fm) was only slightly affected by drought stress. Biofertilizer application improved Fv/Fm in date palm plants under water shortage. The single (AMF1, PGPR B2 and compost C1), bi-(AMF1+C2), and tripartite (AMF2+C1+B2) combinations presented the most effective treatments to increase chlorophyll fluorescence under water deficit conditions compared to non-inoculated and non-amended plants.

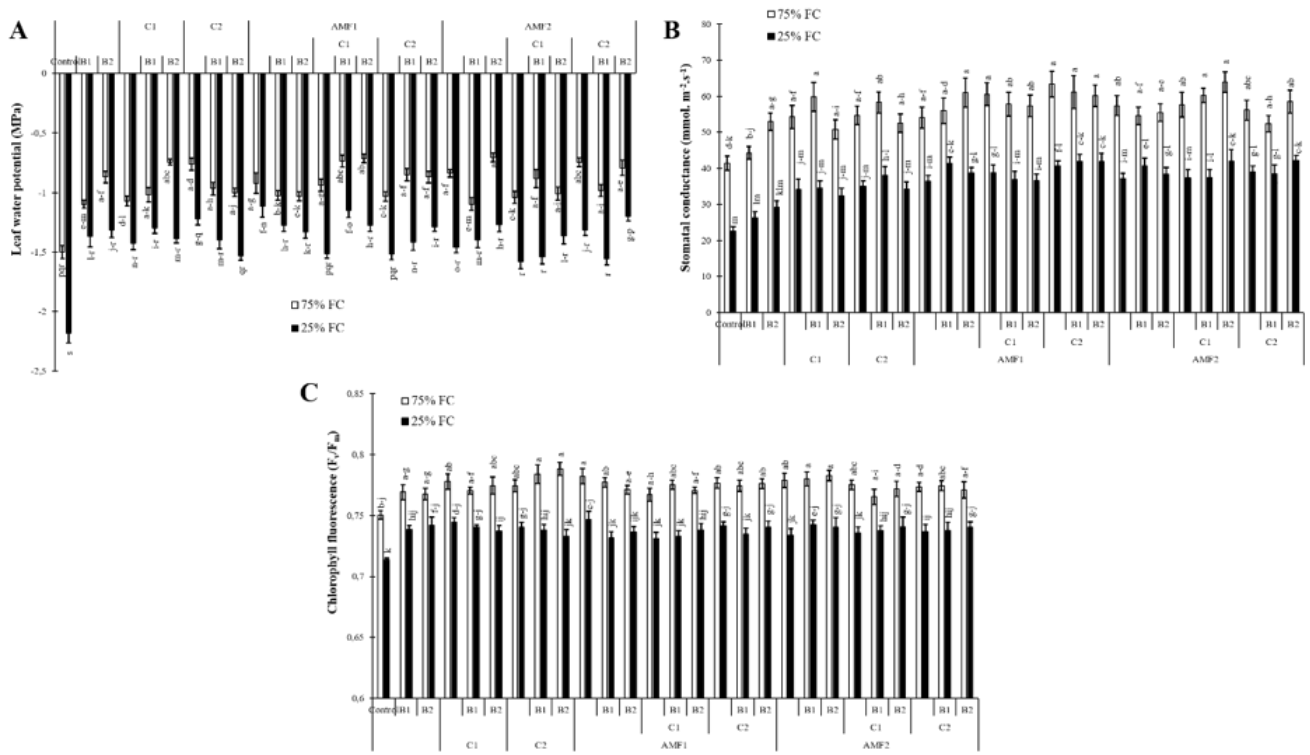

Figure 4. (A) Leaf water potential, (B) stomatal conductance, (C) and chlorophyll fluorescence of date palm plants under two water regimes (75% field capacity (FC); open bars and 25% FC; filled bars) and grown under control (non-amended and non-inoculated) or biofertilizer applications (composts C1 or C2, arbuscular mycorrhizal fungi (exogenous AMF1 and native AMF2), and/or plant growth promoting rhizobacteria (PGPR) (B1 or B2). Data are mean ± SE of six biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

In response to drought stress and inoculation with AMF, PGPR and compost application, chlorophyll a, b, total chlorophyll, and carotenoid content are shown in Figure 5. Under water deficit, the photosynthetic pigment content was reduced. However, the application of AMF, compost, and PGPR especially the combination C2+B1, AMF2+C1+B2, AMF2+C2+B1, and AMF2+C2+B2 increased pigments contents compared to control plants, under water stress conditions. As for carotenoid content, this was positively affected by biofertilizers applied alone (C2 and AMF1) or in combination (AMF2+C1, AMF2+C1+B1, AMF1+B2, and AMF2+C2+B1) as compared with non-inoculated with AMF/PGPR and non-amended with composts, under water deficit.

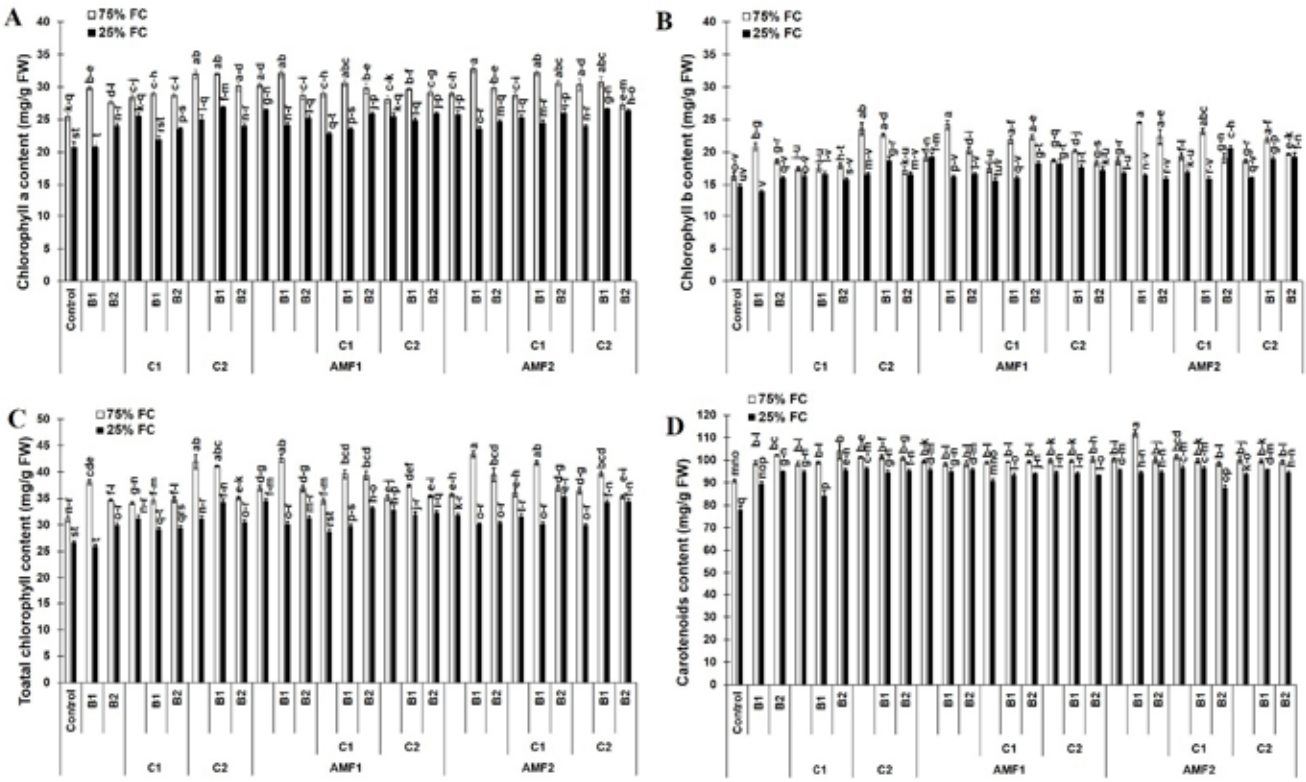

Figure 5. (A) Chlorophyll a, (B) chlorophyll b, (C) total chlorophyll, and (D) carotenoid content in leaves of date palm plants under two water regimes (75% field capacity (FC); open bars and 25% FC; filled bars) and further grown without (control; non-amended and non-inoculated) or with biofertilizers (composts C1 or C2, arbuscular mycorrhizal fungi (AMF, exogenous AMF1 and native AMF2), and/or PGPR (B1 or B2)). Data are mean ± SE of six independent biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

Biochemical traits

Total sugar, protein, MDA and H_2O_2 contents as well as and polyphenol oxidase activities were all significantly affected by water regime and biofertilization and their interaction (Table 5). Results related to the effect of drought stress and biofertilizer applications on sugar and protein content, and POX and PPO activity in date palm plants are presented in Figure 6. Under normal water conditions, both compost and AMF increased sugar and protein content. Exposure to water deficit caused a significant decrease in sugar and protein content (Figures 6A and 6B). The addition of biofertilizers yielded a significant increase in sugar and protein, especially AMF2+C1+B2, AMF1+C2+B2, AMF2, C2 and AMF1, and AMF1+C2+B2, C2+B2, AMF1+C1, AMF2+C1+B1and C2+B1 compared to stressed control plants. Under 75% FC conditions, POX and PPO activity did not differ significantly among the biofertilizers treatments (Figures 6C and 6D). Exposure to drought stress led to a considerable increase in the POX and PPO specific activities as compared to non-treated control plants.

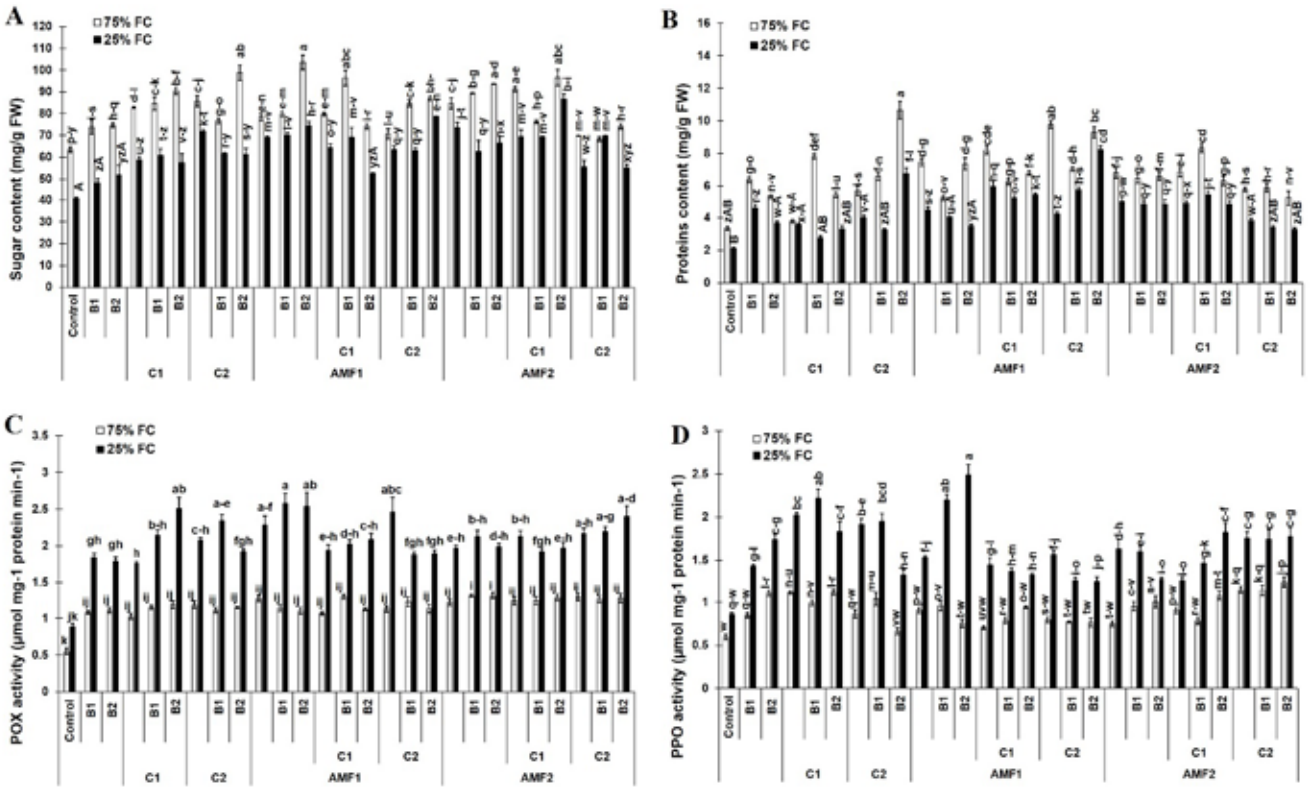

Figure 6. (A) Total soluble sugar, (B) protein, (C) peroxidase (POX), and (D) polyphenol oxidase (PPO) content in date palm shoots under two water regimes (75% field capacity (FC); open bars and 25% FC; filled bars) of the tested control treatments (non-amended and non-inoculated) and biofertilizers treatments (composts C1 or C2, arbuscular mycorrhizal fungi (exogenous AMF1 and native AMF2), and/or plant growth promoting rhizobacteria (PGPR) (B1 or B2). Data are mean ± SE of six biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

To characterize damage caused by drought stress, we carried out MDA and H_2O_2 analyses (Figure 7). The exposure of date palm plants to severe water deficit resulted in an increase in MDA and H_2O_2 content. Under water stress, in contrast, the application of single or combined biofertilizers showed reduced MDA and H_2O_2 content compared to non-inoculated and non-amended controls.

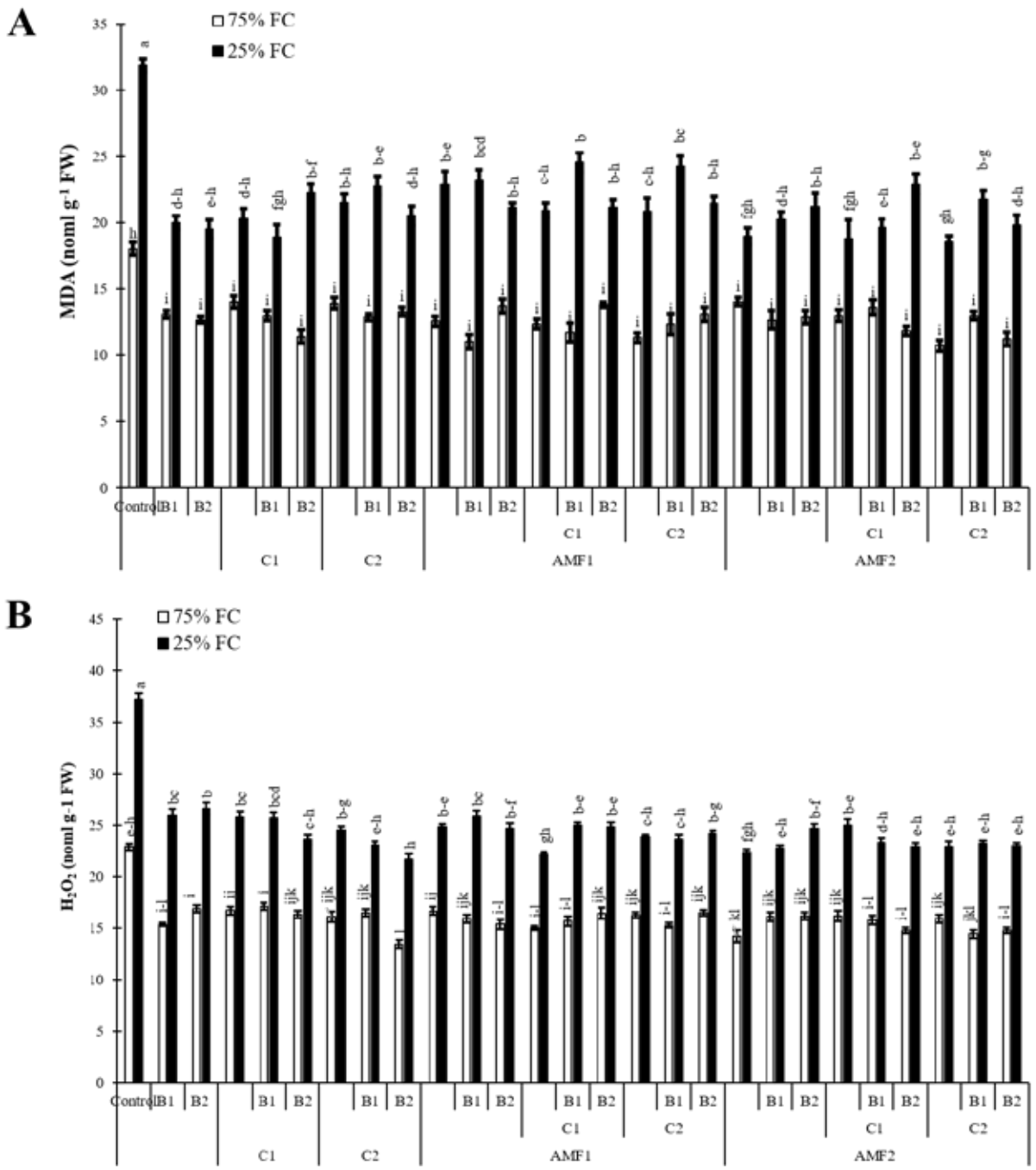

Figure 7. (A) Malondialdehyde (MDA) and (B) hydrogen peroxide (H_2O_2) content in date palm shoots under two water regimes (75% field capacity (FC); open bars and 25% FC; filled bars) of the tested control treatments (non-amended and non-inoculated) and biofertilizers treatments (composts C1 or C2, arbuscular mycorrhizal fungi (AMF, exogenous AMF1 and native AMF2), and/or plant growth promoting rhizobacteria (PGPR) (B1 or B2). Data are mean ± SE of six independent biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

Principal component analysis (PCA)

The PCA approach was used to summarized all the data and identify important parameters and treatments affecting date palm performance under 75 and 25% of FC. Under 75% FC conditions, the PC1 and PC2

explained for 56.47% of the total variance with combined treatments with AMF1, AMF2 and C2 being the most effective which associated with improved date palm growth, nutrient uptake and physiological as well as biochemical traits (Figure 8A). Under 25% FC conditions, the PC1 and PC2 accounted for 59.4% of the total variance (Figure 8B). All treatments including inoculation with AMF1 and AMF2 were the most effective in improving date palm growth, nutrient uptake as well as physiological and biochemical parameters under 25% FC conditions (Figure 8 B). Under both water regimes, the MDA and H_2O_2 contents were the parameters, which were most closely associated with the control treatments (Figure 8A, B).

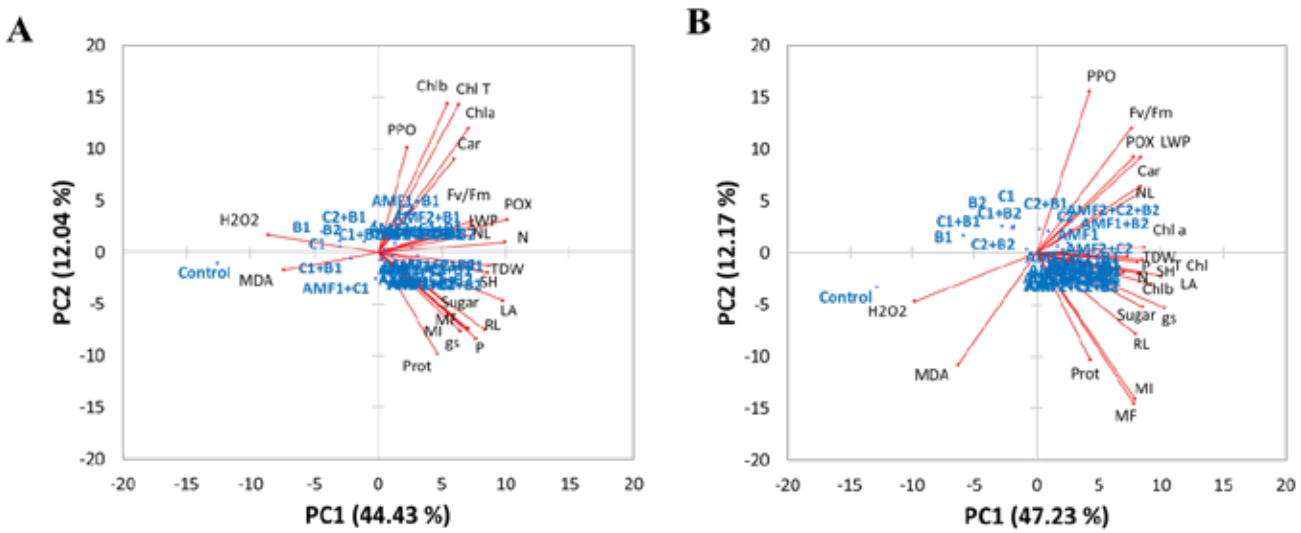

Figure 8. Principal component analysis (PCA) of the different studied traits and treatments under well-watered (A) and drought stress (B) conditions (25% FC).

Chl a: chlorophyll a, Chlb: chlorophyll b, Fv/Fm: chlorophyll fluorescence, gs: stomatal conductance, H_2O_2: hydrogen peroxide, LA: leaf area, LWP: leaf water potential, MDA: malondialdehyde, MI: mycorrhizal intensity, MF: mycorrhizal frequency, N: nitrogen content in plant, NL: leaf numbers, P: Phosphorous content in plant shoot, POX: peroxidase, PPO: polyphenol oxidase, RL: root length, SH: shoot height and T Chl: total chlorophylls.

Results of Field Experiment

Mycorrhizal root colonization frequency and intensity

Mycorrhizal root colonization frequency and intensity were significantly affected by water regime, biofertilization treatments and their interaction (Table 7). Under well-watered and drought conditions, all treatments including inoculation with *R. irregularis* i.e. AMF1, AMF1+B1, AMF1+C1 and AMF1+B1+C1, significantly increased the frequency and intensity of root colonization (Figure 9). Under well-watered conditions, colonization frequencies and intensities ranged between 27% and 90% and 7% and 51%, respectively, while under drought conditions, colonization frequencies and intensities ranged between 18% and 67% and 3% and 22%, respectively.

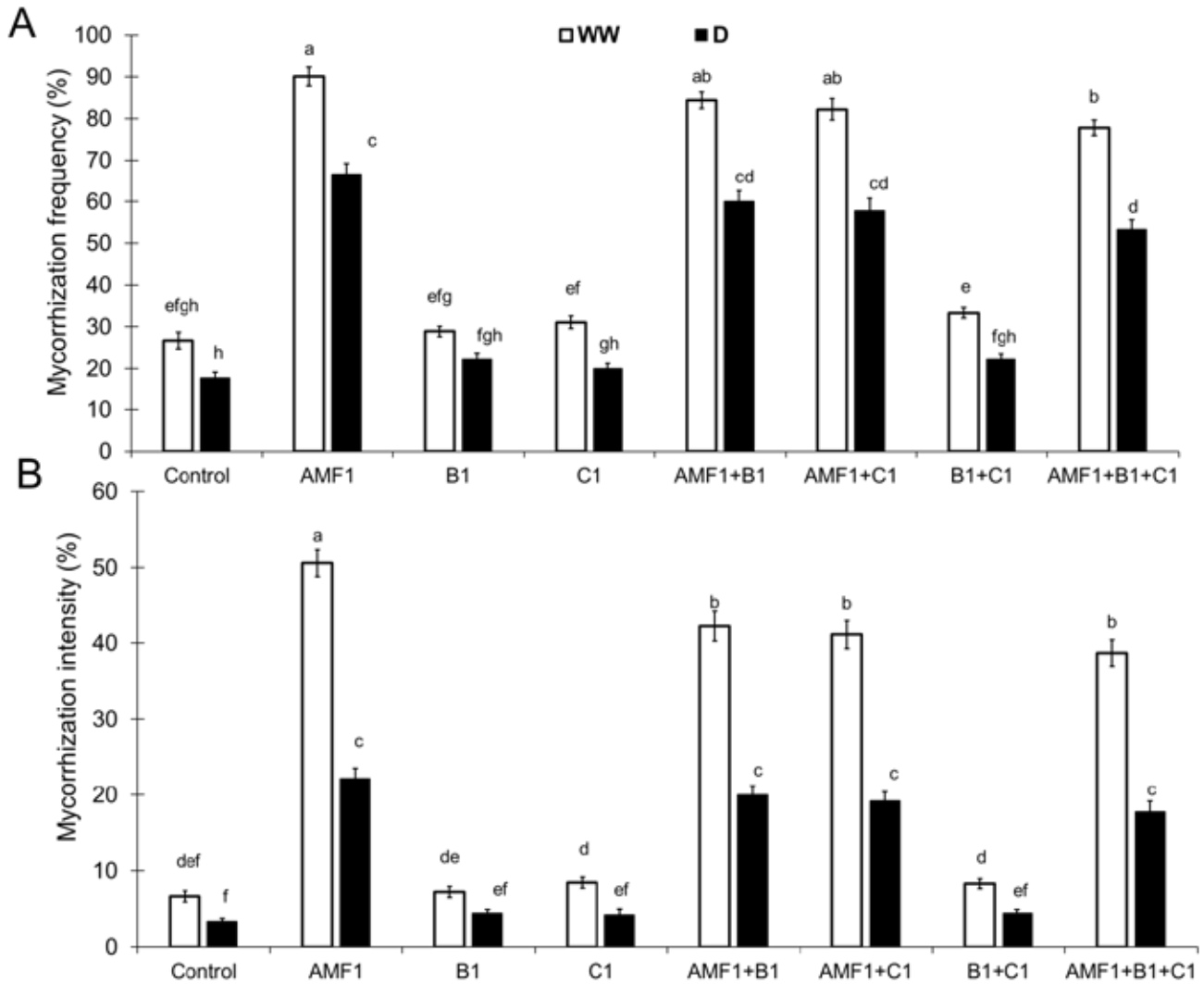

Figure 9. Influence of different water regimes: well-watered and drought on mycorrhization frequency (A) and intensity (B) of date palms amended with compost (C1) and/or inoculated with *Rhizophagus irregularis* (AMF1) or plant growth-promoting rhizobacteria (PGPR) strains (B1) or in non-amended and non-inoculated controls (control). Data are means ± standard error (n=6). Means followed by the same letters are not significantly different at $p< 0.05$ (Tukey's HSD).

Table 7. Result of 2-way ANOVA test for independent variables including testing the water stress (2 levels, well-watered and drought) and inoculation and compost supplementation treatments (T) at 8 levels (control, compost (C1) supplementation, *R. irregularis* (AMF1), plant growth promoting rhizobacteria (B1) consortia inoculations, AMF1+B1, AMF1+C1, B1+C1 and AMF1+B1+C1 combinations) and the water stress x inoculation and compost supplementation treatments on the measured date palm parameters. Where: ns, not significant, *$p< 0.05$, **$p< 0.01$, ***$p< 0.001$.

Parameters	Water regime (W)	Biofertilization treatments(T)	W x T
Number of leaves	70.1 (***)	11.5 (***)	3.1 (ns)
Shoot height (m)	134.3 (***)	17.3 (***)	12.6 (***)
Root length (m)	61.0 (***)	20.6 (***)	8.9 (***)
Leaf area (cm^2)	219.2 (***)	25.5 (***)	4.3 (*)
Biomass dry weight (shoot+ root) (g plant $^{-1}$)	267.1 (***)	47.2 (***)	8.4 (**)
Phosphorus content (shoot+ root) (g P plant $^{-1}$)	528.0 (***)	252.1 (***)	48.4 (***)
Leaf water potential (MPa)	130.0 (***)	17.0 (***)	1.4 (ns)
Stomatal conductance (mmol m^{-2} s^{-1})	313.4 (***)	16.6 (***)	2.3 (ns)
Chlorophyll fluorescence Fv/Fm	54.5 (***)	12.7 (***)	0.9 (ns)
Chlorophyll a (mg. g^{-1}dry weight)	146.5 (***)	46.6 (***)	1.3 (ns)
Chlorophyll b (mg. g^{-1}dry weight)	57.1 (***)	3.2 (***)	0.8 (ns)
Total Chlorophyll (mg. g^{-1}dry weight)	193.0 (***)	26.0 (***)	1.3 (ns)
Carotenoid (mg. g^{-1}dry weight)	73.4 (***)	43.8 (***)	2.1 (ns)
Mycorrhizal frequency (%)	369.7 (***)	258.0.4(***)	7.7 (***)
Mycorrhizal intensity (%)	453.6 (***)	288.0 (***)	19.6 (***)
Total sugar content (shoot + root) (mg. g^{-1}dry weight)	205.1 (***)	42.2 (***)	5.4 (*)
Total soluble proteins (shoot + root) (mg. g^{-1}dry weight)	551.0 (***)	68.2 (***)	7.2 (**)
Peroxidase activity (shoot + root) (**µmol mg^{-1}** protein min^{-1})	609.1 (***)	67.0 (***)	10.6 (***)
Polyphenol oxidase activity (shoot+ root) (**µmol mg^{-1}** protein min^{-1})	949.5 (***)	58.6 (***)	32.8 (***)
H_2O_2 content (shoot + root) (nmol g-^{1}dry weight)	300.3 (***)	69.4 (***)	3.9 (*)

Where: ns, not significant, *P<0.05, **P<0.01, ***P<0.001

Growth assessment and mineral nutrition of date palm

The imposed water regime, biofertilization treatment, and their respective interaction significantly affected the shoot height, root length, leaf area, DM of biomass, and P content of date palm (Table 7). While the compost supplementation exerted the biggest effect under WW conditions, inoculation with AMF1+C1 most efficiently increased root length under D conditions (Table 8). The drought reduction effects on the dry matter biomass were highly significant (Figure 10A). Inoculation with AMF1+B1+C1 and AMF1+C1 triggered the highest dry matter biomass under WW conditions. Under drought, only the AMF1+B1+C1 treatment showed the highest dry matter biomass (Figure 10A).

Table 8. Influence of water regimes on number of leaves, shoot height, root length, and lea area of date palm plants under well-watered and drought conditions of the tested control, compost (C1) supplementation, *R. irregularis* (AMF1), plant growth promoting rhizobacteria (B1) consortia inoculations (B1), and the AMF1+B1, AMF1+C1, B1+C1 and AMF1+B1+C1 combinations. Data are mean ± SE of six independent biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

Water regimes	Treatments	Number of leaves	Shoot height (m)	Root length (m)	Leaf area (cm^2)
Well-watered	Control	5.3 ± 0.33 c	0.28 ± 0.01 efg	0.21 ± 0.01 fg	94.3 ± 2.1 ef
	AMF1	6.3 ± 0.33 bc	0.34 ± 0.01 c-f	0.35± 0.02 ab	125.7 ± 3.0 bc
	B1	6.7 ± 0.33 abc	0.38 ± 0.02 bc	0.27 ± 0.01 c-f	116.5 ± 4.0 cd
	C1	7.0 ± 0.37 ab	0.35 ± 0.01 cde	0.39 ± 0.02 a	119.0 ± 3.9 cd
	AMF1+B1	6.3 ± 0.33 bc	0.32 ± 0.01 c-f	0.29 ± 0.01 b-e	139.3 ± 2.0 ab
	AMF1+C1	7.3 ± 0.33 ab	0.46 ± 0.03 a	0.32 ± 0.02.18 bc	143.0 ± 6.3 ab
	B1+C1	8.0 ± 0.26 a	0.44 ± 0.01 ab	0.28 ± 0.01 c-f	125.0 ± 4.7 bc
	AMF1+B1+C1	8.0 ± 0.37 a	0.47 ± 0.01 a	0.33 ± 0.01.53 abc	150.0 ± 2.1 a
Drought	Control	3.7 ± 0.21 d	0.25 ± 0.01 g	0.19 ± 0.01 g	78.3 ± 3.7 f
	AMF1	6.0 ± 0.37 bc	0.36 ± 0.01 cd	0.25 ± 0.01 d-g	105.5 ± 4.4 de
	B1	5.3 ± 0.33 c	0.32 ± 0.01 c-g	0.22 ± 0.01 fg	94.0 ± 2.0 ef
	C1	6.0 ± 0.37 bc	0.27 ± 0.01 fg	0.23 ± 0.01 efg	90.3 ± 2.6 ef
	AMF1+B1	6.0 ± 0.26 bc	0.31 ± 0.01 d-g	0.31 ± 0.02 bcd	108.2 ± 5.5 cde
	AMF1+C1	6.0 ± 0.37 bc	0.32 ±0.01 c-f	0.32 ± 0.02 bcd	101.5 ± 4.2 de
	B1+C1	6.0 ± 0.26 bc	0.28 ± 0.01 efg	0.22 ± 0.01 fg	108.0 ± 3.2 cde
	AMF1+B1+C1	5.3 ± 0.21 c	0.30 ± 0.01 d-g	0.29 ± 0.01 b-e	104.3 ± 3.4 de

The total P content of date palms is shown in Figure 10B. Plants under WW conditions showed the highest plant (shoot + root)-P contents than the ones under D conditions (Figure 10B). Under drought, co-inoculations of AMF1+B1+C1 and B1+C1 supplementations to date palm trees showed the highest P contents than plants of the control treatments. Under WW conditions, we also observed that co-inoculation of AMF1+B1+C1, AMF1+C1, and B1+C1 supplementation increased the date palm plant (shoot + root)-P contents than other analyzed treatments.

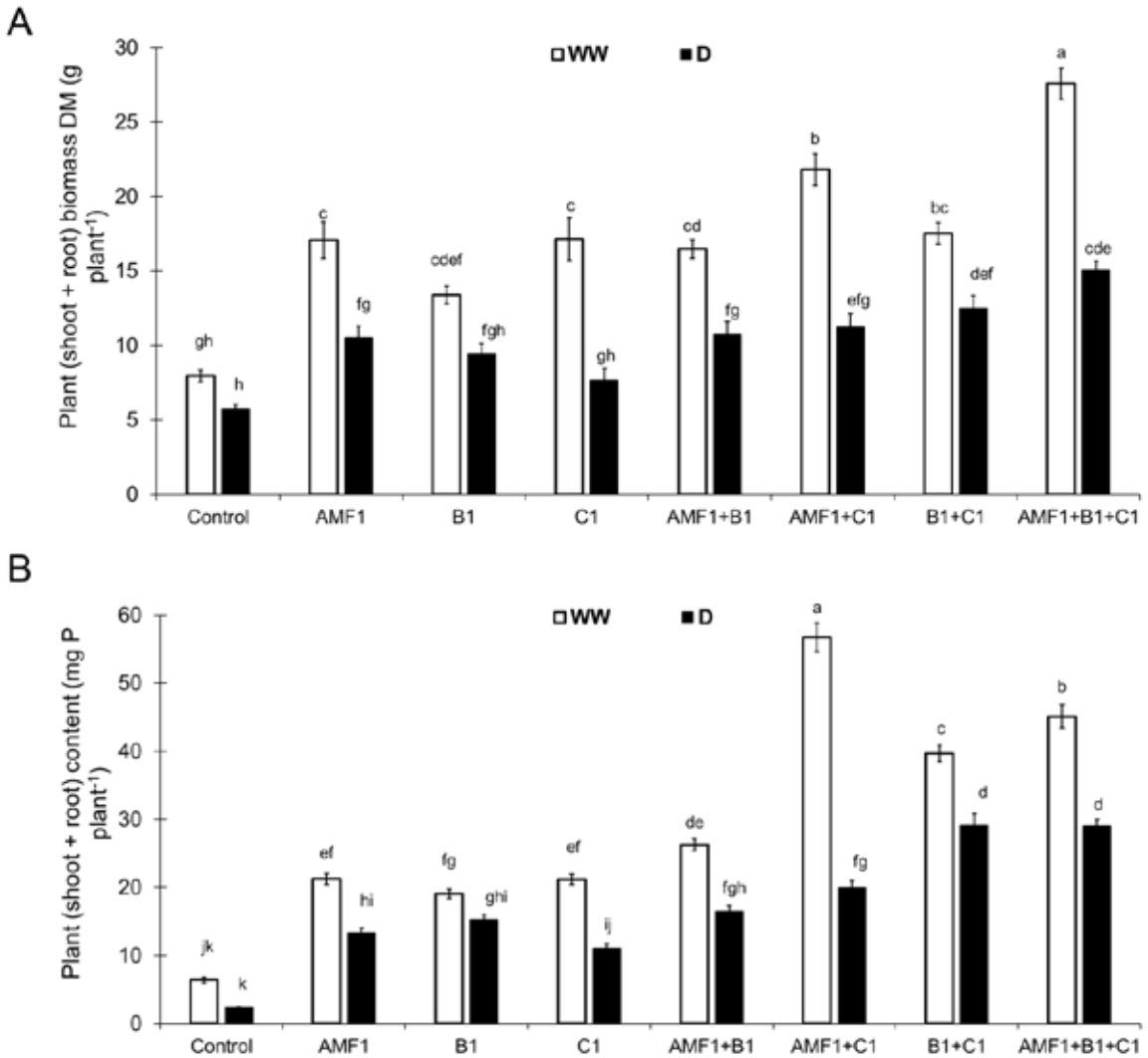

Figure 10. Influence of different water regimes: well-watered and drought on plant dry matter (DM) biomass (A) and total phosphorus (P) content (B) of date palms amended with compost (C1) and/or inoculated with *Rhizophagus irregularis* (AMF1) or plant growth-promoting rhizobacteria (PGPR) strains (B1) or in non-amended and non-inoculated controls (control). Data are mean ± standard error (n=6). Means followed by the same letters are not significantly different at $p< 0.05$ (Tukey's HSD).

Leaf water potential, stomatal conductance, chlorophyll fluorescence, Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid of date palm

Leaf water potential, stomatal conductance, chlorophyll fluorescence (Fv/Fm), chlorophyll a and b, and carotenoid contents were all significantly affected by water regime and biofertilization (Table 9). In general, stomatal conductance, chlorophyll a and total chlorophyll contents of the different biofertilizer treatments decreased in plants subjected to drought compared to plants under WW conditions (Tables 9). Single and co-inoculation with AMF1 and B1, and supplementation with C1 significantly increased almost all parameters, with few exceptions, compared to the controls; only chlorophyll b content was similar across all treatments (Table 9). Under D conditions, leaf water potential and stomatal conductance most efficiently increased when date palms were inoculated with AMF1 and the triple combination AMF1+B1+C1 and with AMF1+B1 and AMF1+B1+C1, respectively (Table 9). Chlorophyll fluorescence (F_v/F_m) was increased in all date palms inoculated with AMF1 and B1+C1 under

D conditions. Chlorophyll a was highest in plants inoculated with AMF1+B1 while total chlorophyll was increased in AMF1, AMF1+B1 and AMF1+C1 under D conditions. Carotenoids most efficiently increased in date palms inoculated with AMF1 and AMF1+C1 when subjected to drought (Table 9).

Total sugar, protein and H_2O_2 contents, peroxidase, and polyphenol oxidase activities in date palms

Total sugar, protein and H_2O_2 contents as well as peroxidase and polyphenol oxidase activities were all significantly affected by water regime and biofertilization and their interaction (Table 7). Under both water regimes, all treatments significantly increased total sugar and protein contents as compared to the controls (Table 10). Under D conditions, AMF1+C1 most efficiently increased total sugar contents, while supplementation with C1 most efficiently increased total protein contents. H_2O_2 contents significantly decreased in all treatments compared to the controls with the triple combination AMF1+B1+C1 being the most efficient under both water regimes. The activity of POX also significantly increased in all treatments under both water regimes, with treatment AMF1 and AMF1+B1 being the most efficient under WW and D conditions, respectively. The activity of PPO was significantly increased only by treatment AMF1 under WW conditions, but by almost all treatments except treatment C1 under D conditions (Table 10).

Table 9. Leaf water potential, stomatal conductance, chlorophyll fluorescence, Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents of the date palm plants under well-watered and drought conditions of the tested control, compost (C1) supplementation, *R. irregularis* (AMF1), plant growth promoting rhizobacteria (B1) consortia inoculations, and the AMF1+B1, AMF1+C1, B1+C1 and AMF1+B1+C1 combinations. Data are mean ± SE of six independent biological replicates. Means followed by the same letters are not significantly different at P< 0.05 (Tukey's HSD).

Water regimes	Treatments	Leaf water potential (MPa)	Stomatal conductance (mmol m^{-2} s^{-1})	Chlorophyll fluorescence	Chlorophyll a (mg. g-1dry weight)	Chlorophyll b (mg. g-1dry weight)	Total chlorophyll (mg. g-1dry weight)	Carotenoids (mg. g-1dry weight)
Well-watered	Control	-1.42 ± 0.07 b	57.7 ± 1.8 c	0.75 ± 0.01 fg	10.7 ± 0.61 fg	11.3 ± 0.51 a-e	19.0 ± 0.64 ef	71.4 ± 1.05 fg
	AMF1	-0.78 ± 0.05 a	68.4 ± 1.9 b	0.80 ± 0.01 a	21.1± 0.70 a	13.1 ± 0.68 ab	26.5 ± 0.75 a	95.2 ± 1.28 abc
	B1	-1.06 ± 0.09 ab	60.0 ± 2.0 c	0.78 ± 0.00 a-e	17.1 ± 0.43 b-e	12.9 ± 0.59 ab	22.1 ± 0.65 b-e	92.9 ± 0.88 a-d
	C1	-0.93 ± 0.08 a	61.3 ± 2.0 c	0.80 ± 0.00 ab	20.2 ± 0.81 ab	12.4 ± 0.55 a-d	25.3 ± 0.66 ab	98.5 ± 1.64 ab
	AMF1+B1	-0.83 ± 0.07 a	71.1 ± 2.0 ab	0.79 ± 0.00 abc	19.8 ± 0.61 abc	11.9 ± 0.55 a-e	24.6 ± 0.93 abc	97.7 ± 2.68 ab
	AMF1+C1	-0.77 ± 0.07 a	72.0 ± 1.8 ab	0.79 ± 0.01 a-d	20.8 ± 0.83 a	13.9 ± 0.56 a	27.1 ± 0.62 a	99.9 ± 3.59 a
	B1+C1	-0.87 ± 0.7 a	71.9 ± 2.2 ab	0.78 ± 0.00 a-e	19.6 ± 0.83 a-d	11.8 ± 0.50 a-e	24.3 ± 0.86 a-d	95.5 ± 1.63 abc
	AMF1+B1+C1	-0.78 ± 0.09 a	73.3 ± 2.0 a	0.79 ± 0.01 a-d	20.0 ± 0.94 ab	12.7 ± 0.67 abc	25.3 ± 0.97 ab	97.7 ± 2.39 ab

Water regimes	Treatments	Leaf water potential (MPa)	Stomatal conductance (mmol m^{-2} s^{-1})	Chlorophyll fluorescence	Chlorophyll a (mg. g-1dry weight)	Chlorophyll b (mg. g-1dry weight)	Total chlorophyll (mg. g-1dry weight)	Carotenoids (mg. g-1dry weight)
Drought	Control	-2.08 ± 0.11 c	41.5 ± 1.5 e	0.73 ± 0.01 g	8.4 ± 0.39 g	9.4 ± 0.67 e	14.7 ± 0.43 g	67.3 ± 1.97 h
	AMF1	-1.08 ± 0.10 ab	49.3 ± 1.7 d	0.78 ± 0.01 a-f	16.6 ± 0.63 cde	10.5 ± 0.53 b-e	21.0 ± 0.47 de	92.9 ± 1.07 a-d
	B1	-1.43 ± 0.09 b	47.2 ± 1.8 d	0.75 ± 0.01 efg	11.9 ± 0.64 f	9.9 ± 0.56 cde	17.0 ± 0.61 fg	79.9 ± 2.42 ef
	C1	-1.38 ± 0.12 b	50.1 ± 2.2 d	0.76 ± 0.01 d-g	16.3 ± 0.57 de	10.5 ± 0.56 b-e	20.6 ± 0.71 e	91.0 ± 1.22 a-d
	AMF1+B1	-1.42 ± 0.09 b	56.4 ± 2.0 c	0.77 ± 0.01 b-f	17.4 ± 0.54 b-e	11.3 ± 0.51 a-e	22.2 ± 0.62 b-e	84.4 ± 1.24 de
	AMF1+C1	-1.45 ± 0.08 b	49.30 ± 1.6 d	0.77 ± 0.01 a-f	15.7 ± 0.78 e	11.7 ± 0.47 a-e	21.4 ± 0.48 cde	92.0 ± 0.87 a-d
	B1+C1	-1.43 ± 0.09 b	50.7 ± 1.7 d	0.76 ± 0.01 c-f	15.3 ± 0.44 e	9.8 ± 0.70 cde	19.2 ± 0.70 ef	87.1 ± 1.97 cde
	AMF1+B1+C1	-1.12 ± 0.09 ab	58.33 ± 2.0 c	0.76 ± 0.01 c-f	15.7 ± 0.48 e	9.8 ± 0.35 de	19.5 ± 0.71 ef	90.4 ± 1.57 bcd

Table 10. Total soluble sugar, protein, peroxidase (POX), polyphenol oxidase (PPO) and hydrogen peroxide (H_2O_2) contents of date palm under well-watered and drought conditions of the tested control, compost (C1) supplementation, *R. irregularis* (AMF1), plant growth promoting rhizobacteria (B1) consortia inoculations, and the AMF1+B1, AMF1+C1, B1+C1 and AMF1+B1+C1 combinations. Data are mean ± SE of six independent biological replicates. Means followed by the same letters are not significantly different at $P < 0.05$ (Tukey's HSD).

Water regimes	Parameters/ Treatments	Sugar content (mg. g^{-1} dry weight)	Proteins content (mg. g^{-1} dry weight)	POX (µmol mg^{-1} protein min^{-1})	PPO (µmol mg^{-1} protein min^{-1})	H_2O_2 (nmol g^{-1} dry weight)
Well-watered	Control	144.0 ± 4.0 f	16.2 ± 0.54 gh	1.7 ± 0.10 i	2.2 ± 0.14 fg	51.0 ± 1.4 b
	AMF1	197.3 ± 6.6 abc	26.2 ± 0.46 bcd	4.2 ± 0.13 e	3.1 ± 0.18 cde	30.7 ± 0.9 ij
	B1	203.4 ± 5.4 ab	25.8 ± 1.01 cd	2.9 ± 0.16 gh	2.4 ± 0.18 efg	37.3 ± 1.3 ghi
	C1	214.0 ± 5.6 a	29.4 ± 0.89 ab	2.4 ± 0.12 hi	1.4 ± 0.11 hi	38.6 ± 1.4 fgh
	AMF1+B1	211.1 ± 5.4 ab	26.8 ± 0.78 bc	3.6 ± 0.12 efg	1.9 ± 0.07 gh	29.9 ± 1.0 j
	AMF1+C1	200.5 ± 5.8 ab	26.9 ± 0.62 bc	3.6 ± 0.25 efg	2.0 ± 0.10 gh	34.23 ± 0.8 hij
	B1+C1	188.5 ± 5.0 bcd	25.3 ± 0.87 cd	2.8 ± 0.14 gh	2.5 ± 0.08 efg	37.87 ± 1.0 gh
	AMF1+B1+C1	208.7 ± 5.2 ab	31.7 ± 0.88 a	3.1 ± 0.18 fgh	1.2 ± 0.05 i	31.04 ± 1.1 ij

Water regimes	Parameters/ Treatments	Sugar content (mg. g^{-1} dry weight)	Proteins content (mg. g^{-1} dry weight)	POX (µmol mg^{-1} protein min^{-1})	PPO (µmol mg^{-1} protein min^{-1})	H_2O_2 (nmol g^{-1} dry weight)
Drought	Control	112.2 ± 4.3 g	11.4 ± 0.57 i	3.0 ± 0.20 gh	2.8 ± 0.10 def	69.1 ± 1.5 a
	AMF1	175.4 ± 2.5 cde	17.4 ± 0.47 fg	6.4 ± 0.28 b	5.0 ± 0.10 b	43.6 ± 1.2 c-g
	B1	167.5 ± 5.8 def	13.1 ± 0.42 h	4.4 ± 0.12 de	6.3 ± 0.20 a	50.2 ± 1.2 bc
	C1	154.3 ± 2.7 ef	23.17 ± 0.74 de	3.9 ± 0.12 ef	3.1 ± 0.13 cde	47.7 ± 1.6 d-g
	AMF1+B1	168.6 ± 2.8 de	20.6 ± 0.75 ef	7.3 ± 0.28 a	5.1 ± 0.25 b	44.8 ± 1.7 b-f
	AMF1+C1	194.4 ± 4.5 abc	20.1 ± 0.59 ef	6.5 ± 0.12 ab	5.3 ± 0.21 b	43.2 ± 1.2 d-g
	B1+C1	154.5 ± 4.1 ef	16.0 ± 0.51 gh	5.3 ± 0.10 cd	3.4 ± 0.13 cd	45.3 ± 1.6 b-e
	AMF1+B1+C1	168.7 ± 3.6 de	20.9 ± 0.76 e	6.0 ± 0.35 bc	3.7 ± 0.15 c	39.2 ± 1.0 e-h

Identification of traits conferring drought tolerance to date palm under water stress conditions

All data were summarized using the PCA approach to identify important parameters and treatments affecting date palm performance under WW and D conditions. Loaded variables and their contribution to both axes of the PCA are showed (Figures 11). Under WW conditions, the PC1 and PC2 explained for 75.6% of the total variance with treatments AMF1+B1+C1 and AMF1+B1 being the most effective which associated with improved date palm growth, nutrient uptake and physiological and biochemical parameters (Figure 11A). Inoculation with B1 alone was the treatment most similar compared to the control. Under D conditions, the PC1 and PC2 accounted for 79.5% of the total variance (Figure 11B). All treatments including inoculation with AMF1 were the most effective in altering date palm growth, nutrient uptake as well as physiological and biochemical parameters under D conditions (Figure 11B). Under both water regimes, the total H_2O_2 content was the parameter, which was most closely associated with the control treatments (Figure 11A, B).

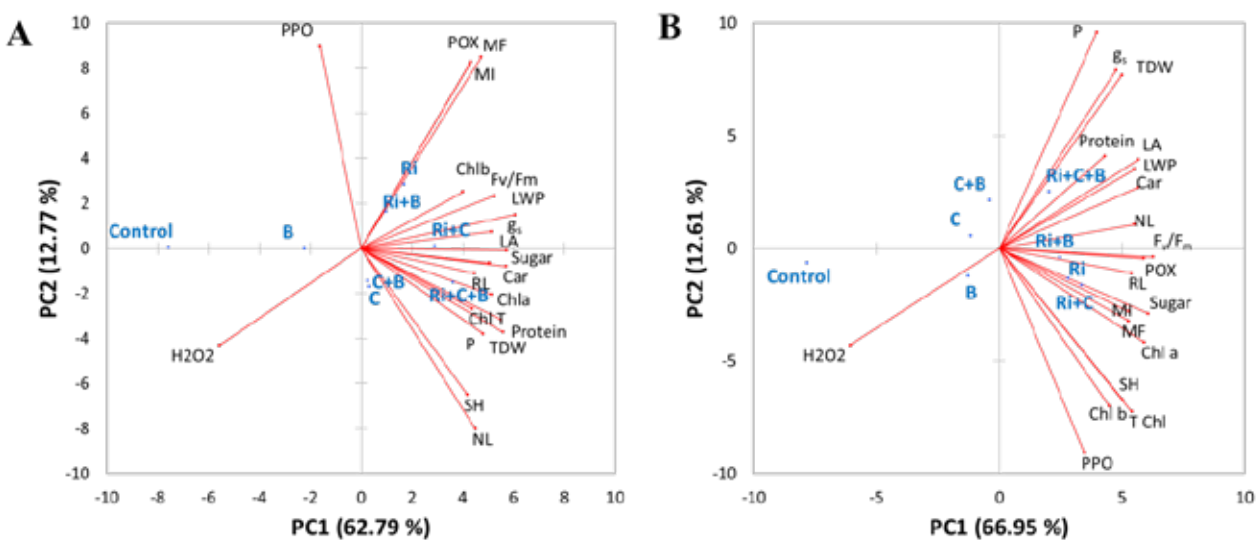

Figure 11. Principal component analysis (PCA) of all investigated traits under (A) well-watered and (B) drought conditions. Car: carotenoid, Chl a: chlorophyll a, Chl b: chlorophyll b, Fv/Fm: chlorophyll fluorescence, gs: stomatal conductance, H_2O_2: hydrogen peroxide, LA: leaf area, LWP: leaf water potential, MF: mycorrhizal frequency, MI: mycorrhizal intensity, NL: leaf numbers, P: plant phosphorous, POX: peroxidase, PPO: polyphenol oxidase, RL: root length, SH: shoot height, TDW: Total dry weight, T Chl: total chlorophyll.

Discussion

To ensure sustainable food production systems with minimal environmental degradation in the era of climate change, agricultural practices require eco-friendly approaches, particularly in arid and semi-arid zones. Therefore, production systems to benefit small farmers exploiting local-based resources such as the use of compost combined with microbial inoculation practices could make significant contributions towards resilient agroecosystem management. A proper understanding on how date palms' agrophysiology and biochemistry are affected by drought stress and on options to mitigate stress damages will help farmers to increase their production and also might benefit breeding programs to develop cultivars/varieties being tolerant against abiotic stresses like drought stress. The present study illustrated the adverse effects of drought stress on date palms in the greenhouse stage and the field conditions as revealed by morphological, physiological, and biochemical traits.

The application of composts alleviated drought stress damage in date palms cultivated in greenhouse and field conditions by improving the plants' water relations as revealed by higher leaf water potentials and stomatal conductance. When organic matter was added via the compost, the soils' organic matter content is enhanced, which in turn increases the water retention and water storage potential of the soil (Somerville et al., 2019a,b). Thus, compost supplementation will support the plants in maintaining their physiological activities, helping the plants to escape from the drought stress (Abd El-Mageed et al., 2018, 2019; Bashir et al., 2020). On *Sorghum bicolor* and *Beta vulgaris*, Abd El-Mageed et al. (2018, 2019) observed that compost application also alleviated the negative effect of drought by regulating the internal plant water status as well as physiological traits and ameliorated the physical and chemical properties of soils. On wheat crops, compost combined with biochar helped to improve the photosynthesis, growth, and yield by mitigating the oxidative stresses in leaves (Bashir et al., 2020). Date palms supplemented with different dosages (5 and 20%) of compost at nursery stage showed growth improvement and enhancement in their nutrition status as revealed by an increase in root length, leaf area, root dry weight, leaf nitrogen, P and potassium contents (Anli et al., 2020b). Also, EL Kinany et al. (2018) reported the growth-promoting effects of compost when date palm was grown in sand amended with 25% compost. Compost also serves as a source of carbon, providing food for microbes promoting their proliferation, which might further translate into enhanced root colonization and plant growth improvement through assimilation/solubilization of soil nutrients such as N, P and K (Xu et al., 2018; Rodríguez-Berbel et al., 2020). The plant growth-promoting effect under drought stress has also been described for many other plants treated by organic amendment including turf grass, sugar beet and marigold (Kanwal et al., 2017; Duo et al., 2018; Abd El-Mageed et al., 2019; Khosravi Shakib et al., 2019).

Inoculation with the PGPR consortia in greenhouse as well as in field conditions contributed to improving date palm performance under drought stress by improving the plants' water relations as shown by enhanced leaf water potentials and stomatal

conductance and helped the plants to maintain photosynthetic activity. The four PGPR strains used in our studies allowed the date palm to mitigate the drought stress, indicating their potential to assist plants to withstand stressful conditions. It is well recognized that PGPRs secrete osmolytes to mitigate drought stress, which acts synergistically with plants internal osmolytes and boost their growth (Kumar and Verma, 2018; Nazir et al., 2018; Khan and Bano, 2019; Kumar et al., 2019; Mishra et al., 2020). Besides, our PGPR strains possess the ability to solubilize P, which translated into an increased P uptake by date palms under both water regimes. Similarly, Meena et al. (2017) and Khan and Bano (2019) reported about the growth-promoting effects of PGPRs on the growth of wheat and other crops grown under water limiting conditions. The use of PGPR considerably reduced the adverse effects of drought on wheat as well as lettuce and many other crops by enhancing the physiological and biochemical aspects (Bacon and White, 2016; Vurukonda et al., 2016; Kanwal et al., 2017; Aini et al., 2019).

Inoculation with the native consortium of AMF (AMF2) and the exotic *R. irregularis* (AMF1) significantly improved date palm growth, development and adaptation to drought stress as highlighted by the PCA, with all AMF2 and AMF1 inoculated date palms clustering together apart from the control in greenhouse and field conditions respectively. All AMF2 and AMF1 inoculated date palms showed an increase in root colonization frequency and intensity, which was more than two and five times higher, respectively as compared to the controls when subjected to drought stress. The increased root colonization was in line with the observed increases in date palm growth, P and N uptake, stomatal conductance and leaf water potential in all treatments including AMF2+C2, AMF2+C2+B2, AMF2+C2+B1 and AMF2+C1+B2 greenhouse as well as *R. irregularis* inoculations, namely AMF1, AMF1+B1, AMF1+C1 and AMF1+B1+C1 in field conditions. By the direct uptake of nutrients and water by the hyphal network, AMFs assist their host plants to sustain their nutritional needs and enhance the plants' drought tolerance (Augé et al., 2014; Frosi et al., 2016; Volpe et al., 2016; Symanczik et al., 2018; Volpe et al., 2018; Zhang et al., 2018; Li et al., 2019; Boutasknit et al., 2020). Inoculation with exogenous and native AMF further increased the activity of POX and PPO and total protein content in date palms subjected to drought stress. Earlier studies have reported the positive benefits of AMF inoculation to enhance the synthesis of antioxidative enzymes and the accumulation of solutes (sugar, proline and protein molecules), maintenance of a water potential gradient and water absorption to preserve plants against water stress damages (Yooyongwech et al., 2016; Rahimzadeh and Pirzad, 2017; Begum et al., 2019; Behrooz et al., 2019). Yooyongwech et al. (2016) and Begum et al. (2020) further reported improved performance of AMF-inoculated plants via up-regulation of the antioxidant metabolism pathway and accumulation of osmolytes in sweet potato and tobacco. Similarly, inoculation with *Funneliformis mosseae* and *R. irregularis* as well as native arbuscular increased enzymatic activities and accumulation of soluble sugars leading to improved growth of sesame (*Sesamum indicum* L.) (Estrada et al., 2013; Gholinezhad et al., 2020). Li et al. (2019) and Ghanbarzadeh et al. (2019) also reported about AMF inoculation effects to alleviate drought stress in C3 (*Leymus chinensis*) and C4 (*Hemarthria altissima*) grasses as well as *Dracocephalum moldavica* plants via altering antioxidant enzyme activities and photosynthesis. The potential of AMF to reduce the drought stress damaging effects in date palms through amelioration of water status, photosynthesis, and antioxidant activities has previously observed (Baslam et al., 2014; Meddich et al., 2015, 2020; Bashir et al., 2020).

The triple combination of *R. irregularis* and the native consortium of AMF with PGPR and composts alleviated drought stress in date palms as revealed by a decreased degradation of total chlorophyll a and b and carotenoids, resulting in a higher accumulation of pigments under drought stress, implying a better performance of the photosynthetic apparatus. This might have resulted from the reduced accumulation of H_2O_2 via increasing in POX and PPO activities. The accumulation of ROS in shoots and roots cells under drought stress can cause important damages and interferes with the plants' photosynthesis machinery resulting in a decrease in photosynthetic activity and chlorophyll concentrations (Becklin et al., 2016; Mo et al., 2016; Li et al., 2019; Fracasso et al., 2020). Also, Siddiqui et al. (2019) reported that the activation of the antioxidant defense system and the production of exopolysaccharides improved the performance of inoculated tomato seedlings under drought stress. Another stress adaptation mechanism is associated with increased accumulation of soluble sugars and proteins that regulates the osmotic potential of cells to develop short- or long-term response to mitigate the stress (Begum et al., 2020; Boutasknit et al., 2020). The triple combination of *R. irregularis* and the native consortium of AMF, PGPR and composts increased soluble sugars and protein contents in leaf and root tissues under drought. Earlier results also reported about an increase in total sugar and proteins contents after inoculation with AMF or PGPR in valerian, lettuce and sesame plants (Vurukonda et al., 2016; Aini et al., 2019; Gholinezhad et al., 2020). Similarly, using compost in combination with PGPRs and AMF in cottony cistus, wheat, walnut, and turfgrass plants helped to alleviate drought stress by higher accumulation of soluble sugar and proline contents (Kanwal et al., 2017; Duo et al., 2018; Ortuño et al., 2018; Behrooz et al., 2019). Up to date, some studies reported about the beneficial effect of AMF and PGPR inoculation combined with compost supplementation to improve plant growth under drought stress in date palms and other crop plants (Baslam et al., 2014; Meddich et al., 2015; Ortuño et al., 2018; Aini et al., 2019; Hao et al., 2019b). Thus, the combined use of microbial inoculants and organic fertilizers should be further evaluated to gain further evidence of their good efficacy. This might help to convince farmers to use these practices to promote resource preservation and improve the resilience of agroecosystems. The obtained results demonstrated that levels of MDA and H_2O_2 were lower and POX and PPO activities were increased under drought stress in treated plants compared to control plants. To explain the low lipid peroxidation damage and the increase of antioxidant activities in AMF-treated plants, two possibilities were suggested by Abbaspour et al. (2012): (1) either inoculated plants with AMF suffered less drought stress owing to a primary drought avoidance effect by symbiosis (e.g., direct water uptake by fungal hyphae from the soil to the host plant) or (2) AMF colonization improved the activities of antioxidant enzymes as a defense to eliminate the ROS. Our results suggest that the application of compost and inoculation with AMF and PGPR could improve the defense against drought stress by reducing and eliminating ROS diffusion and production. Plants treated with AMF/PGPR counteract water deficit-induced oxidative stress by upregulating ROS-scavenging antioxidant compounds and antioxidant enzymatic activities.

In the present work, we identified several physiological, metabolic and growth traits including leaf water potential, stomatal conductance, chlorophyll fluorescence, chlorophyll pigment contents, soluble sugars, and total protein content, being altered under drought stress. These traits could be manipulated in the breeding programs to improve the drought tolerance of new varieties of date palm and adaptation in drought-prone areas. Plant adaptation to stress is associated with high accumulation of soluble sugars and protein that regulate the osmotic potential of cells and develop short- or long-term response to mitigate the stress. The synthesis of soluble sugars and proteins could be a trait of interest in the breeding program to improve the drought tolerance of crops.

The results highlight a physiological and biochemical switching mechanism in microbe association and provide additional confirmation of the hypothesis that microbial association and compost operate at multiple (including photosynthesis machinery, antioxidant system, and osmolytes biosynthesis) levels. Our study showed an improvement in the parameters studied in date palms growing in soil treated by the exogenous and autochthonous biofertilizers mainly the AMF1 strain and the consortium AMF2 alone and their combinations with composts (C1 and C2) and/ or PGPR (B1 and B2) under drought stress, especially AMF2+C2 and AMF2+C2+B2 treatments in greenhouse and AMF1+B1+C1 treatment in open field. This improvement in growth, mineral uptake, and physio-biochemical traits together with the decrease in MDA and H_2O_2 could be due to the synergy between AMF, compost, and PGPR: (1) the composts (C1, C2) with low dose 5% allows good mycorrhizal infectivity, the presence of essential mineral elements such as N, and P for plant growth, (2) the native and exogenous AMF (AMF2, AMF1) hyphal structure might allow the uptake of water and nutrients needed by the plants and/or the changes in the level of phytohormones that participate in symbiosis. Furthermore, the stimulation of AMF symbiosis by root exudates could constitute an important source of organic carbon in the rhizosphere and a route of chemical communication between root plants and the fungi and (3) the PGPR (B1, B2) could enhance phosphate solubilization resulting in increased phosphate available in the soil absorbed by plants through the production of organic acids and phosphatase. Furthermore, PGPR could modulate the tolerance of date palms via other mechanisms, yet to be elucidated, such as phytohormones (auxin, cytokinins), siderophores, and exopolysaccharide production.

It is worthy of note that the use of exogenous (AMF1) and autochthonous biofertilizers (i.e AMF2, C2, C1, B1 and B2) could constitute an original approach to improving the boost in growth and tolerance and may be a suitable combination for date palms in arid climates.

Conclusion

In the present study, we investigated drought stress effects on agrophysiological as well as biochemical parameters of the date palm. Inoculation of the date palms with AMF and PGPR and supplementation with compost ameliorated the date palm performance and mitigated the drought stress negative impact. Thus, biofertilization i.e. using a combination of microbial inoculants and organic fertilizers represents a valuable tool for farmers to improve date palm cultivation under the harsh environmental conditions of the arid and semi-arid ecosystems where date palms represent a major crop. Also, the identification of traits being altered under drought stress such as leaf water potential, stomatal conductance, chlorophyll fluorescence, chlorophyll pigment contents, soluble sugars and total protein content could be manipulated in breeding programs to improve the drought tolerance of new varieties for drought-prone areas. Finally, in our efforts to support farmers producing date palm fruits under adverse climatic conditions, the present study paves the way towards sustainable production using an eco-friendly approach that preserves environmental degradation.

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Chapter 5

Arbuscular Mycorrhizal Fungi in Saline Soil: Alleviating Date Palm under High-Salt Stress

Mohamed Ait-El-Mokhtar[1], Raja Ben Laouane[1], Mohamed Anli[1], Abderrahim Boutasknit[1], Said Wahbi[1]and Abdelilah Meddich[1*]

[1]Laboratoire d'Agro-Alimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Facultédes Sciences Semlalia, Université Cadi Ayyad, BP2390 Marrakech, Maroc.

*Corresponding author: a.meddich@uca.ma

Abstract

The current study was carried out to assess the response of date palm (*Phoenix dactylifera* L.) to salinity and to examine the possible role of arbuscular mycorrhizal fungi (AMF) in enhancing the salt tolerance. Plants were grown under non-saline or saline conditions (0 and 240 mM NaCl) with and without AMF inoculation while biochemical, mineral as well as growth parameters were measured in this study. Plant growth parameters including plant height, leaf area and shoot and root dry weight were negatively affected by salinity. However, mycorrhizal plants showed higher growth parameters under saline condition compared to non inoculated salt-affected plants, although root colonization by AMF structures was reduced by salinity. Mycorrhiza mitigated the reduction of K, P and Ca content induced by salinity. Ca/Na and K/Na ratios were also improved in colonized plants. Otherwise, AMF symbiosis improved physiological parameters through elevating stomatal conductance, photosynthetic efficiency and leaf water potential under salinity stress. In the same conditions, mycorrhizal inoculation significantly enhanced concentrations of photosynthetic pigments and protein content. Furthermore, salt stress caused high lipid peroxidation and increased H_2O_2 content; however, the application of AMF reduced these two parameters in salt-affected plants while activities of antioxidant enzymes (superoxide dismutase, catalase, peroxidase and ascorbate peroxidase) were increased by salt stress and were further enhanced in plants treated with AMF. Overall, it is evident that soil salinity induced detrimental effects on date palm plants, which were lower in mycorrhizal plants than in the non-mycorrhizal ones. In conclusion, colonization with AMF may protect date palm seedlings against the negative salinity influence by mitigating the salt induced oxidative stress.

Keywords: Date palm, AM fungi, salinity tolerance, antioxidant enzymes, nutrient uptake.

Introduction

Plants are often exposed to many environmental stresses inducing growth and metabolism perturbations (Barnawal et al., 2014). Salinity is one of the most important and adverse agricultural and eco-environmental problems, which is restricting growth and development of plant especially in arid and semi-arid regions (Alqarawi et al., 2014; Yaish and Kumar, 2015). Furthermore, it drastically reduces agricultural yield by over than 20% (Porcel et al., 2012). Increasing industrialization, use of saline water for irrigation and effects of climate change further aggravates this issue (Roy et al., 2014; Shrivastava and Kumar, 2015). It has been estimated that salt-affected soils occupy around 8% of the earth's surface and are increasing world wide (Hajiboland 2013). The increase in soil salinity causes disruption in physiological and bio-chemical activities such as ion homeostasis, seed germination, osmotic adjustment, photosynthesis and respiration processes and nitrogen metabolism (Sheng et al., 2008; Evelin et al., 2009; Kaya et al., 2009; Porcel et al., 2012). Osmotic stress is a primary stress induced by the excess of Na^+ and Cl^-. Salinity generates also a secondary stress known as oxidative stress and is due to the accumulation of reactive oxygen species (ROS) (Gill and Tuteja, 2010; Noctor et al., 2014). Salt stress enhances the production as well as accumulation of toxic ROS including superoxide, hydroxyl and peroxide radicals, and they induce deleterious effects to normal metabolism and growth (Mittler 2002; Aroca et al., 2013). The produced toxic ROS disrupt normal metabolism through denaturation of proteins, mutagenesis of nucleic acids and lipid peroxidation as the immediate effects of these molecules (Triantaphylides et al., 2008; Estrada

et al., 2013). To detoxify and eliminate the generated ROS, plants are well equipped with different defense mechanisms. Enhancement of antioxidant enzymes activities is one of the prime defense mechanisms that help plants to handle stress-induced oxidative damage. Antioxidant defense system includes many enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Liu et al., 2014). These antioxidants scavenge the ROS and protect macromolecules from damage. Efficient sequestration and compartmentalization of toxic ions into other cellular compartments as vacuole or apoplast is another protective mechanism to avert stress induced damage (Azooz et al., 2011; Liu et al., 2014).

Scientists are exploring alternatives that may increase the crop production under saline conditions. The use of arbuscular mycorrhizal fungi (AMF) as a biological process to mitigate the harmful effects of salt stress on plants is of such alternatives (Ruiz-Lozano et al., 2012; Santander et al., 2019). AMF belong to the phylum Glomeromycota (Schubler et al., 2001) and are known to exist in saline soils (Aliasgharzadeh et al., 2001; Wilde et al., 2009). AMF symbiosis is known to alleviate salt stress by enhancing the rhizospheric soil characteristics (Ahanger et al., 2014; Hodge and Storer, 2014), improving nutrient acquisition, especially Phosphorus (P) (Alqarawi et al., 2014; Krishnamoorthy et al., 2016), reducing the uptake of Sodium (Na^+) and Chlore (Cl^-) (Al-Karaki 2006; Daei et al., 2009), enhancing water uptake (Aroca et al., 2013; Ahanger et al., 2014), improving photosynthetic activity and chlorophyll content (Kaya et al., 2009; Hidri et al., 2016) and increasing the synthesis and effectiveness of antioxidant molecules (Ruiz-Lozano et al., 2016; Ghouchani et al., 2017). However, AMF can themselves be negatively affected by soil salinity (Wu et al., 2010; Shekoofeh et al., 2012). For this reason, selection of the suitable AMF that increase plant performance in saline environment is important for the success of rehabilitation of lands affected by salinity. AMF species that are native to a particular soil are more effective symbionts than non-native AMF (Estrada et al., 2013; Pellegrino and Bedini, 2014), presumably due to adaptation of those fungi to edaphic and environmental factors (Querejeta et al., 2006; Estrada et al., 2013).

Phoenix dactylifera L., commonly known as date palm, is one of the most economically important perennial plants in arid and semi-arid areas of the Middle East and the North Africa (Chao and Krueger, 2007) where it is extensively cultivated for food and many other commercial and ecological purposes. Date fruits are a good source of essential nutrients such as sugars, proteins, fibers, minerals, antioxidants and vitamins (Vayalil 2012; Kamal-Eldin and Ghnimi, 2018). Despite numerous studies on date palm responses to salinity (Kurup et al., 2009; Sperling et al., 2014; Yaish et al., 2015; Meddich et al., 2018) relatively little is known about the effect of AMF in alleviating salt stress by this plant and the underlying mechanisms. In this context, this experiment was carried out to examine the efficiency of AMF on growth rate, photosynthetic efficiency, water status and biochemical responses of date palm seedlings grown either under normal or salinity stress. Different parameters related to salinity stress such as pigment content, antioxidants and nutrient uptake have been assessed in the present study.

Materials and methods

Biological material and treatments

Seeds of *Phoenix dactylifera* cv. Boufeggous were surface sterilized by 10% bleach for 10 min and were rinsed five times with sterile distilled water. Germination was carried out in plastic bowls containing a sterile sandy substrate with incubation for 3 weeks at 38°C in the dark. Two-month palm seedlings (leaf stage) are later transplanted into plastic pots containing 2.3 kg of river sand (0.001% P (Phosphorus), 0.001% K (Potassium), 0.006% Mg (Magnesium), 0.012% Fe (Iron), 0.01% Ca (Calcium), 0.002% Na (Sodium), 0.002% Si (Silicon), 0.01% Al (Aluminum), EC: 0.29 $dScm^{-1}$ and pH: 9.31) previously sterilized for 3 h at 180°C.

Arbuscular Mycorrhizal Fungi (AMF) were obtained from Aoufous mycorrhizal consortium (AMC), isolated from the soil surrounding the roots of *P. dactylifera* from the palm grove of Tafilalet. The AMC contains a mixture of indigenous species *Glomus* sp. (15 spores g^{-1} soil), *Sclerocystis* sp. (9 spores g^{-1} soil), and *Acaulospora* sp. (1 spore g^{-1} of soil) (Meddich et al., 2015).

AMC was multiplied by trap culture in pots using *Zea mays* L. as host plant under greenhouse-controlled conditions for 3 months. AM fungal inoculum consisted of a mixture of rhizospheric soil from the trap culture containing spores, hyphae and mycorrhizal root fragments. The inoculum was subjected to a most probable number test (Sieverding 1991) to determine potential infectivity and equalize application doses. Sources of inoculum contained 1056 infective propagules/100 g inoculum. The mycorrhizal inoculum was added to the corresponding pots, when transplanting seedlings, as 10 g of trap soil culture (approximately 11 spores/g trap soil, M = 78.8%). Non-inoculated pots received the same amount of autoclaved mycorrhizal inoculum.

Transplants were irrigated regularly with distilled water for 5 months after germination (3 months after AMF inoculation), and then it was subjected to two salinity levels of different NaCl concentrations (0 and 240 mM). To avoid osmotic shock, the NaCl concentration was gradually increased on alternative days (Estrada et al., 2013). It took two weeks, to reach the desired 240 mM NaCl level. The pots were watered with distilled water and saline solution (as needed) to maintain the level of salinity treatment near the target level. A plastic bag was put underneath each pot to collect excess water due to drainage. This water was reapplied to the respective pot. All seedlings were grown for 10 months without any fertilizer treatment.

Experimental design and growth conditions

The experimental design consisted of four treatments crossing two mycorrhizal inoculations levels (-AMF and +AMF) with two soil salt levels (0 and 240mM NaCl). Pots were arranged in a completely randomized block design. Each treatment had ten replicates (pots) for a total of 40 pots.

Plants were grown for 10 months in a greenhouse at the Faculty of Science Semlalia, Cadi Ayyad University, Marrakesh, Morocco, with a 16/8 h day/night cycle and average temperature of 25.5°C, relative humidity average of 68.5%, and light of 410 μm^{-2} s^{-1}.

Determination of Root Colonization and Plant Biomass Production

At harvest (10 months after seeds germination), the root system was separated from the shoot and the biometrical data (plant height, number of leaves, leaf area and dry weight of shoots and roots) were determined. The leaf area was determined using scanner LC 4800 and the software Winfolia v. 2004. A fraction of the roots, from the lateral root system, were carefully washed, cleared with 10% of KOH at 90 °C for 30 min, then acidified with 1% HCl for 10 min and were stained with Trypan blue at 90 °C for 20 min (Phillips and Hayman, 1970). Fine roots of 1.0 cm in length were examined under a Zeiss Axioskop 40 microscope at 40–100× magnification. A minimum of 30 segments for each replicate sample were observed to assess structural colonization of AMF associated with roots. The number of root segments forming AM was counted with criss cross lacing method and used to calculate AMF infection (McGonigle et al., 1990). AMF infection frequency and intensity were calculated using the following equations:

$$\text{AMF infection frequency (Fa) (\%)} = \left(\frac{\text{Infected root segments}}{\text{Total root segments}}\right) x100$$

$$\text{AMF infection intensity (Ma) (\%)} = \frac{(95n5+70n4+30n3+5n2+n1)}{\text{Total root segments}}$$

Where “n5” means the number of root with infection level of 5 (infection rate 90–100%). “n4” is the root number at level 4 infection (infection rate 50–90%). “n3” is root number at level 3 (infection rate at 10–50%). “n2” is root number at infection level 2 (infection rate 1–10%). “n1” is root number at level 1 (infection rate 0–1%).

Nutrient analysis

To determine the mineral contents, oven-dried shoots and roots were powdered, and the powder was digested with 98% H_2SO_4 and 30% H_2O_2. The phosphorus (P) content was estimated using the Olsen method (Olsen and Dean, 1965). The contents of Na^+, K^+ and Ca^{2+} in plant were estimated by flame photometry (JENWAY, PFP7) as described by Wolf (1982).

Photosynthetic efficiency

Measurements were carried out on the third youngest, fully expanded and attached leaf from five plants per treatment. An average of four records from different parts of each individual leaf was considered for each replicate. Chlorophyll fluorescence parameters were recorded using a portable fluorometer (Opti-sciences OSI 30p) for dark adapted leaves. Leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. Initial (F_0), maximum (F_m), variable ($F_v=F_m- F_0$) fluorescence as well as F_v/F_m ratio were recorded (Baker 2008).

Leaf relative water content, leaf water potential and stomatal conductance

Leaf samples were taken from two plants per replicate (the third leaf from the top) to determine fresh weight (FW), dry weight (DW) and turgid weight (TW). Values of FW, TW, and DW were used to calculate leaf relative water content (LRWC) using the equation below (Barrs and Weatherly, 1962):

$$\text{LRWC (\%)} = \frac{(FW - DW)}{(TW - DW)} x100$$

The leaf water potential was measured using a pressure chamber according to Scholander et al. (1965). Stomatal conductance (gs) was measured on five replications per treatment from 9:30 to 10:40 am at a sunny day before harvest and by using a porometer system (Leaf Porometer LP1989, Decagon Device, Inc., Washington, USA) following the user manual instructions. Stomatal conductance measurements were taken in the second youngest leaf from five different plants from each treatment.

Photosynthetic pigments, malondialdehyde and hydrogen peroxide contents

Photosynthetic pigments were extracted from leaf samples in 80% acetone as described by Arnon (1949). The extracted material was centrifuged at 10,000×g for 10 min. The optical density of the supernatants was recorded at 480, 645, and 663 nm using a UV-visible spectrophotometer (UV-3100PC spectrophotometer). A blank with 80% acetone served as the control.

Malondialdehyde (MDA) content in leaves and roots were determined according to the method of Dhindsa et al. (1981). 0.25 g of fresh material were homogenized in 10 mL of 0.1% TCA and centrifuged at 18,000xg for 10 min. Two milliliter aliquot of supernatant was mixed with 2 mL of 20% TCA containing 0.5% TBA. The mixture was heated at 100 °C for 30 min, quickly cooled and then centrifuged at 10,000×g for 10min for clarification. The absorbance of the supernatant was read at 532 nm. The unspecific turbidity was corrected by A600 subtracting from A532.

Hydrogen peroxide content in leaves and roots were determined according to Velikova et al. (2000) method. 0.25 g of fresh material was homogenized in a cold mortar with 5 mL 10% (w/v) TCA and then centrifuged at 15,000xg for 15 min at 4°C. The supernatant was then recovered to determine the content of H_2O_2. 0.5 mL of potassium phosphate buffer (10 mM, pH 7) and 1 mL of iodic potassium (1 M) were added to 0.5 mL of the supernatant. The absorbance at 390 nm was recorded after 1 hour of incubation in the dark. The blanks were made by replacing sample extract by 10% TCA.

Antioxidant enzyme assays

Assay of enzymes were performed according to optimized protocols described elsewhere (Benhiba et al., 2015). Fresh leaves or roots (0.5 g) were frozen in liquid nitrogen and then ground at 4°C in 5 mL solution containing 0.1 M potassium phosphate buffer (pH 7.0), 0.1 g polyvinylpolypyrrolidone (PVPP), and 0.1 mmol ethyl enediamine tetra acetic acid (EDTA). The homogenate was centrifuged at 18,000xg and 4°C for 10 min, and the supernatants were kept at -20°C for subsequent biochemical assays. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by the method of Beyer and Fridovich (1987) by measuring the ability of enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD was defined as the amount of enzyme required to inhibit the reduction rate of NBT by 50% at 25°C. The activity of SOD was expressed at unit min^{-1} mg^{-1} protein. The activity of catalase (CAT, EC 1.11.1.6) was determined as a decrease in absorbance at 240 nm for 3 min following the decomposition of H_2O_2 (Aebi 1984). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 20 mM H_2O_2 and 100 µL of enzyme extract in a 2 mL volume. The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured as a decrease in absorbance at 290 nm for 1 min (Amako et al., 1994). The assay mixture contains 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM H_2O_2, 0.1 mM ascorbate and 100 µL of enzyme extract. The ascorbate peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol test by following the change of absorbance at 470 nm. The activity was measured for 3 min in a reaction

solution containing 100 mM potassium phosphate buffer (pH 7.0), 20 mMguaiacol, 10 mM H_2O_2 and 0.15 mL enzyme extract (Polle et al., 1994). The protein concentration in the different extracts was measured following the protocol of Bradford (1976) with bovine serum albumin (BSA) as a standard.

Statistical analysis

Data presented are mean values based on five replicates ± standard error (S.E.) per treatment. Statistical analyses were carried out with the software package SPSS 10.0 for Windows. AMF inoculation, salinity level and their interaction effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using Dunkan's test calculated at $P<0.05$.

Results

Growth and mycorrhizal colonization

The salt stress caused significant ($P<0.01$) (Table 1) decrease in all the growth parameters such as plant height, number of leaves, leaf area and shoot and root dry weight (Table 2). Maximum decrease was observed for root dry weight and leaf area (40.98% and 39.20% respectively). The use of AMF alleviated the negative effect of salinity on the growth parameters significantly. Effectively, AM seedlings under salt conditions were significantly ($P<0.01$) taller with more leaves and leaf area, shoot and root dry weight increment were significantly larger than corresponding non-AM seedlings. Interactions among salinity × AMF treatment were significant ($P<0.05$) (Table 1).

Table 1. Result of two-way ANOVA test for independent variables including Salinity treatment and AMF inoculation and interaction among them.

	Tissue	Salinity	AMF	Salinity x AMF
Plant height	-	***	***	*
Number of leaves	-	**	***	*
Leaf area	-	***	***	***
AMF infection frequency	-	***	***	***
AMF infection intensity	-	***	***	***
Leaf relative water content	-	***	***	***
Leaf water potential	-	***	***	Ns
Stomatal conductance	-	***	***	***
Fv/Fm	-	***	***	**
P uptake	-	***	***	***
Na uptake	-	***	*	***

	Tissue	Salinity	AMF	Salinity x AMF
K uptake	-	***	***	Ns
Ca uptake	-	***	***	Ns
Chlorophyll a	-	***	***	Ns
Chlorophyll b	-	***	***	*
Total Chlorophyll	-	***	***	Ns
Carotenoids	-	***	***	***
Dry Weight	Shoot	***	***	**
	Root	***	***	**
K/Na	Shoot	***	***	***
	Root	***	***	***
Ca/Na	Shoot	***	**	***
	Root	***	***	**
H_2O_2	Shoot	***	***	Ns
	Root	***	***	Ns
MDA	Shoot	***	***	Ns
	Root	***	***	**
Protein	Shoot	***	***	***
	Root	***	***	***
SOD	Shoot	***	***	Ns
	Root	***	***	Ns
CAT	Shoot	***	***	Ns
	Root	***	***	Ns
POD	Shoot	***	***	Ns
	Root	***	***	Ns
APX	Shoot	***	***	Ns
	Root	***	***	Ns

ns non significant; *Significant at P<0.05; **significant at P<0.005; ***significant at P<0.001***; Non-inoculated date palm plants did not show any colonization with AMF in roots. In non-salinized plants, mycorrhizal inoculation treatment produced active colonization in root systems of date palm seedlings (Fa=100%, Ma=74%). The level of colonization in roots of mycorrhizal plants decreased significantly (P<0.001) (Table 1) with the application of NaCl stress. Indeed, AMF infection frequency and intensity decreased by 43.38% and 63% respectively (Table 2). Interactions between salinity and AMF treatment were significant for these two parameters (P<0.001) (Table 1).

Table 2. Influence of different salinity levels (0 mM and 240 mM) on biometrical data (plant height, number of leaves, leaf area and dry weight) and on AMF infection of non-mycorrhizal (control) and mycorrhizal ten months old plants. Values of each parameter labeled by different letters indicate significant differences assessed by Dunkan's test after performing two-way ANOVA (P<0.05).

NaCl treatment	AMF treatment	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Shoot dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	AMF infection frequency (Fa) %	AMF infection intensity (Ma) %
0 Mm	-AMF	30.6±0.39c	3.2±0.42c	18.95±0.68c	1.55±0.06c	1.11±0.10c	0.00±0.00b	0.00±0.00c
	+AMF	35.3±0.54a	5.2±0.42a	32.40±0.65a	4.99±0.89a	3.36±0.53a	100±0.00a	74.00±4.16a
240 Mm	-AMF	27.3±0.40d	3.1±0.32c	11.52±1.20d	1.09±0.14d	0.66±0.09d	0.00±0.00b	0.00±0.00c
	+AMF	31.5±0.34b	4.1±0.32b	20.62±0.55b	3.51±0.47b	1.68±0.22b	100±0.00a	27.38±4.44b

Water and physiological parameters

Table 3 shows the effect of salt stress and AMF application on leaf relative water content (LRWC), leaf water potential, stomatal conductance and photosynthetic efficiency parameters of date palm plants. The interaction between salinity and AMF had significant effect (P<0.01) on these parameters except leaf water potential (Table 1).

All these parameters were significantly lowered by salt stress (P<0.001) mostly leaf water potential and stomatal conductance which decreased by 32.77 and 38.37% respectively (Table 3). However, AMF application significantly enhanced water and physiological parameters (P<0.001) compared with the non-mycorrhizal plants under salt stress. The maximum values were obtained for stomatal conductance and photosynthetic efficiency (111.66 and 115.82% respectively).

Table 3. Influence of different salinity levels (0 mM and 240 mM) on water and on physiological parameters of non-mycorrhizal (control) and mycorrhizal ten months old plants. Values of each parameter labeled by different letters indicate significant differences assessed by Dunkan's test after performing two-way ANOVA (P<0.05).

NaCl treatment	AM treatment	Leaf relative water content (LRWC) %	Leaf water potential (ψh) (bar)	Stomatal conductance (gs) (mmol m^{-2} s^{-1})	Photosynthetic efficiency (Fv/Fm)
0 Mm	-AMF	73.72±1.35b	-24.9±0. 87c	17.63±1.25c	0.47±0.03c
	+AMF	82.81±1.78a	-17.34±1.04a	43.96±3.53a	0.78±0.03a
240 mM	-AMF	50.03±0.76d	-30.87±1.98d	10.87±1.80d	0.34±0.01d
	+AMF	65.92±1.14c	-22.27±1.14b	21±1.52b	0.73±0.01b

Nutrient Contents

As shown in Figure1, phosphorus uptake by plants was significantly (P<0.001) decreased by salinity (43.15%). Uptake of Na was enhanced two times in salt- affected plants compared to control plants. As expected, K and Ca uptake of salt-affected plants decreased, by 44.57% and 31.11% respectively, in comparison to the control. Colonization with AMF increased the content of P, K and Ca and decreased Na content of salt-affected plants (Figure 1). In addition, the ratios of K/Na and Ca/Na were significantly (*P*<0.001) reduced by salinity (Table 4). However, arbuscular mycorrhizal (AM) plants had greater values for K/Na and Ca/Na in both shoots and roots compared to the non-mycorrhizal plants (Table 3). The interaction between salinity and AMF inoculation had significant effect on Na and P uptakes and K/Na and Ca/Na ratios (*P*<0.05) (Table 1).

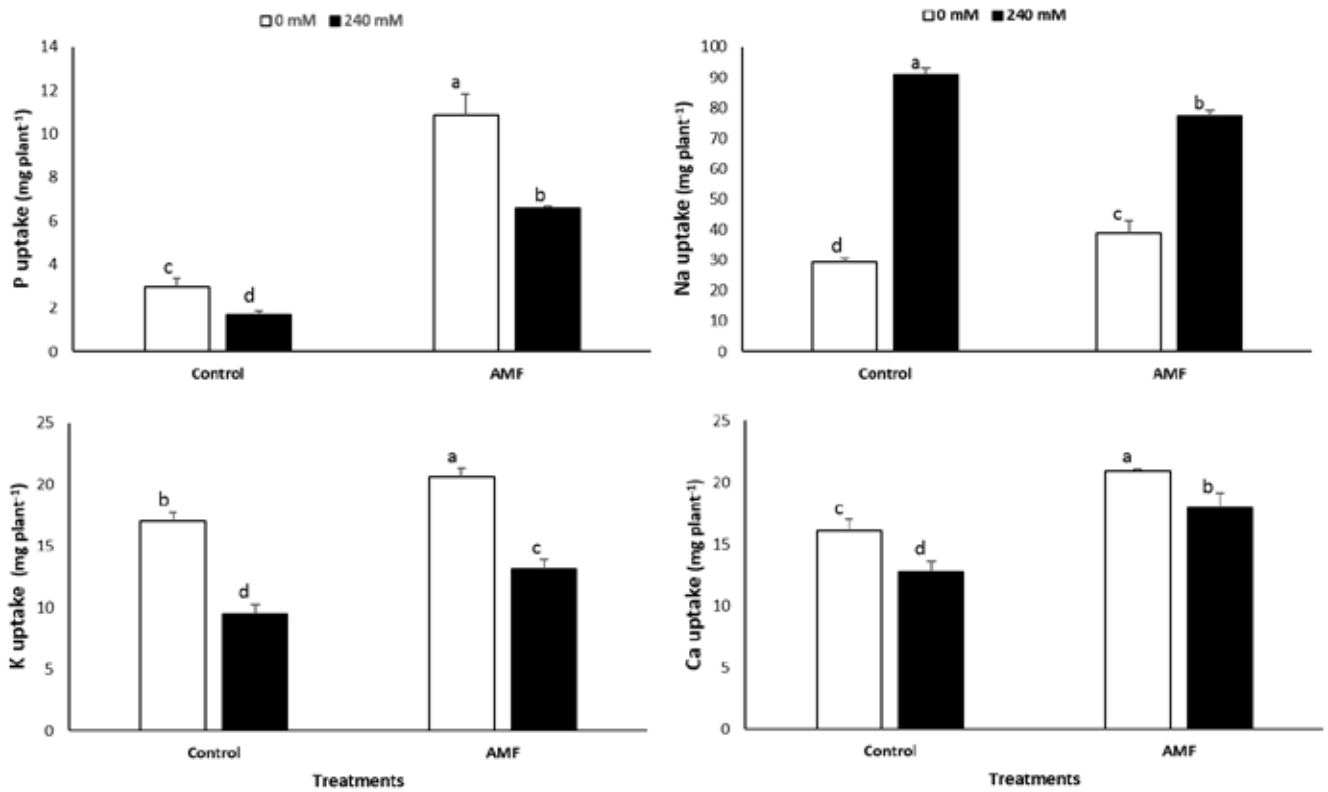

Figure 1. Influence of different salinity levels (0 mM and 240 mM) on mineral content of non-mycorrhizal (control) and mycorrhizal ten months old plants. Bars of each parameter labeled by different letters are significantly different (*P*<0.05) by Duncan's test.

Table 4. Influence of different salinity levels (0 mM and 240 mM) on K/Na and Ca/Na ratios of non-mycorrhizal (control) and mycorrhizal ten months old plants. Values of each parameter labeled by different letters indicate significant differences assessed by Dunkan's test after performing two-way ANOVA (P<0.05).

NaCl treatment	AMF treatment	Shoot		Root	
		K/Na	Ca/Na	K/Na	Ca/Na
0 mM	-AMF	1.95±0.05a	1.27±0.12a	0.23±0.002a	0.36±0.01b
	+AMF	1.54±0.05b	1.00±0.04c	0.23±0.02a	0.40±0.04a
240 mM	-AMF	0.16±0.01d	1.14±0.002b	0.06±0.005c	0.14±0.01d
	+AMF	0.26±0.003c	0.26±0.02d	0.11±0.009b	0.22±0.01c

Photosynthetic Pigments

Data presented in Figure 2 showed that photosynthetic pigments concentrations were significantly (P<0.001) decreased in the presence of salt stress. Effectively, when plants were grown in the presence of salinity, pigments decreased, Chla by 53.63%, Chlb by 43.45%, Carotenoids by 36.95% and total chlorophyll content by 49.89%, respectively, compared to non salt-affected plants. However, AMF application positively counteracted the negative effect of salt stress and induced stimulatory effect on all plant photosynthetic pigments concentration compared with non-mycorrhizal plants.

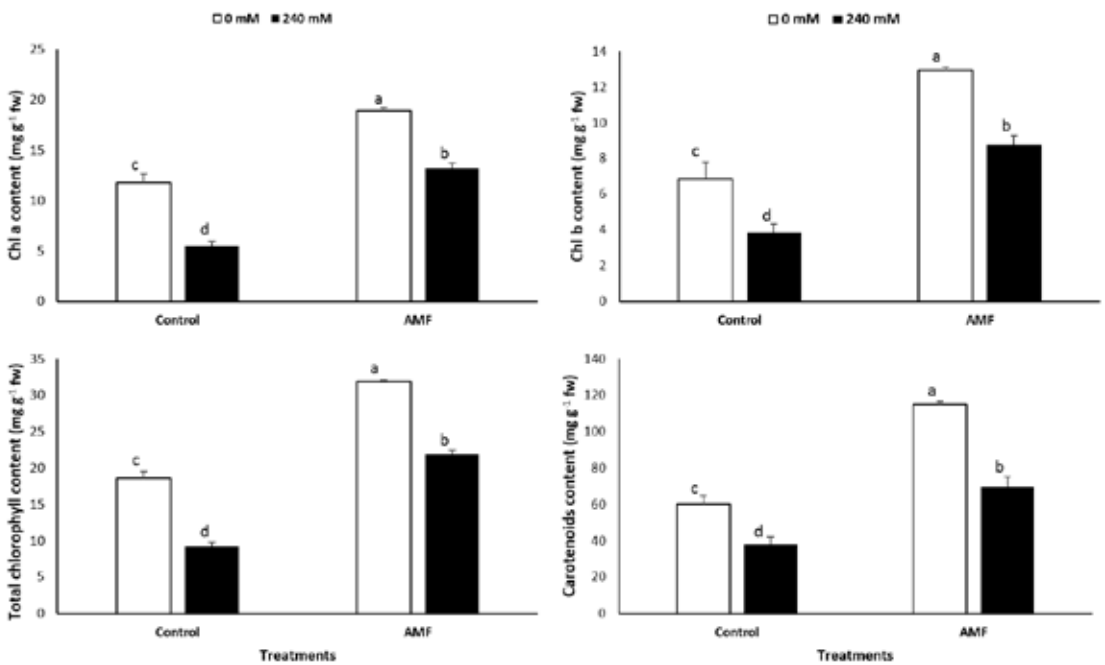

Figure 2. Influence of different salinity levels (0 mM and 240 mM) on Chlorophyll a, b and Carotenoids concentration of non-mycorrhizal (control) and mycorrhizal ten months old plants. Bars of each parameter labeled by different letters are significantly different (P<0.05) by Duncan's test.

H_2O_2, MDA and protein contents

The results related to the effect of salt stress on H_2O_2, MDA and protein contents in date palm seedlings are presented in table 5. Salinity significantly (P<0.001) increased H_2O_2 and MDA contents in both shoots and roots as compared to control. In contrast, salt stress decreased significantly (P<0.001) protein content in all plants. Application of AMF had shown a significant decrease in H_2O_2 and MDA

contents, while it had significant (P<0.001) positive effect on protein content and showed 34.53% greater protein as compared with the control plants. The interaction between salinity and AMF inoculation had significant effect on protein and root MDA contents (P<0.01) (Table 1).

Table 5. Influence of different salinity levels (0 mM and 240 mM) on the concentration of hydrogen peroxide, MDA and protein in the shoot and roots of non-mycorrhizal (control) and mycorrhizal ten months old plants. Values of each parameter labeled by different letters indicate significant differences assessed by Dunkan's test after performing two-way ANOVA (P<0.05).

NaCl treatment	AMF treatment	Shoot			Root		
		H_2O_2 nmol g^{-1}fw	MDA µmol g^{-1}fw	Protein g^{-1}fw	H_2O_2 nmol g^{-1}fw	MDA µmol g^{-1}fw	Protein g^{-1}fw
0 mM	-AMF	17.85±0.47b	23.05±1.30b	21.23±0.75b	36.83±2.01c	11.58±0.64b	11.67±0.47c
	+AMF	11.82±1.41c	16.69±0.79	26.57±1.27a	24.86±1,99d	6.88±0.79c	14.00±0.41a
240 mM	-AMF	24.81±2.40a	27.18±0.30a	16.04±1.02d	51.33±2.21a	14.29±0.30a	8.61±0.57d
	+AMF	19.47±1.07b	21.56±1.37b	20.08±0.31c	41.72±1.40b	8.43±0.30c	12.38±0.25b

Antioxidant Enzymes Activity

Salinity significantly increased activity of the studied antioxidant enzymes in shoots and roots (Figures 3 and 4). The activity of these enzymes was more important in root part compared to the shoot one. Activity of POD was mostly enhanced by salinity followed by SOD, CAT and APX. AMF colonization was accompanied by an enhancement of activity of SOD, CAT, POD and APX (Figures 3 and 4) in both salt- affected and control plants. Treatments effects on antioxidant enzymes activity were considered significant at (P<0.001) (Table 1).

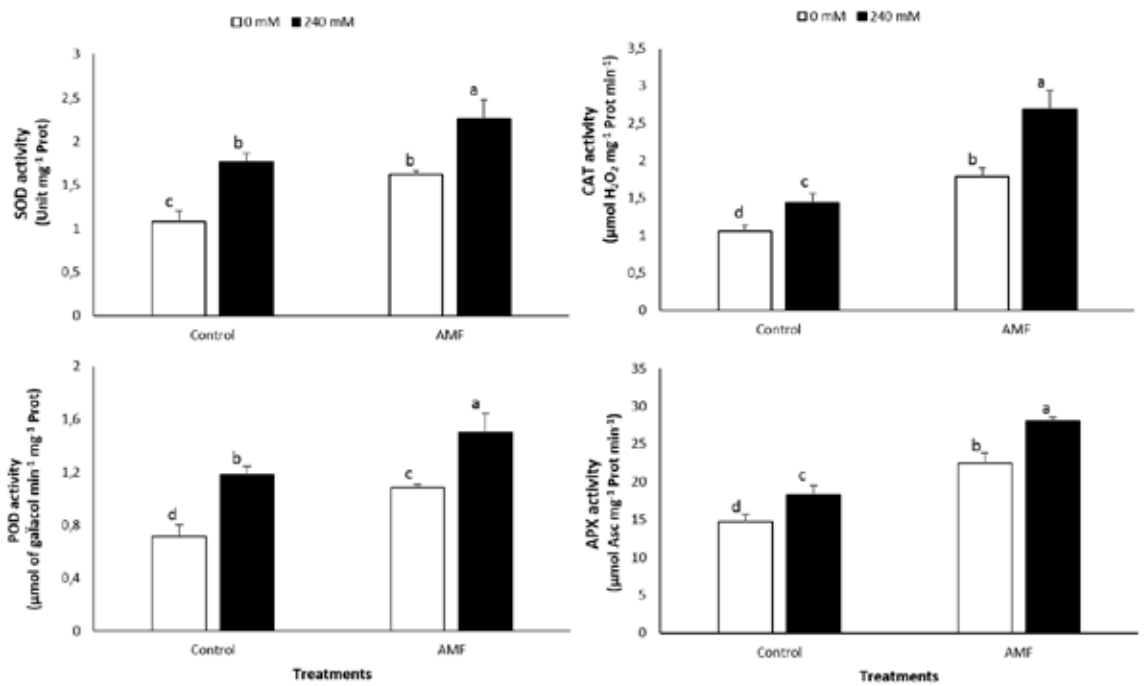

Figure 3. Influence of different salinity levels (0 mM and 240 mM) on the specific activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) activities in the shoot of non-mycorrhizal (control) and mycorrhizal ten months old plants. Bars of each parameter labeled by different letters are significantly different (P<0.05) by Duncan's test.

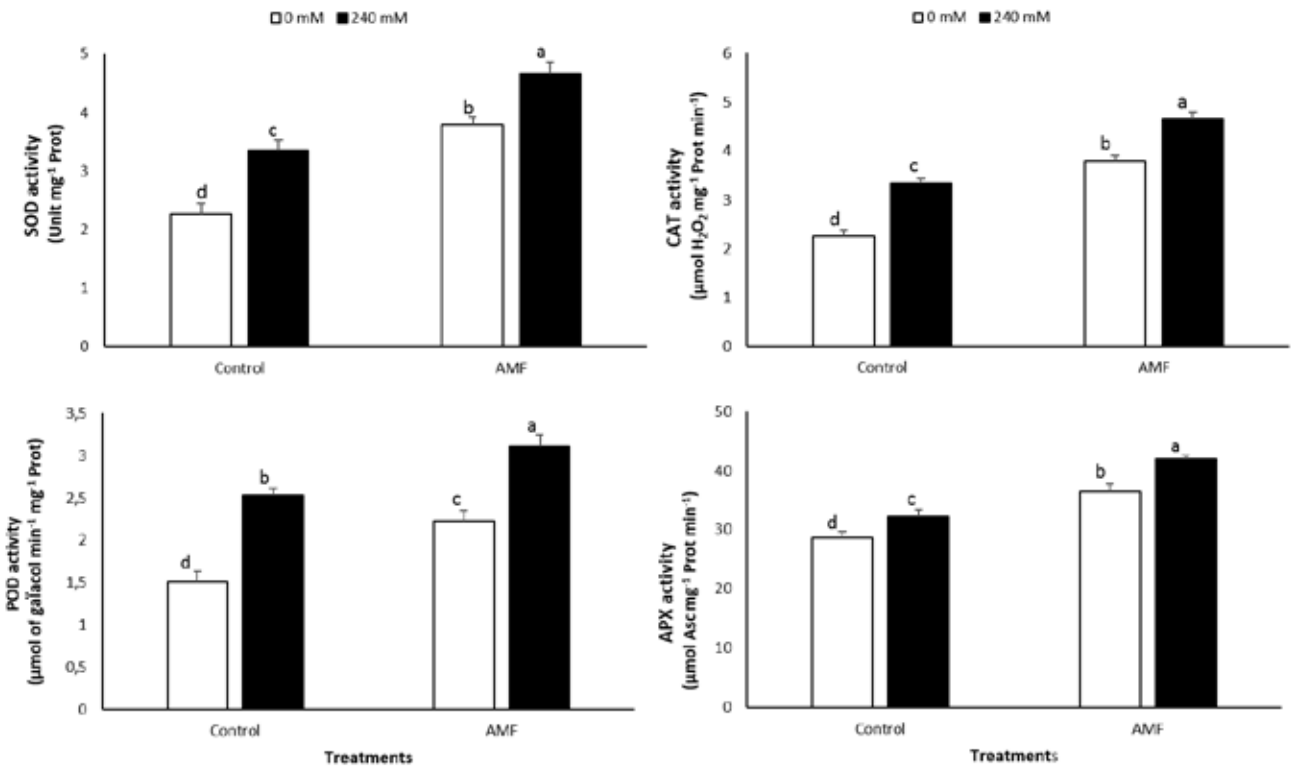

Figure 4. Influence of different salinity levels (0 mM and 240 mM) on the specific activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) activities in the root of non-mycorrhizal (control) and mycorrhizal ten months old plants. Bars of each parameter labeled by different letters are significantly different (P<0.05) by Duncan's test.

Discussion

Arbuscular-mycorrhiza can frequently increase host resistance to salt stress despite the fact that salinity attenuates AMF growth in different ways (Porcel et al., 2012; Evelin and Kapoor, 2014). Thus and as expected, root colonization rates declined under saline conditions, which has been attributed to an inhibition in spores germination and hyphal growth, or decrease in the spread of mycorrhizal colonization (Sheng et al., 2008; Hajiboland et al., 2010; Yarahmadi et al., 2018). Salt stress can also enhance H_2O_2 accumulation in the AM roots, which might ultimately induce the arbuscular degradation (Hause and Fester, 2005).

In the present investigation, a clear decline in date palm growth parameters was observed under saline conditions. Plant salt tolerance has frequently been assessed as the biomass production (da Silva et al., 2008). Although root biomass has been reported to be generally less affected by excess salinity than aboveground organs (Munns and Tester, 2008), in the present study, root dry weight was more severely affected by salinity than shoot dry weight, as roots are the first to encounter excess salinity in the soil solution. The decrease in growth is in accordance with the studies on other date palm cultivars (Kurup et al., 2009; Sperling et al., 2014). Salinity generally reduces plant growth due to osmotic stress and salt-excess effects (Munns and Tester, 2008; Rasool et al., 2013). In addition, salinity disrupted many metabolic processes, which delay growth and development of plants (Evelin et al., 2009; Porcel et al., 2012). Colonization with AMF significantly improved different growth parameters in the salt-stressed

plants. The effect of AMF on dry matter was more pronounced in shoot biomass than root biomass which may be because of mycorrhiza produced higher allocation of carbohydrates to the shoot than root tissues (Shokri and Maadi, 2009). Enhanced growth of mycorrhizal date palm seedlings grown in saline soils has been related partly to mycorrhiza-mediated enhancement of host plant P nutrition (Kaya et al., 2009; Alqarawi et al., 2014).

Salinization and AMF symbiosis not only affected date palm growth, but also its composition of mineral nutrients. In this study, we observed that NaCl treatment caused the content of Na^+ to increase significantly, and the P, K^+ and Ca^{2+} to decrease significantly in non-mycorrhizal date palm plants. In contrast, AMF application delayed salt stress effects on nutrient uptakes. Early studies reported that plants treated with NaCl in combination with AMF showed a clear enhancement in the uptake of P, K^+ and Ca^{2+} in the AM seedlings, as AMF inoculation has reduced the uptake of Na^+(Yarahmadi et al., 2018; Sallaku et al., 2019). The main impact of salt stress is imbalance of several important ions (Bhosale and Shinde, 2011; Garg and Pandey, 2015). Zheng et al. (2008) reported that the ionic balance has a determinant role in photosynthesis and other metabolic activities of the cell. This effect can be linked to the production of secondary metabolites (such as antibiotics and plant hormones), which promote physiological processes by increasing the absorption of essential macro and micro nutrients, thus improving plant growth (Garg and Manchanda, 2009; Kaschuk et al., 2010). Haque and Matsubara (2018) reported that removel of Na^+ absorption through roots in AM plants could be a mechanism of tolerance to salt stress. Other mechanisms that allowed mycorrhizal plants to present better salinity tolerance was the increase of K^+ and Ca^{2+} uptake. Potassium ions are vital for the plant to their involvement in the osmotic balance and has a role in the opening and closing of the stoma, and is a key factor in protein biosynthesis (Tomar and Agarwal, 2013). Whereas, calcium acts as a second messenger and during salt stress, its concentration is enhanced to transduce signals (Evelin et al., 2009). Previous studies reported an increase of Ca^{2+} uptake in mycorrhizal plants under salt stress (Yano-Melo et al., 2003; Hajiboland et al., 2010). In addition, the improvement of P uptake by the application of AMF could also be a reason for improving the tolerance of date palms to salinity, as shown in our study. This may be attributed to the role of P uptake in helping the vacuolar membranes to preserve its integrity, and facilitate the compartmentalization of Na^+ ions in the vacuoles (Cantrell and Linderman, 2001; Bothe 2012). As well, salinity inhibits the uptake of essential mineral elements, such as K^+, Ca^{2+} and P, because of the antagonistic relationship of Na^+ (Kohler et al., 2009; Hashem et al., 2016). Our study showed that AMF colonization enhanced the ratio of K^+/Na^+ and Ca^{2+}/Na^+ in salt affected plants. A body of evidence support a high K^+/Na^+ ratio under salinity in AM-inoculated plants (Giri et al., 2007; Wu et al., 2010). Maintenance of a higher cytosolic K^+/Na^+ ratio is an important aspect for the maintenance of physiological cellular functioning (Ahanger et al., 2015) to mitigate stress-induced deleterious changes. Our results showed also a higher Ca^{2+}/Na^+ ratio in date palm seedlings inoculated with AMF in the stressed condition. The same result was reported by Wu et al. (2010) in citrus and by Hajiboland et al. (2010) in tomato inoculated plants under salt stress. This result suggested that Ca^{2+}/Na^+ ratio may potentially reduce the antagonistic effect of Na^+ and therefore enhanced salt tolerance of mycorrhizal plants.

Salinity induced disturbance and damage to the photochemical reactions of photosynthesis, especially to the PSII (Sheng et al., 2008; Hidri et al., 2016). In the present study, salt stress also affected negatively chlorophyll contents (Chla, Chlb, Carotenoids and total chlorophyll). This negative effect is due to the destructive impact of salt stress on chloroplast (Zörb et al., 2009) and disruption of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute et al., 2018). A decrease in the uptake of minerals necessary for chlorophyll biosynthesis may also reduce the chlorophyll content (Sheng et al., 2008). Nevertheless, our study demonstrated that the toxic effect of salinity could be alleviated by AMF symbiosis since AM plants manifested a better photosynthesis capacity, as well as an increment in chlorophyll content under saline conditions. The increase in the activity of PSII in stressed AM plants, was most likely attributed to some metabolites accumulation (e.g. glycine betaine) which may protect the PSII complex and the CO_2-fixing enzymes (Yang et al., 2008). Increasing in photosynthetic pigments due to AMF application is because of improved mineral uptake especially magnesium, an essential component of chlorophyll molecule (Sheng et al., 2008). Likewise, AM seedlings had higher ability for CO_2 assimilation due to elevated stomatal conductance. Augé (2000) suggested that increased sink strength of AM roots could be a reason for the promoting observed effect of AMF colonization on stomatal conductance (gs). Furthermore, Augé et al. (2008) suggested that higher leaf water potential observed in AM plants under salt stress was due to changes in root morphology and fineness.

The application of NaCl stress and AMF affected the water status of date palm seedlings. Indeed, the LRWC decline in plants exposed to saline conditions, which is partly due to the effect of the salt on the electrical potential of the plasma membrane that alter not only ions uptake but also that of water, generating water stress (Munns 2002). However, the AMF colonization has raised the LRWC of salinity stressed AM plants. The application of AMF allows the plants to have a higher water content compared to non-AM plants (Sheng et al., 2008). This may be attributed to the enhanced hydraulic conductivity mediated by AMF symbiosis on the plant roots even under saline conditions (Kapoor et al., 2008).

Our findings demonstrated that salinity stress induced oxidative damage through increased lipid peroxidation and H_2O_2 content in date palm seedlings, which corroborate with the findings of Hajiboland et al. (2010) for *Solanumlycopersicum* L., Rasool et al. (2013) for *Cicer arietinum* L. and Alqarawi et al. (2014) for *Ephedra aphylla*. Roots were the organs that directly experienced the saline environment; thus, they exhibited higher accumulation of ROS than leaves. ROS reacts with unsaturated lipid membranes resulting in the loss of its integrity (Pedranzani et al., 2016). However, AM plants always showed lesser H_2O_2 and MDA contents involving lower accumulation of ROS and lower membrane damage in mycorrhizal plants. Reduced H_2O_2 and MDA contents in AMF colonized plants may be explained by the significant increase in antioxidant activities and phosphate metabolism (He et al., 2007; Tang et al., 2009).

A significant increase in activity of antioxidant enzymes such as SOD, CAT, POD and APX of date palms under salt stress was observed in the present study. In concurrence with our results, increased antioxidant activity in salt stressed plants is the result of several workers (Mittal et al., 2012; Nounjan et al., 2012; Rasool et al., 2013). SOD acts as the first line of defense and mediates scavenging of superoxide radicals into water and hydrogen peroxide (Mittler 2002) and constitutes an important contributor to maintenance of membrane stability in plant cells. H_2O_2 produced is thereafter converted into water and oxygen either by CAT and APX (Mittler 2002). Hydrogen peroxide can be removed also by POD in the apoplast of lignifying tissues (Ros-Barceló et al., 2006), since hydrogen peroxide is used as an electron donor to metabolize phenolic compounds.

In our study, mycorrhizal date palm plants maintained higher activities of antioxidant enzymes as compared to the control plants under saline conditions. Increased activities of antioxidant enzymes in AM plants corroborate with the findings of He et al. (2007) and Abdel Latef and Chaoxing (2011) in *Solanum lycopersicum*, Huang et al. (2008) in *Avenanuda*, Wu et al. (2010) in *Poncirus trifoliate*. In Sulla carnosa, Hidri et al. (2016) reported that salinity-stressed AMF-inoculated seedlings maintained higher activities of SOD and APX as compared to salt-stressed uninoculated seedlings. Ghouchani et al. (2017) also showed increase in antioxidant activity in wheat plants colonized by AMF under NaCl stress. In the present report, higher activity of antioxidant enzymes in mycorrhizal compared with non-mycorrhizal plants was associated with lower accumulation of H_2O_2 and lipid peroxidation indicating a lower oxidative burden and less membrane damage in the colonized plants. Consequently, inoculated date palm plants are more tolerant than non-inoculated ones due to the higher antioxidant activity.

Conclusion

Salinity stress resulted in reduced growth of date palm seedlings due to its evident effects on the physiological and biochemical traits. Salinity enhanced production of hydrogen peroxide and lipid peroxidation inducing loss of membrane integrity and simultaneously decreased the uptake of essential nutrients. AMF symbiosis allayed the deleterious impact of salinity on physiological and biochemical parameters, through enhancing relative water content, photosynthetic efficiency and pigments content. In addition, AMF mitigated the salt stress-induced changes by improving the antioxidant defense system, thus preserving cells components from oxidative damage. Furthermore, mycorrhizal symbiosis hampered the excess uptake of Na^+ and improved uptake of important ions to ensure better growth under saline conditions. Our findings allow us to deduce that salt stress-induced toxic effects on growth, water status, photosynthesis, antioxidant system and mineral nutrients in date palms can be alleviated by AMF and that the potential use of AMF could be a practical option to improve crop production under saline conditions in arid and semi-arid areas.

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Chapter 6

Optimizing growth and tolerance of date palm (*Phoenix dactylifera* L.) to drought and vascular fusarium-induced wilt (*Fusarium oxysporum*) by application of Arbuscular Mycorrhizal Fungi (AMF)

Abdelilah Meddich[1], Mohamed Ait El Mokhtar[1], Widad Bourzik[2], Marouane Baslam[3,4], Hafidi Mohamed[5] and Abdallah Oihabi[6]

[1]Laboratoire d'Agro-Alimentaire, Biotechnologies et Valorisation des Bioressources Végétales (AGROBIOVAL), Faculté des Sciences Semlalia, Université Cadi Ayyad, BP2390 Marrakech, Maroc.
[2]Service de l'Environnement Wilaya de la Région Marrakech-Safi Maroc.
[3]Laboratory of Biochemistry, Faculty of Agriculture, Niigata University, Niigata, Japan.
[4]Department of Life and Food Science, Graduate School of Science and Technology, Niigata University, Niigata, Japan.
[5]Laboratoire de Biotechnologies Microbiennes, Agrosciences et Environnement (BioMAgE), Faculté des Sciences Semlalia, Université Cadi Ayyad, BP511 Marrakech, Maroc.
[6]. X Full University Professor and Independent International Consultant.

***Corresponding author: a.meddich@uca.ma**

Abstract

Date palm (*Phoenix dactylifera* L.) is an important agricultural and commercial crop in the North of Africa and Middle Eastern countries of Asia. Date palm tree could be used for generations to come due to its remarkable nutritional, health and economic value in addition to its esthetic and environmental benefits. During the last decade, date palm plantations were subjected to degradation due to an extensive exploitation and to drastic environmental conditions. The major problems of drought and salinity have become more intense over time and their negative impacts on palm crop are marked by decreasing the production of *phoenix dactylifera*. Furthermore, *fusarium* wilts (bayoud) are economically important soil-borne diseases that result in significant crop losses and damage to natural ecosystems. Bayoud is a vascular wilt caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), and it is the most serious fungal disease threatening date palm plantations. This vascular disease combined with the problems of drought caused huge losses in our palm groves destroying more than 12 million trees and reducing the total areas from 150 000 to 44 000 Ha approximately. Plant-microbe interactions can be either beneficial or detrimental and a fast and accurate assessment of the surrounding organisms is essential for the plant's survival. Arbuscular mycorrhizal fungi (AMF) are a major component of soil biofertility and its use can improve crop resistance to biotic and abiotic stresses. This study highlights the importance of AMF in increasing tolerance of date palm to both *Fusarium oxysporum* f. sp. *albedinis*; a root-infecting fungal pathogen that causes wilt disease on a broad range of plant species, and to water-deficit. Initially, date palm seedlings were inoculated with four AMF spores: *Glomus monosporus, Glomus clarum, Glomus deserticola* and Consortium "Aoufous" (indigenous AMF), and cultivated for 14 months. Our results revealed that after this period, mycorrhizal infection rates were higher and slightly affected by water stress. The inoculation by the Consortium Aoufous, *G. monosporus* or *G. clarum* increased biomass production of date palm instead of the attacks by the fungal pathogen *F. oxysporum*, whatever the water regime. AMF allowed leaf water parameters to be maintained in *F. oxysporum*-inoculated plants or not under water-limiting conditions. The mortality rate among the date palm trees infected by *F. oxysporum* was lower in mycorrhizal plants than nonmycorrhizal plants. Results showed also that AMF decrease the deleterious effect of *F. oxysporum* on date palm, nevertheless the bioprotection effect against the plant pathogen was dependant on the type of AMF species. It therefore seems that the indigenous AM fungal communities "Aoufous" take advantage to improve crop resistance to those harsh biotic and abiotic conditions.

Keywords: Bioprotector agents, date palm, *Fusarium oxysporum* f. sp. *albedinis*, vascular wilt, water stress, indigenous community, mycorrhizal symbioses, tolerance.

Introduction

The date palm, *Phoenix dactylifera* L., is one of the most economically important perennial plants in arid and semi-arid areas of the Middle-East and the North Africa, where it is widely cultivated for food and many other commercial purposes. This tree is considered as a symbol of life in the desert. It is one of the oldest trees from which man has derived benefit, and it has been cultivated since ancient times (Chao and Krueger, 2007). The only indigenous wild desert plant definitely domesticated in its native harsh environments appears to be the date palm (Zohary and Hopf, 2000). Date fruits are a good source of essential nutrients, including sugars, proteins, fibers, trace elements, etc., and form an important part of the daily diet (Benmeddour et al., 2013). Over the last century, several constraints limited the expansion of the date palm culture. In North Africa, particlarly in Morocco, its culture and production are adversely affected by bayoud disease and drought apart from salinity (Oihabi 2001; Meddich et al., 2015).

Bayoud disease, caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), appeared to be the most important disease of date palm (Saaidi 1990; Oihabi 1991). The impact of this disease is very serious in North Africa, especially in Morocco where losses are increasing and may be a threat for palm-groves around the world (Daayf et al., 2003). It has affected nearly all Moroccan palm groves as well as those of western and central Algerian; it has killed more than 12 million in Morocco and 3 million in Algeria and has accelerated desertification (Djerbi 1998). Unfortunately, one of the causes of the high rate of spread of parasites and diseases is human intervention, by transporting infested young or adult date palm trees from infested to clean areas (Meddich and Boumezzough, 2017).

Drought is one of the main factors negatively affecting the productivity of agricultural or natural ecosystems (Passioura 2007; Ciais et al., 2005) and the diversity of plant species (Engelbrecht et al., 2007). It has also a global impact on carbon gain (Buermann et al., 2007). Desertification processes in arid areas are the result of drought since plant growth is seriously limited in arid sites by water disponibility. Changes in plant physiology, nutrient acquisition and metabolism induced by drought are highly limiting factors for plant growth (Evelin et al., 2009).

Arbuscular mycorrhizal fungi (AMF) are considered an integral component of plant communities in both natural and agricultural ecosystems (Smith and Read, 1997; Redecker et al., 2000). In recent years, studies indicated that AMF confers numerous benefits to host plants including improved plant growth and mineral nutrition, tolerance to diseases and stresses such as drought, temperature fluctuation, metal toxicity and salinity (Oihabi and Meddich, 1996; Harrison 1997; Al-Karaki 2000; Vigo et al., 2000; Borowicz 2001; Cantrell and Linderman, 2001; Dell'Amico et al., 2002; Al-Karaki et al., 2004; Asghari et al., 2005; Sannazzaro et al., 2006; Giri et al., 2007; Fan et al., 2011; Meddich et al., 2015). Many crops could potentially benefit from mycorrhizal symbiosis, although the degree to which plant benefit from mycorrhizae might vary greatly due to variation in plant species dependency on mycorrhizae (Janos 2007). In the case of date palm, the limited development of the root system (low densities of root hairs), along with field observations of high levels of mycorrhizal colonization, suggest that they benefit greatly from mycorrhizal relationship. It becomes especially more important under harsh environemental conditions prevailing in arid and semi-arid regions.

Thus, our study is the first of its kind on the combined application of indigenous AMF to improve the tolerance of date palm (*Phoenix dactylifera* L.) to *Fusarium oxysporum* f. sp. *albedinis* (Foa) and water-deficit stress. Integrating AMF for the development of date palm might be considered as an appropriate strategy to reverse the land degradation trend and encourage sustainable patterns for the development of oasis zones in Morocco.

Materials and methods

Multiplication of AMF and cultivation of date palms subjected to water stress and *Fusarium* attacks

Mycorrhizal fungi were obtained from different soils after trapping and multiplication on host plant, and are as follows: *Glomus monosporus* reference strain from INRA Dijon France (Dr. Plenchette); *Glomus Clarum* and *Glomus deserticola* which are strains selected from the Laboratory of Biotechnology of the University of Yaoundé in Cameroon (Dr. Ngawa) ; and the mycorrhizal consortium Aoufous (MCA) from the palm grove of Tafilalet containing a mixture of indigenous species *Glomus* sp. (15 spores / g soil), *Sclerocystis* sp. (9 spores / g soil) and *Acaulospora* sp. (1 spore / g of soil) (Meddich 2001).

The endomycorrhizal fungi cannot be grown separately from the plant. The inoculum was therefore used in the form of plants of barley (*Hordeum vulgare* L.) mycorrhized by the above AM fungi. Barley seeds were disinfected and placed in germinating condition within the vermiculite (previously sterlizedat 200 °C for 3 h) watered with sterile distilled water. After a week of germination, the barley plants were planted in plastic pots (13 × 09 cm) containing soils with different fungi to be tested. These plants were watered regularly with distilled water with a 30 ml weekly intake of modified nutrient solution of Long Ashton (Plenchette et al., 1982). After 3 months of culture, the mycorrhized roots of barley were disinfected for 10 min (Strullu1986), rinsed three times for 10 min with sterile distilled water and cut into fragments of 1–2 mm long. In all cases, the frequency of infection (F) of barley root was determined by the technique described by Trouvelot et al. (1986). An average of over 82% frequency of infection was maintained for the prepared inocula (Aoufous consortium 100%; *G. monosporus*: 95.55%; *G. clarum*: 86.67% and *G. deserticola*: 82.22%).

Procedure for water stress application

The cultures of date palm were performed in black plastic buckets of five liter with an inside diameter of 16 cm and a height of 20 cm, equipped with a drainage device for removing excess water and allow to determine the soil field capacity. The methodology for the application of water stress is described by Tobar et al. (1994) and Meddich et al. (2000). P1 is the weight of the bucket full of dry soil. Then the soil was watered to saturation, and allowed to drain under gravity, to a constant weight. The soil is hence at field capacity. P2 is the weight of the bucket after the flow of excess water. The difference (P2–P1) corresponds to the volume required for obtaining the field capacity of the used soil (100% FC). For equivalent humidity of the soil, of 75% and 25% of field capacity, we added, to the series of buckets containing dry soil, water volumes corresponding to 0.75 × (P2–P1) and 0.25 × (P2–P1) respectively. P3 and P4 weight of the buckets at 75% and 25% of field capacity, respectively, are then presented. During the experiment, in both water treatments used, buckets were returned by successive weighing and addition of distilled water, to the weight corresponding to water treatments imposed (75% or 25% of field capacity). Throughout the experiment, the buckets were weighed twice a day (counting balance of 70.041 M, accuracy 0.1–5 g and 30 kg weighing capacity) and lost water was replaced (Tobar et al., 1994).

Inoculation of the host plant by AMF and growing conditions

The seeds of the date palm variety Bouffgouss were disinfected and put into germination in plastic bowls containing a sterile sandy substrate previously washed with distilled water. Then they were incubated in an oven at 38°C for 3 weeks. Palm seedlings are then transplanted at the age of 2 months (leaf stage) at 6 plants per black plastic bucket containing 4 kg of sand–peat mixture (2:1 v/v) previously sterilized for 3h at 180°C.

The inoculation by symbiotic fungi was carried out by supplying 2.8 g (fresh weight) of barley roots mycorrhizal and disinfected (Strullu 1986) near the root system of each palm plant. The physicochemical parameters of the sand–peat mixture used are: water pH 7.31; total phosphorus 0.041%; organic carbon 0.82%; total nitrogen 0.13% and conductivity 148µs/cm. The water treatments were applied at transplanting inoculated date palm seedlings (eight weeks after germination). For soil fertilization, a weekly intake of 30 ml of the nutrient solution Long Ashton amended (Plenchette et al.,1982) was performed for all treatments. The buckets were then placed in a clear plastic greenhouse (average temperature 24.5 °C, relative humidity average of 69.12% and light 330 μm^{-2} s^{-1}).

Ten treatments (non-inoculated control and uninfected by Foa, non-mycorrhized and infected by Foa, complex Aoufous, complex Aoufous with Foa, *G. monosporus*, *G. monosporus* with Foa, *G. desertícola*, *G. desertícola* with Foa, *G. clarum* and *G. clarum* with Foa). For each treatment two water treatments (75% and 25% of field capacity) were used. The combination of each water and fungal treatment consisted of 10 repetitions of six plants. Thus, 1,200 plants were used.

Isolation and infection of the host plant by pathogenic fungi *Fusarium oxysporum*

The fungal pathogen Foa used was isolated from infected palm taken from date palms suffering from Bayoud in the palm of Draa (Figure1). Its preservation was performed on sand and its pathogenicity is regularly confirmed by infection of young plants grown from seed of the susceptible cultivar JHL Bayoud (Oihabi1991).

The inoculum consists of a spore suspension obtained by successive washings with sterile distilled water to an agar culture Foa aged 10 days. Four months after the installation of mycorrhizal inoculation of date palms by Foa isolate is provided in the form of a spore suspension concentration to 2.10^6 spores / ml against the roots of young palms at 5 ml per plant.

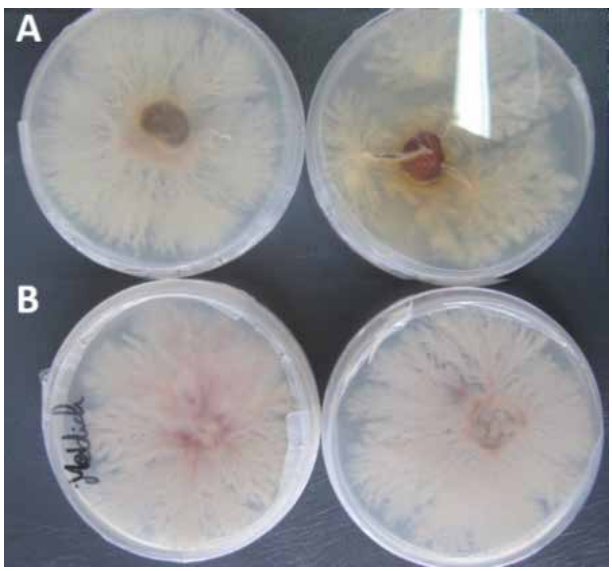

Figure 1. Photographs of *Fusarium oxysporum* f.sp. *albedinis* (foa) isolated from rachis tissues of diseased adult date palms (A) and from roots of diseased young palms trees (B) (not associated with AMF).

Measured parameters

After 48 weeks of mycorrhization, samples from thirty date palms per treatment were performed to assess the effect of water stress on growth and water features of mycorrhized plants and non-mycorrhized ones as well as the growth and development of mycorrhizal fungi.

Mycorrhization Settings

Palm plant roots were processed (Phillips and Hayman, 1970) and stained with trypan blue 0.01% in lactoglycerol. The review of the status of the mycorrhization root system was performed according to the method described by Trouvelot et al. (1986) to characterize the development and aggressiveness of mycorrhizal fungi on the basis of water regimes.

Determination of growth and water and physiological parameters

- The response of plants palm mycorrhization was estimated by determining the number of formed leaves, leaf area and biomass production.
- Dry mass (DM) was measured after drying in an oven at 105 ◦C for 24 h. Leaf area was determined on the leaf of the same rank for all treatments.
- The water content of the aerial part (WC) was determined by the difference between the mass of fresh material (FM) and the dry matter (DM), expressed in grams of water per gram of DM (g/g DM).
- The relative water content (RWC) was also measured on the leaf at the same level for all plants and for all treatments using the following equation:

$$RWC\% = \frac{FM\text{-}DM}{FMsat\text{-}DM} \times 100$$

where MFsat corresponds to the saturated fresh materials

- The stomatal resistance was determined in well-developed leaf samples of the same rank with the help of a promoter LI-1600.
- The content of mineral elements (P, Ca, Mg, Na, K, Cu and Mn) was determined on the aerial parts after mineralization, by atomic absorption spectrophotometer.

Extraction of peroxidase and polyphenol oxidase enzymes was made from 200 mg of fresh root material according to the protocol described by Jaiti et al. (2007). The peroxidase and polyphenol oxidase activities were determined by spectrophotometry at 470 nm and 410 nm respectively. These enzymatic activities are expressed as enzymatic units per gram fresh weight (U/GMF). The phenol extraction is performed according to the protocol described by El Hadrami et al. (1997). The content of phenolic compounds was determined according to their oxidation by the Folin–Ciocalteu reagent. The optical density at 760 nm is determined and the levels of soluble phenols were expressed in mg catechin equivalent/g fresh weight.

Determination of the incidence of Fusarium

The number of dead young plants affected by Bayoud has been recorded after 32 weeks of infection with Foa. Typical symptoms of pathogen attack include coiling followed by drying of the leaves of young seedlings and the presence of localized browning in the attacked roots. The presence of the pathogen in the tissues was confirmed by microbiological isolations performed on the roots of dead plants.

Statistical analysis

All results were analyzed statistically with the CO-STAT soft-ware (Statistical Software, New Style Anova). The study includes an analysis of variance followed by Newman–Keuls test at the 5%.

Principle results

Effect of water stress and attacks by Foa on colonization of palm roots by AMF

The percentage of colonization of the palm root system by AMF is slightly affected by soil water deficiency during 48 weeks of mycorrhization (Figure 2). This frequency of mycorrhization is not affected by Foa with the exception of *G. deserticola*. For the two studied water regimes (75 and 25% FC), mycorrhizal frequency remained high (> 48%) for all mycorrhizal fungi tested. Mycorrhizal isolates from Aoufous consortium, *G. monosporus* and *G. clarum* were more infectious (F> 67%) than those of *G. deserticola*, even in the presence of Foa and irrespective of soil moisture regime.

The intensity of colonization of the palm roots by the different AMF decreases for the severe water regime (25% FC) (Figure 3). In the case of the favorable water regime (75% FC), contrary to the percentage of colonization, mycorrhization intensity is significantly reduced in the presence of Foa for the different AMF used. This intensity of colonization is higher in the case of infection by Aoufous complex, *G. monosporus and G. clarum* than in the case of *G. deserticola* regardless if the conditions of water supply are favorable or limiting. *Glomus deserticola* was most affected by water stress and Foa infection, with a root colonization intensity that does not exceed 8%.

Figure 4 shows some AMF structures developed by the Aoufous consortium at the date palm roots.

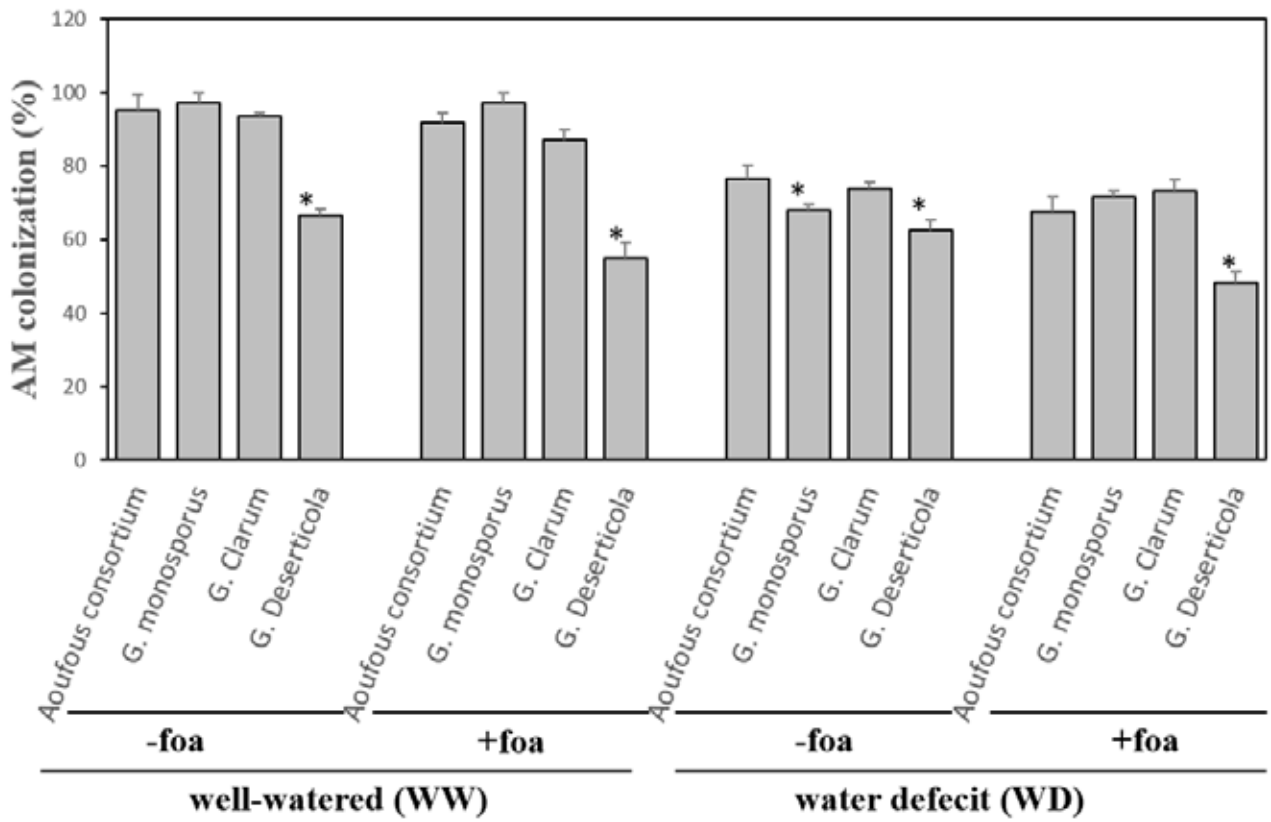

Figure 2. Mycorrhizal colonization of date palm associated with Consortium Aoufous, *Glomus monosporus, Glomus clarum, Glomus deserticola*, inoculated (+foa) or not (-foa) with *Fusarium oxysporum* f. sp. *Albedinis* and subjected to well-watered (WW) or water deficit (WD) conditions. Values represent the mean ± SD (n=10 plants). Asteriks indicate significant differences between treatments according to Student's t-test ($p \leq 0{,}05$).

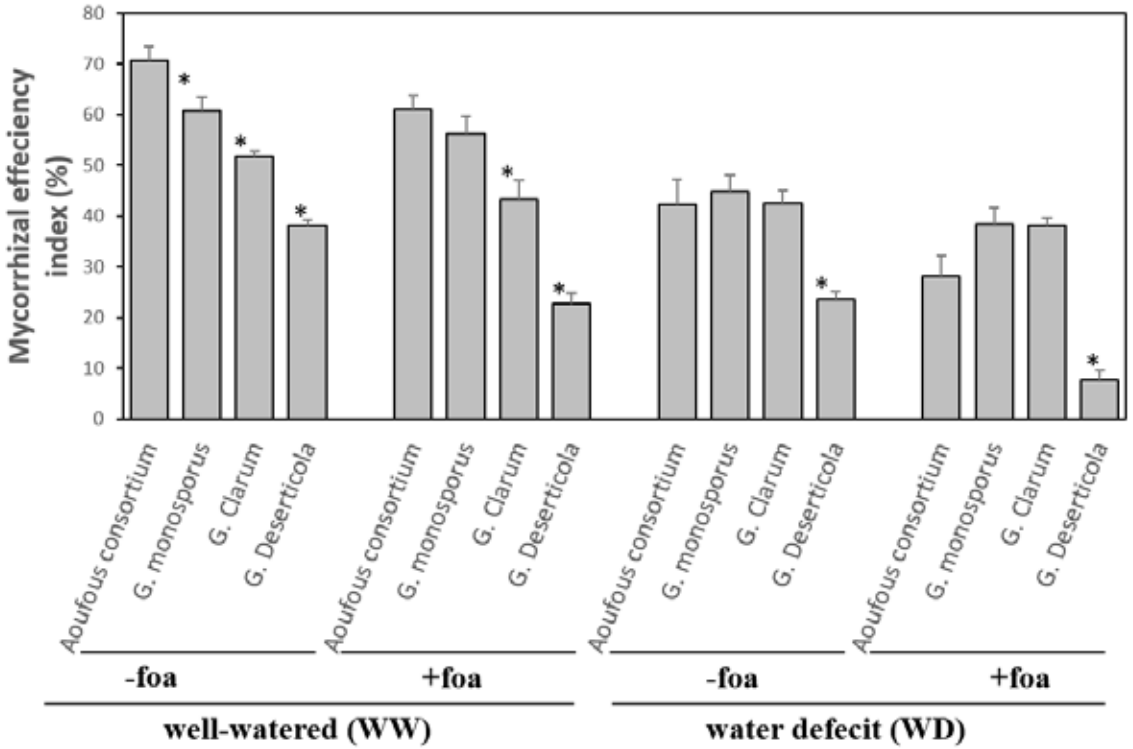

Figure 3. Mycorrhizal efficiency index (MEI) of date palm associated with Consortium Aoufous, *Glomus monosporus, Glomus clarum, Glomus deserticola,* inoculated (+foa) or not (-foa) with *Fusarium oxysporum* f. sp. *albedinis* and subjected to well-watered (WW) or water deficit (WD) conditions. Values represent the mean ± SD (n=10 plants). Asteriks indicate significant differences between treatments according to Student's t-test ($p \leq 0,05$).

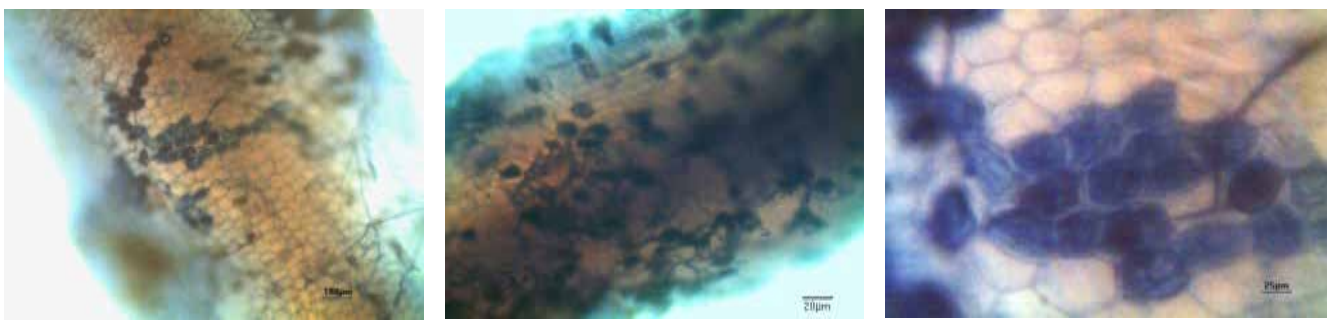

Figure 4. AMF structures developed by the Aoufous Consortium in date palm.

Effect of AMF on the growth and water parameters of date palm subjected to water stress and attacks by Foa

Mycorrhization of the palm trees allowed the formation of a number of leaves significantly greater than that produced by non-mycorrhized plants, and regardless of water regime imposed on the ground (Table 1). The reduction in the number of leaves formed is clearly significant in the case of non-mycorrhized control plants infected with Foa subjected to water deficiency. Plants inoculated with Aoufous consortium and *G. monosporus* showed significant improvement in their leaf area compared to control plants or those mycorrhized with *G. deserticola*. Infection with Foa induces a significant decrease in the leaf area of mycorrhized and non-mycorrhized plants. The number of leaves formed and leaf surface of date palm decreased significantly when the soil available water reserves decreases.

In general, the non-mycorrhized palm plants have been more sensitive with respect to changes in soil moisture than mycorrhized plants. For both soil moisture regimes, inoculated plants showed a water content (WC) similar to that of the control (Table 1). No significant differences were recorded at the WC in the palm mycorrhizal and non mycorrhizal infected or not by Foa and that the water conditions are favorable or limiting. In the opposite, the mycorrhized date palm with Aoufous consortium and *G. monosporus* maintain a higher relative water content (RWC), for both water regimes soil. The application of Foa significantly reduced RWC in non-mycorrhized and mycorrhized plants by G. *Deserticola* reaching low values of 48 and 38%, respectively, when combined with water deficit (25% FC). When the drying of the soil increases (25% FC), the stomatal resistance (R) increases significantly in non mycorrhizal plants inoculated or not by Foa compared to plants mycorrhizal with the tested AMF isolates, except those mycorrhized with *G. deserticola* added Foa and subject to the same severe water treatment (Table 1). Thus the values of resistance of the stomata are 3.36, 3.18 and 2.95 s / cm, respectively, in the non-mycorrhized date palm added Foa, non mycorrhized palm uninfected by Foa and mycorrhized palm with *G.* deserticola and added Foa. While these values are only of 2.12, 2.10 and 2.22 s / cm in uninfected palm by Foa and colonized respectively with the complex Aoufous, *G. monosporus* and *G. clarum*.

Moreover, production of aerial dry matter increased significantly by the colonization of the palm by different mycorrhizal fungi and regardless if the water supply is favorable (75% FC) or limiting (25% FC) (Figure 5). The Aoufous complex, *G. monosporus* and *G. clarum* allow the production of a larger aerial dry mass to face severe water regime. Non-mycorrhizal plants or those inoculated with *G. deserticola* showed low weight dry matter either in the presence or absence of the pathogen Foa at the severe water regime (25% FC).

Table 1. Number of leaves (NL), Leaf area (LA) and Relative water content (RWC) of date palm associated with Consortium Aoufous, *Glomus monosporus*, *Glomus clarum*, *Glomus deserticola*, or not (control) under well-watered or drought stressed conditions and inoculated (+foa) or not (-foa) with *Fusarium oxysporum* f. sp. *albedinis*.

	Traitement	75%	25%
NL	Consortium Aoufous	6.00±0.000a	5.00±0.000bc
	Consortium Aoufous+Foa	4.00±0.000cd	4.00±0.000cd
	Glomus monosporus	5.00±0.000b	4.00±0.000bc
	Glomus monosporus+Foa	4.33±0.577bc	3.67±0.577cd
	Glomus clarum	5.00±0.000bc	4.33±0.577bc
	Glomus clarum+Foa	4.67±0.577bc	4.00±0.000d
	Glomus deserticola	4.33±0.577cd	2.67±0.577e
	Glomus deserticola+Foa	3.33±0.577cd	2.33±0.577f
	Control	4.00±0.000cd	3.00±1.000f
	Control+Foa	3.67±0.577d	2.33±0.577f

	Traitement	75%	25%
LA (cm2)	Consortium Aoufous	50,25±1.391a	35.50±1.299de
	Consortium Aoufous+Foa	38.25±1.044b	28.00±1.500e
	Glomus monosporus	48.00±1.464a	33.33±1.474bc
	Glomus monosporus+Foa	31.75±1.147cd	27.00±1.000de
	Glomus clarum	49.00±1.00bc	31.00±0.790de
	Glomus clarum+Foa	35.25±1.070de	28.75±1.035f
	Glomus deserticola	29.00±1.732de	23.45±1.149f
	Glomus deserticola+Foa	26.54±1.164de	19.75±1.205g
	Control	27.00±1.447de	22.00±0.460fg
	Control+Foa	20.05±0.926fg	19.25±1.089g
W.C (g/ gMS)	Consortium Aoufous	2.81±0,06a	2.91±0,04a
	Consortium Aoufous+Foa	2.99±0,05a	3.04±0,03a
	Glomus monosporus	3.15±0,15a	2.97±0,23a
	Glomus monosporus+Foa	3.14±0,47a	3.03±0,21a
	Glomus clarum	2.86±0,09a	2.64±0,18a
	Glomus clarum+Foa	2.63±0,20a	2.92±0,17a
	Glomus deserticola	2.80±0,05a	2.93±0,16a
	Glomus deserticola+Foa	2.99±0,11a	2.87±0,27a
	Control	2.96±0,22a	2.85±0,04a
	Control+Foa	2.99±0,23a	2.69±0,08a
RWC (%)	Consortium Aoufous	83.43±1.974b	71.79±1.043def
	Consortium Aoufous+Foa	75.44±2.158c	70.88±1,141f
	Glomus monosporus	82.42±1.035a	68.92±1.545d
	Glomus monosporus+Foa	70.26±2.576de	68.74±1.573f
	Glomus clarum	76.88±1.764c	69.23±1.985g
	Glomus clarum+Foa	70.15±1.592ef	66.01±1.779h
	Glomus deserticola	71.29±0.551d	60.47±1.410g
	Glomus deserticola+Foa	50.10±1.831i	38.26±1.210j
	Control	75.20±2.585bc	61.15±1.001g
	Control+Foa	53.45±1.552h	47.77±1.948i
R (s/cm)	Consortium Aoufous	2.21±0,01hij	2.12±0,02j
	Consortium Aoufous+Foa	2.48±0,20defg	2.33±0,14fghij
	Glomus monosporus	2.14±0,02ij	2.10±0,02j
	Glomus monosporus+Foa	2.61±0,12de	2.29±0,02fghij
	Glomus clarum	2.39±0,02efgh	2.22±0,02hij
	Glomus clarum+Foa	2.37±0,05fghi	2.26±0,10ghij
	Glomus deserticola	2.39±0,01efgh	2.48±0,02defg
	Glomus deserticola+Foa	2.53±0,19def	2.95±0,14c
	Control	2.33±0,02fghij	3.18±0,02b
	Control+Foa	2.64±0,10d	3.36±0,06a

The values followed by the same letter are not significantly different P <0.05 (Newman and Keuls test).

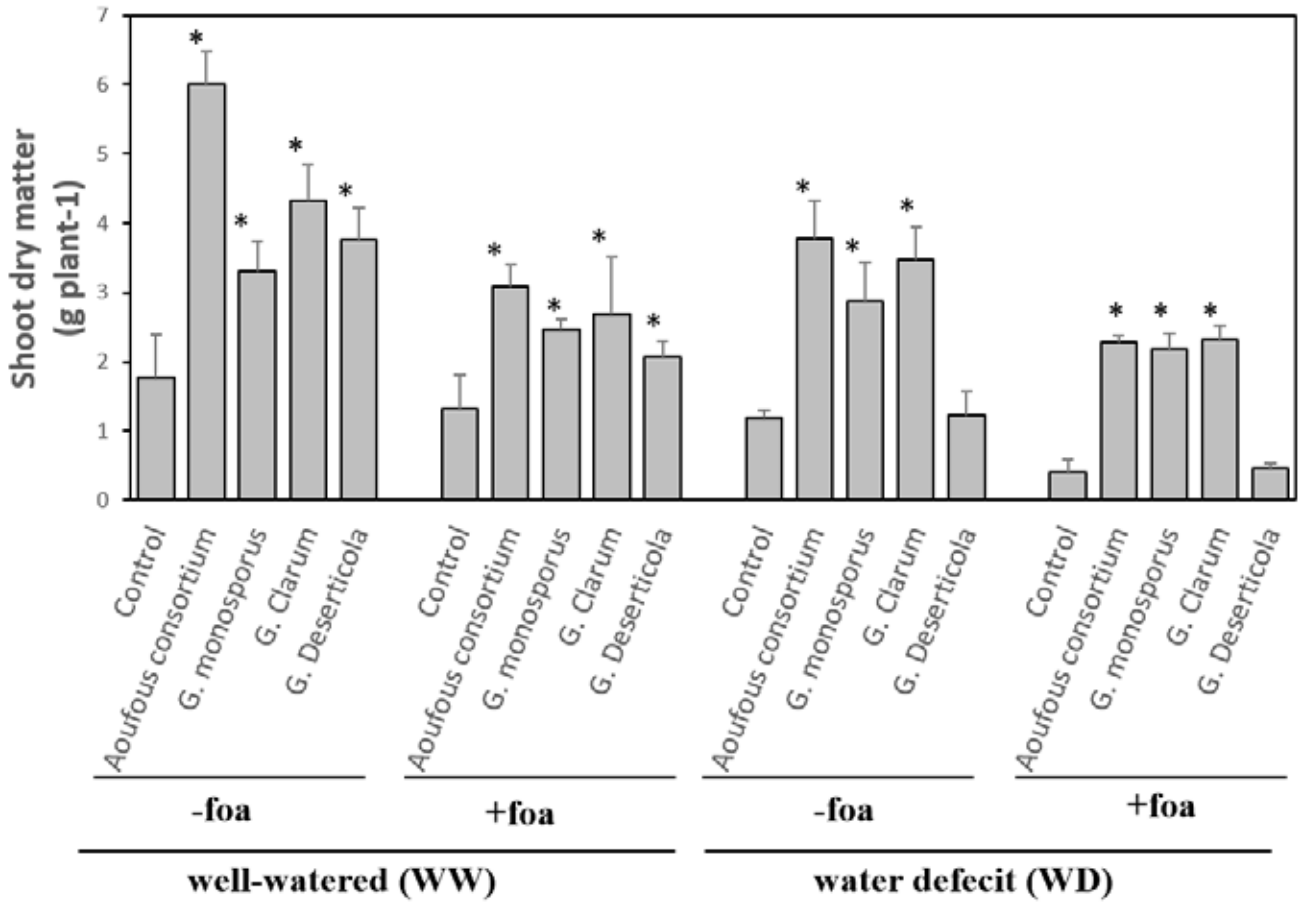

Figure 5. Shoot dry matter of date in mycorrhizal (with Consortium Aoufous, *Glomus monosporus, Glomus clarum, Glomus deserticola*) plants under well-watered or drought stressed conditions and inoculated (+foa) or not (-foa) with *Fusarium oxysporum* f. sp. *albedinis*. Values represent the mean ± SD (n=10 plants). Asteriks indicate significant differences between treatments according to Student's t-test ($p \leq 0,05$).

The aerial parts of mycorrhized plants accumulate higher contents of P, Ca, Mg, K, Mn than in non-mycorrhized plants regardless of the soil water regime (Table 2). The Na and Cu contents in mycorrhized plants are significantly higher under the favorable water regime. Generally, the Aoufous consortium, *G. clarum* and *G. monosporus* have improved the nutritional status of plants whatever the water regime. In the presence of *F. oxysporum*, the mineral content of the aerial parts of plants subjected to water regimes (75 and 25%) are reduced. Similarly, the contents of P, Mg, K, Cu and Mn are reduced by 50% in plants mycorrhized with *G. monosporus* and infected by *F. oxysporum* compared to those inoculated by the same mycorrhizal fungus without *F. oxysporum* under favorable water regime (75% FC).

Table 2. Effect of mycorrhiza and water deficit on the ionic content of the aerial part of the date palm infected or not by Foa.

		P (mg/plant)	Ca (mg/plant)	Mg (mg/plant)	Na (mg/plant)	K (mg/plant)	Cu (mg/plant)	Mn (mg/plant)
75% FC	Complexe d'Aoufous	9.32±0,50cd	2.43±0,20de	6.03±0,06b	0.50±0,03b	4.40±0,05c	0.050±0,008c	0.011±0,002b
	Complexe d'Aoufous+Foa	7.43±0,66fg	2.58±0,22d	4.92±0,10c	0.42±0,04bcd	3.62±0,06e	0.044±0,004cd	0.009±0,002bcd
	Glomus monosporus	17.05±0,73a	1.80±0,20f	9.14±0,28a	0.68±0,07a	7.40±0,2a	0.080±0,01a	0.018±0,001a
	Glomus monosporus+Foa	8.75±1,05def	1.62±0,20fg	5.15±0,26c	0.46±0,03bc	4.19±0,19d	0.045±0,005cd	0.010±0,001bc
	Glomus clarum	10.39±0,54bc	3.55±0,19a	4.22±0,20d	0.43±0,02bcd	2.42±0,12g	0.040±0,006cde	0.008±0,001cde
	Glomus clarum+Foa	8.83±0,76def	2.17±0,20e	3.72±0,24e	0.37±0,03de	2.18±0,07h	0.034±0,006de	0.0067±0,0006ef
	Glomus deserticola	10,45±0,78bc	2,97±0,28bc	6.04±0,19b	0.68±0,08a	5.34±0,11b	0.060±0,01b	0.010±0,002bc
	Glomus deserticola+Foa	7.65±0,56efg	2.48±0,19de	4.19±0,17d	0.49±0,04b	4.05±0,09d	0.041±0,004cde	0.0071±0,0007def
	Control	3,40±0,53ij	1.12±0,10hi	2.16±0,15h	0.19±0,01hi	1.68±0,03ij	0.020±0,001fg	0.003±0,0001hi
	Control+Foa	3.24±0,41ij	1.26±0,13gh	1.45±0,21i	0.18±0,01hi	1.63±0,03j	0.017±0,003g	0.0028±0,0004hi
25% FC	Complexe d'Aoufous	10.88±0,83b	1.54±0,17fg	4.06±0,09d	0.33±0,04ef	3.45±0,11e	0.040±0,006cde	0.006±0,0002efg
	Complexe d'Aoufous+Foa	9.09±0,87cde	1.30±0,10gh	3.32±0,28fg	0.28±0,02fg	2.90±0,17f	0.030±0,002ef	0.005±0,0005fg
	Glomus monosporus	4.62±0,46hi	3.22±0,12b	3.04±0,07g	0.33±0,02ef	3.61±0,13e	0.040±0,005cde	0.007±0,0003def
	Glomus monosporus+Foa	3.62±0,58ij	2.61±0,20d	2.40±0,10h	0.25±0,02fgh	2.92±0,12f	0.029±0,003ef	0.0055±0,0005fg
	Glomus clarum	8.26±0,53defg	2.73±0,21cd	3.48±0,07ef	0.43±0,03bcd	1.85±0,05ij	0.037±0,001cde	0.0053±0,0003fg
	Glomus clarum+Foa	6.90±0,95g	2.31±0,11de	3.40±0,10ef	0.40±0,05cde	1.81±0,11ij	0.030±0,004ef	0.0040±0,001gh
	Glomus deserticola	5.08±0,95h	0.97±0,18hi	2,11±0,18h	0.28±0,01fg	1.91±0,17i	0.020±0,001fg	0.002±0,0002hi
	Glomus deserticola+Foa	2.62±0,41jk	0.93±0,12hi	1.18±0,16i	0.16±0,02i	1.09±0,13k	0.012±0,003g	0.0011±0,0001i
	Control	1.65±0,38k	1.05±0,04hi	0.79±0,08j	0.21±0,01ghi	1.05±0,06k	0.016±0,002g	0.0010±0,0002i
	Control+Foa	1.29±0,31k	0.72±0,17i	0.71±0,07j	0.16±0,01i	0.84±0,04l	0.011±0,002g	0.0008±0,0001i

Means followed by the same letters are not significantly different $P < 0.05$ (Neumans and keuls test).

Both peroxidase activity and polyphenol oxydase increased significantly when soil water deficiency is increasing and that is regardless if the palm plants are mycorrhized or not (Table 3). When faced with a favorable water regime (75% FC), mycorrhized palm plants by different fungal isolates showed a highly significant increase in peroxidase activity (POX) and polyphenol oxydase (PPO) compared with non-mycorrhized control plants. Indeed, mycorrhization of date palm by *G. monosporus*, *G. clarum* and *G. deserticola* has significantly stimulated the POX and PPO compared to the control. For POX, the highest activities are recorded in the case of inoculation with *G. monosporus* and *G. clarum*. However, for PPO activities are much higher in the case of the mycorrhization with *G. deserticola* under favorable water supply conditions. During the drying of the soil (25% FC), the effect on the POX and PPO activity by mycorrhization was dependent on the fungal isolates. Indeed, POX activities are greater in mycorrhized plants by *G. deserticola*, while the PPO activities were significantly higher in mycorrhized plants by complex Aoufous and *G. monosporus* compared to non-mycorrhized plants. The application of severe water stress (25% FC) causes a significant increase in levels of soluble phenols in mycorrhized and control plants except in the case of inoculation with *G. deserticola*. The largest concentrations are found in the case of infection with *G.* clarum and *G. monosporus*. Faced with favorable water regime (75% FC), the levels of phenols in mycorrhized plants were significantly elevated compared to control plants except in the case of mycorrhization by *G. monosporus*.

Table 3. Effect of water deficit and AMF on peroxidase, polyphenoloxydasiques activities and the contents of phenols palm.

	Treatment	75% FC	25 % FC
Peroxidase (Unité / g MF)	Consortium Aoufous	418.89±6,23h	1124.17±6,65c
	Glomus Monosporus	750.00±6,58e	1428.33±4,10b
	Glomus Clarum	723.33±9,68f	1060.00±6,96d
	Glomus Deserticola	576.11±6,92g	1780.00±3,31a
	Control	281.67±6,34i	1427.50±7,74b
Polyphenoloxydase (Unité / g MF)	Consortium Aoufous	606.67±5,08h	1816.67±6,79a
	Glomus Monosporus	736.67±3,92g	1588.33±8,55b
	Glomus Clarum	850.00±3,25f	1181.67±6,88d
	Glomus Deserticola	1070.00±6,58e	1290.00±9,33c
	Control	366.67±3,71i	1176.67±6,79d
Phenols (Equivalentcatechine / g MF)	Consortium Aoufous	952.38±7,11g	1022.22±2,23f
	Glomus Monosporus	615.87±6,09i	1629.52±4,88b
	Glomus Clarum	1401.97±7,48c	2101.59±5,67a
	Glomus Deserticola	1177.78±5,53e	1026.03±7,36f
	Control	660.32±3,36h	1347.30±7,45d

Means followed by the same letters are not significantly different $P < 0.05$ (Neumans and keuls test).

Effect of AMF on the severity of the attacks by Foa

The mortality of date palm infected with Foa in terms of tested AMF and soil moisture regimes was evaluated, after a period of 32 weeks (Figure 6). Regardless of the soil water regime, plant mortalities stabilized between 11 and 22% in mycorrhized plants by the Aoufous consortium, *G. monosporus* and *G. clarum*. After 8 months of Foa infection, mortality rates were 61 and 78% respectively in *G. desertícola* mycorrhized and non-mycorrhized plants under favorable water regime (75% FC) (Figure 6). These mortality rates increased to 67% and 89% respectively in plants inoculated with *G. deserticola* and non-mycorrhized plants subjected to soil water deficiency.

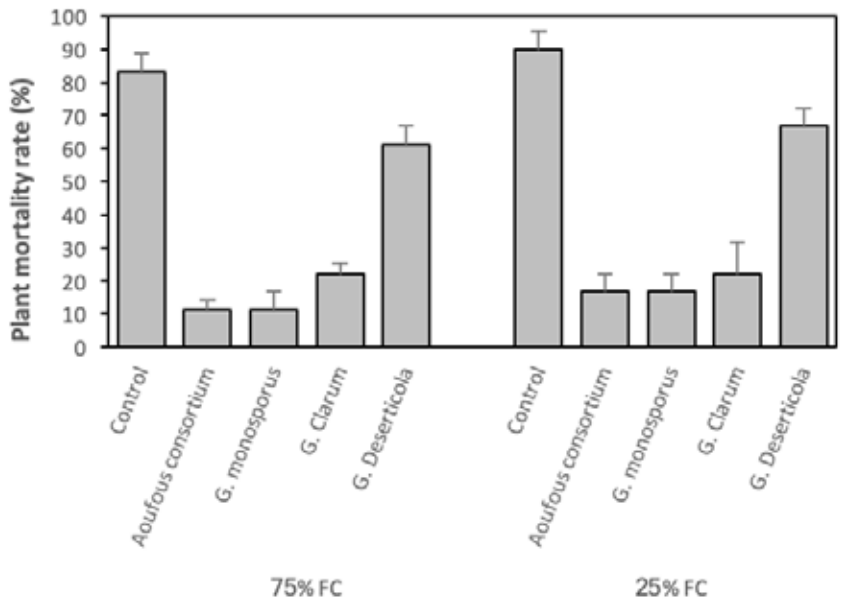

Figure 6. Plant mortality rate of date palm in mycorrhizal (with Aoufous consortium, *G. monosporus*, *G. clarum*, *G. deserticola*) plants under well-watered or drought stressed conditions after 8 months of inoculation with Foa. Values represent the mean ± SD (n=10 plants). Asteriks indicate significant differences between treatments according to Student's t-test (p≤0,05).

Discussion

Establishment of mycorrhizal simbiosisunder water stress

The fungal isolates of the Aoufous consortium and selected *Glomus* showed a greater colonization of the roots of the date palm. The severe water regime (25% FC) has slightly affected frequency and colonization of these AMF. In our previous work (Meddich et al., 2000, Meddich 2001), we observed a significant reduction in infectivity and colonization parameters for clover and barley roots subjected to water deficit and mycorrhizated by the same inoculum (Aoufous consortium and *G. monosporus*). This suggests the presence of variability in the AMF infectivity parameters depending on the host plants tested and the environmental conditions. As a result, an exchange of signals between the two plant-AMF partners exists before there is contact between them. On the plant side, the organic compounds contained in the former root-sucking buds influence the development of arbuscular mycelia (Koske 1982; Gianinazzi-Pearson et al.,1996). Low molecular weight molecules and proteins necessary for the establishment of mycorrhizal symbiosis have also been identified in angiosperms (Delaux et al., 2013). On the fungi side, Maillet et al. (2011) found that *G. intraradices* secretes symbiotic signals in the form of a mixture of lipochitooligosaccharides, which stimulates the formation of mycorrhizal symbiosis in certain plant families, including the fabaceae, asteraceae and umbelliferae. Subsequently, the mycelia of the AMF isolates colonize the cortical cells and give rise to the fungal arbuscules representing the preferred site of the metabolic exchanges between the fungus and the host plant (Gianinazzi-Pearson and Gianinazzi, 1986, 1988; Gianinazzi-Pearson et al., 1996).

Growth and water parameters of date palm under water stress

Mycorrhization by the Aoufous consortium and selected *Glomus* provides a good palm plant growth. Thus, inoculation with these AMF increased the number of leaves produced and the leaf area of the plant on two water regimes used. Foa infection results in a slight decrease in these growth parameters in mycorrhizal plants. The reduction of this growth was remarkable in non-mycorrhized plants or those mycorrhized by *G. deserticola*, subjected to the severe water regime. Similarly, the dry matter production was improved compared to control plants. Identical responses were reported for lettuce and maize inoculated with *G. mosseae* and *G. intraradices* (Kothari et al., 1990; Tobar et al., 1994; Sheng et al., 2008; Baslam and Goicoechea, 2012). We also noted the importance of AMF in improving the mineral nutrition of date palm. Mycorrhizal palms showed higher levels of P, Ca, Mg, K and Mn than those of non-mycorrhizal plants, whatever the water regime imposed on soil. The application of Foa reduced the mineral content of aerial parts of date palm. These reductions in nutritional status remained similar to those noted in the aerial growth of palms infected by Foa. This reflects the relationship between plant growth, mineral and water nutrition by increasing certain macroelements and microelements from the roots to the leaves of mycorrhizal plants. Some positive effects of mycorrhizal symbiosis on the growth and health of date palms have been reported (Al-Karaki 2013; Baslam et al., 2014; Meddich et al., 2015). Studies have revealed that (i) the AMF has promoted the growth of date palm seedlings in nursery conditions (Shabbir et al., 2011) compared to controls treated with chemical fertilizers (Symanczik et al., 2014) (ii) increasing the availability of nutrients in soil cultures (Al-Karaki et al., 2007) and (iii) improving the absorption of water and nutrients in saline conditions (Bearden and Peterson, 2000).

Also, mycorrhized plants showed a low stomatal resistance compared to control plants in absence or in the presence of water stress. High stomatal conductance in mycorrhized plants could improve CO2 fixation in mesophyll (Brown and Bethelenfalvay, 1987). Improving CO2 fixation consequently contributes to a marked increase in photosynthesis ofthe plant (Lawlor 1987; Zuccarini and Okurowska, 2008). Plants inoculated with the Aoufous consortium showed a higher RWC under water stress than the control plants. This reflects the ability of AMF to maintain well-hydrated host plant tissues. AM symbiosis may enhance osmotic adjustment in plants which could contribute to maintaining higher leaf water status in AM plants during drought and keeps the plants protected against oxidative stress, and these cumulative effects increase the tolerance of plants to abiotic stress. The plant genetic analyzes carried out by our team (Zézé et al., 2007, 2008) revealed the expression of three types of aquaporin genes, intrinsic proteins, in clover roots mycorrhized by the Aoufous consortium and *G. monosporus* and subjected to water stress (30% FC), which would contribute to a better distribution of water circulation in plant tissues. This better distribution of water circulation in the plant may explain, in part, this tolerance in the presence of AMF.

Water stress leads to the overproduction of active oxygen species (AOS) responsible for oxidative stress that causes destructive physiological disruptions to plants (Reddy et al., 2004). It is now known that plants protect against the damage caused by this oxidative stress by detoxification mechanisms of these AOS which can be enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and monodehydroascorbate reductase) and non-enzymatic (flavanones, anthocyanins, carotenoids and ascorbic acid) (Yang et al., 2008 and Guo et al., 2010). In this study, water stress induced a significant increase in the activity of one of these antioxidant enzymes, peroxidase (POX) in the roots of *P. dactylifera*. Similar results were observed in Abiesfabri (Guo et al., 2010), Lactuca sativa (Baslam and Goicoechea, 2012), Zea mays (Porcel and Ruiz-Lozano, 2004), Cupressus atlantica (Zarik et al., 2016) under the same conditions of water deficit or also by application of other abiotic stresses such as salinity (Maya and Matsubara, 2013) and high temperatures (Zhu et al., 2010). Indeed, endomycorhization can stimulate the synthesis pathway of phenolic compounds (Krishna and Bagyaraj, 1984), phytoalexins (Morandi and Gianinazzi-Pearson, 1986) and POX (Spanu and Bonafante-Fasolo, 1988 and Chandrasekaran et al., 2014). Peroxidase activities may be involved in the catabolism of auxins (Gaspar et al., 1982), linkage of parietal compounds (Grabber et al., 2002 and Price et al., 2003), cell elongation (Cosgrove 2001), the formation of suberin (Keren-Keiserman et al., 2004), lignin (Lewis and Yamamoto, 1990, Kiefer-Meyer et al., 1996 and Boerjan et al., 2003) and the defense against pathogens (Passardi et al., 2005). On the other hand, we have noted an activation of polyphenoloxidases (PPO), enzymes involved in the oxidation of phenolic compounds into quinones that participate in the linkage of the different constituents of the wall and consequently increase its rigidity (Avdiushko et al., 1993 and El Modafar 2010). Other studies have reported the involvement of polyphenol oxidases in plant resistance to pathogens (Mohammadi and Kazemi, 2002 and Cooper et al., 2004). Mycorrhization promotes stimulation of POX and

PPO activities during water stress (25% FC), we also noted an exaltation of these defense mechanisms that remains dependent on the fungal isolate. This dissimilar behavior of AMF in relation to enzymatic activities of plants has often been reported (Azcon-Aguilar and Barea, 1996; Azcon and Tobar, 1998; Calvente 2003). Our study (Zarik et al., 2016) showed weak superoxide dismutase (SOD) and catalase (CAT) activities in mycorrhizal *Cupressus atlantica* under water stress (25% FC). On the other hand, the POX activity was very pronounced in the same mycorrhizal plants of Cupressus atlantica subjected to the same severe water regime. CAT is not the only enzyme capable of destroying H_2O_2. POX can also play this role and trap with more affinity H_2O_2, which was produced by SOD dismutase during the period of water stress. The application of a severe water regime (25% FC) to non-mycorrhizal palms induces a significant increase in the phenol contents. The study of Zhang and Liu (2015) has described the activation of phenylalanine ammonia-lyase (PAL) as a key enzyme in the phenylpropanoid biosynthesis pathway responsible for phenol synthesis in plants. Currently, it is evident that many antioxidants including secondary metabolites, especially phenols, play a key role in adapting plants to biotic and abiotic stresses (Burritt and MacK-enzie, 2003, Hernández et al., 2004 and Liu et al., 2006).

The use of mycorrhizal fungi that are resistant to wáter stress and are able to help the plants, which they are associated to tolerate adverse weather and soil conditions (Sharifi et al., 2007; Jahromi et al., 2008; Fini et al., 2011; Navarro et al., 2011; Sheng et al., 2011; Baslam and Goicoechea, 2012; Augé et al., 2014; Taffouo et al., 2014; Zhanget al., 2014, Meddich et al., 2015) could be one of the most promising biological means. It is also interesting to note that indigenous strains (Aoufous complex) have been effective in improving the tolerance of the host plant to wáter stress. Such indigenous and adapted fungal isolates to adverse conditions could be an effective biological means for plants living constantly under increased abiotic stress.

Interactions between AMF and pathogens

Irrespective of soil moisture, mortality rates in palm trees infected with Foa remained lower in mycorrhized plants than in non-mycorrhized plants after 32 weeks of infection. The effect of prior mycorrhization by AMF is remarkable on the expression of bayoud disease. Indeed, Oihabi (1991) found that the simultaneous inoculation of *G. mosseae* and the pathogen does not allow the expression of a protective effect in the young plants of the palms cultivated on calcined clay. On the other hand, Caron et al. (1986) observed a reduction in root necrosis of the tomato due to *Fusarium oxysporum* f. sp. *radicis-lycopersici*, when *G. intraradices* is applied 5 weeks before the pathogen. In addition, Bartschi et al. (1981) reported that the inoculation of *Chamaecyparis lawsonia* by a natural AMF mixture 6 months prior to the infestation with *Phytophthora cinnamoni*, a root rot agent, greatly reduces the mortality rate of the host, whereas simultaneous inoculation has no effect. Jalali and Tharija (1981) showed that mycorrhization allows a 53% reduction in the incidence of chickpea *fusarium* wilt. In our case, the application of Foa 4 months after the establishment of mycorrhization by the Aoufous consortium and *G. clarum* affected only slightly the growth parameters of the young plants, this for the two water regimes applied to the soil, while non-mycorrhized plants subjected to water stress and Foa attack showed higher mortality rates. Thus, the effectiveness of prior mycorrhization may be related to colonization and protection of the root system by AMF prior to penetration by the pathogen. Ismail and Hijri (2012) have shown that

potato inoculation by *G. irregulare* significantly reduces the negative effects of *Fusarium sambucinum* on biomass and tuber production. These authors attribute these effects to the role of AMF in the positive regulation of the expression of the majority of the defense genes (ChtA3, gluB, CEVI16 and PR-1) in the host plant root. In the same way, Ismail et al. (2011, 2013) showed that *G. irregulare* significantly inhibited the growth of *F. sambucinum* while modulating the expression of genes producing toxins (trichothecenes) by this pathogen.

Other studies have shown the beneficial role of AMF in the protection of other host plants towards several diseases (Linderman 1994; Azcon-Aguilar et Barea, 1996; Thygesen et al., 2004; Jung et al., 2012; Xiao et al., 2014).

Mycorrhizal date palm could be better prepared to overcome attacks of pathogens than non-mycorrhizal ones. It seems that AMF can induce some structural, physiological and/or biochemical changes in plants in response to fungal infection *F. oxysporum*. The overall data show that AMF can be used as biocontrol agents in triggering date palm defense against its pathogen and confers a promising strategy for effective control of the vascular fusarium-induced wilt disease and therefore resistance to those harsh biotic and abiotic conditions.

Conclusions

According to our results, the association of date palm with AMF benefited growth under well-watered conditions, being the native consortium Aoufous the most effective in increasing shoot biomass. However, when plants were undergoing restriction of water, both the native and exotic AMF *G. monosporus* and *G. clarum* appeared as the most beneficial fungus for improving plant growth. It appears that after 48 weeks of mycorrhization, Aoufous consortium and selected *Glomus* are aggressive and colonizers, even under conditions of water stress. These same AMF allowed a marked improvement in growth parameters and plant health despite Foa attacks and soil drying. Only plants colonized by *G. deserticola* were more susceptible to attack by *F. oxysporum* and to water stress. The indigenous Aoufous strains have been shown to improve the tolerance of date palm to water stress and *F. oxysporum* attack. The same indigenous mycorrhizal isolate increased the biomass and other water and physiological parameters of date palm subjected to water stress. The use of such fungal isolates, which are indigenous and adapted to unfavorable conditions, could constitute an integrated solution to alleviate the constraints of Moroccan oases, namely drought and *F. oxysporum* attack.

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Chapter 7

Olive Mill Wastewaters as Bio-Insecticide Agent to Control the New Pest (Potosia Opaca) in Date Palm cultivation: Potential of Biopesticides for Integrated Crop and Pest Management

Abdelilah Meddich[1*], Abderrahim Boutasknit[1], Mohamed Anli[1], Meriame Ait Ahmed[1], Abdelilah El Abbassi[1], Hanane Boutaj[1], Mohamed Ait-El-Mokhtar[1] and Ali Boumezzough[2]

[1]Laboratory of Agro-Food, Biotechnologies and Valorization of Vegetal Bioresources, Faculty of Semlalia Sciences, Cadi Ayyad University, 40 000 Marrakesh, Morocco.
[2]X Full Professor University Marrakech, Morocco.

* **Corresponding author: a.meddich@uca.ma**

Abstract

The date palm is one of the most economically important perennial plants of the North Africa and in Morocco, where it is extensively cultivated for food and many other commercial purposes. Palm trees are threatened by many pests such as *Potosia opaca* newly identified in Morocco, especially in Marrakesh and Errachidia regions. In addition, olive mill wastewaters (OMW) are an environmental problem in olive oil producing countries such as Morocco. Generally, these effluents are drained into ecosystems without any pre-treatment. To reduce their negative impact and to get benefits in particular from their high phenolic content, OMW were used as bio-insecticides in crude form. OMW is known for its antimicrobial activity and biocide properties to control plant pests. The results showed that crude OMW were effective against this pest causing a weight loss similar to Cordus insecticide (17% vs. 15%) and mortality almost similar to Kemaban insecticide. OMW's biocide potential was related principally to their high phenolic content. Based on HPLC analysis, ten phenolic molecules were identified, including two which were revealed as the major monomeric phenolic compounds in OMW, hydroxytyrosol with a content of 0.248 g/L and tyrosol with 0.201 g/L. In this chapter, the potential use of OMW as bio-insecticides for the control of *P. opaca* in date palm is discussed. The palm health status is also reported. To reduce chemical and synthetic pesticide use, considerable economic profits are likely to arise from the development of sustainable strategies involving the recycling and reuse of OMW against plant pests.

Keywords: Olive mill wastewaters, *Potosia opaca*, date palm trees, insecticidal activity, biological control.

Introduction

The date palm trees have many important socio-economic and ecological roles in oases ecosystems (Sedghiani et al., 2017). In North Africa and in Morocco, the oases are facing several constraints related to urbanization, drought, salinity, desertification, poor soils in organic matter and nutrients, genetic erosion, aging, diseases like Bayoud palm caused by *Fusarium oxysporum* f. sp *albedinis* (Awad 2006; Meddich et al., 2015a, 2015b; Ziouti 1998) and pests attacks (Howard 2001; El-Shafie 2012). Palm trees are strongly threatened by the red weevil caused by *Rhynchophorus ferrugineus*, which causes huge economic losses (Mahmoud and Gabarty, 2017). The red weevil causes millions of dollars in economic losses each year, interms of agricultural production or costs related to pest control (FAO, 2017). In the Gulf countries and the Middle East, $ 8 million is spent every year to cut down contaminated trees (FAO, 2003, FAO, 2017). In Spain, red palm weevil has appeared since 1999 and damaged almost 20,000 palms of *Phoenix dactylifera* (Sanchez 2017). In the North of Morocco and more precisely in Tangier, the number of *Phoenix canariensis* prospected during 2009-2016 is 244 393 (Ben Ayad 2017). The number of *P. canariensis* infested with *R. ferrugineus* was 904, which 896 were incinerated (Ben Ayad 2017), whereas no *P. dactylifera* has been infested with *R. ferrugineus*. In Morocco, *Potosia opac*a var. cardui Gyllenhal has been observed for the first time by Meddich and Boumezzough (2017). Indeed, in Marrakesh and Errachidia regions, it attacks *P. dactylifera* L. and *P. canariensis* by consuming their wood, which causes faster degradation. Thus, to remedy the damage caused by *P. opaca*, most farmers were forced to use synthetic pesticides. However, the intensive use of these pesticides are generally effective in protecting crops (Joseph and Taylor, 2017), but they are toxic to wildlife and to organisms from different levels of the ecosystems (Herrero-Hernández et al., 2013; Palma et al., 2014; Leromina et al., 2014; Jovana et al., 2014). Over time, the permanent use of insecticides may be accompanied

by the development of resistant strains in some treated species. Biocontrol strategies for pests need to be investigated and developed to provide an ecological substitute or alternative approach to the conventional methods. Environmental engineering techniques are currently used to control pests such as olive oil mill waste waters (OMW), which is essential for crop protection (Haouache and Boughdadi, 2014; El-Abbassi et al., 2017). Most of the OMW phenolic compounds derived from olive polyphenols have many other biological properties (Shi et al., 2017; Belaqziz et al., 2017), as well as biocide activities (Mishra et al., 2018) and phytotoxic effects (El Hassani et al., 2009). Due to their particular characteristics, these effluents are a serious problem for the Mediterranean region, which annually produce around 30 million m^3 of OMW with a damaging effect on the environment (El Hassani et al., 2009) and accounts for approximately 95% of olive oil production in the world (El-Abbassi et al., 2017). In addition, different physicochemical methods have been proposed to treat OMW, including natural and forced evaporation (Masi et al., 2015), electro-coagulation (Hanafi et al., 2010), oxidation by ozone and Fenton reagent (Lafi et al., 2009) as well as their agricultural spreading (Belaqziz et al., 2016), which is an alternative among the suggested solutions. However, the agronomic application of OMW is limited by the doses to be applied and the risk of polyphenols accumulation in the soil after consecutive applications (Belaqziz et al., 2016; Mechri et al., 2008). In parallel with researches made on the treatment of OMW, many valorization studies have been carried out aiming the recovery of OMW phenolic compounds. Recent studies tried to take its advantage from the antimicrobial and phytotoxic properties by using it as biopesticide for crops protection (Yangui et al., 2010; Larif et al., 2013; Lykas et al., 2014) or as insecticides to control *P. opaca* larvae (Boutaj et al., 2019). This contribution summarized the quality of palm health status, OMW characteristics and itsapplication as insecticides to control *P. opaca* larvae in date palm, especially in *P. dactylifera* L.

Materials and Methods

Study Area and Plant Material

This study was conducted during the period of 2014-2016 in the oasis of Marrakesh located in the central region of Morocco and the oasis of Errachidia situated in the southeastern part of the country. The majority of their territory presents arid climate, hot in summer and cold in winter. Palms (*Phoenix*) constitute one of the important botanical families, and include some of the world's most important economic plants. In North Africa regions, dates production provides jobs for estimated 50 million people (El Hadrami et al., 2009). It plays by now an undeniable role in maintaining human populations in arid regions where natural resources are limited and living conditions are difficult (El Hadrami et al., 2009; Ehsine et al., 2014). Like in Zagora, Errachidia, Ouarzazate which are located in the Draa-Tafilalet region. It is built on terraces, crowned by an old Glaoui kasbah of a hill and surrounded by a major oasis of palm grove. This includes in particular the biosphere reserve of the oases of south-eastern Morocco (El Rhaffari et al., 2002), which forms an agglomeration of ksours (small castles). This area is located in an environment covered by vegetation and many dunes were transformed into regs (vast stone expanses) where the vestiges of khetaras (old local irrigation system) are still visible. As Marrakesh city, the area is under a semi-arid climate regime characterized by relatively cold and humid winters and hot and dry summers with a large diurnal temperature range (Tahraoui et al., 2006). This study was performed on *P. dactylifera* and *P. canariensis*. 60-year-old trees with a diameter of 70–90 cm were selected for larvae sampling (Figure1). Analyzed leaves were 4–5 m long with 80–100 segments on each side of the spine. No specific permissions were required for these locations and activities. The field studies did not involve endangered or protected species. The pathogen presence was visually confirmed. No plants were available at very early stage of infection.

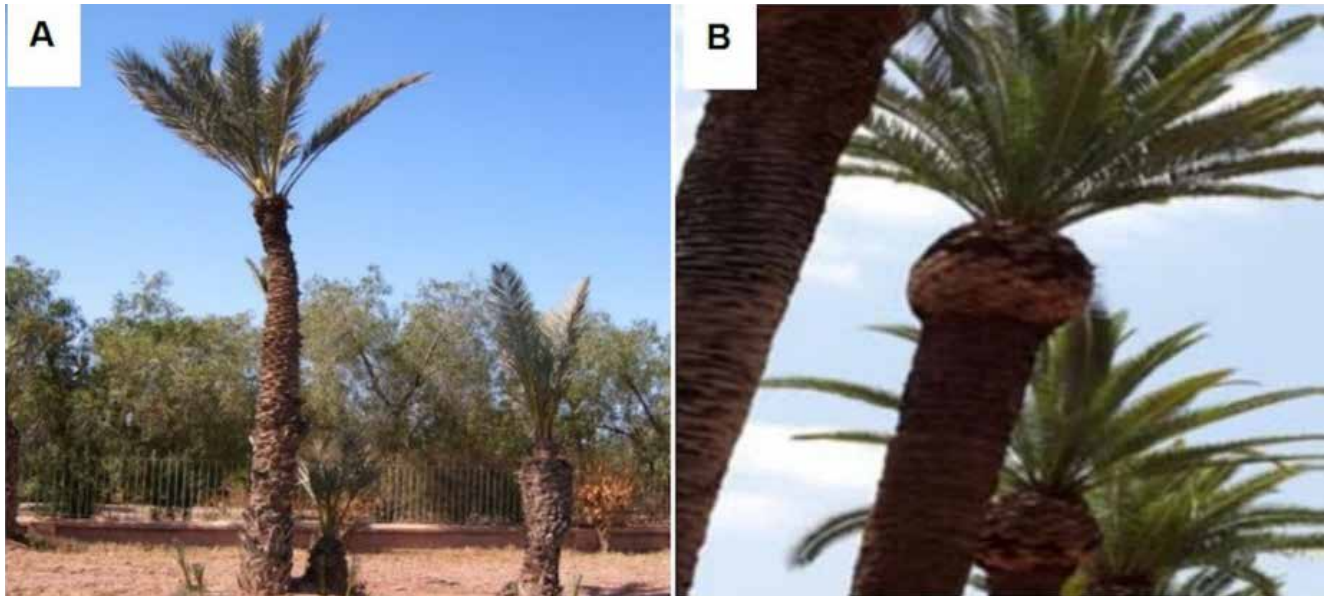

Figure 1. Pictures of *P. dactylifera* (A) and *P. canariensis* (B) in Marrakesh city.

Phytopathological analysis and fungi isolation

The present study used augers to perform localized sampling and perform microbiological isolates. To deepen the diagnosis of P. *canariensis*, we made samplings of rachis, leaflets palms, dryand green leaf bases at the crown. We have carried out cultures and incubation of extracts of rachis (1cm) and palm leaves puny on selective and non-selective media.

Sampling techniques and prospecting the crown of Palm

During the exploration of the palm crown, using a scaffold (Figure 2A), a number of larvae (white grubs) sluggish andarched with strong mandibles were harvested at the base of green and/or deadrachis. Similarly, insect larvae were removed from the base of green and dried palms for laboratory breeding in incubators with controlled conditions of temperature, humidity and photoperiod. Dead rachis (Figure 2B) were brought back to the laboratory to explore them further and also put them in terrariums to ensurethe follow-up and development of the larvae trapped inside in the hope of having imagos (adult forms). Identification of larvae was performed according to the key proposed by Mico and Galante (2003).

Figure 2. Exploration of the *P. canariensis* palm crown using a scaffold (A); Base of leaves (B).

Breeding of larva and nymphs

The collected larvae were immediately placed into breeding boxes; transparent, with openings in lateral side and on the top of the boxes to ensure oxygenation and avoid asphyxiation. The openings on the boxes are closed with afabric scrim (muslin type). Rectangular boxes were used forrearing larvae harvested from *P. canariensis* and *P. dactylifera*. The breeding substrate was composed of a

mixture of untreated natural soil and debris of dead wood, rotted wood and sawdust. Care was taken not to import diseases on bringing boxes of dry dung in breeding substratein order to increase its acidity, which disadvantages the development of diseases. The breeding substrate was constantly renewed as soon as the feces of larvae appear in large quantities on the surface and more debris and wood in the rearing environment was observed. This breeding operation continued in incubators refrigerated and illuminated with controlled temperature and humidity. However, larvae are lucifugous (escape behavior of light); the optimum temperature is between 25 and 30 °C. The nymphal hulls were placed in boxes with slightly damppeat. The duration of pupation varies according to the temperature supported by the larvae and also the male or female sex. The infected nymphal hulls were removed as soon as possible from the breeding environment to avoid pathological contamination of the rest of the cocoons.

Chemical characteristics of palm trees waste

Date palm wastes were used as food for *P. opaca* larvae. The sampling of these wastes was carried out in April 2016 in Marrakesh area (Morocco). Table 1 summarizes the main chemical traits of date palm wastes.

Table 1. Chemical characteristics of date palm trees waste.

Parameters	Mean ± SD
pH	7.03 ± 0.17
TOC (%)	40.8 ± 2.47
NTK (%)	1.06 ± 0.08
C/N	38.6 ± 4.88
Ashes (%)	29.8 ± 1.58
NH_4^+ (mg/g)	738 ± 30.1
NO_3^- (mg/g)	0.7 ± 0.08
Available phosphorus (µg/g)	9 ± 0.8
NH_4^+/NO_3^- (×1000)	1.05 ± 0.75

TOC: Total Organic Carbon, TKN: Total Kjeldahl Nitrogen.

Main chemical traits of OMW

The collection of OMW was carried out in a semi-modern three-phase olive mill installed in Marrakesh (Morocco) and the samples were conserved at 4°C. The determination of the volatile matter (VM) was performed by differentiating between the dry matter (DM) obtained by evaporation at 105°C and the ash residue obtained from calcination at 550°C over a two-hour period.

Determination of phenolic compounds

OMW phenolics extraction

OMW total phenolic compounds were obtained by liquid-liquid extraction according to the method described by El Abbassi et al. (2012). HCl (2M) was added to OMW samples (5 ml) to adjust pH to pH (2.0). OMW were defatting using n-hexane and two extractions were performed with ethyl acetate. The aqueous ethyl extracts were dried at 40°C under reduced pressure via a rotary evaporator and then recovered in methanol (5 ml).

Total phenolic content

Estimation of the total phenol content was determined by the Folin-Ciocalteu calorimetric method (Abraham et al., 1998) where gallic acid was used as the standard. Therefore, it was measured as gallic acid equivalent (GAE) and expressed as g of GAE/L of OMW.

OMW phenolic compounds identification

HPLC analysis was conducted at the Center for Analysis and Characterization (Cadi Ayyad University, Marrakesh, Morocco) with C18 column (Eurospher II 100-5, 250 x 4.6 mm) in gradient system (eluting solution A = acetonitrile; eluting solution B = *o*-phosphoric acid/ water (pH=2.6), 5/95 v/v). A volume of 10 µl was injected at a flow rate of 1 mL/min and pressure of 117 bar. The characterization of phenolic compounds was carriedout using their UV-Vis diode-array detector at a spectrum of 280 nm and their identification was performed by comparing their retention time (RT) with standards. Then these compounds were quantified through the calibration curve of the corresponding standards. The results obtained are expressed in g/L.

Larvae cultures

Sampling of larvae of *P. opaca* var. cardui Gyllenhal (Figure 3) was conducted according to section sampling techniques. The larvae were reared in round boxes (8 cm x 5 cm) containing a mixture of palm waste (150 g). The larvae culture was maintained in darkness at an optimal temperature of 25-30°C inside an incubator. The experiments were conducted in the same conditions as those for the cultures.

Figure 3. *Potosia opaca* larvae isolated from *P. canariensis and P. dactylifera palms.*

Spray toxicity Bioassay

The insecticidal activity of crude OMW and the two commercial insecticides (used as positive controls, Cordus and Kemaban 48 EC) against *P. opaca* larvae was assessed by a spray toxicity bioassay conducted using 5 g of palm compost in plastic boxes. Cordus and Kemaban are composed of 50 and 48% chlorpyrifos-ethyl, respectively. Chlorpyrifos is an insecticide, acaricide and organophosphate miticide used mainly against insect pests. Concentrations of 0.5 and 1 µL/mL of Cordus and Kemaban were dissolved in distilled water. Many preliminary tests have been performed to select the doses to be used for positive controls. For each solution and crude OMW, a volume of 5 ml was sprayed on the surface of the palm compost every 24 hours for 6 days. Based on dry matter, the dose of OMW was calculated to be 94.86 g/L (≈95 mg/mL) (Table 2). The cumulative dosage of OMW polyphenols applied over a six days treatment was calculated. For each treatment, six larvae were placed in each box using ten replicates. Weight loss and mortality of positive controls and OMW were recorded 8 hours per day every 2 hours. Negative control was treated with distilled water. Any larva which was shaken and not moving was considered dead. Observations were made on all treated larvae until their death.

Statistical analysis

All results were analyzed statistically with the CO-STAT software (Statistical Software, New Style Anova). The study includes an analysis of variance followed by the Newman and Keuls test at the 5% threshold.

Probit analysis (Finney 1971) was conducted to estimate lethal times (LT_{50} and LT_{90}) with their 95% confidence interval by SPSS 20.0 Statistical software; LT values were considered significantly different when their respective 95% confidence interval did not overlap.

Results

Microbiological analysis of crown palm

In Laboratory, the microbiological analysis showed fungal formations (blackish, whitish and greenish spots) observed on the inoculated Petri dishes (Meddich and Boumezzough, 2017). After observation under the microscope, it has been found as saprophytic fungi in particular the Dimaties, with blackish spots; those take advantage of the damage already noted and the genus *Fusarium* with a non-virulent strain developed in whitish and pinkish spots with some conidia. The genus *Trichoderma is* wide spread, in greenish spots and is often a key fungus in symbiosis and in biological control.

Sampling techniques and prospecting the crown of Palm

During the exploration of the palm crown (*P. canariensis* and *P. dactylifera*), a number of larvae (grubs) soft arched with strong mandibles were harvested at the base of the green spine and / or dead. The first diagnosis of these larvae showed that they have a powerful mandibles, form “melolonthoides”, sub-cylindrical strongly arched, whitish, with head, stigma and brownish legs; the head was always perpendicular to the body axis, with blackish posterior end; related to the larvae of beetles Scarabeidae (3 pairs of legs, antennae with 3 items). Maximum width of head capsule: 4.6-4.9 mm. Cranium; Colour light yellow. Dorsoepicranium with 2 groups of short setae more or less arranged in 2 rows

on each side; Clypeus with 2 anterior setae and 2 external setae on each side. Labrum; Trilobed, narrower than clypeus; clithra present Epipharynx. Plegmatium absent. Corypha with 4 long setae flanked by 2–3 sensilla on each side. Haptomeral region with slightly curved, transverse row of 15–18 heli above which are 7–9 sensilla. Acanthoparia with 7–9 short and stout setae, decreasing in size posteriorly. Chaetoparia well-developed, covered with longitudinal rows of long, stout setae and many smaller and finer setae near the gymnoparia. Haptolachus with 4 sensilla (2 on the base, 2 on left margin). Sensorial cone present Antenna. 4-segmented.Apical segment with 2 dorsal and 3 ventral sensory spots (1 lateroexternal, and 1 laterointernal). Legs.Tarsunguli cylindrical bearing 10–11 setae. Antenna.4-segmented.Apical segment with 2 dorsal and 3 ventral sensory spots (1 lateroexternal, and 1 laterointernal). Tegilla composed of short, acute setae and sparse long setae. Legs.Tarsunguli cylindrical bearing 10–11 setae. Larvae of the Scarabeidae Coleoptera live normally and develop on decomposing organic matter such as manure, compost being degraded and mated. These larvae were either rhizophagous (melolonthoids), saprophytophagous or saproxylophagous (Cetoniidae). Initial research has shown that in the forms of phytophagous and saproxylophagous larvae, egg-laying can include about one hundred eggs placed directly in the soil or in the wood, sometimes with the aid of an auger (Tauzin 2007). According to field investigations, Meddich and Boumezzough (2017) observed the theft of a number of Scarabeidae beetles from the Cetoniidae family, but linking the larvae harvested at the level of the palm crown and the adults captured on the flight seems to be an illusory thing especially since all the larvae of Coleoptera, Scarabaeidae were similar and that the systematic identification at the generic and specific level requires a rearing of the larvae under appropriate conditions of temperature and humidity for obtaining the adult. The ultimate stage of development of this Scarabeidae and whose characteristics were indispensable for the identification of specimens. The evolution of the larvae is carried out at the base of the palms and rachis weakened and attacked by saprophytic fungi, which can lead to the appearance of rot and the dieback of the weakened palm (Meddich and Boumezzough, 2017). Their presence at the top of the palm can also be explained by the fact that this beetle found an ideal biotope for the proliferation of larvae. Probably the adult manages to lay his eggs at the base of the weakened rachis and continues its development process while damaging the foliar bases as well as the heart of the palm. The larvae of this insect developing in the crown of the stem can infect the whole leaf mass and cause the crown tilt, which can be fatal to the palm. It should be noted that important attacks are observed at the palms base, at the crown; attacks marked by the formation of galleries with a rejection of sawdust and dejections of white grubs (Figure 4) (Meddich and Boumezzough, 2017).

Figure 4. Larvae founded in the palm crown (*P. canariensis*) (A and B larvae), (C) Leaf base.

Breeding of larva and nymphs

After one month of larval rearing, the majority of stage III (L3) larvae are transformed into nymphs (the last stage before adult release) (Meddich and Boumezzough, 2017). At the end of the last stage of development, the larva becomes more yellowish (accumulation of adipose tissue to the detriment of the stercoral volume of the rectal sac). The premymphal phase lasts a few days during which the elderly larva (L3) no longer feeds and migrates to the bottom of the substratum to build a pupal cocoon, which it generally chooses to make against a support (Figure 5).

Figure 5. Observed cocoons (A) and nymphs (B).

Pupation occurs in the dorsal position since the larvae moveon the back, which distinguishes them from Oryctes larvae (Rhinoceros). In general, its cycle development from the egg to the imago known to be set on one year (Tauzin 2007). However, its metabolic activity is closely related to the ambient temperature, the low temperatures slow down this passage, which may last 2 years (Tauzin 2007). The oases, Moroccan as already said are relatively warm, which could privilege this passage. The nymph of brown-orange color has appendages entirely free and folded down on its ventral surface. During this critical phase of development, the insect does not feed, its mobility is very limited and it is very dependent on the conditions of the environment (temperature, humidity and predation). During this passive stage of development, the nymph gradually acquires a darker color. This pigmentation is perfected in the days before the moult. On the occasion of this final metamorphosis, the insect takes a ventral position, in order to facilitate the deployment of wings and elytra. Its tissues harden progressively in the presence of oxygen from the air. After gaining greater rigidity, the adult perforates its cocoon and migrates to the surface of the substrate in order to begin its phase of aerial life. The adult that has just hatched is sometimes still a little soft and often presents colors less sustained and clearer than its older congeners (Figure 6) (Meddich and Boumezzough, 2017).

Figure 6. Adult observed in his damaged cocoon (A), B and C Free adults.

Examination of adults under binocular loupe and using a dichotomous key and reference collections from the laboratory led us to the species of Coleoptera Scarabeidae: *Potosia opaca* var. Cardui Gyllenhal. This species varies greatly in size (from 14 to 24 mm), its morphology with the sides of the pronotum, which can be weakly indented before the posterior angles, or not indented. Its coloring; on the top, the general color black or dark, passes more rarely to the dark green (typical form) and even to bronze and green with coppery metallic reflection. On the underside, the color is sometimes black, sometimes bluish, or sometimes greenish or white.

Systematic

Hexapoda (Insecta)
Coleoptera,
Scarabeidae
Cetoniini
Potosia opaca Fabricius

Olive mill wastewater characteristics

Physicochemical characteristics of crude OMW

Table 2 shows the physicochemical characteristics of the OMW according to Boutaj et al. (2019). In addition, these effluents have an acidic pH of 4.7, a high electrical conductivity of 23.5 mS/cm, a residual oil of 2.2 g/L, a high polyphenol content of 8.38 g GAE/L of crude OMW, and an average dry matter content of 94.86 g/L.

Table 2. Physicochemical characteristics of crude OMW.

Parameters	Mean ± SD
Ph	4.7 ± 0.11
EC (mS/cm)	23.50 ± 0.50
TOC (g/L)	26.23 ± 1.40
DM (g/L)	94.86 ± 1.66
TSS (g/L)	21.79 ± 0.50
Ash (g/L)	11.35 ± 0.67
TPC (g GAE/L)	8.38 ± 0.14
Residual Oil (g/L)	2.20 ± 0.30

EC: Electrical Conductivity, TOC: Total Organic Carbon, DM: Dry Matter, TSS: Total Suspended Solids, TPC: Total phenolic content.

Identification and Quantification of OMW Phenolic Compounds

As described by Boutaj et al. (2019), OMW present a high phenol content. Table 3 summarizes the qualification and quantification of these principal phenolic compounds identified by HPLC analysis. Based on comparisons of their retention times and UV spectra with standards analysed under the same conditions, ten free compounds were provisionally identified and quantified in crude OMW (Table 3). HPLC analysis revealed that the two main monomeric phenolic compounds in OMW were hydroxytyrosol (0.248 g/L) and tyrosol (0.201 g/L).

Table 3. OMW phenolic compounds determined by HPLC.

Peak	Retention time (min)	Area (mAU)	Concentration (g/L)	Compounds
1	3.950	654.660	0.146	Gallic acid
2	9.967	3191.702	0.248	Hydroxytyrosol
3	13.317	2005.142	0.201	Tyrosol
4	14.450	53.766	0.122	Hydroxybenzoic acid
5	15.167	186.037	0.128	4-dihydroxybenzoic acid
6	16.117	102.685	0.124	Vanillic acid
7	17.317	42.461	0.122	Caffeic acid
8	22.250	53.637	0.122	Coumaric acid
9	30.933	39.469	0.122	Oleuropein
10	44.417	674.747	0.147	Quercetin

Olive mill wastewater as bio-insecticides against *P. opaca*

Weight Loss of Treated Larvae

The effect of OMW spray on the larvae was significantly important compared to the control larvae sprayed with distilled water (Figure 7). Over time, larvae treated with OMW showed a significant weight loss from 2.38 to 2.02 g after 216 h. In contrast, the negative control was increased from 2.38 to 2.45 g after 168 h, and then decreased slightly from 2.41 to 2.39 g from 192 to 456 h. In comparison with the crude OMW and the negative control, the two positive controls (Cordus and Kemaban) showed a significant difference (Figure 7). Indeed, Cordus is a very effective insecticide resulting from the combination of two active substances, whose weight loss was similar to that of OMW for the two doses applied. The greatest weight loss over the first three days compared to the other treatments was achieved at a dose of 1 µL/mL, which resulted from the slow decrease in weight. Then, a more or less similar weight loss was observed for both doses. Kemaban insecticide is formed by a single active substance and resulted in significant weight loss at both doses compared to control and other treatments (Cordus and OMW) (Figure 7). Significant weight loss was seen during the first 4 days at a dose of 1 µL/mL of Cordus compared to 0.5 µL/mL of Kemaban. Thereafter, weight loss was found to be almost similar until 174 h, and then became slight and stable at a dose of 1 µL/mL until 224 h. The stability was the result of larval death.

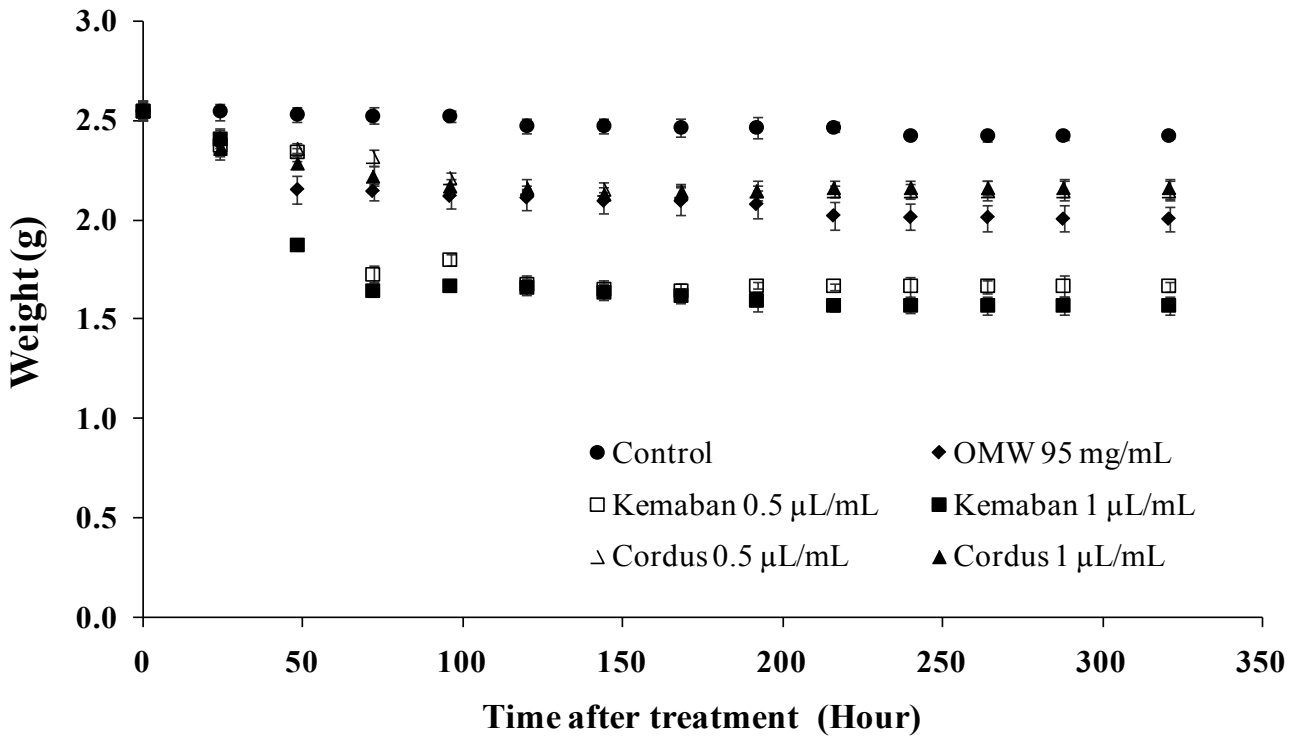

Figure 7. Weight loss of *P. opaca* larvae treated with crude OMW and different doses of commercial insecticides.

Mortality Rate of Treated Larvae

Mortality due to crude OMW was 33, 67 and 100% at 216, 224 and 456 h, respectively (Table 4). In contrast, Kemaban exhibited 33% mortality at 192 and 174 h with application of doses of 0.5 and 1 µL/mL, respectively. The mortality rate was 100% when larvae were treated with Kemaban at 218 and 216 h for 0.5 and 1 µL/mL, respectively. Otherwise, mortality of larvae treated with Cordus was significantly higher compared to other treatments. After 144 and 146 h, the effect began with doses of 1 and 0.5 µL/mL, respectively. However, larvae treated with OMW, Kemaban and Cordus started to die for the first time from day 9, 8 and 6 respectively. In contrast, the two commercial insecticides caused 100% mortality within 8 (Cordus) and 9 (Kemaban) days for all doses tested, compared to OMW which showed 33 and 100% mortality after 9 and 19 days, respectively.

Table 4. Percentage of dead *Potosia opaca* larvae for each treatment.

	Time after treatment (Hour)										
Treatment	**0**	**144**	**146**	**174**	**192**	**198**	**216**	**218**	**224**	**288**	**456**
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crude OMW											
95 mg/Ml	0.00	0.00	0.00	0.00	0.00	0.00	33.33	33.33	66.67	66.67	100

	Time after treatment (Hour)										
Treatment	**0**	**144**	**146**	**174**	**192**	**198**	**216**	**218**	**224**	**288**	**456**
Cordus											
0.5 µL/mL	0.00	0.00	33.33	66.67	66.67	66.67	100	100	100	100	100
1 µL/mL	0.00	33.33	33.33	66.67	66.67	100	100	100	100	100	100
Kemaban											
0.5 µL/mL	0.00	0.00	0.00	0.00	33.33	33.33	33.33	66.67	100	100	100
1 µL/mL	0.00	0.00	0.00	33.33	33.33	66.67	66.67	100	100	100	100

Spray toxicity Bioassay

Boutaj et al. (2019) reported that OMW showed insecticidal activity against *P. opaca* larvae with LT_{50} and LT_{90} values of 245 h and 324 h, respectively (Table 5). Furthermore, a correlation was found between the treatments required to kill 50% and 90% of the population (TL_{50} and TL_{90}) and the duration of exposure. It was found that the highest efficacy was observed in Cordus with LT_{50} of 160 h for a dose of 1 µL/mL and 173 h for a dose of 0.5 µL/mL and LT_{90} of 199 h for a dose of 1 µL/mL and 211 h for a dose of 0.5 µL/mL. Median lethal times (LT_{50} and LT_{90}) generally decrease when insecticide concentrations increase. The least effectiveness was observed in *P. opaca* larvae for crude OMW. However, the results were close to Kemaban at a dose of 0.5 µL/mL.

Table 5. LT_{50} and LT_{90} values of OMW and two positive controls applied by using spray toxicity bioassay against *Potosia opaca* larvae.

Treatments	LT_{50}(h) (95% CL)[a]	LT_{90} (h) (95% CL)[a]	Slope ± SE[b]	χ^2	Df[c]
Crude OMW					
95 mg/mL	245.39 (224.25-326.42)	323.86 (281.69-568.58)	0.01 ± 0.01	2.17	4
Cordus					
0.5 µL/mL	172.85 (128.56-201.58)	211.00 (188.72-383.17)	0.02 ± 0.01	1.98	4
1 µL/mL	160.02 (66.13-184.92)	199.23 (177.22-430.20)	0.02 ± 0.01	1.30	4
Kemaban					
0.5 µL/mL	208.01 (173.03-249.35)	233.91 (217.58-623.46)	0.04 ± 0.24	2.52	4
1 µL/mL	197.53 (164.23-237.69)	228.65 (209.02-507.80)	0.02 ± 0.02	2.07	4

Notes: [a]95 % lower and upper confidence limits are shown in parenthesis,[b]SE: Standard error, [c]Df: Degree of freedom.

Discussion

The palm represents the symbolism of life in the arid and semi-arid area and being one of the oldest domesticated trees with multifold socioeconomic status (Zohary and Hopf, 2000; Chao and Krueger, 2007). The *P. dactylifera* is a former species, which constitutes the pivot of the oasis agriculture in the south of Morocco. Out of an overall area estimated at 84,500 ha in 1948, the Moroccan palm groves in 1994 covered an area of 44,450 ha occupied by a total of 4.42 million palm trees (FAO, 2003). This population is currently estimated at 5.12 million palms on an area of 48,000 ha. The importance of the palm by province showed that the provinces of Ouarzazate (1,873,000 palm trees), Errachidia (1,250,000), Tata (Bani) (800,000), Marrakesh (799,000), Tiznit (139,140), Guelmime (138,000) and Figuig (125,500) were the most important and thus constitute the largest phoenicultural areas (quoted by Sedra (2003) and updated by Meddich (2014). Meddich and Boumezzough (2017) note that the prospected and infected *P. canariensis* palms were planted and located in the North-East palm grove of Marrakesh. This will allow exchanges of contamination between the two palm species studied. For the oasis one, the problem will cause a lot of damage and a serious socio-economic problem. Moroccan palms suffer from invasions by larvae and adults of *Rhinoceros Borer*; this is the case in Tunisia, the Middle East and Iran (Ehsine et al., 2009, 2014). In Morocco, no *R. Borer* larvae or adults were found; also, the colonization of the wounds by the saprophytic fungi can lead to the appearance of rot and the dieback of the weakened palm. According to INRA (Institut National de Recherche Agronomique) reports and their research axis developed on *P. opaca* in oases newly identified in Morocco by Meddich and Boumezzough (2017). Tauzin (2007) indicated the presence of this species in Anti-Atlas (Ifni, Tiznit), middle and southern of Morocco. The presence of *P. opaca* in the crown of the *P. canariensis* and *P. dactylifera* palm which can be explained only by the fact that adults have found an ideal biotope for egg laying and larval development. Meddich and Boumezzough (2017) showed that *P. opaca* occured in decaying wood of *P. dactylifera*, also, where they consumed the wood and promote more rapid decay and laid their eggs in the hollows of branches. The finding of Meddich and Boumezzough (2017) was supported by Mico and Galante (2003). Besides, adults of the Cetoniidae fly above the vault of the trees (including the palm tree) and feed on nectar plants and fruit trees and probably the inflorescences of the palm trees. In this way, it can be assumed that this species has undergonea mutation by changing biotope and passing from saprophagous larvae (dead organic matter, compost) to saproxylophagous larvae (dead woods, rachis and dead and / or alive palms of the *P. canariensis* and *P. dactylifera*). As finding by Meddich and Boumezzough (2017), *P. opaca* larvae was found of all studied sites. The phytophagous species (Scarabeidae) were active at night. In the broad sense, saproxylophages cause damage by attacking either roots or leaves. Larvae (white worms) were generally the most harmful to palms. However, Meddich and Boumezzough (2017) conclude that the degradation of the Moroccan palm grove may be linked to the attack of *P. opaca* larvae. They observed that the frequency of palms prospected and infected with *P. opaca* remains higher in the Marrakesh palm grove than in the south of Morocco. Date palm grove of Marrakesh is more confronted with anthropogenic constraints such as urban extension and air pollution by dust exceeding the tolerance threshold (Meddich 2014; OREDD, 2013). Suspended particle concentrations in certain areas of Marrakesh are slightly above the limit value for health protection in Morocco (OREDD, 2013). This may favor the creation of biotopes necessary for the development of *P. opaca* larvae in this city.

Many researches carried out in other countries have highlighted the danger of the red palm weevil for both species *P. dactylifera* and *P. canariensis*. In the Arab countries, the efforts deployed to control *R. ferrugineus* were based mainly on modified cultural practices, the application of traditional insecticides and traps that uses pheromones to lure *R. ferrugineus* (Abraham et al., 1998). Moreover, the control of *P. opaca* insect pests involves spreading chemicals (in the form of toxic pellets) as uniformly as possible in areas where the larvae are causing damage. The elimination of diseased palms (attacked by larvae) can reduce the spread of the pest and consequently limit the damage.

For preventive treatments of the aerial parts and the crown of the palm:

- Use Imidacloprid (200 g/l) by spraying at the crown (stipe, palm and heart) 2 to 3 times. It is a systemic insecticide for the selective control of scarabeidae beetles.
- Spray acetamiprid 100g / l at 2-3 times / year.
- Also, use entomo-pathogenic nematodes with a dose of 180 million nematodes per 100 L of water, which act by reducing the size of the larvae and consequently affect the number of female adults capable of laying the eggs.
- Trapping control using pheromones. The use of such specific substances for sexual confusion may be a means.

Efforts now focus on the development of integrated pest management methods based on biological control and pheromone traps rather than on conventional insecticides (Murphy and Briscoe (1999). Since it is an internal tissue borer, *R. ferrugineus* is difficult to control in the early stage of attack (Abraham et al., 1998; Ferry and Gomez, 2002). Initial efforts to control red palm weevil in the Kingdomof Saudi Arabia using chemical insecticides were failed (Bokhari and Abozuhairah, 1992). An integrated pest management strategy, developed in India, has successfully suppressed the pest in the date plantations in the Kingdom of Saudi Arabia (Abraham et al., 1998). The strategy is modeled on the lines of tackling the pest on coconut. The pheromone traps have been used successfully to monitor and mass attract the pest, and it could be considered as the core of in any integrated pest management (Vidhyasagar et al., 2000; Faleiro et al., 2002; Al-Saoud 2013).

Recognizing this real danger, farmers and consumers turned their efforts to environmental and eco-friendly practices by using as well as consuming biological and healthy products. The polyphenols are natural molecules contained in OMW from the olive fruit which could be an alternative and an asset for pests' control. However, total polyphenols amount in OMW may vary substantially based on a multitude of factors, such as the climatic conditions and olive variety and fruit ripening stage as well as the harvest period (Yousfi et al., 2006; Ben Ahmed et al., 2007; Gómez-Rico et al., 2008). The OMW phenolic compounds content, can be considerably affected by the technological processes used for olive oil extraction (Butinar et al., 2006). In this context, the phenolic compounds content of OMW which, presented potential insecticidal activity has been assessed and investigated by Boutaj et al. (2019) in view to develop new valorization strategies. Additionally, an application of a hydroxytyrosol-rich OMW extract by spraying it against olive psyllid (*Euphyllura olivina*), in a drip-irrigated olive orchard for evaluating the insecticidal activity of OMW, was carried out in 2008 and 2009 (Larif et al., 2013). The extract from OMW had a strong insecticidal activity against this insect when the applied concentration was 2 g/L. In addition, the authors observed a significant biocide effect depending on OMW phenolic extracts concentration on *E. olivina* larvae as well as adults. Indeed, OMW showed similar toxicity to

the Kemaban insecticide at 0.5 µL/mL dose. Nevertheless, it is clear that the obtained results were attributed to the chemical molecules that contain the two commercial insecticides. Cordus presents two active molecules and Kemaban presents a single active molecule, namely chlorpyriphos ethyl and cypermethrin and chlorpyriphos ethyl, respectively. These molecules act on the spread of nerve impulses along the axon (cypermethrin action) and inhibit the acetylcholine esterase by blocking the transmission of the nerve flux (chlorpyriphos ethyl action) (McCarthy 2003; Tian et al., 2008). The main mechanisms which explain the OMW's biocide effect on invasive species in general including insects are not clarified. It has been suggested that the transmission of the nerve flux may be blocked by the high phenolic compounds content in OMW (Danellakis et al., 2011; Campani et al., 2017). A significant inhibition of acetylcholine esterase activity in a marine mollusk (*Mytilus galloprovincialis*) has been reported by Danellakis et al. (2011) after exposition to OMW. While, Campani et al. (2017) reported that the inhibition of acetylcholine esterase may be attributed to the potential presence in OMW of organophosphates and carbamates, two pesticides which, are strong inhibitors of acetylcholine esterase activity and commonly used to treat the olive fruit fly (*Bactroceraoleae*). However, the authors did not dismiss the inhibition of acetylcholine esterase possible which, could be explained, by the phenolic compounds as well as metals and ammonia contained in OMW. Thus, the used OMW crude showed a toxic effect on *P. opaca* larvae. Danellakis et al. (2011) noted that the toxicity is provided by the phenolic compounds and trace metals contained in OMW. Furthermore, Barbera et al. (2013) showed that during their growth cycle, no phytotoxic effects were observed when OMW were applied on crops. This is related to plant phenological stage, the application modalities and the applied doses. The absence of harmful residues is the main advantage of OMW application to control plant pests as well as pathogens. Therefore, we can assume that there will be no need for a pre harvest interval on crops, after the application of OMW.

Conclusions and future directives

In this chapter, we present a new eco-friendly approach to control the spreading of *P. Opaca* which started in Morocco. Microbiological analyses show the presence of saprophytic fungi and genus *Fusarium* with a non-virulent strain. On the other hand, the two insecticides used separately and crude OMW are toxic on *P. opaca* var. Cardui Gyllenhal larvae. These results are promising and suggest the possibility of using OMW due to their high content of phenolic compounds as a means of biological control to overcome environmental problems caused by synthetic pesticides. The OMW and their phenolic extract compounds could be used in agricultural systems. Moreover, focused field researches (each plant-pathogen system) could be carried out to understand and evaluate the effects of OMW on specific in situ pest problems. Based on the main findings, it is clear that OMW may contribute to improve the date palm protection against *P. opaca* and could be used as bio-insecticides. Nevertheless, OMW could be used safely as a challenge to control plant pest without affecting negatively the soil and plants. Besides, the use of OMW combined with other pest bio-control practical methods which could be a sustainable approach to minimize the potential risks. In this context and to understand the beneficial effects of OMW, more investigations could be required to assess the feasibility of OMW application in bio-controlled systems at large-scale, and determining the limitations and advantages on the long term. Further, research works are needed to test, besides crude OMW, pretreated OMW (ultrafiltered or heated) and its phenolic extracts as biodegradable pesticides.

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General conclusion and Prospects

- The soils of Tafilalet palm grove, which are poor in N and in available P, have shown greater mycorrhizal potential and infectivity capacity than those of the palm grove of Marrakech.
- The mycorrhizal fungi used (Aoufous consortium and *Glomus*) were infectious and slightly affected by the drying out of the soil. These same AMF allowed a marked improvement in growth parameters and plant health despite Foa attacks and soil drying.
- The indigenous Aoufous strains have been shown to improve the tolerance of date palm to water stress and *F. oxysporum* attack. The same AMF increased the biomass and other water, biochemical and physiological parameters of date palm subjected to salt and water stress. Mycorrhizal symbiosis hampered the excess uptake of Na^+ and improved uptake of important ions to ensure better growth of date palm under saline conditions.
- AMF symbiosis may enhance osmotic adjustment in plants which could contribute to maintaining higher leaf water status and RWC in AM plants during drought and keeps the plants protected against oxidative stress, and these cumulative effects increase the plant drought tolerance.
- Mycorrhizal plants could be better prepared to overcome attacks of pathogens than non-mycorrhizal ones. It seems that AMF can induce some structural, physiological and/or biochemical changes in date palm in response to fungal infection *Fusarium oxysporum* f. sp. *albedinis*.
- The overall data show that AMF can be used as biocontrol agents in triggering date palm defense against its pathogen and confers a promising strategy for effective control of the vascular *fusarium*-induced wilt disease and therefore resistance to those harsh biotic and abiotic conditions.
- The use of such fungal isolates, which are indigenous and adapted to unfavorable conditions, could constitute an integrated solution to alleviate the constraints of Moroccan oases, namely salinity, drought and *F. oxysporum* attack.
- The use of mature composts has clearly favored the growth of date palms and underlying crops tested (alfalfa, wheat, maize, tomato, lettuce, leek and garlic). A variation of this plant growth of date palm was noted as a function of the dose of applied compost (GW). The 20% dose of these biofertilizers has reduced date palm development and AMF infectivity.
- The application of compost at low doses alone and in combination with AMF could play an important role in promoting date palm growth and development, especially via increased nutrient uptake.
- The dual-inoculation of PGPR/AMF amended with composts in combination boosted the biomass of date palm under water deficit conditions to a greater extent than in non-inoculated and/or non-amended plants.
- The tripartite combination improved water and physiological parameters

by elevating stomatal conductance, RWC, ψ_w, photosynthetic pigments (chlorophyll and carotenoids content), and photosynthetic efficiency in date palm vitroplants grown in greenhouses and in the field.

- The use of such effective organic and biological amendments could constitute a biotechnological means in improving growth and plant adaptation to soil and climatic constraints of the environment. Thus, the input of chemical fertilizers can be reduced or even completely replaced by adding organic matter to the systems when transplanting date palms into field soils generally characterized by low soil organic matter contents and fertility.
- Palm trees in Moroccan oasis are facing a new threat which is *Potosia opaca* larvae (Coleoptera). These larvae could inflict several damages to palm trees and when enters the palm by the crown, it causes the greatest damage since this will stop the growth of new fronds. The larvae found can also cause damage to the heart of palm trees and will ultimately kill the palm.

- In this context, olive mill wastewaters (OMW) can be used as a biopesticide and contributes to palm trees (*Phoenix dactylifera* L.) protection against *Potosia opaca*, avoiding the use of synthetic insecticides.

- In summary, we have elucidated the positive impacts of biofertilizers used such as composts- AMF-PGPR on the tolerance of date palm to water and salt stress and *F. oxysporum* attack. The results obtained are encouraging both under controlled conditions and in the open field as well as in different soil and climatic conditions.

- It is interesting in perspective to evaluate the impact of this bio-fertilizer technology on the behavior of adult date palms and the quality of dates in open field experimentation.

- The present study has therefore the potential to offer a new approach through the implementation of an integrated bio-fertilizer technology. This will then allow the development of sustainable strategies needed to rapidly improve the growth of date palm and underlying crops. The transfer of this technology in the open field will have a positive impact on the oasis environment by generating socio-economic and environmental benefits such as improving farmers' incomes, reducing poverty and preserving natural resources. Finally, the molecular tool under development will be useful for identification of different species and strains constituting our selected mycorrhizal and bacetrian consortia.

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KHALIFA INTERNATIONAL AWARD FOR DATE PALM AND AGRICULTURAL INNOVATION

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