Effect of PGPR and mixed cropping on mycorrhizal status, soil fertility, and date palm productivity under organic farming system

Mohamed OU-ZINE (mohamed.ouzine1@usmba.ac.ma)
Sidi Mohamed Ben Abdellah University
Sarah SYMANCZIK
Forschungsinstitut für Biologischen Landbau
Said El KINANY
Ecole Nationale d'Agriculture de Meknès
Larbi AZIZ
Ecole Nationale d'Agriculture de Meknès
Mustapha FAGROUD
Ecole Nationale d'Agriculture de Meknès
Ali ABIDAR
Ecole Nationale d'Agriculture de Meknès
Paul MÄDER
Forschungsinstitut für Biologischen Landbau
El Hassan ACHBANI
National Institute of Agricultural Research
Abdellatif HAGGOUD
Sidi Mohamed Ben Abdellah University
Rania EL HILALI
Ecole Nationale d'Agriculture de Meknès
Mustapha ABDELLAOUI
Office Régional de la Mise en Valeur Agricole de Tafilalet
Rachid BOUAMRI
Ecole Nationale d'Agriculture de Meknès

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Abstract

A field study was carried out for two years at an organic farm under arid climate in Morocco to investigate the effect of an integrated biofertilization approach on Arbuscular Mycorrhizal Fungal (AMF) abundance and infectivity, soil fertility, yield, and fruit quality of date palm. The biofertilization approach included three management practices namely application of compost, inoculation with a consortium of native PGPR strains originally isolated from date palms of Drâa-Tafılalet region (*Pseudomonas koreensis*, *Serratia nematodiphila*, *S. marcescens*, and *Klebsiella* sp.) and using mixed-cropping with sorghum. Accordingly, four treatments were established in this study: 1) mixed-cropping with sorghum, 2) PGPR inoculation, 3) sorghum + PGPR, and 4) control (without sorghum or PGPR). All treatments received compost as organic amendment. Results revealed that mixed-cropping with sorghum significantly increased AMF colonization intensity and spore density by more than 50% and 29%, respectively. Sorghum association also resulted in a significant increase in organic matter concentrations of up to 2.95% against 2.45% in monocropping soils. The integrated biofertilization approach resulted in the highest yield with an increase rate of 10.6% and 12.1% in the first and the second year, respectively compared to date palms receiving compost alone. Similarly, the mineral composition and quality characteristics of date fruits were significantly improved. The enhancement of soil fertility and date palm productivity under harsh environmental conditions represents a first step towards the adoption of sustainable practices in the region and in similar areas.

Introduction

Date palm (*Phoenix dactylifera* L.) is considered as one of the oldest fruit crops in the world (Chao and Krueger 2007). For several decades, it has been cultivated mainly in North Africa and the Middle East, and currently, it is cultivated in 30 countries across the world (El Bouhssini and Faleiro 2018). The Moroccan oasis agroecosystem is characterized by harsh environmental conditions in which the date palm has to cope with several threats including soil and water salinity, drought, desertification, bayoud disease, and low soil fertility (Jaradat 2011; Ou-zine et al. 2021; Ou-zine et al. 2022). Currently, the Moroccan “Green Generation” program aims the development of organic farming system to reach 10 000 hectares, 75% of which are dedicated to date palms (ONCA, 2021).

Accordingly, there is a need to change farming practices towards more sustainable practices. In addition to the application of organic amendments namely compost, such practices might include the management of beneficial soil microorganisms such as plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) (Barea et al. 2011; Manaut et al. 2015). Soil microorganisms including PGPR play an important role in nutrient cycling and organic matter (OM) mineralization and, thereby, in improving the soil fertility status (Abbott and Murphy 2007). Indeed, soils generally contain high concentration of mineral nutrients but their availability and accessibility for the crops are low; PGPR are able to make them more available via dissolving organic (e.g. phytates) and inorganic phosphorus by liberating enzymes and acids (Yin et al. 2015), release potassium from organic and insoluble materials (Meena et al. 2013), produce siderophores, which are high affinity iron chelating compounds (Sivasakthi et al. 2014), and solubilize other nutrients such as zinc (Kumar et al. 2019). These bacteria also exhibit inhibition of pathogens (Li et al. 2020), and secret plant hormones such as auxins and cytokinins (Mohite 2013). Accordingly, due to these aptitudes, the application of PGPR shows a significant effect on yield of different plant species such as raspberry (Orhan et al. 2006), strawberry (Pırlak and Kose 2009), apples (Karlidag et al. 2007), banana (Kavino et al. 2010), date palm (Naser et al. 2016) and several other crops (Abbasi et al. 2011; Shahid et al. 2019; Li et al. 2020). According to Zafar et al. (2012), PGPR introduced into a soil are usually not sufficient to compete with native bacterial strains. Hence, inoculation of native bacteria isolated from the target ecosystems is necessary to ensure their successful integration and to take advantage of their beneficial properties (Igual et al. 2001; Zafar et al. 2012). Besides PGPR, AMF are key microorganisms in agricultural soils that maintain crop productivity and environmental quality (Van der Heijden et al. 1998; Hazzoumi et al. 2015; Cozzolino et al. 2015). AMF can improve soil structure (Rillig and Mummey 2006) and soil water retention (Querejeta 2017), mitigate abiotic stresses such as salinity, drought, and high temperatures (Plouznikoff et al. 2016), and help plants to overcome biotic stress (Brito et al. 2019). In addition, they were shown to improve mineral nutrition and plant growth (Latef and Chaoxing 2011), increase crop yield (Regvar et al. 2003; Celebi et al. 2010; Gao et al. 2020) and enhance the soil attributes and tree seedlings survival (Asmelash et al. 2016). Thus, theoretically, AMF inoculation may result in significant impact on the ecosystem especially on the plant-soil system. However, AMF inoculation of agricultural fields is expensive because of high prices of the products. Further, products are not always effective as they mainly just include single AMF strains. And last, it is difficult to introduce AMF into existing plantations i.e. bringing the AMF in close contact with the fine roots of adult trees. Therefore, the adoption of another method to increase the activity of native AMF will represent a valuable alternative to the direct inoculation. Previous studies have revealed that mycorrhizal colonization was higher in plants cultivated in mixed culture compared to those cultivated in monoculture (Ouahmane et al. 2006; Duponnois et al. 2011). Muleta et al. (2008) found higher spore densities in the rhizosphere of coffee trees planted in mixed culture compared to coffee trees in monocultures. Similarly, Ouahmane et al. (2006) declared that *Lavandula multifida* could acts as AMF nurse plants in association with trees such as *Cupressus* spp. and that this association improves the propagation of native AMF in the soil and enhances the growth of associated cultures. The adoption of this strategy, based on the use of nurse plants in association with perennial trees, could play a key role by promoting soil- and plant-associated microorganisms, particularly AMF (Ouahmane et al. 2006, Ingleby et al. 2007), and thus indirectly facilitating the transfer of nutrients and improving the growth of neighboring trees (Battie-Laclau et al. 2019). To the best of our knowledge, no study has investigated the effect of associated nurse plants on mycorrhizal colonization of date palm and AMF abundance in soil.

Therefore, this study aimed at investigating i) the effect of inoculation of date palm rhizosphere with indigenous PGPR on nutrient assimilation, ii) the impact of sorghum cultivated in mixed-cropping system with date palm on soil nutrients as well as on AMF density and infectivity, and iii) the interactive effect of PGPR and mixed cropping on soil fertility and date palm nutrition and productivity under field conditions. Accordingly, we hypothesized that the application of native PGPR consortium could improve the mineral nutrition of date palm and its productivity, and that mixed-cropping with sorghum would increase date palm root mycorrhization and spore density of preexisted AMF.

Material and methods

Experimental sites description

A field experiment was carried out during two growing seasons at an organic farm (31°29'46.4"N 5°02'04.8"W) located in Tinjdad belonging to Drâa Tafilalet region in Southeastern Morocco. The initial physicochemical properties of the soil are presented in Table S1. The experiments were carried out on 15-years old date palms of the cultivar Majhoul planted at 8 m × 8 m density in plots of 4 meters.

Organic amendment used

The organic amendment used in this study is Moroccan compost produced by a private company in Meknes and certified as a bio-product that can be used in the organic farming. Its physicochemical properties are presented in Table S2.

PGPR identification and inoculum preparation

Four bacteria were isolated, among others, from date palm rhizosphere and they were *in vitro* screened for activities involved in plant growth promotion and tested for their compatibility (Table S3) (El Kinany et al., 2021).

PGPR isolates used in this study were cultured in YPGA medium (yeast extract 5 $g.L^{-1}$, peptone 5 $g.L^{-1}$, glucose 10 $g.L^{-1}$, and agar 15 $g.L^{-1}$) and incubated at 28 ± 2°C for 24 hours. Colonies were recovered for the extraction of the genomic DNA. Polymerase chain reaction amplification of the 16S rRNA region of the bacteria was performed using universal primers (27F/1492R) (Weisburg et al. 1991) according to the following program; 5 min at 94°C, 35 cycles; 94°C for 1 min, 52°C for 1 min followed by 72°C for 1 min and a final step of 10 min at 72°C. The amplicon was revealed in 1% electrophoresis gel. The PCR products were then sequenced and the obtained sequences were edited and aligned using BioEdit software (version 7.0.5.3). The sequences were checked for similarity using the Blast program and then deposited in GenBank. The partial 16S rRNA gene sequences of the isolated bacterial strains were aligned and the bootstrapped Neighbour-joining relationships were estimated with MEGA X software (Kumar et al. 2008).

Before their application, the four bacteria were separately grown on YPGA medium, suspended in sterile physiological saline solution (NaCl 0.85%) to reach a concentration of 10^8 $CFU.ml^{-1}$, and finally well mixed together to obtain the final used inoculum.

Treatments and experimental design

Four treatments were established in this study described as follows: compost, compost + nurse plant; compost + PGPR, and compost + nurse plant + PGPR. All treatments were additionally supplemented with 5 kg/tree of fish meal (8.42% N) applied once in January. Compost was incorporated homogeneously into the soil at 0.2 m depth and at 1.5 m distance from the date palm trunk (15 ton of compost / ha).

Plants inoculated with PGPR were received an inoculum of 250 ml/tree (10^8 $CFU.ml^{-1}$) containing a consortium of the four bacteria inoculum. The total inoculum volume was applied in the date palm rhizosphere at 0.2 m depth on five spots surrounding the date palm at 1.5 m distance from the trunk. In treatments with nurse plant, sorghum seeds were sown around the date palm tree and mown twice a year. The mown sorghum biomass was then used as mulch to cover the soil. Date palm trees were selected as uniform as possible in vigor and they were arranged in a randomized-block design with three replicated blocks.

Soil analysis

Soil samples were collected from 0-0.6 m depth in January (after treatment application) and in October (after the harvest) using a soil auger. Each sample was a composite of five soil cores randomly collected at a distance of 1.5 m from each date palm trunk. Chemical properties of the soils including pH, OM, N, phosphorus (P), potassium (K), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) were assessed. Soil pH was measured in water suspension 1:5 (v/v) using a glass electrode (Rayment and Higginson 1992). The OM concentration was calculated after the assessment of the organic carbon by titration method of Walkley and Black (1934). Total N and P concentrations were measured according to Kjeldhal technique and Olsen extraction method followed by spectrophotometry, respectively, and K, Fe, Mn, Cu, and Zn were measured using atomic absorption spectroscopy (Estefan et al. 2013).

Leaf mineral nutrients' concentration

The middle part of the median leaves were sampled from the four directions around the tree in September of each growing season and kept in an isothermal cooler. The sampled leaves were washed, rinsed with distilled water, and then air-dried at 70°C for 72 h. The dried leaves were ground, digested, and prepared for analysis. Total N was assessed by the Kjeldahl method following the procedure suggested by AOAC (1995). Phosphorus and K were measured by the method of Chapman and Pratt (1961) and by the flame photometer (Jackson 1958), respectively. Iron, Mn, Cu, and Zn were measured using the atomic absorption spectrophotometer (Estefan et al. 2013). Boron (B) was measured by dry ashing (Chapman and Pratt 1961) and subsequently measured by colorimetry using azomethine H (Bingham 1982).

Assessment of spores density and colonization of date palm and sorghum roots with AMF

Date palm and sorghum roots and soil samples were collected from each treatment in October of each season. Roots were rinsed with tap water, cut into 10 mm fragments, bleached for 45 min at 90°C in 10% KOH, rinsed with distilled water, and then submerged in 1% HCl for 3 min and immediately stained with 0.05% (w/v) Trypan blue (Phillips and Hayman 1970). Stained roots were observed under optical microscope to assess the colonization frequency corresponding to the ratio of colonized versus non-colonized root fragments, and the colonization intensity corresponding to the proportion of cortical cells colonized by AMF (Trouvelot et al. 1986). Spore density was assessed in 100 g of soil, collected at a depth of 0-0.30 m, by using the wet-sieving and sucrose gradient techniques followed by enumeration of extracted spores under a binocular stereomicroscope (Brundrett et al. 1996).

Yield and fruit quality measurements

At harvesting time (mid-September to mid-October in both years), fruits of each date palm tree were harvested and weighted using an electronic balance to evaluate the total yield per tree (kg/tree).

From each tree, fruit samples were randomly collected from all bunches to determine fruit weight (g) and flesh percentage (%) by using an electronic balance, and fruit length and diameter (mm) by using an electronic digital caliper. To assess nutrient concentrations, fruits were cut into pieces and dried at 70°C in the oven until constant weight. About 0.5 g of dried fruit sample was digested in nitric acid and hydrogen peroxide and then ultra-deionized water (20 mL) was added. The extracts were filtrated through Whatman filter paper and submitted to the analysis by inductively coupled plasma-optical emission spectrometry to determine the concentrations of P, K, Mg, Ca, Fe, Zn, Mn, Cu, and B in fruits as described by Al Juhaimi et al. (2014) whereas total N was determined by Kjeldahl method (AOAC 1995).

Statistical analysis

Data were analyzed using two-way-ANOVA at P = 0.05. Duncan test was used for statistically significant results at P = 0.05. The values expressed with percentage were first arcsin transformed before statistical analyses. All analyses were performed using the SPSS software, version 24.0.

Results

Effect of treatments on soil fertility and plant nutrition

The analysis of soil properties (Tables 1 and 2) showed an improvement of the organic and mineral fractions as compared to the initial analysis of the used field due to the application of compost (Table S1). Considering the effect of associated nurse plants during the second year, a significant increase in OM concentrations of up to 2.95% was noted in plots grown in mixed culture compared to 2.45% in plots with date palms only. Unsurprisingly, results showed that soil N concentration was decreased on average by 100% under mixed-cropping compared to date palms grown alone at the end of the study. Similarly, P and K concentrations in soil were lower under mixed-cropping compared to date palm monoculture system. On the other hand, mineral nutrition of date palm especially N, P, and K were improved by 17, 82, and 31%, respectively by adopting the integrated biofertilizer treatment as compared to the control (Compost alone). The applied PGPR were identified as *Pseudomonas koreensis* DPR-6M, *Serratia nematodiphila* DPR-8M, *Serratia marcescens* DPR-2M, and *Klebsiella* sp. DPR-9M and deposited in the GenBank database under the accession numbers MZ413920, MZ355924, MZ356167, MZ356236, respectively (Figure S1). Data showed that the rate of OM degradation (calculated as the difference between OM content in January and October of each year) in soils receiving compost and PGPR was slightly higher than OM degradation rate in soils amended with compost alone; it was 0.61% against 0.42%, respectively. The application of the four strains significantly improved the Mn uptake by more than 74% during the first year and the Fe uptake by around 7% during the second year.

Table 1
Influence of treatments on soil physicochemical properties of the organic farm

Parameters										
Treatment	Sampling period	OM	pH	N	P	K	Cu	Mn	Fe	Zn
		%		%	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$
Compost	January (1st season)	1.49 ± 0.15a	9.23 ± 0.05a	0.12 ± 0.02a	244.9 ± 8.6a	833.2 ± 31.2c	1.01 ± 0.10a	5.83 ± 0.28a	8.63 ± 0.17a	3.58 ± 0.02a
Compost + N. plant		1.48 ± 0.11a	9.25 ± 0.22a	0.12 ± 0.01a	237.8 ± 6.5a	930.6 ± 40.6b	0.98 ± 0.07a	5.91 ± 0.19a	8.82 ± 0.18a	3.63 ± 0.18a
Compost + PGPR		1.49 ± .0.06a	9.28 ± 0.16a	0.11 ± 0.02a	242.2 ± 9.92a	962.1 ± 14.8ab	0.95 ± 0.05a	6.05 ± 0.36a	8.91 ± 0.44a	3.55 ± 0.14a
Compost + N. plant + PGPR		1.38 ± 0.08a	9.46 ± 0.38a	0.10 ± 0.02a	243.9 ± 5.94a	977.1 ± 16.0a	1.03 ± 0.05a	6.13 ± 0.17a	8.75 ± 0.12a	3.57 ± 0.10a
F_{ANOVA}		2.35 ns	1.44 ns	1.08 ns	0.38 ns	30.70***	0.68 ns	0.63 ns	0.50 ns	0.27 ns
Compost	October (2nd season)	1.01 ± 0.05a	8.77 ± 0.06a	0.09 ± 0.01a	193.0 ± 14.73a	770.0 ± 30.0a	0.80 ± 0.10a	5.33 ± 0.71a	7.53 ± 0.21a	1.90 ± 0.12a
Compost + N. plant		0.92 ± 0.10a	8.40 ± 0.20b	0.08 ± 0.01a	150.7 ± 8.02b	726.7 ± 20.8a	0.73 ± 0.15a	5.43 ± 0.58a	7.27 ± 0.49a	1.43 ± 0.21b
Compost + PGPR		0.88 ± 0.03a	8.87 ± 0.06a	0.07 ± 0.02a	167.0 ± 27.73ab	726.7 ± 30.6a	0.73 ± 0.15a	5.63 ± 0.35a	7.03 ± 0.95a	1.73 ± 0.17ab
Compost + N. plant + PGPR		0.90 ± 0.07a	8.83 ± 0.06a	0.09 ± 0.03a	152.3 ± 19.86b	716.7 ± 15.3a	0.70 ± 0.10a	5.73 ± 0.32a	6.97 ± 0.23a	1.54 ± 0.22b
F_{ANOVA}		3.56 ns	10.64**	0.63 ns	2.48 ns	4.25 ns	4.40ns	1.69 ns	0.80 ns	4.04 ns
Compost	January (1st season)	2.53 ± 0.27b	8.50 ± 0.20a	0.16 ± 0.03a	396.0 ± 23.3a	1456.7 ± 63.5a	0.85 ± 0.02a	8.49 ± 1.64a	17.97 ± 3.05a	2.80 ± 0.33b
Compost + N. plant		2.98 ± 0.25a	8.50 ± 0.10a	0.19 ± 0.02a	390.0 ± 47.5a	1570.0 ± 85.4a	0.83 ± 0.13a	8.70 ± 1.42a	16.75 ± 2.02a	3.84 ± 0.16a
Compost + PGPR		2.45 ± 0.22b	8.63 ± 0.32a	0.17 ± 0.01a	382.3 ± 34.1a	1493.3 ± 65.1a	0.75 ± 0.13a	7.15 ± 0.55a	15.34 ± 1.91a	3.68 ± 0.02a
Compost + N. plant + PGPR		2.95 ± 0.13a	8.63 ± 0.21a	0.18 ± 0.01a	392.3 ± 25.0a	1486.7 ± 71.0a	0.82 ± 0.12a	8.20 ± 0.14a	19.55 ± 0.72a	3.14 ± 0.24b
F_{ANOVA}		47.66***	0.37 ns	2.16 ns	0.07 ns	1.36 ns	1.46 ns	0.95 ns	1.89 ns	10.86**
Compost	October (2nd season)	1.34 ± 0.29a	8.47 ± 0.25a	0.13 ± 0.01a	267.2 ± 25.5a	1083.3 ± 71.0a	0.52 ± 0.18a	3.98 ± 0.79a	6.05 ± 0.28a	1.75 ± 0.39a
Compost + N. plant		1.57 ± 0.23a	8.43 ± 0.15a	0.06 ± 0.01b	198.0 ± 14.5a	943.3 ± 47.3b	0.60 ± 0.07a	3.72 ± 0.51a	6.03 ± 0.13a	1.81 ± 0.38a
Compost + PGPR		1.26 ± 0.24a	8.40 ± 0.10a	0.12 ± 0.03a	261.3 ± 16.6a	1033.3 ± 47.3ab	0.58 ± 0.11a	3.91 ± 0.18a	6.12 ± 0.23a	1.70 ± 0.26a
Compost + N. plant + PGPR		1.62 ± 0.21a	8.47 ± 0.15a	0.07 ± 0.01b	166.0 ± 46.1a	926.7 ± 70.2b	0.62 ± 0.07a	3.89 ± 0.32a	6.17 ± 0.30a	1.79 ± 0.20a

OM: organic matter, N: nitrogen, P: phosphorus, K: potassium, Cu: copper, Mn: manganese, Fe: iron, and Zn: zinc. Data represent means ± standard deviation (n = 3). Means followed by the same letter are not statistically different according to Duncan test at P = 0.05, ns: not significant, * $p < 0.05$; ** $0.001 \le p < 0.01$; *** $p < 0.001$.

Parameters									
F_{ANOVA}	2.45 ns	0 .12 ns	15.44**	9.63 ns	5.24*	0.47 ns	0.14 ns	0.19 ns	0.06 ns

OM: organic matter, N: nitrogen, P: phosphorus, K: potassium, Cu: copper, Mn: manganese, Fe: iron, and Zn: zinc. Data represent means ± standard deviation (n = 3). Means followed by the same letter are not statistically different according to Duncan test at P = 0.05, ns: not significant, * p < 0.05; ** 0.001 ≤ p < 0.01; *** p < 0.001.

Table 2
Effect of treatments on concentration of mineral nutrients in the date palm leaves

		N	**P**	**K**	**Mg**	**Ca**	**Zn**	**Cu**	**Mn**	**Fe**	**B**
Season	**Treatment**	% of dry matter					mg.kg^{-1} of dry matter				
1st season	**Compost**	1.02 ± 0.07c	0.08 ± 0.00b	0.85 ± 0.07b	0.19 ± 0.04b	0.37 ± 0.06b	8.83 ± 0.75b	3.47 ± 0.12b	46.7 ± 8.39b	252.0 ± 51.1a	24.7 ± 5.86a
	Compost + N. plant	1.55 ± 0.08a	0.12 ± 0.01a	1.06 ± 0.23b	0.34 ± 0.03a	0.46 ± 0.17b	11.2 ± 0.70a	4.70 ± 0.26a	81.3 ± 12.9a	269.3 ± 19.5a	39.3 ± 8.50a
	Compost + PGPR	1.01 ± 0.03c	0.08 ± 0.01b	0.80 ± 0.13b	0.20 ± 0.04b	0.38 ± 0.06b	9.57 ± 0.60b	3.90 ± 0.36b	59.3 ± 3.21b	280.0 ± 38.6a	28.7 ± 1.15a
	Compost + N. plant + PGPR	1.35 ± 0.05b	0.13 ± 0.02a	1.43 ± 0.05a	0.35 ± 0.05a	0.65 ± 0.10a	11.7 ± 0.47a	3.40 ± 0.36b	91.3 ± 9.71a	292.7 ± 28.8a	35.0 ± 7.00a
	F_{ANOVA}	54.13**	37.00***	15.52**	20.56***	6.57*	11.52**	9.40**	12.94**	0.69 ns	3.96 ns
2nd season	**Compost**	1.49 ± 0.05c	0.11 ± 0.01b	0.94 ± 0.11b	0.41 ± 0.07a	0.56 ± 0.02a	9.67 ± 0.58a	3.03 ± 0.06a	84.0 ± 31.8a	314.7 ± 11.4b	51.0 ± 2.00a
	Compost + N. plant	1.72 ± 0.07ab	0.18 ± 0.02a	1.19 ± 0.08a	0.48 ± 0.11a	0.59 ± 0.05a	10.0 ± 2.65a	2.87 ± 0.23a	86.3 ± 5.51a	316.7 ± 5.13b	50.3 ± 4.16a
	Compost + PGPR	1.59 ± 0.06bc	0.10 ± 0.02b	0.91 ± 0.06b	0.42 ± 0.02a	0.57 ± 0.03a	9.33 ± 0.58a	2.97 ± 0.06a	83.7 ± 12.0a	336.7 ± 7.77a	50.7 ± 8.50a
	Compost + N. plant + PGPR	1.75 ± 0.06a	0.20 ± 0.01a	1.23 ± 0.04a	0.47 ± 0.03a	0.60 ± 0.03a	10.0 ± 1.95a	3.07 ± 0.12a	87.0 ± 5.00a	347.6 ± 10.6a	49.7 ± 1.53a
	F_{ANOVA}	9.96**	35.62***	23.00***	1.09 ns	0.79 ns	0.10 ns	1.04 ns	0.03 ns	10.39**	0.034 ns

N: nitrogen, P: phosphorus, K: potassium, Mg: magnesium, Ca: calcium, Zn: zinc, Cu: copper, Mn: manganese, Fe: iron, and B: boron. Data represent means ± standard deviation (n = 3). Means followed by the same letter are not statistically different according to Duncan test at P = 0.05, ns: not significant, * p < 0.05; ** 0.001 ≤ p < 0.01; *** p < 0.001.

Table 3
Effect of treatments on date palm yield and quality characteristics of date fruits

Season	Treatment	Yield (kg/date palm tree)	Length (mm)	Diameter (mm)	Fruit weight (g)	Flesh (%)
1st season	**Compost**	50.0 ± 2.01b	43.5 ± 0.13d	28.1 ± 0.10c	20.5 ± 0.28b	94.3 ± 0.13a
	Compost + N. plant	54.8 ± 3.52a	46.2 ± 0.16b	29.1 ± 0.08b	21.3 ± 0.33ab	94.5 ± 0.27a
	Compost + PGPR	50.7 ± 3.87b	44.7 ± 0.18c	29.2 ± 0.10b	20.8 ± 0.12b	94.3 ± 0.40a
	Compost + N. plant + PGPR	55.3 ± 5.30a	47.22 ± 0.14a	29.5 ± 0.24a	21.9 ± 0.73a	94.6 ± 0.18a
	F_{ANOVA}	6.14*	1267.42***	57.66***	5.73*	0.99 ns
2nd season	**Compost**	53.9 ± 4.18c	47.5 ± 0.58c	27.8 ± 0.13c	22.6 ± 0.24b	94.5 ± 0.09a
	Compost + N. plant	56.9 ± 4.22b	48.6 ± 0.25b	29.4 ± 0.30b	23.9 ± 0.54a	94.7 ± 0.19a
	Compost + PGPR	54.4 ± 3.96bc	48.2 ± 0.20b	29.1 ± 0.05b	22.7 ± 0.35b	94.6 ± 0.23a
	Compost + N. plant + PGPR	60.4 ± 3.44a	49.4 ± 0.19a	30.3 ± 0.30a	23.9 ± 0.36a	94.8 ± 0.32a
	F_{ANOVA}	16.56**	18.22**	70.36***	10.52**	0.82 ns

Data represent means ± standard deviation (n = 3). Means followed by the same letter are not statistically different according to Duncan test at P = 0.05, ns: not significant, * $p < 0.05$; ** $0.001 \leq p < 0.01$; *** $p < 0.001$.

Plant mycorrhizal colonization and spore density

The results of date palm mycorrhization and AMF spore density are presented in Figs. 1 and 2, respectively. Statistical analysis showed that mixed-cropping with sorghum increased mycorrhizal root colonization as well as AMF spore density from the first season of the experiment. Indeed, the mycorrhization intensity and spore density increased by 28% and 33%, respectively in the first year and by more than 50% and 29%, respectively in the second one. In parallel to the evaluation of date palm mycorrhization, we assessed AMF colonization in sorghum roots during the two growing seasons (Fig. 3). Results showed that sorghum root colonization increased significantly from the first to the second year and reached on average a final colonization frequency and intensity of 67.8% and 37.8%, respectively.

Yield, fruit quality characteristics and mineral composition of date fruits

Yield data, presented in Table 4, showed a significant difference between the treatments in both years (Compost + Nurse Plant + PGPR > Compost + Nurse Plant > Compost + PGPR > Compost). Indeed, the integrated biofertilization approach resulted in the highest yields with an increase of 10.6% and 12.1% in the first and the second year, respectively compared to date palms receiving compost alone. Similarly, fruit quality characteristics, namely fruit length, fruit diameter, and fruit weight were significantly affected by treatments in both years (Table 4). At the end of the experiment, fruit length, diameter and weight increased by 4.0%, 9.0%, and 5.8%, respectively when plants where cultivated following the integrated biofertilization approach as compared to compost alone. Looking at the mineral composition of date fruits, we observe most significant effects in the second season (Table S4). The cultivation following the integrated approach resulted in highest concentration of most nutrients except N and Cu.

Discussion

Effect of mixed cropping system and PGPR on soil fertility

The significant increase in OM concentration in soils where date palms and sorghum were grown together compared to the sole application of compost is explained by the effect of associated sorghum. The accumulation of organic carbon regenerated from the sorghum biomass might have led to the increase in soil OM concentrations especially in the second year. This improvement due to mulching was previously stated by several authors (e.g. Medcalf 1956; Mehlich 1966; Ramakrishna et al. 2006). Wijanarko and Purwanto (2017) reported that the utilization of corn and peanut biomass as plant residue mulch improved the soil OM content. On the other hand, data showed that soil N, P and K concentrations were decreased in soils under mixed-cropping compared to date palms alone. It's evident that the assimilation of nutrients increased under mixed-cropping due to the higher plant density and root volumes leading to a decrease in soil macroelement concentrations. In addition, the decrease in N concentration might also be explained by N immobilization by microorganisms as N is required for the mineralization of sorghum biomass. Indeed, the applied organic materials were found to increase the microbial activity and, thereby, increase the N immobilization (Choi et al. 2001).

The rate of OM degraded in amended soils with compost and inoculated with PGPR consortium was slightly higher than the one in soils amended with compost alone. Bacteria are known by their capability to mineralize soil OM by releasing hydrolytic enzymes (Pii et al. 2015). In our study, the microbial inoculant applied corresponds to autochthone bacteria isolated from local conditions. This is very interesting in terms of competition with preexisting soil bacteria and of adaptation to the climatic conditions and edaphic properties, which ensures their integration and, thus, leading also to take more advantage of their beneficial traits (Igual et al. 2001; Zafar et al. 2012). Previous study showed that the application of similar bacteria namely *P. koreensis*, *S. nematodiphila*, *S. marcescens*, and *Klebsiella* sp. were able to colonize the rhizosphere and exert positive effects on soil and/or crops (Dastager et al. 2011; Singh et al. 2015; Devi et al. 2016; Kang et al. 2019).

Effect of mixed-cropping system on AMF infectivity and spore abundance

Mixed-cropping with sorghum increased mycorrhizal root colonization as well as AMF spore density in both years. In agricultural fields, inoculation with AMF is infrequent due to the limited availability of inoculum, which cannot be readily produced in artificial culture (Hailemariam et al. 2013), and to the potential incompatibility of introduced strains with local soil characteristics (Duponnois et al. 2013), which can lead to the disappearance of the introduced strains, as well as to economic reasons as commercial inoculum are expensive. In addition, inoculation of plants with AMF may influence the richness and diversity of native AMF communities (Mummey et al. 2009). For these reasons, and in order to valorize the native AMF inhabiting the soil of date palm groves and to establish an agroecological practice that can be easily adopted by farmers, we have chosen to cultivate sorghum in association with date palm as a principal culture, serving as nurse plant to promote the propagation of native AMF. Our results showed that mixed-cropping with sorghum effectively increased spore density and mycorrhizal root colonization of date palm in the agroforestry system, where the perennial tree was being associated with an annual crop (date palm/sorghum) as compared to date palm in monoculture. Earlier studies revealed that mycorrhizal root colonization might be increased when different plant species are grown together (Ouahmane et al. 2006; Duponnois et al. 2011). Muleta et al. (2008) found higher spore densities in agroforestry coffee systems compared to monoculture coffee systems. Results obtained by Shukla et al. (2012) showed that crops (*Phaseolus mungo* and *Triticum aestivum*) sown together with trees (*Albizia procera* and *Eucalyptus tereticornis*) significantly increased the root colonization with indigenous AMF. Similarly, the number of AMF spores was significantly higher in soil collected under *Cupressus atlantica*/ *Lavandula stoechas* dual cultivation than in soil collected under single cultivation (Duponnois et al. 2011). The improvement of mycorrhizal status of date palm and the increase in AMF spore density could also be attributed to the effect of sorghum residue mulch. Indeed, Okon et al. (2010) found that the use of leaf mulch obtained from different plants increased AMF infectivity. Several studies suggested that an increase in OM concentrations stimulates the sporulation of some AMF (Gryndler et al. 2005; Oehl et al. 2009).

The steady increase in sorghum root colonization noted over the two seasons indicates an increase in AMF propagation in the rhizosphere, and thus suggesting the successful use of sorghum as nurse plant. Accordingly, the date palm rhizosphere may be considered as an important niche of AMF and a source of inoculum. The natural association of date palm with AMF has been previously confirmed by Bouamri et al. (2006) and Bouamri et al. (2014) in Drâa-Tafilalet region where our study was carried out.

Effect of treatments on mineral nutrition and productivity of date palm

Data revealed that mixed-cropping with sorghum improved date palm nutrition compared to date palms grown in monoculture system. This improvement could be due to an indirect facilitating effect of sorghum, leading to an increased nutrient uptake of date palm via the improvement of the mycorrhizal status and AMF activity. Battie-Laclau et al. (2019) declared that the improvement of plant colonization by AMF through plant associations maximize the facilitative effects and minimize the competition between perennial trees and annual crops. Sorghum, used principally as nurse plant for AMF, was mown at the end of each cycle and used secondarily as mulch to cover plots where it was grown. This agroecological practice allowed to improve the OM rate and to compensate for the potential loss of nutrients assimilated by sorghum. As a result, the enhancement of mineral nutrition of date palm associated with sorghum could also be explained by the positive impact of sorghum residue mulch leading to the release of nutrients during mineralization processes. Furthermore, the utilization of plant residue mulch has been shown to conserve soil moisture (Tuure et al. 2021), reduce soil temperature (Wang et al. 2019) increase the biological activity of soils (Jabran 2019), improve the root growth (Akhtar et al. 2019), and inhibit weed proliferation (Iqbal et al. 2020), thereby it could have contributed to the improved nutrition of date palms grown under mixed-cropping with sorghum.

The application of PGPR significantly improved microelements' nutrition of date palms especially iron. As shown in Table S3, the applied PGPR have the potential to produce siderophores, which are high affinity iron chelating compounds (Sivasakthi et al. 2014). Therefore, the improvement of Fe acquisition observed in date palms inoculated with bacteria could be explained, partially, by the capability of these bacteria to secrete siderophores in field conditions. A recent study showed that *P. koreensis* is a plant growth promoting rhizobacteria with significant effect on promoting plant growth and suppressing post-emergence damping off due to its capability to produce siderophores (Ghazy and El-Nahrawy 2021).

Results showed that the integrated biofertilization approach most effectively improved yield, fruit quality and fruit nutrient concentrations, which most likely might be explained by the interplay of PGPR, enhancement of date palm mycorrhization mediated by mixed-cropping with sorghum and mineralization of sorghum residues. Already earlier studies reported about beneficial effects of PGPR on fruiting trees. Aslantaş et al. (2007) showed that PGPR improve yield and yield components of apple trees by producing plant growth promoting metabolites and dissolving P. Equally, Mia et al. (2005) found that PGPR inoculation significantly increased banana bunch yield and fruit physical attributes including length and diameter, and pulp/peel ratio. Many studies reporting about positive impacts on yield and yield components by PGPR, but most of them studied the effect of single strain inocula only (Li et al. 2020). A recent study showed that consortium inocula exerted superior effects on plant growth than single strain inocula (Gómez-Godínez et al. 2019). Accordingly, we selected four bacteria and applied them as consortium to take advantage of their combined activities and to avoid limitations of single strain inocula such as environmental incompatibility as reported by Gómez-Godínez et al. (2019). Besides PGPR, also AMF were shown to improve date palm performance especially under drought stress. Benhiba et al. (2015) observed AMF mediated enhancement in date palm growth and increased contents of soluble sugars, proteins, and K in date palm leaves grown under long-term drought stress. Also Souna et al. (2010) showed that the mycorrhization enhanced the growth of date palm seedlings with approximately 26% caused by an improved hydric and mineral nutrition due to the higher assimilation capacity of AMF hyphae.

Conclusions

Mixed-cropping with sorghum is an agroecological practice that can be easily applied by farmers to improve the low fertile soils, mycorrhizal status, and date palm performance in the oasis agroecosystem. PGPR are involved in the nutrient cycling within the tested oasis soils through the improvement of soil OM decomposition and Fe uptake. This study revealed for the first time the effect of mixed-cropping system on soil OM content and mineral nutrition of date palm as well as AMF abundance and infectivity. For a good management of date palm fertilization, further researches should focus on effect of mixed-cropping and compost on the microbial biomass evolution (including AMF) and nitrogen and carbon mineralization dynamics.

Declarations

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

All data generated or analyzed during this study are included in this article and its supplementary information file.

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Figures

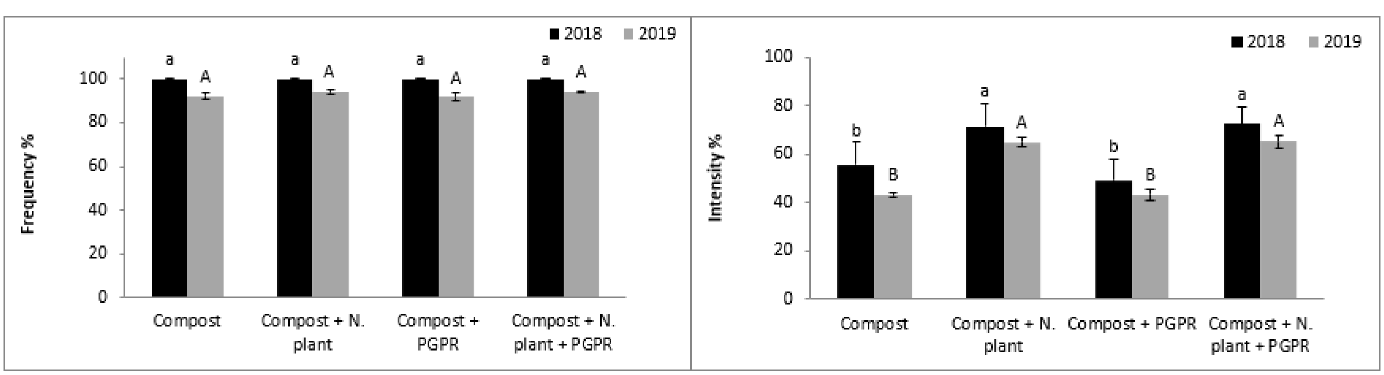

Figure 1

Colonization of date palms by arbuscular mycorrhizal fungi (AMF) in 2018 and 2019. Data with the same letter are not statistically different according to Duncan test at P= 0.05. Data represent means +/- standard deviations (n=3).

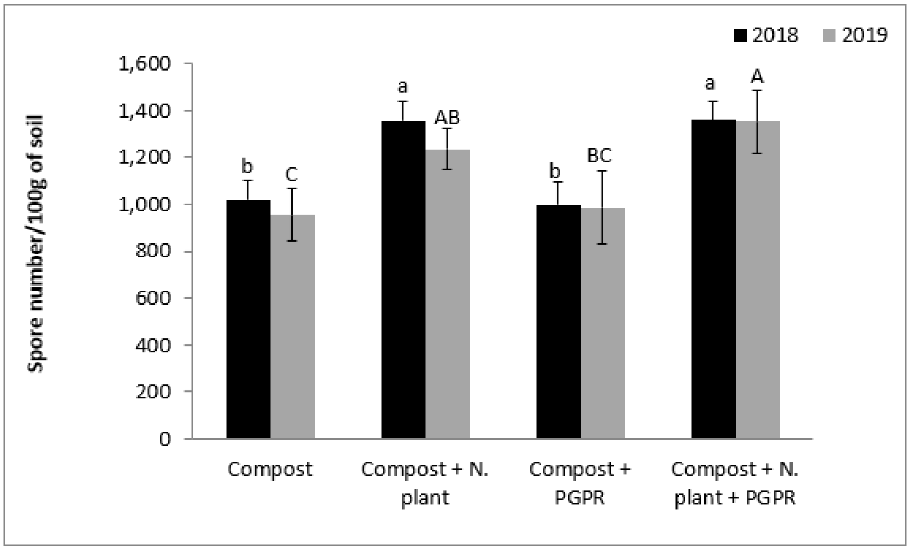

Figure 2

AMF spore density in the organic farms during the two growing season. Data with the same letter are not statistically different according to Duncan test at P= 0.05. Data represent means +/- standard deviations (n=3).

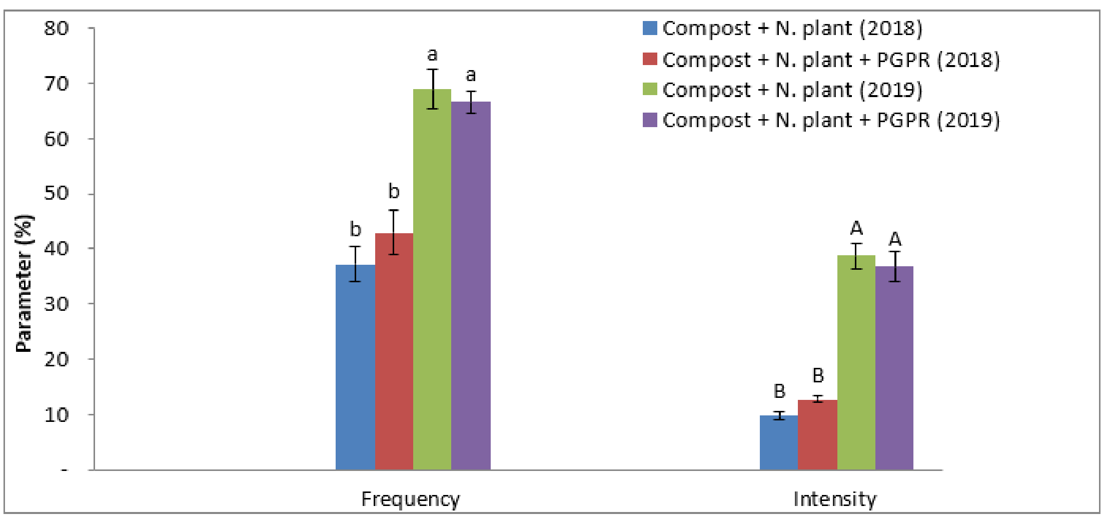

Figure 3

AMF colonization frequency and intensity of sorghum roots. Data with the same letter are not statistically different according to Duncan test at P= 0.05; lowercase letters for frequency and uppercase letters for intensity. Data represent means +/- standard deviations (n=3).

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