Impact of *Streptomyces* spp. and *Glomus* spp. on growth of date palm (*Phoenix dactylifera* L.) and suppression of fusarium wilt

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SAMMARY
This study investigated the ability of *Streptomyces* spp. and *Glomus* spp. isolates to control the fusarium wilt pathogen of date palm (*Phoenix dactylifera* L.), *Fusarium oxysporum* f.sp. *albedinis*, as well as the effect of these isolates on plant growth in the presence and absence of the pathogen. The genus *Glomus* is the most abundant fungi in rhizosphere of date palm in Biskra from Algeria. *Streptomyces* spp. BI21 showed the largest zone of inhibition against *F. oxysporum* f.sp. *albedinis* (35 ± 3 mm). In the absence or presence of the pathogen, the *Streptomyces* spp. BI21 and *Glomus* spp. A01 isolates increased all plant growth parameters of date palm. The initial results indicate the arbuscular mycorrhizal fungi and *Streptomyces* as a promising biocontrol agent for promoting growth of plants and also controlling palm date Fusarium wilt disease.
Keywords: Biocontrol, *Glomus* spp., *Streptomyces* spp., Rhizosphere, Fusarium wilt

RESUME
Cette étude a examinée la capacité des isolats de *Streptomyces* spp. et de *Glomus* spp. pour lutter contre la fusariose chez le palmier dattier (*Phoenix dactylifera* L.), *Fusarium oxysporum* f.sp. *albedinis*, ainsi que l'effet de ces isolats sur la croissance des plants en présence et en absence d'agent pathogène. *Glomus* est le genre du champignon mycorhizien le plus abondant dans la rhizosphère des palmiers dattier de Biskra en Algérie. *Streptomyces* spp. BI21 a montré la plus grande zone d'inhibition contre *F. oxysporum* f.sp. *albedinis* (35 ± 3 mm). En absence ou en présence de l'agent pathogène, les deux isolats *Streptomyces* spp. BI21et *Glomus* spp. A01 améliorent tous les paramètres étudiés de croissance du palmier dattier. Les résultats préliminaires indiquent que les champignons mycorhiziens arbusculaires et les *Streptomyces* sont des agents de lutte biologique prometteurs pour favoriser la croissance des plantes et également pour lutter contre la fusariose.
Mots clés : Lutte biologique, *Glomus* spp., *Streptomyces* spp., Rhizosphère, Fusariose

1. INTRODUCTION

Date palm (*Phoenix dactylifera* L.), a dioecious species that belongs to the Arecaceae family. It has a long history of cultivation and utilization in North Africa and the Middle East. The Bayoud disease, caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), represents a major limiting factor of date palm culture in Morocco and constitutes a serious threat to the date palm plantations in Algeria and all other countries (El Modafar, 2010).

Foa is an invasive pathogen responsible for wilt and cortical rot diseases in more than a hundred cash crops, including date palm (*Phoenix dactylifera* L.). Bayoud disease, caused by this pathogen, destroyed two-thirds of date palm in North Africa, especially in Morocco (more than 10 million trees) and in the western and central parts of Algeria in 20th centuries (Shabani and Kumar, 2013).

Applying chemical pesticides is generally considered as the most effective and fastest strategy for plant disease management, however, no effective chemical product is available for Fusarium wilt in palm date. In addition, the application of chemical pesticides causes long-lasting adverse effects on ecosystems and human health (Karlidag et *al*., 2007; Sowndhararajan et *al*., 2012; Sathiyabama and Charles, 2015). The resistance of numerous pathogenic bacteria and fungi to commonly used bioactive secondary metabolites is presently an urgent focus of research, and new antifungal and antibacterial molecules are necessary to combat these pathogens (Kumar et *al*., 2011).

Hence, it is necessary for searching and developing non-hazardous biologically compatible alternatives. Such, biological control through application of microorganisms antagonistic to plant pathogens is an alternative and a sustainable strategy for plant protection. Inhibition or killing of harmful pests by biocontrol agents is biologically safe, target specific and without creating any environmental pollution (Karthik et *al*., 2015).

These fungi establish the arbuscular mycorrhizal association with roots of 80%–90% of vascular plants and provide an 87 array of benefits to ecosystems (Smith and Read, 2008). Arbuscular Mycorrhizal Fungi (AMF) live in symbiosis with most crop plants and represent essential elements of soil fertility and plant nutrition and productivity, facilitating soil mineral nutrient uptake and protecting plants from biotic and abiotic stresses (Battini et *al*., 2016). AMF can improve the growth of many plants by many pathways, known to increase nutrient uptake (Zhang et *al*., 2011), improve drought tolerance (Auge et *al*., 2015), improve salt resistance (Zhang et *al*., 2018), and reduce plant vulnerability to pathogens (Souna et *al*., 2010; Wehner et *al*., 2011; Veresoglou and Rillig, 2012). The use of plant growth promoting Rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) is a better alternative to solve this problem. They play an important role to increase in soil fertility, plant growth promotion, and suppression of phytopathogens for development of ecofriendly sustainable agriculture (Gupta et *al*., 2015).

The main objective of this study was to evaluate the effect of *Streptomyces* and AMF on growth of date palm seedlings cultivated at presence of *Fusarium oxysporum* f. sp. *albedinis* under controlled conditions.

2. MATERIALS AND METHODS

2.1. Sampling and cultivation of *Streptomyces* spp. and AMF species

Streptomyces spp. BI21 and *Glomus* spp. 01 were isolated from the rhizospheric soil of indigenous legume *Astragalus gombo* Coss. & Dur. and date palm (*Phoenix dactylifera* L.) respectively growing in the desert region Biskra of Algeria (Fig. 1). *Streptomyces* spp. BI21 was maintained in yeast extract-malt extract agar medium (Ghadbane et *al*., 2015).

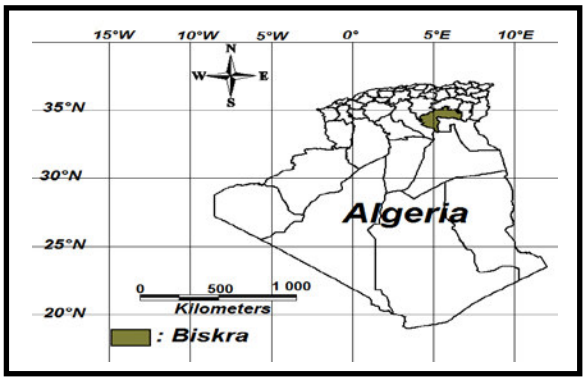

Figure 1: A map showing the sites from which the rhizosphere soil samples of *Astragalus gombo* and *Phoenix dactylifera* L. were collected

2.2. Determination of AMF root colonization

Roots from date palm (*Phoenix dactylifera* L.) were stained with trypan blue in glycerol after clearing with potassium hydroxide (Phillips and Hayman, 1970). AMF root colonization was estimated by light microscopy as described by Trouvelot et *al*. (1986). Overall frequency of mycorrhization (F%), percentage of root cortex colonization (M%) were calculated with the MycoCalc program (http://www.dijon.inra.fr/ mychintec/Mycocalc-prg/download.html).

2.3. Spore isolation and identification of AM fungi

One hundred grams of air dried soil sample was used for spore isolation. AMF spores were isolated using the wet sieving and decanting method of Gerdemann and Nicolson (1963). AM fungi were identified following the current taxonomic criteria (Schenck and Pérez 1990; Morton and Redecker 2001; Schüßler et *al*. 2001; Oehl and Sieverding 2004; Walker and Schüßler 2004), and using information from International Culture Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi on the internet (http://www.invam.caf.wdu.edu). Spores were first mounted in water, and morphological characteristics were measured. Melzer's reagent and cotton blue were used in the identification.

2.4. AMF species and cultivation

Glomus spp. isolate A01 was used as inoculum for AMF colonization studies. The fungus was maintained and replicated using a monoxenic culture with transformed date palm (*Phoenix dactylifera* L.) roots according to the method established by (Declerck et *al*.,1998). Infected roots were kept growing in petri dishes containing modified Strullu-Romand (MRS) agar medium (Pérez-de-Luque et *al*., 2017). For inoculation of date palm in rhizotrons, the content of a petri dish was blended and mixed with 50 ml sterile distilled water. The concentration of spores was measured and adjusted to approximately 500–600 spores per ml, and 5 ml were added on the root of each plant in the rhizotrons.

2.5. In vitro Antagonistic Bioassay

Streptomyces spp. BI21 and *Glomus* spp. A01 were used to antagonistic bioassay against *Fusarium oxysporum* f.sp. *albedinis*. The antimicrobial activity of isolates was performed by using the cross streak modified method (Ghadbane et *al*., 2015). ISP2 plates were prepared and inoculated with isolates by a single streak in the Petri plate and incubated at 30°C for 7 days. The plates were then inoculated with *Fusarium oxysporum* f.sp. *albedinis* by a single streak at 90° angles to *Streptomyces* spp. BI21 and *Glomus* spp. A01 strains and incubated at 24 °C of 7–10 days. Antagonism was observed by the inhibition of the test organism. All experiments were carried out in three replicates.

2.6. Production of lytic enzymes

Chitinase activity of *Streptomyces* spp. BI21 and *Glomus* spp. A01 isolates was examined using the modified method described by Gupta et *al.,* (1995) and Kawase et *al.,* (2004). The chitinase enzyme activity of the selected isolates was tested in nutrient agar medium containing 1% colloidal chitin. Chitinase production was assessed by visual examination of cleared zones developed around colonies incubated at 28 °C for 7 days. Protease activity of *Streptomyces* spp. BI21 and *Glomus* spp. A01 isolates was done as per the protocols of Bhattacharya et *al.,* (2009). Microorganisms were streaked on casein agar medium and incubated at 28 °C for 7 days. At the end of the incubation, the plates were observed for halo zone around the colonies, which indicates the presence of protease.

2.7. Determination of Potential Plant Growth Promoting traits of *Streptomyces* spp. BI21 and *Glomus* spp. A012

- Determination of indole acetic acid production

IAA production by bacterial and fungal strains was estimated based on the method of Gordon and Weber (1951) and Khamna et *al*., (2009). 500 µl of microbial culture was inoculated in 50 ml of nutrient broth added with 0.1% DL-tryptophan and incubated at 30±0.1°C for 2 days in the dark. After incubation, the bacterial cultures were centrifuged at 10,000 rpm for 10 min. Salkowski reagent (4 ml) was added to one ml of collected supernatant and after 30 min incubation pink color developed which indicated production of IAA. To quantify IAA, absorbance was taken at 535 nm by using UV/Visible spectrophotometer. The IAA concentration was estimated with a standard curve of IAA.

- Qualitative estimation of phosphate solubilization

Phosphate solubility was conducted qualitatively according to the method described by Franco-Correa et *al.,* (2010). *Streptomyces* spp. BI21 and *Glomus* spp. A01 isolates were spot-inoculated

onto minimal medium based on the Pikovskaya (PVK) medium described by Pikovskaya (1948). This medium contained (per liter): glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; KCl, 0.2 g; yeast extract, 0.5 g; $MnSO_4 \cdot H_2O$, 0.002 g; and $FeSO_4 \cdot 7H_2O$, 0.002 g, supplemented with agar 10 g. The dishes were incubated at 28 °C for 7 days. A positive reaction was indicated by clear zones around the colonies.

2.8. Biological control assay

The biocontrol efficacy and growth promoting effects of *Streptomyces* spp. and *Glomus* spp. isolates were studied in growth room conditions with temperature 24 °C, 16 h light / 8 h dark photoperiod and relative humidity of 80 %. Pre-germinated seeds of date palm cultivar Daghlet Nour were grown in pots containing sand–peat-soil mixture (1:1:1 v/v/v). Soils were previously sterilized by autoclave (120 °C for 20 min) to prevent colonization by native microorganism. A fresh suspension of *Streptomyces* spp. BI21 approximately 1×10^6 CFU/ml in 1 ml , *Glomus* spp. A01 6×10^2 spores/ml with 0.01 % Tween-20 and 1 ml of *F. oxysporum* f.sp. *albedinis* approximately 1×10^5 CFU/ml were added to the planting mixture immediately before planting. Plants without *Streptomyces* spp.BI21, *Glomus* spp. A01 and *F. oxysporum* f.sp. *albedinis* served as control. Seeds were placed on the surface of the planting mix approximately 2 cm from to the pot (five seedlings per pot) and covered with a 0.5 cm layer of the sterilized sand and vermiculite mixture. Plants were fertilized weekly with a Murashige and Skoog (MS) solution Murashige and Skoog (1962), and watered as needed. The germination index and growth parameters, shoot height, shoot fresh weight, root fresh weight and root length were determined after six months. The proportion of root colonized by *Glomus* spp. A01 including frequency of mycorrhization (F%) and percentage of root colonization (M%) was determined by Trouvelot et *al*. (1986) method. The experiment was conducted with three replicates per treatment.

2.9. Statistical Analyses

All results were analyzed statistically with SAS software 9. Statistical differences between means were determined by one-way ANOVA with Duncan's multiple range with the level of significance established at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Identification of Actinobacteria and AMF species

The actinobacteria strain was isolated from the rhizosphere of *Astragalus gombo* grown in the Biskra, Algeria. The ISP2 agar medium added with supplemented with streptomycin and amphotericin B was selected for actinobacteria isolation from rhizospheric soil samples. Actinobacteria BI21 isolate was confirmed as *Streptomyces* spp. based on morphological and cultural characteristics, according to the methods described in Bergey's Manual of Systematic Bacteriology (Williams et *al*., 1989) and International Streptomycete Project (ISP) (Shirling and Gottlieb, 1966).

Arbuscular Mycorrhizal fungal spores were isolated from rhizosphere of date palm grown in Biskra using wet sieving and decanting technique. The structures of AMF were demonstrated in the date palm root (Fig. 2). Intercellular hyphae, hyphal coils, vesicle, arbusculate were detected in all date palm root samples observed. The vesicles are also of various shapes: oval, oblong. The presence of different mycorrhizal structures in the rhizospheric soil collected from the studied sites confirmed the mycorrhizal status of date palm, considered as a mycothrophic species (Bouamri et *al*., 2006). The roots of date palm were receptive to arbuscular mycorrhizal fungi (Khaliel and Abu Heilah, 1985). Endophytes were also observed in the roots of the date palm: septate hyphae and sclerotia in cells of the cortical parenchyma (Sghir et *al*., 2015).

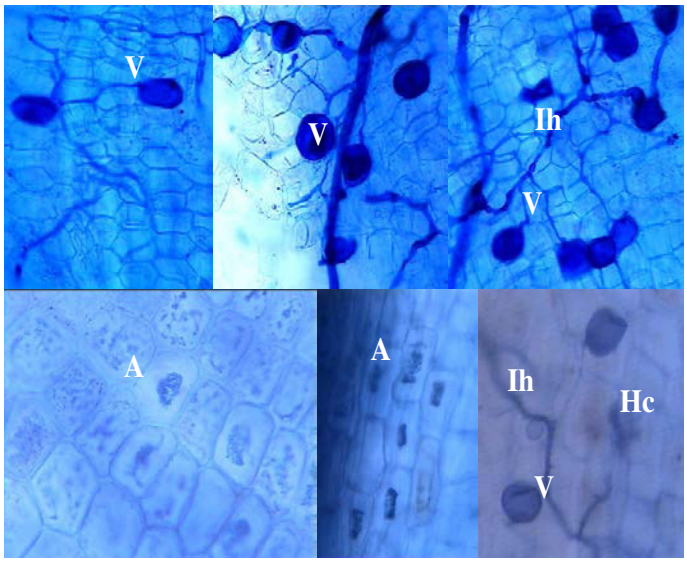

Figure 2: Anatomy of palm roots. Ih: Intercellular hyphae, Hc: Hyphal coils, V: Vesicle and A: Arbusculate.

A total of 26 morphotypes of AMF corresponding to four genera were recorded from the rhizosphere of date palm (Fig. 3). From the 26 morphotypes, a total of 10 species belong to the genus *Glomus* (Fig.4), two to the genus *Scutellospora*, four each to the genera *Gigaspora and Rhizophagus*, and six to the genus *Acaulospora* (Fig. 3). AMF belonging to the genus *Glomus* seem to be dominant in the rhizosphere of date palm in Biskra. This provides strong support for the conclusions of other workers that AMF belonging to *Glomus* tend to be dominant in arid ecosystems (Shi et *al*., 2006; Jaiti et al., 2007). The soil of the desert regions rich with endomycorrhizae provides favorable conditions for the growth and development of date palm trees by facilitating their access to minerals and water, and increased tolerance to abiotic stress conditions (drought, salinity of water or soil) and biotic (attacks of pathogenic microorganisms) (Sghir et *al*., 2014).

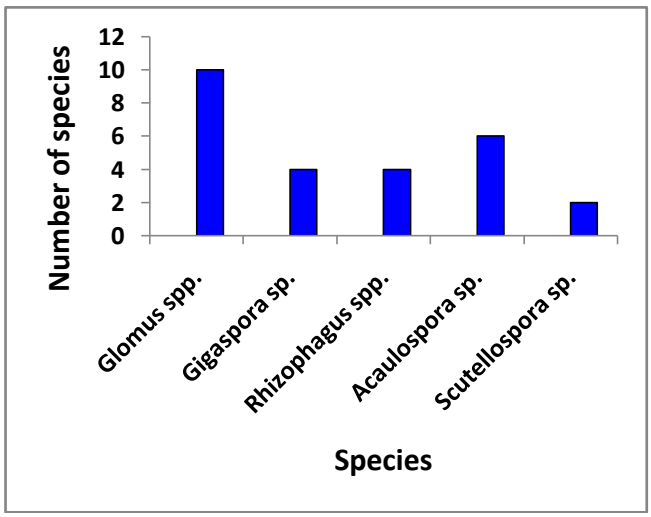

Figure 3: AMF species in 100 g in rhizospheric soil of date palm growing in Biskra.

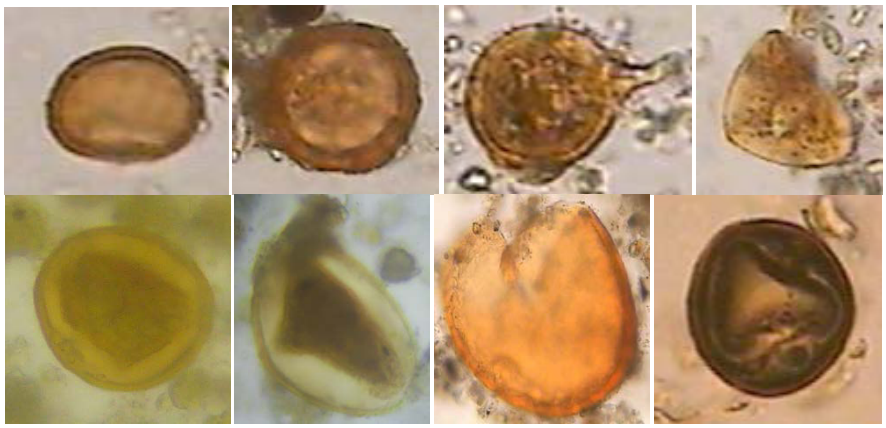

Figure 4: Spores of *Glomus spp* in the rhizosphere of date palm growing in Biskra.

3.2. Antagonisms

The actinobacteria was initially screened to determine their antifungal activity by following cross streak method. *Streptomyces* spp. BI21 strain exhibiting the ability to produce both clear zones of inhibition and metabolites against the tested pathogenic fungus *Fusarium oxysporum* f. sp. *albedinis* ((Foa) was considered antagonistic. *Streptomyces* spp. BI21 showed highest inhibition growth against Foa with large zone of inhibition 35 ± 3 mm. However, *Glomus* spp. strain A01 appeared to have non inhibitory activity compared with Streptomyces spp. BI21 against Foa (Table 1).

Table 1: Plate assay for elucidation of functional traits of antagonistic and plant growth promoting *Streptomyces* spp. BI21 and *Glomus* spp. A01

Isolates	**Activity**				
	Chitinase	Indole acetic acid (IAA)	Protease	Phosphate solubilization	Antimicrobial
BI21	+	+	-	+	+
A01	-	+	+	+	-

- = Negative result
+ = Positive result

3.3. Chitinase and protease production by *Streptomyces* spp. BI21 and *Glomus* spp. A01

Streptomyces spp. BI21 induced complete clearance of chitin from the agar plates, even at locations up to 1,5 cm from bacterial colonies. However, *Glomus* spp. A01, was growth on the chitin agar, but no clearance of chitin. Proteolytic activity was detected for only *Glomus* spp. A01 isolate (Table 1). It has been reported that antifungal mechanism of antagonists has been attributed to the action of hydrolytic enzymes such as chitinase (Quecine et *al*., 2008) and protease (Naing et *al*., 2014).

3.4. Screening of Phosphate Solubilizing *Streptomyces* spp. BI21 and *Glomus* spp. A01

Qualitative estimation of P solubilization by *Streptomyces* spp. BI21 and *Glomus* spp. A01 isolates grown on Pikovskaya medium showed the development of a clear solubilization zone around the colony. In this study, *Streptomyces* spp. BI21 and *Glomus* spp. A01 were able to solubilize phosphate which was confirmed by appearance of largest halos around their colonies (translucent areas) in PVK agar medium (Table 1). Phosphorus is considered as growth-limiting macronutrient. Phosphate solubilizing microorganisms have been employed in agriculture and horticulture and have been considered very important due to their potential of ecological amelioration (Naik et *al*., 2008). The *Streptomyces* spp. BI21 and *Glomus* spp. A01 isolates tested seems to have the ability of solubilizing P sources in soil. Among the several potential mechanisms for phosphate solubilization

those involving the production of chelating compounds, like organic acids or by means of a modification of pH of the medium by the secretion of organic acids or protons are the more often described in the literature (Pikovskaya 1948; Richardson et *al*., 2009; Franco-Correa et *al*., 2010).The microorganism capable to solubilize phosphate offer great potential for agricultural applications as they increase the level of available P in soil for uptake by plants. The potential for phosphate solubilization of the target *Streptomyces* and *Glomus* supports future research for quantitative analysis.

3.5. Production of Plant Growth Promoting Hormone Indole Acetic Acid

The ability of *Streptomyces* spp. BI21 and *Glomus* spp. A01 to produce IAA was detected by the development of pink color in ISP2 and PDA culture medium respectively after the addition of salkowski reagent to the culture. The tow isolates were able to produce high levels of IAA (Table 1).

Interestingly, *Streptomyces* spp. strains BI21 produced highest amount of IAA as compared to *Glomus* spp. A01. Root exudates are important sources of natural tryptophan, which may enhance microbial biosynthesis of IAA and other auxins in the rhizosphere (Khamna et *al*., 2009). The level of IAA produced by such PGPR bio-inoculants will be critical in effecting beneficial root response and hence biomass productivity as well as plant cell division and proliferation (Kochar et *al*., 2011). Strains *Streptomyces* spp. and its IAA production could be recommended as useful bio-inoculants based on the plant growth parameters monitored.

3.6. Biocontrol potential and plant growth promotion assay

The effect of the actinobacteria and AMF was tested on date palm by inoculation assay in growth room conditions, using the representative isolate *Streptomyces* spp. BI21 and *Glomus* spp. A01. The biocontrol potential of these microorganisms was assessed according to their antimicrobial activity and activities of fungal cell wall degrading enzymes including protease and chitinase. The plant growth promoting potential of the strains was assessed according to their activities of solubilizing phosphate and siderophore, as well as their production of IAA. Microbial antagonists are widely used for the biocontrol of fungal plant diseases. Many species of actinobacteria, particularly those belonging to the genus *Streptomyces*, are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi and enhanced the growth of plant.

The germination rate was higher in treated seeds with *Streptomyces* spp. BI21 and *Glomus* spp. A01 in comparison to inoculated with Foa or un-inoculated control, and the lowest value for this indicator was reported in plants inoculated with Foa. This may be attributed to plant growth promoting activities. Although the germinating seeds derive most of the nutrients from reserve food material, plant growth substances like indole acetic acid, produced by *Streptomyces* spp. BI21 and *Glomus* spp. A01 may act as stimulants. A collective effect of many factors, such as production of antifungal substances (Souagui et *al*., 2015). and phytohormone (Spaepen and Vanderleyden, 2011). by strains, might be involved in increasing seed germination and controlling plant pathogenicity (Al-Askar et *al*., 2015; Sabaratnam and Traquair, 2015).

Table 2: Effect of *Strptomyces* spp. BI21 and *Glomus* spp. A01 and *Fusarium oxysporum* f.sp. *albedinis* (Foa) strains on the, root length , root fresh weight, shoot height, shoot fresh weight, germination index and mortality of date palm (*Phoenix dactylifera* L.).

Strains	Root length (cm)	Root fresh weight (g)	Shoot height (cm)	Shoot fresh weight (g)	Germination index (%)	Plant mortality (%)
BI21	35 ± 3.0^{b}	3.8 ± 0.2^{b}	27.7 ± 0.6^{c}	4.1 ± 0.2^{b}	83.7 ± 3.2^{bac}	0.5 ± 0.8^{c}
A01	28 ± 2.7^{dc}	2.7 ± 0.2^{d}	25.0 ± 1.0^{d}	3.5 ± 0.2^{c}	87.7 ± 2.5^{ba}	0.8 ± 0.2^{c}
BI21+ A01	45.3 ± 3.5^{a}	4.6 ± 0.2^{a}	35.0 ± 2.0^{a}	5.1 ± 0.3^{a}	89.0 ± 2.7^{a}	0.4 ± 0.5^{c}
BI21+ Foa	29.7 ± 1.5^{c}	3.4 ± 0.3^{c}	25.0 ± 1.0^{d}	3.6 ± 0.2^{c}	$81.0\ \pm3.6^{bc}$	11.0 ± 2.7^{b}
A01+ Foa	23.3 ± 2.1^{d}	2.1 ± 0.1^{e}	21.0 ± 1.0^{e}	2.8 ± 0.3^{d}	$88.0\pm3.6b^{a}$	7.3 ± 0.6^{cb}

BI21+ A01+ Foa	37.3±2.5^{b}	4.0±0.1^{b}	30.0±1.0^{b}	4.3±0.2^{b}	80.0±5.0^{c}	10.3±1.5^{b}
Foa	12.3±2.5^{e}	0.8±0.2^{g}	8.7±1.5^{g}	1.1±0.1^{f}	20.0±5.0^{e}	97.7±2.5^{a}
Control	23.3±3.2^{d}	1.4±0.1^{f}	16.3±1.5^{f}	1.9±0.1^{e}	89.3±5.1^{d}	0.3±0.6^{c}

The values presented in the table are means ± standard errors. The same letter in the row indicates not significantly different values based on one-way ANOVA and Duncan's test ($P < 0.05$). Control: Plants without *Streptomyces* spp., *Glomus* spp. and Foa strains.

After 6 months, co-inoculation of *Streptomyces* spp. BI21 with *Glomus* spp. A01 in absence or in presence of pathogenic fungus Foa increased root fresh weight and root length, which differed significantly from control or other treatments (Table 2). Other researchers have also found that effectiveness of microbial inoculation depends on specific combinations of associated microorganisms and functional compatibility with the host (Azcón, 1993). Phytohormone production is among the mechanisms described for the rhizobacterial stimulating effect (Probanza et *al*., 1996). The date palm subjected to the dual inoculation treatment with *Streptomyces* spp. BI21 and *Glomus* spp. A01, and inoculation with these isolates alone exhibited a significantly higher shoot height and shoot fresh weight, than the non-inoculated controls or the inoculated with pathogenic fungi Foa. However, the Foa treatment caused a significant reduction in plant growth compared to the control plants or to the other treatments (Table 2). Bending et *al*., (2002), suggested that growth promotion of pine by mycorrhizas fungus and bacteria resulted from production of hormones, including indolacetic acid, which promote the formation of lateral roots. All the date palm seedlings, inoculated with *Streptomyces* spp. BI21, *Glomus* spp. A01 and Foa developed disease symptoms. Six month after BI21+Foa inoculation, 11 ± 2.65 % of the control plants died. With the application of A01 + Foa or BI21+ A01 + Foa, there was a significant reduction in plant mortality. Similar observations were reported by Morin *et al*. (1999). Beneficial rhizobacteria can directly influence the physiology of the plants and in addition to interacting directly to beneficially influence the mycorrhizal relationship and/or plant growth, specific bacteria together with AMF have been studied to create a more indirect synergism that supports plant growth including nutrient acquisition, inhibition of plant pathogenic fungi, and enhancement of root branching (Patil et *al*., 2013).

3.7. AMF colonization

The mycorrhizal colonization of the inoculated date palm is shown in Fig 5. All roots of date palm were successfully colonized by the AMF, and produced typical intraradical structures (arbuscules, vesicles, hyphae). AMF colonization rates were significantly affected by *Glomus* spp. A01, *Streptomyces* spp. BI21 and Foa and their interaction (Fig. 5).

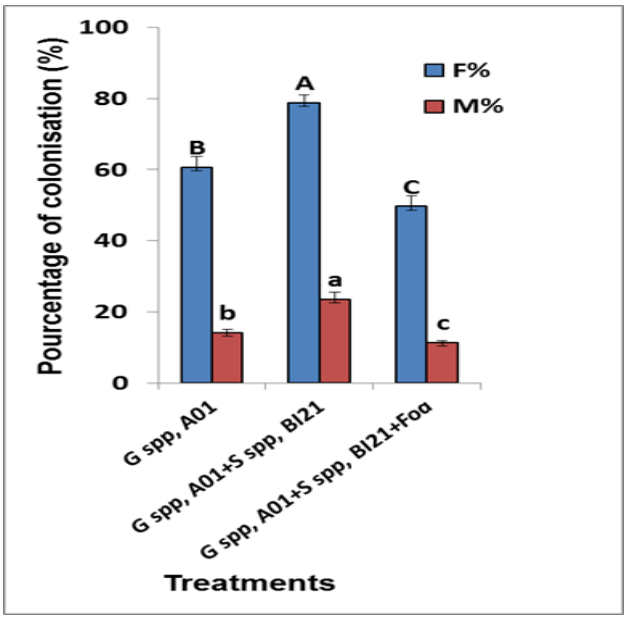

Figure 5: Frequency and intensity of root colonization by *Glomus* spp. A01 in date palm inoculated with A01, BI21 and Foa. The values followed by the same letter are not significantly different $P<0.05$ (Duncan's multiple range test).

The mycorrhizal colonization, estimated by the mycorrhizal frequency (F%) and mycorrhizal intensity (M%), showed differences between the infected and noninfected plant. As against, control plants which were grown in a sterilized soil showed no mycorrhizal colonization. The mixture of two *Glomus* spp. A01, *Streptomyces* spp. BI21, showed a significantly higher level of colonization than *Glomus* spp. A01. It is very interesting to observe that the dual inoculation *Streptomyces* spp. and *Glomus* spp. increased mycorrhizal infection. The higher proportion of mycorrhizal colonization was consistent across mycorrhizal structure such as arbuscules, vesicles and external mycelium. However, the lowest mycorrhizal dependency of date palm plants was recorded at the presence of Fao (Fig. 5). *Streptomyces* spp. BI21 stimulated the development of mycorrhizal roots after 6 months, doubling the mycorrhizal rate of roots by *Glomus* spp. A01 relative to the control (Fig. 5). Increase in mycorrhizal colonization of the roots or efficiency of mycorrhizal symbiosis caused by inoculation with bacteria was observed earlier by El-Shanshoury et *al*., (1989) and Gryndler et *al*., (2002). In natural conditions, bacteria associated with mycorrhizal fungi colonize the surface of extraradical hyphae or, at least in some fungal taxa, live in the cytoplasm as endobacteria (Bonfante and Anca, 2009).

4. CONCLUSION

In the present study, the plant growth promoting traits of *Glomus* spp. A01 and *Streptomyces* spp. BI21have been evaluated. The combinations of *Glomus* spp. A01 and *Streptomyces* spp. BI21 may increase the plant growth and resistance to pathogens. The inoculation of date palm with *Glomus* spp. A01 and *Streptomyces* spp. BI21 may help plants to obtain P and AIA which is beneficial for sustainable nutrient management and reducing the dependency on chemical fertilizers. The results confirmed the potential of applying mycorhizal and PGPR biotechnology in sustainable date palm culture in arid areas.

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