

## Biological control of root rot, wilt diseases complex in offshoot date palm and improvement of growth parameters in New Valley Governorate, Egypt

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### Abstract

Root Rot wilt disease complex were detected in different date palm offshoots in nurseries and new orchards in New Valley Governorate. Pathogenicity tests showed that *Fusarium oxysporum*, *F. solani* and *F. moniliforme* were pathogenic to date palm offshoots (. Saidy) but they differed in their pathogenic capabilities. The effect of *Bacillus subtilis* (BSM1), *B. megaterium* (BMM5), *B. cereus* (BCM8), *Trichoderma viride* (TVM2), *T. harzianum* (THM4) as bioagents against root rot/ wilt disease complex of date palm offshoots under natural infection in nursery cultivated in two location (El-Kharga and El-Dakhla) were studied. The obtained data indicated that all treatments reduced significantly disease severity compared with untreated offshoots (control) in both locations. *Bacillus megaterium* and *T. viride* recorded the highest protection against disease severity, while *B. cereus* and *T. harzianum* gave the lowest ones in this respect. Under laboratory conditions, all bio-agents inhibited growth of the pathogenic fungi with different percentages. *Bacillus megaterium* and *B. subtilis* recorded the highest percentages of growth inhibition, while *T. harzianum* gave the lowest one. On the other hand, treatments significantly improved growth parameters of date palm offshoots viz. offshoot height, number of leaves, leaflet number leaf<sup>-1</sup>, nick leaf thickness in both locations. *Bacillus megaterium* and *T. viride* recorded the highest all growth parameters whether in El-Kharga and El-Dakhla, while *B. cereus* and *T. harzianum* gave the lowest one. further more

Bio-control agents significantly increased chlorophyll a, b and carotenoids in leaf in both locations. *Bacillus megaterium* and *T. viride* recoded the highest contents of chlorophyll a, b and carotenoids. Also, all treatments increased nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg) Chlorophyll a, b and carotenoid contents in leaves compared with control in both locations. *Bacillus megaterium* recorded the highest levels of N, P, Ca contents in offshoot leaves, while *B. cereus* increased K, Na contents and Mg in both locations.

**Key words:** Biological control agents (BCAs), Date palm offshoots, Growth parameters, Root rot wilt diseases.

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### Introduction

The date palm (*Phoenix dactylifera* L.) is one of the major fruit trees in Egypt (El- Assar *et al.*, 2005). Date fruit consumption is an important source of supplying mineral and vitamin in a balanced nutrition regime (Al-Shahib and Marshall, 2003). Date palm trees and offshoots are attacked by several soil borne pathogenic fungi at different regions around the world causing severe losses and deterioration of trees and new offshoots. *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *F. equiseti*, *F. Semitectium* ,and *Rhizoctonia solani* had been reported in different countries and they caused root rot and wilt diseases in young and adult date palm trees (El-Morsi *et al.*, 2009 and Maitlo *et al.*, 2013).

Agricultural practices for management of soil borne pathogens in the field includes cultural practices, crop rotation, fungicide applications, methyl bromide fumigation, soil solarization

and use of resistant or tolerant varieties. At present, no single method provides an adequate control of soil borne diseases (Hausbek and Lamour, 2004). Using of chemicals to control soil borne pathogens causes several negative effects, such as: i) development of pathogen resistance, ii) hazards to humans, iii) damage to beneficial organisms and iv) environmental pollution. Moreover, many chemicals will be banned in near future. However, for sustainable production, pathogens still need to be controlled in order to ensure healthy plant establishment and growth (Gerhardson, 2002). Therefore, developments of various beneficial micro-organisms or biological control agents' methods are urgently needed in order to provide an alternative to chemical control. Among different biological approaches, use of microbial antagonists like yeasts, fungi and bacteria could be promised, effectively, safely and eco-friendly in controlling many of soil borne pathogens (Gravel *et al.*, 2004). Many biological control agents such as *Trichoderma* spp. and *Bacillus* spp. could be effectively used in suppressing diseases caused by *Fusarium* spp. as reported by many workers (Abdel- Monaim, 2010 and Perveen and Bokhari, 2012). Modes of action for beneficial micro-organisms include direct parasitism of plant pathogens, competition for space or nutrients, or production of antibiotics, enzymes or plant hormones (Lugtenberg *et al.*, 2003). This led to promote plant growth during the growing season as reported by Wahyudi *et al.* (2011). However, up to date, a few antagonist microorganisms have been identified as potential, effective biological control agents (BCAs) against soil borne pathogens (Spadaro and Gullino, 2005). Also, these bioagents increased significantly due to root growth and increased of plant growth in date palm and other many crops (Abdel-Monaim, 2010 and Perveen and Bokhari, 2012). On the other hand, PGPR as biofertilization played an important role in plant nutritional requirements; since biofertilizers were reported to enhance crop productivity through improving plant nutrition, enhancement of nutrients availability, nitrogen fixation, and phosphate solubilization, increase leaf contents of chlorophyll, carotenoids and plant hormone production (Lazarovits and Nowak, 1997 and Yaso *et al.*, 2007 and Isfahani and Beshara, 2012).

The main objective of the present study was to evaluate the effectiveness of isolated micro-organisms from soil under New Valley governorate conditions as biological control agents (BCAs) against the incidence of root rot and wilt diseases caused by *F. oxysporum*, *F. solani* and *F.moniliforme* and its impact on vegetative growth of date palm offshoots cv. Saigy under field conditions. In addition, its effect on chlorophylls, carotenoids and mineral contents in offshoot leaves were also investigated

## Materials and methods

### Isolation of the causal fungi

Roots samples from naturally infected date palm offshoots (cv. Saigy) were collected from different locations of New Valley governorate during growing season 2015. Infected roots were washed several times with tap water to remove the attached soil particles. The samples were then cut into small pieces, rinsed several times in sterilized distilled water, disinfected by 0.1% sodium hypochlorite solution for one minute, followed by washing three times with sterilized water samples were dried between folds of sterilized filter paper. The sterilized fragments were aseptically transferred to Petri dishes containing 20 ml of potato dextrose agar (PDA) medium, and incubated at 25 C for 5 days. The isolated fungi were purified using the single spore technique and / or the hyphal tip method. The isolates were identified based on published descriptions (Nelson *et al.*, 1983 and Booth, 1985) of morphological and cultural characteristics mycelium, conidiophores, conidia and colony morphology.

### Pathogenicity tests

The pathogenic capability of the isolated fungi was carried out under greenhouse conditions in El-Kharga Agric. Res. Station, New Valley, Egypt. Date palm seeds (cv. Saigy) were

treated with dry heat at 45°C for 2 hours to activate seed germination and then planted in Plastic pots (30 cm in diameter) pots were filled with autoclaved soil (2 kg pot<sup>-1</sup>) at rate one seed pot<sup>-1</sup>. After 6 months from planting, pots were inoculated with the pathogenic fungal by using homogenized culture technique (Muthomi *et al.*, 2007). Disks taken from 1- wk-old culture tested fungi were inoculated in 75 mL of potato dextrose broth (PDB) in a 250 mL flask and incubated at 25 ± 1°C. The obtained fungal cells were collected on whatman filter paper No. 1, rinsed with sterile distilled water, placed in a waring blender with a small amount of sterile water, and blended for 2 min at high speed. Sterile distilled water was then added to each inoculum suspension to give a final concentration of 10<sup>6</sup> colony forming units (CFU/mL) that was used for soil infestation. Five pots were used as a replicates for each fungal isolate along with check treatment (un- infested soil). The pots were irrigated as needed.

The severity of root rot/wilt complex was determined after 90 days (Abdou *et al.*, 2001) using a rating scale of 0-5 on the basis of root discoloration or leaf yellowing: 0: no root discoloration or leaf yellowing; 1: 1-25% root discoloration or one leaf yellowed; 2: 26-50% root discoloration or more than one leaf yellowed; 3: 51-75% root discoloration plus one leaf wilted; 4: up to 76% root discoloration or more than one leaf wilted; and 5: completely dead plants. For each replicate a disease severity index (DSI) similar to that described previously (Liu *et al.*, 1995) was calculated as follows:

$$DSI = \frac{\sum d}{d \max \times n} \times 100$$

Whereas: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

#### ***In Vitro* Studies**

The used antagonistic organisms *Bacillus subtilis* B. *megaterium* B. *cereus* *Trichoderma viride* and *T. harzianum*: these isolates provided by Plant Pathol. Res. Inst., Agric. Res. Center *Trichoderma* isolates and the tested pathogenic fungi (*F.oxysporum*, *F. solani* and *F. moniliforme*) were cultured on PDA medium for 7 days at 25±1°C. A disc (0.7 cm diameter) of the antagonistic fungal colony was cut and placed opposite to the colony of the pathogenic fungal isolates on PDA medium. On the other hand, *Bacillus* isolates were streaked at opposite ends of PDA plates near edge and incubated at 25±1 °C for 24 hr. Then a mycelial disc (0.7 cm) of the tested fungi was placed in the center of each plate. For control treatment, the agar plug of only pathogen isolates was placed on PDA plates. The inoculated plates incubated at 25±1 °C until colony of control grew to full plate. At this point, colony diameter was measured using ruler. Percentage of growth inhibition of pathogen was calculated using the formula below:

$$\% \text{ Inhibition} = (A-B)/A \times 100$$

Where: A = Colony diameter of pathogen in control, B = Colony diameter in treated plates

#### ***In Vivo* studies**

##### **Preparation of formulated antagonistic fungi and bacteria**

Inoculum of antagonistic bacteria *viz.* *B. subtilis* (BSM1), *B. megaterium* (BSM5) and *B. cereus* (BCM8) were produced in 100 ml of potato dextrose broth (PDB) medium (pH7) in 250 ml conical flasks, on an orbital shaker at 125 rpm and 28±1°C for 3 days. Bacterial cells were harvested by centrifugation (10,000 × g for 20 min) and washed twice with sterile 0.1 M MgSO<sub>4</sub>. Bacterial concentration in the suspension was adjusted to proximately 5 × 10<sup>8</sup> cells per ml by measuring absorbance at 600 nm (A<sub>600</sub>) in a spectrophotometer and using standard curves for each bacterial isolate. While, inoculum of the antagonistic fungi *viz.* *T. viride* (TVM2) and *T. harzianum* (THM4) was prepared by culturing on 50.0 mL potato dextrose broth (PDB) medium (pH5) in 250 mL Erlenmeyer flasks for 10 days at 25±2°C followed by

washing and blending in sterilized water. Colonies forming units (cfu) were adjusted to  $10^6$ cfu/mL using haemocytometer slide.

### **Effect of biological control agents (BCAS) on root rot / wilt and growth parameters under field conditions:**

Field experiments were carried out at New Valley Agric. Res. Station Farm and Directorate of Agriculture in El-Dakhla, New Valley governorate during 2016 season, to evaluate the efficiency of the tested biological control agents (*Bacillus subtilis*, *B. megaterium*, *B. cereus*, *Trichoderma viride* and *T. harzianum*) individually for controlling root rot and wilt diseases of date palm offshoots (cv. Saïdy) as well as its effect on growth parameters. The chosen field test area was naturally infested with the causal organisms of root rot and wilt pathogens. The experimental design was a complete randomized block with four replicates. The experimental unit area was 2 m<sup>2</sup> (1 × 2 m). Each unit included 2 date palm offshoots (3- years-old). Soil of the planting with offshoots were drenched three time at 15 – day intervals with bio- control agents inocula prepared as above at rate 3 L per offshoot. Untreated soil was used for control. The disease severity was assessed for each treatment after 6 months from the least of application treatments. After the end of this experiment the following estimations were recorded: root numbers offshoot<sup>-1</sup>, offshoot height (cm), number of leaves offshoot<sup>-1</sup>, leaflet numbers leaf<sup>-1</sup> and Leaf thickness (cm)

### **Estimation of chlorophyll and carotenoid contents**

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949). Fresh leaf material of 500 mg was grounded with 10 ml of 80 percent acetone at 4 C and centrifuged at 2500 rpm for 10 minutes at 4 C. This procedure was repeated until the residue became colour less. The extract was transferred to a graduated tube and made up to 10 ml with 80 per cent acetone and assayed immediately. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with a spectrophotometer (U-2001- Hitachi) against 80 per cent acetone as blank. Chlorophyll content was calculated using the formula of Arnon (1949) and expressed in milligram per gram fresh weight.

Chlorophyll 'a' (mg/ml) =  $(0.0127) \times (A.663) - (0.00269) \times (A.645)$

Chlorophyll 'b' (mg/ml) =  $(0.0229) \times (A.645) - (0.00468) \times (A.663)$

Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight.

Carotenoid (mg/g) =  $A.480 + (0.114 \times A.663 - 0.638 \times A.645)$

### **Leaf mineral contents**

The leaf samples were washed with tap water, rinsed twice in distilled water and air dried in an oven at 70 C. The dried leaves were ground and digested with H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub> according to Evanhuis and De Waard (1980). Suitable aliquots were taken for the determination of the mineral content. Nitrogen was determined by the Kjeldahl method (A.O.A.C, 1995). Phosphorus was determined by the ascorbic acid method according to Murphy and Riley (1962). Potassium and sodium were determined with a flame photometer. The Ca, Mg contents were measured using atomic absorption spectrophotometer. The concentrations of N, P, K, Ca, Na and Mg were expressed as percentages.

### **Statistical analysis**

All experiments were performed twice. Analyses of variance were carried out using MSTAT-C, 1991 program version 2.10. Least significant difference (LSD) was employed to test for significant difference between treatments at  $P \leq 0.05$  (Gomez and Gomez, 1984).

## Results and discussions

### Isolation, Identification of the causal organism (S) and pathogenicity tests

Five *Fusarium* species were isolated from date palm offshoots showing root rot and wilt symptoms. These species were identified as *Fusarium oxysporum*, *F. Equiseti*, *F. solani*, *F. Semitectium* and *F. moniliforme*. The pathogenicity tests indicated that all tested fungi significantly caused root rot and wilt diseases in date palm offshoots cv. Saigy (Fig. 1). *Fusarium oxysporum* was the most pathogenic fungi as it recorded (89.26%) root rot/wilt severity followed by *F. solani* and *F. moniliforme*, which were caused 82.18% and 73.26% disease severity, respectively. On the contrary, *F. equiseti* and *F. semitectium* were the least pathogenic ones recording the lowest percentages of these criteria. These results are in harmony with those reported by (El-Morsi *et al.*, 2009 and Maitlo *et al.*, 2013).

### Effect of biological control agents (BCAs) on root rot and wilt severity under field conditions

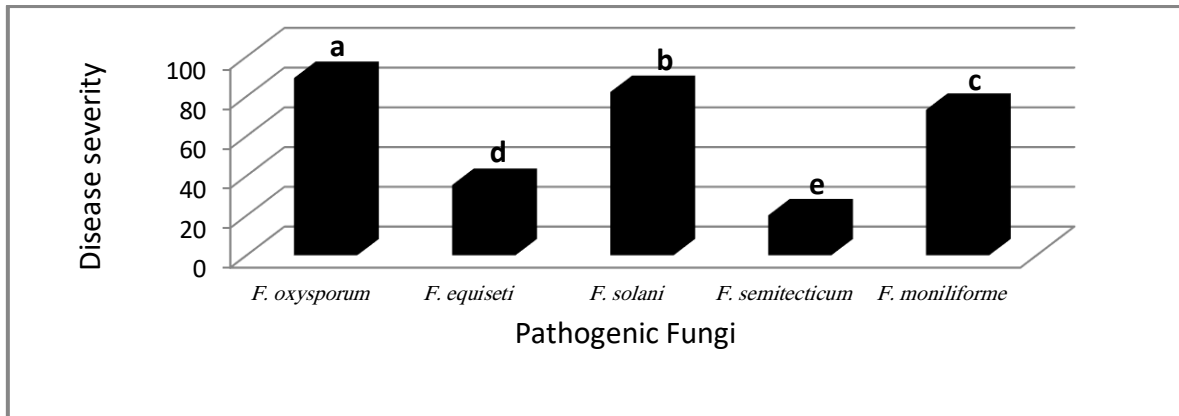
Data presented in Fig. 2 showed that all tested biological control agents (BCAs) significantly decreased root rot and wilt disease complex under naturally infection in cultivated field in El-Kharga and El-Dakhla. Efficiency of the tested BCAs for controlling this disease on date palm offshoots cv. Saigy was varied. However, *B. megaterium* and *T. viride* were the most effective BCAs for decreasing root rot and wilt severity, being 13.47, 18.96 and 18.83, 25.36% compared with 75.38, 86.36% disease severity in control in both locations, respectively. Meanwhile, *B. cereus* and *T. harzianum* were the lowest effect ones they recorded 36.70, 40.80 and 39.36, 43.36 % disease severity, respectively.

Control of root rot and wilt diseases in date palm offshoots depends mainly on fungicides application (El-Morsy *et al.*, 2012). Meanwhile, fungicides always undesirable due to high cost, probability of development of resistant strains and potential hazards to the environment. An option for reducing pollution caused by the use of synthetic agrochemical in date palm offshoots disease management is a bio-control using of antagonist microorganisms belonging to the *Bacillus* spp. and/or *Trichoderma* spp., because they are considered the most efficient for their inhibitory properties (El-Mohamedy and Ahmed, 2009) and , stimulation of plant growth (Wahyudi *et al.*, 2011).

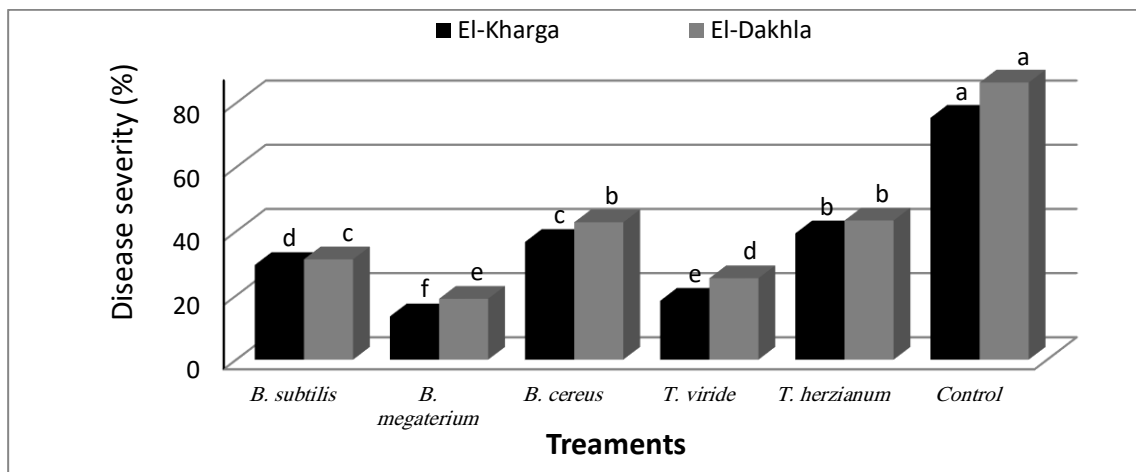
### In vitro screening inhibitory effect of biological control agents (BCAs)

*Bacillus subtilis*, *B. megaterium*, *T. viride* and *T. harzianum*, strains were evaluated for antagonistic effect against *F. oxysporum*, *F. solani* and *F. moniliforme* on Petri dishes containing PDA medium. Fig. 3 showed that the bio-agent strains succeeded in reducing the radial growth of the tested pathogenic fungi. *B. megaterium* and *B. subtilis* were active more than the other tested bioagents. The inhibition percent of radial growth of tested fungi viz., *F. oxysporum* (68.49 and 55.26%), *F. solani* (77.36 and 70.14%), and *F. moniliforme* (52.08%) were reduced by *B. megaterium* and *B. subtilis*, respectively. On contrary, *T. harzianum* gave the lowest ones in this respect. Generally, *Bacillus* strains were more effective than *Trichoderma* strains in inhibition of radial growth of tested fungi and the greatest reduction occurring in *F. solani* followed by *F. oxysporum*, while *F. moniliforme* was less effective.

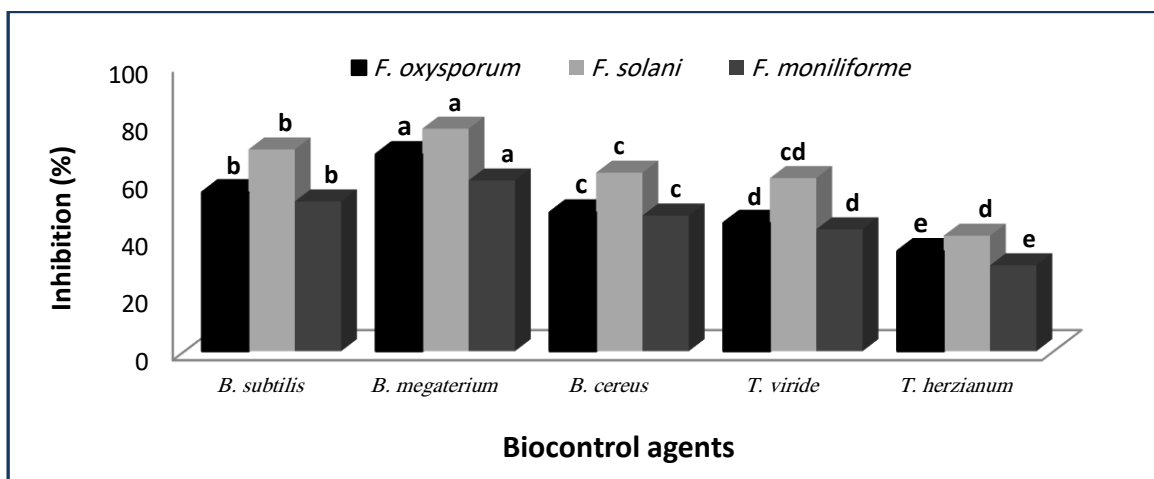




**Fig 1.** Pathogenic ability of five *Fusarium* species on root rot and wilt severity of date palm offshoots (cv. Saigy) under greenhouse conditions. Different letters indicate significant differences among pathogenic fungi within the same column according to least significant difference test ( $p \leq 0.05$ ).



**Fig 2.** Effect of biological control agents (BCAs) on disease severity of root rot/ wilt disease of date palm offshoots cv. Saigy under field conditions cultivated in El-Kharga and El-Dakhla. Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).



**Fig. 3.** Inhibitory effect of some biological control agents (BCAs) isolates on mycelial growth of date palm offshoots pathogenic fungi. Different letters indicate significant differences among biological control agents (BCAs) within the same colour column according to least significant difference test ( $p \leq 0.05$ ).

### Effect of bio-control agents on growth parameters

Data present in Table 1 and 2 showed that all tested biological control agents (BCAs) significantly increased all growth parameters *viz.* root numbers offshoot<sup>-1</sup>, offshoot height (cm), number of leaves offshoot<sup>-1</sup>, leaflet number leaf<sup>-1</sup> and nick leaf thickness of date palm offshoots (var. Saidy) compared with control whether in El-kharga or El-Dakhla. *B. megaterium* and *T. viride* recorded the highest values of all growth parameters they increased their root numbers offshoot<sup>-1</sup> from 4.33, 3.33 in control to 23.67, 20.33 and 21.00, 18.67, offshoot height from 110.59, 106.00 in control to 235.33, 218.69 and 222.32, 230.14 cm and number of leaves offshoot<sup>-1</sup> from 1.17, 1.36 to 5.83, 6.05 and 5.17, 6.36 in both locations, respectively. Also, both treatments increased leaflet number leaf<sup>-1</sup> from 26.25, 28.36 in control to 121, 125.36 and 100.17, 105.36, leaf thickness from 0.65, 0.72 in control to 1.52, 1.63 and 1.42, 1.36 cm in both locations, respectively. On the other hand, *B. cereus* and *T. harzianum* gave the lowest values of in all growth parameters. Our study showed that date palm offshoots treated with biological control agents (BCAs) appeared higher reduction in root rot and wilt severity compared to untreated control plants. (Mogle and Mane, 2010, Nihorimbere *et al.*, 2010) these results are in line with those reported by Biological control agents (BCAs) such as *Bacillus* and *Trichoderma* species help in solubilization of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates and improve soil structure and organic matter content (Al-Taweil *et al.*, 2009). Antagonistic microorganisms retain more soil organic N and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients (Baset *et al.*, 2010). *Bacillus* and *Trichoderma* also have been known to produce compounds which promote plant growth directly or indirectly *viz.*, hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), solubilize phosphorous, *Trichoderma* spp. could elucidate to produce trichotoxins promoting plant and antifungal activity (Shobha and Kumudin, 2012 and Al-Rajhi, 2013).

The mechanism of antagonistic microorganisms action on pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Zaghloul *et al.*, 2007), produce siderophores and hydrogen cyanide (Soleimani *et al.*, 2005), production of secondary metabolites such as Phenazine -1-Carboxylic acid (PCA), 2,4-Pyrrolnitrin, Oomycin (Knudsen, 1995) and production of antibiotics (Ehteshamul-Haque and Ghaffar, 1993).

**Table (1)** Effect of biological control agents (BCAs) on growth parameters of date palm offshoots Var. Saidy under field conditions in El-Kharga.

Treatments	Root numbers offshoot <sup>-1</sup>	offshoot height (cm)	Number of leaves offshoot <sup>-1</sup>	Leaflet number leaf <sup>-1</sup>	Nick leaf thickness
<i>Bacillus subtilis</i> (BSM1)	16.33 c	198.19 c	4.33 c	88 c	1.22 c
<i>B. megaterium</i> (BMM5)	23.67 a	235.33 a	5.83 a	121 a	1.52 a
<i>B. cereus</i> (BCM8)	12.00 d	172.58 d	3.67 cd	68.5 d	1.09 d
<i>Trichoderma viride</i> (TVM2)	21.00 b	222.32 b	5.17 b	100.17 b	1.42 b
<i>T. harzianum</i> (THM4)	10.67 e	165.36 e	3.00 d	62.67 e	1.18 cd
Control	4.33 f	110.59 f	1.17 e	26.25 f	0.65 e

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $P \leq 0.05$ ).

**Table (2)** Effect of biological control agents (BCAs) on growth parameters of date palm offshoots Var. Saidu under field conditions in El-Dakhla.

Treatments	Root numbers offshoot <sup>-1</sup>	offshoot height (cm)	Number of leaves offshoot <sup>-1</sup>	Leaflet number leaf <sup>-1</sup>	Nickleaf thickness
<i>Bacillus subtilis</i> (BSM1)	17.00 c	205.47 c	5.04 b	82.47 c	1.13 c
<i>B. megaterium</i> (BMM5)	20.33 a	218.69 b	6.05 a	125.36 a	1.63 a
<i>B. cereus</i> (BCM8)	10.67 d	163.34 e	4.25 c	70.25 d	1.02 d
<i>Trichoderma viride</i> (TVM2)	18.67 b	230.14 a	6.36 a	105.36 b	1.36 b
<i>T. harzianum</i> (THM4)	9.67 d	149.67 d	2.55 d	66.35 e	1.09 cd
Control	3.33 e	106 f	1.36 e	28.36 f	0.72 e

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $P \leq 0.05$ ).

### Photosynthetic pigment contents

Data in Tables (3 and 4) indicated that all bio-agents significantly increased chlorophyll a b and carotenoid contents leaves compared with control in both experimental locations (El-Kharga and El-Dakhla). *Trichoderma viride* and *B. megaterium* recorded the highest chlorophyll a b and carotenoids contents in leaves in both locations, while *B. cereus* gave the lowest values of chlorophyll a b and carotenoids contents in leaves in both locations. These results were same with those reported by (Isfahani and Beshara, 2012). Amro *et al* (2014) on "Hayany" date palm indicated that using EM at 90 ml / palm/ year enriched with potassium sulphate at 1.5kg/palm/year treatment as soil application enhanced leaf chlorophyll content, retained fruit percentage, yield, fruit quality and leaf minerals content.

**Table (3)** Effect of biological control agents (BCAs) on chlorophylls a, b and carotenoids (mg/gf.w.) of date palm offshoots cv. Saidu under field conditions in El-Kharga.

Treatments	Chlorophyll-a (mg/g fresh weight)	Chlorophyll-b (mg/g fresh weight)	Carotenoids (mg/g fresh weight)
<i>Bacillus subtilis</i> (BSM1)	0.89	0.77	0.68
<i>B. megaterium</i> (BMM5)	1.09	0.92	0.77
<i>B. cereus</i> (BCM8)	0.79	0.65	0.58
<i>Trichoderma viride</i> (TVM2)	1.12	0.99	0.89
<i>T. harzianum</i> (THM4)	0.85	0.75	0.65
Control	0.62	0.49	0.36

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $P \leq 0.05$ ).

**Table (4)** Effect of biological control agents (BCAs) on chlorophylls a, b and carotenoids (mg/g f.w.) of date palm offshoots cv. Saidu under field conditions in El-Dakhla.

Treatments	Chlorophyll-a (mg/g fresh weight)	Chlorophyll-b (mg/g fresh weight)	Carotenoids (mg/g fresh weight)
<i>Bacillus subtilis</i> (BSM1)	0.96	0.85	0.75
<i>B. megaterium</i> (BMM5)	1.12	0.96	0.82
<i>B. cereus</i> (BCM8)	0.85	0.72	0.63
<i>Trichoderma viride</i> (TVM2)	1.15	0.95	0.92
<i>T. harzianum</i> (THM4)	0.92	0.78	0.69
Control	0.68	0.52	0.42

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $P \leq 0.05$ ).

### Mineral contents

Data present in Tables 5 and 6 showed that all treatments significantly increased mineral contents in date palm offshoots *viz.* N, P, K, Ca, Na and Mg compared with control in both locations (El-Kharga and El-Dakhla). *B. megaterium* recorded the highest levels of N, P and



Ca in date palm offshoots, while *B. cereus* recorded the highest levels of K, Na and Mg contents in date palm offshoots in both locations.

**Table (5)** Effect of biological control agents (BCAs) on nitrogen (%), phosphorus (%), potassium (%), sodium (%) and calcium (%) of date palm offshoots cv. Saïdy under field conditions in El-Kharga.

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sodium (%)	Calcium (%)	Magnesium (%)
<i>Bacillus subtilis</i> (BSM1)	1.16	1.36	0.42	0.16	0.019	0.61
<i>B. megaterium</i> (BMM5)	1.42	1.97	0.49	0.22	0.029	0.65
<i>B. cereus</i> (BCM8)	1.09	1.25	0.58	0.28	0.015	0.75
<i>Trichoderma viride</i> (TVM2)	1.36	1.85	0.53	0.23	0.025	0.68
<i>T. harzianum</i> (THM4)	1.16	1.12	0.42	0.13	0.011	0.39
Control	0.76	0.86	0.31	0.09	0.009	0.31

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $P \leq 0.05$ ).

**Table (6)** Effect of biological control agents (BCAs) on nitrogen (%), phosphorus (%), potassium (%), sodium (%) and calcium (%) of date palm offshoots cv. Saïdy under field conditions in El-Dakhla.

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sodium (%)	Calcium (%)	Magnesium (%)
<i>Bacillus subtilis</i> (BSM1)	1.25	1.42	0.39	0.24	0.020	0.69
<i>B. megaterium</i> (BMM5)	1.65	1.95	0.48	0.23	0.035	0.70
<i>B. cereus</i> (BCM8)	1.02	1.21	0.58	0.30	0.019	0.84
<i>Trichoderma viride</i> (TVM2)	1.45	1.77	0.52	0.25	0.028	0.75
<i>T. harzianum</i> (THM4)	1.21	1.24	0.44	0.14	0.012	0.42
Control	0.81	0.90	0.34	0.11	0.010	0.32

Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $P \leq 0.05$ ).

Biofertilization methods played an important role in plant nutritional requirements; since biofertilizers were reported to enhance crop productivity through improving plant nutrition, enhancement of nutrients availability, nitrogen fixation, phosphate solubilization, and plant hormone production (Lazarovits and Nowak, 1997). Abd –Elmoniem and Radwan (2003) reported that, Micro-organisms in bio-fertilizers maximize the availability of nutrients in the soil and improve their uptake and utilization.

### Conclusion

Results of the present study could suggest that soil drench with biological control agents (BCAs) can be used as a safe control measure of the disease on date palm offshoot and as a stimulant of vegetative growth parameters.

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## المكافحة الحيوية لمرض أعفان الجذور والذبول وتحسين صفات النمو لفسائل نخيل البلح في محافظة الوادي

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### الملخص العربي

وجد مرض أعفان الجذور والذبول والذي يصيب الفسائل في العديد من مشاتل وبساتين نخيل البلح المزرعة حديثاً في محافظة الوادي الجديد، وتبين من اختبارات القدرة المرضية أن فطريات الفيوزاريوم اوكسيسبورم والفيوزاريوم سولاني والفيوزاريوم منيليفورم هي الفطريات المسببة لهذا المرض وإن اختلفت في قدرتها المرضية.

تم دراسة تأثير المعاملة ببكتريا الباسيلس ساتلس والباسيلس ميجاتيرم والباسيلس سيرس وفطريات التريكوودرما فيرداي والتريكوودرما هيرزيانم كعوامل للمكافحة الحيوية على شدة الإصابة بهذا المرض على فسائل نخيل البلح (صنف صعيدي) تحت ظروف العدوى الطبيعية في المشتل في كلاً من واحة الخارجة والداخلة، وأيضاً على النمو الميسليومي للفطريات الممرضة المختبرة تحت ظروف المعمل.

وأظهرت النتائج المتحصل عليها تحت ظروف المعمل إن كل العوامل الحيوية لها القدرة على تثبيط نمو الفطريات الممرضة بدرجات متفاوتة وكانت بكتريا باسيلس ميجاتيرم والباسيلس ساتلس أكثر العوامل الحيوية قدرة على تثبيط نمو الفطريات الممرضة في حين إن فطر التريكوودرما هيرزيانم أعطى أقل قدرة تثبيطية لتلك الفطريات.

وأظهرت النتائج المتحصل عليها تحت ظروف الحقل إن كل المعاملات السابقة أدت إلى خفض شدة الإصابة بالمرض مقارنة بالفسائل الغير معاملة (كنترول). وأن المعاملة بفطر التريكوودرما فيرداي وباسيلس ميجاتيرم أفضل المعاملات في خفض شدة الإصابة بالمرض في حين أن المعاملة بفطر التريكوودرما هيرزيانم وبكتريا الباسيلس سيرس كانت أقلها تأثير في هذا الصدد.

من ناحية أخرى أدت المعاملات السابقة إلى تحسين صفات النمو والتمثلة في طول الفسائل وعدد الأوراق لكل فسيلة وعدد الوريقات في كل ورقة وسمك الورقة وكانت المعاملة بفطر التريكوودرما فيرداي وباسيلس ميجاتيرم أفضل المعاملات في تحسين صفات النمو في حين كانت المعاملة بفطر التريكوودرما هيرزيانم والباسيلس سيرس أقلها تأثير على صفات النمو.

من ناحية أخرى تبين ان كل عوامل المكافحة الحيوية ادت الى زيادة صبغات التمثيل الضوئي (كلورفيل أ ب والكاروتينات) مقارنة بالفسائل الغير معاملة اعطت بكتريا باسيلس ميجاتيرم والتريكوودرما فيرداي اعطت اعلى محتوى من الكلورفيل أ ب والكاروتينات. ايضاً ادت المعاملة بتلك العوامل الحيوية الى زيادة محتوى الاوراق من العناصر الغذائية منها عناصر النتروجين والفوسفور والبوتاسيوم والصوديوم والكالسيوم والمغنسيوم وكانت معاملة بكتريا الباسيلس ميجاتيرم اكثر تلك المعاملات فاعلية في زيادة محتوى الاوراق من عنصر النتروجين والفوسفور والكالسيوم في حين ان بكتريا الباسيلس سيرس اعطت اعلى مستوى من محتوى الاوراق من عناصر البوتاسيوم والصوديوم والمغنسيوم في موقعي الدراسة.

الكلمات الدالة: عوامل المكافحة البيولوجية ، فسائل نخيل البلح، قياسات النمو، أمراض الذبول وتعفن الجذور