



# Management of root rot and wilt diseases of date palm offshoots using certain biological control agents and its effect on growth parameters in the New Valley Governorate, Egypt

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## Abstract

Root Rot and wilt diseases were observed on different date palm offshoots in nurseries and new orchards in New Valley Governorate, Egypt. Pathogenicity tests showed that *Fusarium oxysporum*, *F. solani* and *F. moniliforme* were the causal agents of root rot and wilt diseases on date palm offshoots (cv. Saidy). In addition, the isolates of the three *Fusarium* spp. 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 (Al-Kharga and El-Dakhla) and also, on growth of the tested pathogenic fungi under laboratory were evaluated. Under laboratory conditions, all the tested bioagents inhibited the growth of the pathogenic fungi with different percentages. *Bacillus megaterium* and *B. subtilis* showed the highest percentage of fungal growth inhibition, while *T. harzianum* gave the lowest ones. At the field level, the application of bioagents significantly reduced the disease severity compared with untreated offshoots (control) in both locations. *Bacillus megaterium* and *T. viride* showed the highest protection against fungal causal agents, while *B. cereus* and *T. harzianum* gave the lowest ones in this respect. Moreover, the bioagents significantly improved the growth parameters of offshoots viz. plant height, number of leaves plant<sup>-1</sup>, leaflet number leaf<sup>-1</sup>, and leaf thickness. *Bacillus megaterium* and *T. viride* recorded the highest values of all growth parameters in El-Kharga and El-Dakhla locations, while *B. cereus* and *T. harzianum* gave the lowest ones.

**Key words:** Biological control agents, Date palm offshoots, Growth parameters, Root rot and 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 elements in a balanced nutrition regime (Al-Shahib & Marshall, 2003). Date palm trees and offshoots are attacked by several soil borne pathogenic fungi causing severe losses and deterioration of trees worldwide (El-Morsi et al., 2009). *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *F. equiseti*, *F. semitectium*, *Rhizoctonia solani* have been reported in different countries to cause root rot and wilt diseases in young and adult date palm trees (Maitlo et al., 2013; El-Morsi et al., 2009). Agricultural practices of soil borne pathogens management in the field include crop rotation, fungicide applications, methyl bromide fumigation, soil solarization and the use of resistant or tolerant varieties (ref.). At present, no single method provides an adequate control of soil borne diseases (Hausbek & Lamour, 2004). Using chemicals to control soil borne pathogens causes several negative effects, such as: i) development of pathogen resistance, ii) harmful effects on humans, iii) bad impact on beneficial organisms and iv) environmental pollution. Moreover, many chemicals will be banned in the near future (ref). However, for sustainable agriculture, pathogens still need to be controlled in order to ensure healthy plant growth and productivity (Gerhardson, 2002). Therefore, finding of various beneficial micro-organisms are urgently needed in order to provide an alternative strategy to chemical control.

Among different biological approaches, the use of the microbial antagonists like yeasts, fungi and bacteria offers an effective, safely and eco-friendly strategy to control many of soil borne pathogens (Gravel et al., 2004). Strains of *Trichoderma* spp. and *Bacillus* spp. have been proven to be effective in suppressing plant diseases caused by *Fusarium* spp. (Perveen & Bokhari, 2012; Abdel-Monaim, 2010). Modes of action of beneficial micro-organisms in suppressing plant diseases include direct parasitism of pathogens, competition for space or nutrients, or the production of antibiotics, enzymes and plant hormones (Lugtenberg et al., 2003). Wahyudi et al. (2011) reported that the application of bioagents can promote plant growth during the growing season. However, up to date, only a few antagonistic microorganisms have been identified as potential and effective biological agents (BCAs) against soil borne pathogens (Spadaro & Gullino, 2005). Also, these bioagents increased significantly due to root growth and increased plant growth in date palm and other many crops (Perveen & Bokhari, 2012; Abdel-Monaim, 2010). The main objective of the present study was to evaluate the effectiveness of isolated micro-organisms from the New Valley Governorate soils (BCAs) against the root rot and wilt diseases caused by *Fusarium* spp. in date palm offshoots cv. saidy under field conditions.

## Materials and methods

**Isolation of the causal fungi:** Roots samples from naturally infected date palm offshoots (cv. saidy) were collected

from different locations of New Valley Governorate during growing season 2012. 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 in three changes of sterilized water and 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 either the single spore technique or the hyphal tip isolation method. The fungal isolates were identified based on published descriptions of morphological and cultural characteristics mycelium, conidiophores, conidia and colony morphology of *Fusarium* according to Nelson *et al.* (1983) and Booth (1985).

**Pathogenicity tests:** The pathogenic capabilities of the isolated fungi were carried out under green house conditions in El-Kharga Agric. Res. Station, New Valley, Egypt. Date palm seeds (cv. saidy) were treated with dry heat at 45°C for 2 hours to activate seed germination then planted in 30 cm in diameter plastic pots (one seed per pot) filled with autoclaved soil (2 kg pot<sup>-1</sup>). After 6 months post planting, seedlings were inoculated with the pathogenic fungi using homogenized culture technique (Muthomi *et al.*, 2007). Disks were taken from one-week-old fungal culture of the tested fungi and transferred to 75 mL of potato dextrose broth in a 250 mL flask and incubated at 25 ± 1°C for time. Then,

the fungal cultures were collected on Whatman No. 1 filter paper, 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 fungal suspension to give a final concentration of 10<sup>6</sup> colony forming units (CFU)/mL to be ready for soil infestation. Five pots (replicates) were used for each fungal isolate along with negative control treatment, un-infested soil. The pots were irrigated as needed. The severity of wilt was determined 90 days post inoculation 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 (Abdou *et al.*, 2003). For each replicate, a disease severity index (DSI) was calculated according to Liu *et al.* (1995) as follows:

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

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

**In vitro Studies:** The used antagonistic organisms were *B. subtilis* (BCM1), *B. megaterium* (BMM5), *B. cereus* (BCM8), *T. viride* (TVM2) and *T. harzianum* (THM4) provided by Dr.

Montaser F. Abdel-Monaim, Plant Pathol. Res. Inst., Agric. Res. Center (Abdel-Monaim, 2010). *Trichoderma* isolates and the tested pathogenic fungi (*F. oxysporum*, *F. solani* and *F. moniliforme*) were cultured on PDA medium for 7 days at  $25\pm 1$  °C. A 0.7 cm in diameter disk 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  $25\pm 1$ °C for 24 hr. Then a 0.7 cm in diameter mycelial disc 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 without biocontrol agents. The inoculated plates were incubated at  $25\pm 1$  °C until colony of control grew to full plate. At this point, colony diameter was measured using a ruler. Percentage of growth inhibition of pathogen was calculated using the following formula:

$$\% \text{ of growth inhibition} = (A-B)/A \times 100$$

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

### *In vivo* studies

**Preparation of formulated antagonistic fungi and bacteria:** Inocula of antagonistic bacteria (*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 for 3 days and 28°C. Bacterial cells were harvested by

centrifugation ( $10,000 \times g$  for 20 min) and washed twice with sterile 0.1 M MgSO<sub>4</sub>. Bacterial suspension was adjusted to proximately  $5 \times 10^8$  cells per ml by measuring absorbance at 600 nm (A<sub>600</sub>) in a spectrophotometer and using standard curves for each bacterial isolate. For the inocula of the antagonistic fungi (*T. viride* (TVM2) and *T. harzianum* (THM4)) were prepared by culturing fungi on 50.0 mL PDB medium (pH5) in 250 mL Erlenmeyer flasks for 10 days at  $25\pm 2$ °C. Fungal cells were collected by centrifugation and the pellets were washed twice with sterilized water, then blended 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 fungi and plant 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 2014 season, to evaluate the efficiency of the tested biological control agents (*Bacillus subtilis*, *B. megaterium*, *B. cereus*, *Trichoderma viride* and *T. harzianum*) on controlling root rot and wilt diseases of date palm offshoots (cv. Saïdy) as well as their effect on plant 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 3-year-old date palm offshoots. The soil with planted-offshoots was drenched three times at 15-day intervals with inocula of

biocontrol agents at rate of 3 L per offshoot. Untreated soil was drenched three times at 15-day intervals with water. The disease severity was assessed for each treatment after 6 months from the last of application treatments. After the end of this experiment the following estimation were taken:

- Root numbers plant<sup>-1</sup>
- Plant height (cm)
- Number of leaves plant<sup>-1</sup>
- Leaflet numbers leaf<sup>-1</sup>
- Leaf thickness (cm)

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

## Results

**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. semitectum* and *F. moniliforme*. The pathogenicity tests indicate that all the tested fungi significantly caused root rot and wilt diseases in date palm offshoots var. Saïdy (Fig. 1). *Fusarium oxysporum* was the most pathogenic fungi as they recorded percentage root rot/wilt severity (89.26%) followed by *F. solani* and *F. moniliforme* where caused 82.18% and 73.26% disease severity, respectively. On the contrary, *F. equiseti* and *F. semitectum* were the least pathogenic ones recording the lowest percentages of these criteria.

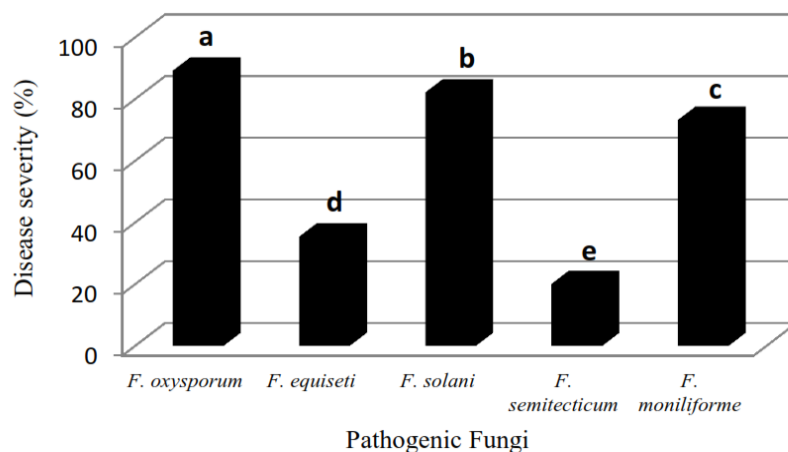


Fig 1. Pathogenic ability of five *Fusarium* species on root rot and wilt severity of date palm offshoots (var. Saïdy) under greenhouse conditions. Different letters indicate significant differences among pathogenic fungi within the same column according to least significant difference test ( $p \leq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

**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. 2 show that the bio-agent strains succeeded in reducing the radial growth of the tested pathogenic fungi. *B. megaterium* and *B. subtilis* was active more than the other tested bioagents for reducing the radial growth of the tested pathogenic fungi. The percent inhibition 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 more than Trichoderma strains inhibition of redial growth of all the tested fungi and the greatest reduction occurring in *F. solani* followed by *F. oxysporum*, while *F. moniliforme* less affected ones.

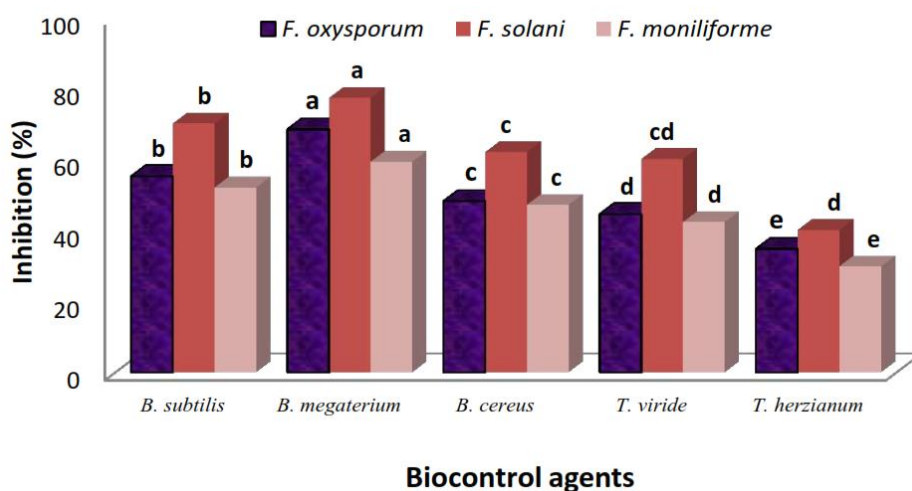


Fig. 2. Inhibitory effect of some biological control agents (BCAs) isolates on mycelial growth of date palm offshoots pathogenic fungi. Different letters indicate significant differences among biological control agents (BCAs) within the same colour column according to least significant difference test ( $p \leq 0.05$ ).

**Effect of biological control agents (BCAs) on root rot and wilt severity:** Data are presented in Fig. 3 showed that all tested biological control agents (BCAs) significantly decreased root rot and wilt disease complex caused by soil borne pathogens under natural infection in field cultivated in El-Kharga and El-Dakhla. The efficiency of the tested BCAs in controlling this disease on date palm offshoots var. saidy was varied.

However, *Bacillus megaterium* and *T. viride* was 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* gave the lowest effect ones while recorded 36.70, 40.80 and 39.36, 43.36 % disease severity, respectively.

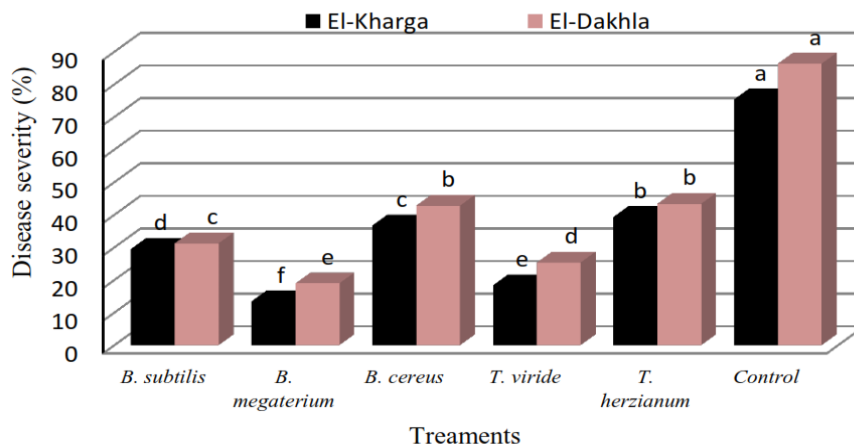


Fig 3. Effect of biological control agents (BCAs) on disease severity of root rot / wilt disease of date palm offshoots var. Saily under field conditions cultivated in El-Kharga and El-Khla. Different letters indicate significant differences among treatments within the same column according to least significant difference test ( $p \leq 0.05$ ).

**Effect of bio-control control agents on growth parameters:** Data are presented in Table 1 and 2 showed that all tested biological control agents (BCAs) significantly increased all growth parameters viz. root numbers plant<sup>-1</sup>, plant height, number of leaves plant<sup>-1</sup>, leaflet number leaf<sup>-1</sup> and 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 increased of all growth parameters, where increased of root numbers plant<sup>-1</sup> from

4.33, 3.33 in control to 23.67, 20.33 and 21.00, 18.67, plant height from 110.59, 106.00 in control to 235.33, 218.69 and 222.32, 230.14 cm and number of leaves plant<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 in . On the other hand, *B. cereus* and *T. harzianum* gave the lowest ones in all growth parameters.

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 plant <sup>-1</sup> | Plant height | Number of leaves plant <sup>-1</sup> | Leaflet number leaf <sup>-1</sup> | Leaf thickness |
|--------------------------------|----------------------------------|--------------|--------------------------------------|-----------------------------------|----------------|
| <i>Bacillus subtilis</i> (BSM) | 16.33 c                          | 198.19 c     | 4.33 c                               | 88 c                              | 1.22 c         |
| <i>B. megaterium</i> (BMM)     | 23.67 a                          | 235.33 a     | 5.83 a                               | 121 a                             | 1.52 a         |
| <i>B. cereus</i> (BCM)         | 12.00 d                          | 172.58 d     | 3.67 cd                              | 68.5 d                            | 1.09 d         |
| <i>Trichoderma viride</i> TVM) | 21.00 b                          | 222.32 b     | 5.17 b                               | 100.17 b                          | 1.42 b         |
| <i>T. harzianum</i> (THM)      | 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. saidy under field conditions in El-Dakhla.

| Treatments                     | Root numbers<br>plant <sup>-1</sup> | Plant<br>height | Number of<br>leaves plant <sup>-1</sup> | Leaflet<br>number leaf <sup>-1</sup> | Leaf<br>thickness |
|--------------------------------|-------------------------------------|-----------------|---|--------------------------------------|-------------------|
| <i>Bacillus subtilis</i> (BSM) | 17.00 c                             | 205.47 c        | 5.04 b                                  | 82.47 c                              | 1.13 c            |
| <i>B. megaterium</i> (BMM)     | 20.33 a                             | 218.69 b        | 6.05 a                                  | 125.36 a                             | 1.63 a            |
| <i>B. cereus</i> (BCM)         | 10.67 d                             | 163.34 e        | 4.25 c                                  | 70.25 d                              | 1.02 d            |
| <i>Trichoderma viride</i> TVM) | 18.67 b                             | 230.14 a        | 6.36 a                                  | 105.36 b                             | 1.36 b            |
| <i>T. harzianum</i> (THM)      | 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 ( $P \leq 0.05$ ).

## Discussion

Date palm is one of the most important tropical fruit crops in Egypt and over the world. Several soil borne fungi attack date palm plants during its various growth stages from seedling till maturity causing root rot and wilt diseases. In the present investigation, an extensive survey was conducted throughout different locations in the New Valley Governorate to determine the main causal pathogens of these diseases. The obtained fungal isolates belonging to five *Fusarium* species were isolated from diseased date palm offshoots viz. *Fusarium oxysporum*, *F. equiseti*, *F. solani*, *F. semitectum* and *F. moniliforme*. Pathogenicity tests proved that all the obtained *Fusarium* species were pathogenic and virulent for date palm offshoots (cv. saidy). The most aggressive fungi were *F. oxysporum*, *F. solani* and *F. moniliforme*. These results are in harmony with those reported by other researchers (Maitlo et al., 2013; El-Morsi et al., 2009). Control of root rot and wilt diseases in date palm offshoots depends mainly on fungicide application (El-Morsy et al., 2012). Meanwhile, fungicides are 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 agrochemicals in date palm offshoots disease management is bio-control by 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 & Ahmed, 2009), stimulation of plant growth (Wahyudi et al., 2011). In this study, effective root colonization of different *Bacillus* species viz. *B. subtilis* (BSM1), *B. megaterium* (BMM5), *B. cereus* (BCM8) and two *Trichoderma* species viz. *T. viride* (TVM2) and *T. harzianum* (THM4) individually is important to achieve improved plant growth of date palm offshoots and/or induced resistance to root rot and wilt diseases. The obtained data indicate that all biological control agents (BCAs) decreased severity of root rot and wilt diseases in date palm offshoots in field cultivated in El-Kharga and El-Khala, also increased all growth parameters viz. plant height, number of leaves plant<sup>-1</sup>, leaflet number leaf<sup>-1</sup> and leaf thickness compared with control. *Bacillus megaterium* and *T. viride* were the better biological control agents (BCAs) for controlling root rot/wilt diseases and



improved growth of date palm offshoots than the other tested biological control agents (BCAs). On the other hand, all the tested biological control agents (BCAs) reduced mycelial growth of the pathogenic fungi *in vitro*. *Bacillus megaterium* and *B. subtilis* recorded the highest reduction in this respect. Our study showed that date palm offshoots treated with biological control agents (BCAs) caused a higher reduction in root rot and wilt severity compared to the untreated control plants (Mogle & Mane, 2010; Nihorimbere et al., 2010) and promote the growth of a wide range of plants (Al-Rajhi, 2013; Wahyudi et al., 2011). 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 the release of the nutrients (base et al., 2010). *Bacillus* and *Trichoderma* have also 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 & Kumudin, 2012). 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 & Ghaffar, 1993).

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