Novel Research in Microbiology Journal (2018), 2(2): 37-47
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/NRMJ.2018.6619

Management of date palm root rot diseases by using some biological control agents under organic farming system

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Received: 7 March, 2018; Accepted: 22 April, 2018; Published online: 26 April, 2018

Abstract

In the current work an attempt was made to find out the most suitable bioagents that have the ability to protect Date palm cv. Zaghloul (*Phoenix dactylfera* L.) from some soilborne fungal diseases. Several soilborne fungi were isolated from root rots of date palm trees located in the farms of El-Beheira Governorate, Egypt, including; *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*, and their pathogenicity were confirmed on date palm seedlings in the greenhouse. These fungi cause economic losses in date palm yield and a wide range of other cultivated plants. Many different antagonistic isolates (bioagents) *i.e. Trichoderma album*, *T. harzianum*, *T. viride* and *T. hamatum* were isolated from the rhizosphere soil of healthy date palm trees. For comparison of results, bio-commercial preparations mainly "Bio-zeid (*T. album* 10×10^6 cfu/ ml)" and "Plant guard (*T. harzianum* 30×10^6 cfu/ ml)" were also used to detect their antagonistic potential against the mycopathogens of date palm. *In vitro* antifungal efficacy of the bioagents was evaluated against all the pathogens, where *T. harzianum* was the most effective as it caused 87.10, 81.55, 77.60 and 68.55% reduction in the radial growth of *F. solani*, *F. oxysporum*, *R. solani* and *M. phaseolina*, respectively. *In vivo* assays under field conditions, all tested biotic treatments significantly reduced severity of root rot diseases caused by the concerned pathogens. Moreover, they increased the percentages of survived date palm plants in infested soils during both successive growing seasons of 2016 and 2017, where *T. harzianum* was the most effective bioagent as it showed an increase in date palm survival of about 82.35 and 86.67% at both seasons, respectively. In addition, all bioagents enhanced the growth parameters of date palm, *i.e.* plant height (cm), number of leaves/ plant and number of leaflets/ leaf, compared with the control treatment. Thus, these effective bioagents could be used as biofungicides to control the root rot diseases of date palm in the field; accordingly, we could displace the use of non-ecofriendly and health hazards synthetic fungicides.

Key words: Date palm (*Phoenix dactylfera* L.), soilborne fungi, root rot, *Trichoderma* spp., biofungicides

1. Introduction

Date palm is one of the most valuable domesticated fruit trees because of its significance in human societies, health benefits, and the range of subsistence products from its fruits and other parts of the large palm (Johnson *et al*., 2015). According to Pariona, (2017), Egypt leads the world date palm production with about 1.1 million metric tons annual production and generated about $41.8 million from export of fresh date fruits.

Date palm trees and offshoots are attacked by several soilborne pathogenic fungi causing severe losses and worldwide deterioration of these trees (Baraka *et al*., 2011; Arafat *et al*., 2012). According to Maitlo *et al*., (2013); Ziedan *et al*., (2013), *M. phaseolina*, *Phoma* sp., *F. oxysporum*, *F. solani*, *F. moniliforme*, *F. equiseti*, *F. semitectum*, *R. solani* and *Thielaviopsis paradoxa* have been reported in different countries to cause root rot diseases in young and adult date palm trees.

Agricultural management of soilborne pathogens in the field include crop rotation, fungicides applications, methyl bromide fumigation, soil solarization and the use of resistant or tolerant varieties. However, no single method provided an adequate control of soilborne diseases (Hausbek and Lamour, 2004). Use of synthetic chemicals to control soilborne pathogens caused several negative effects on the plants such as: i) development of pathogen resistance, ii) harmful effects on humans, iii) bad impact on beneficial organisms, and iv) environmental pollution. However, soilborne pathogens still need to be controlled in order to ensure healthy plant growth and productivity in sustainable agriculture. Kaewchai and Soytong, (2010) stated that biological control of plant pathogens by using microorganisms has been considered more natural and environmentally acceptable alternative to the use of chemicals.

Trichoderma spp. are common saprophytic fungi which were found in almost all soils and among rhizosphere microflora. They have been used as potential biocontrol agents because of their ability to reduce the incidence of diseases caused by many soilborne pathogens (Abdel- Monaim, 2010; Perveen and Bokhari, 2012). Several strains of *Trichoderma* spp. have been isolated and were regarded as effective biocontrol agents against several phytopathogenic fungi under greenhouse and field conditions (Omomowoa *et al*., 2018).

Several reports on using nonpathogenic fungi as biocontrol agents were recorded such as; *Chaetomium* species for control *Thielaviopsis* bud rot of *Hyophorbe lagenicaulis* (Soytong *et al*., 2005), whereas, *T. viride*, *T. polysporum*, *T. hamatum* and *T. aureoviride* significantly reduced growth of *Ceratocystis paradoxa* (Eziashi *et al*., 2007). Modes of action of these beneficial microorganisms in suppressing plant pathogens include; direct parasitism of pathogens, competition for space and nutrients, the production of antibiotics, enzymes, and plant growth promoting hormones (Lugtenberg *et al*., 2003). Moreover, in the study of Abdel-Monaim, (2010); Perveen and Bokhari, (2012), these bioagents significantly increased root and plant growth of date palm and of many other crops.

The objectives of our work were; to decrease the use of chemical fungicides in agricultural fields of date palm to enhance the growth and yields of date palm. In addition, we made a trial to obtain the most promising bioagent that has the ability to protect date palm cv. Zaghloul plants from certain soilborne fungal pathogens.

2. Materials and Methods

2.1. Isolation and identification of the root rot pathogens of date palm trees

About 3-10 root pieces showing necrosis and/ or root rot symptoms were collected from naturally infected date palm trees (cv. Zaghloul) from the top 5 cm of the soil level. These trees were from different locations of EL-Beheira Governorate, Egypt during the growing season of 2016. According to Maciá-

Vicente *et al*., (2008), infected roots were washed, air dried, surface sterilized in 1% sodium hypochlorite solution for 3 min., washed several times with sterilized dist. water and then dried between two sterilized filter papers. The sterilized root fragments were aseptically transferred to the surface of plates of Potato dextrose agar medium (PDA). Plates were incubated at 25°C for 5 d and were examined daily. The developed mycelial growth was picked up and transferred onto new PDA medium. Purification of each isolated fungus was carried out using mycelium tip culture (Al-Sa'di *et al*., 2007).

Identification of the isolated fungi was carried out according to their cultural and microscopical characteristics described by Singh, (1982); Barnett and Hunter, (1987). Colony morphology, conidiophores and conidia of *Fusarium* were identified according to Nelson *et al*., (1983); Booth, (1985). Stock cultures were maintained on PDA slants and kept at 5°C till further studies.

2.2. Isolation and identification of the antagonistic mold fungi

Rhizosphere soil of healthy roots of date palm trees were used to isolate different antagonistic mold fungi using the method of Ahmed, (2005). Ten g of rhizosphere soil was added aseptically to 90 ml sterile dist. water (to make stock dilution of 10^{-1}), shacked on rotary shaker for 15 min. and then serially diluted up to 10^{-4}. PDA medium supplied with 66.7 mg/ l rose bengal and 250 mg/ l streptomycin (Johnson *et. al*., 1960) was used for isolating the antagonistic fungi. One ml of each dilution was aseptically transferred to sterilized petri plates containing 20 ml of melted PDA medium, and then spread using sterile glass rod. Three plates were used for each dilution. All plates were incubated at 25±1ºC for 4 d. Separate colonies of the isolated fungi were selected, sub-cultured and identified according to their morphological and microscopical characteristics (Rifai, 1969).

2.3. Pathogenicity assay

This experiment was carried out in a greenhouse located in Environmental Studies and Research Institute, University of Sadat City, Minufiya Governorate, Egypt. Date palm seeds (cv. Zaghloul) were treated with dry heat at 45°C for 2h to activate their germination then planted in 30 cm diameter plastic pots (one seed per pot) filled with steam pasteurized soil. After 6 months of planting, seedlings were inoculated separately with each of the fungal pathogens using homogenized culture technique (Muthomi *et al*., 2007). Disks were taken from one-week-old cultures of the tested fungi and transferred to 75 ml of potato dextrose broth (PDB) in a 250 mL flask, and then incubated at 25 ± 1ºC for 6-7 d. The fungal mycelia were separated using sterile Whatman no. 1 filter paper, rinsed with sterile dist. water and then blended with a small amount of sterile water in a waring blender for 2 min. Sterile dist. water was then added to each fungal suspension to have a final concentration of 6×10^{6} cfu/ ml for soil infestations. Ten pots were used for each fungal isolate along with non-infested negative control soil.

The severity of root rot symptoms was determined 90 d following pathogens inoculation using a disease rating scale of 0-5 on the basis of root discoloration and leaf yellowing as follows; 0: no root discoloration or leaf yellowing, 1: 1-25% root discoloration or one yellow leaf, 2: 26-50% root discoloration or more than one yellow leaf, 3: 51-75% root discoloration plus one wilted leaf, 4: up to 76% root discoloration or more than one wilted leaf, and 5: completely dead plants. For each replicate, a disease severity index (DSI) was calculated according to Abdullah *et al*., (2003); Ilias, (2000) as follow:

$$\mathrm{DSI} = \frac{\sum \mathrm{ab}}{\mathrm{N.K}} \mathrm{X}\,100$$

Where:

Σab = sum of the product of assessed plants with their corresponding score scale.

N = total number of assessed plants.

K = highest score scale.

2.4 *In vitro* antagonistic potency of the isolated mold fungi against root rot mycopathogens

The antagonistic activity of the different fungal isolates on the radial growth of the mycopathogens was conducted under laboratory conditions using poisoned food technique. *T. album*, *T. hamatum*, *T. harzianum* and *T. viride* isolated from rhizosphere soil of healthy date palm trees, and commercial preparations of "Bio-zeid (*T. album* 10×10^6 cfu/ ml)" and "Plant guard (*T. harzianum* 30×10^6 cfu/ ml)" were evaluated during this assay. Unless otherwise stated, the PDA medium was used for all the growing fungi. Culture filtrate of each of the fungal antagonists was prepared separately according to (Calistru *et al*., 1997). 1 ml of sterile cell free-filtrate of each of the antagonistic fungi and the commercial preparation suspensions were separately added to warm PDA and then poured into petri plates (10 ml/ plate). Each of these treated plates was inoculated separately at the center with discs cut from the periphery of 5 d old cultures of each of *F. solani*, *F. oxysporum*; *R. solani*, and *M. phaseolina*. Plates inoculated with pathogens only without antagonists served as positive control treatment, whereas, non-treated plates served as negative control. Inoculated plates were incubated at 25± 1°C for 7 d. Five plates were used for each treatment (Mishra, 2010).

The experiment was terminated when the mycelial mats covered the surface of PDA in negative control plates. All plates were observed, and percentage of reduction in the radial growth of the pathogenic fungi was calculated using the formula suggested by Ahmed, (2005); Ahmed, (2013) as follows:

$$\%\ \text{Reduction in linear growth} = 100 - \left[\left(\frac{G2}{G1}\right) \text{X}\ 100\right]$$

Where; G1: growth of the pathogenic fungus in positive control plates, G2: growth of pathogen in plates treated with fungal antagonists.

2.5. Evaluation of efficacy of the bioagents to control root rot of date palm plants under field conditions

2.5.1. Preparation of inocula of the bioagents

In this assay, each of the antagonistic fungi was grown in PDB medium under dark conditions for 10 d at 25± 2ºC (Ahmed, 2013). All cultures were individually blended in an electrical blender for 2 min. then used as inocula at concentrations of 30×10^6 cfu/ ml.

2.5.2. *In vivo* assay

Field experiments were conducted in naturally infested farms with each of the fungal pathogens of root rot diseases separately, located in EL-Beheira Governorate, Egypt, during the two successive growing seasons of 2016 and 2017. These assays were carried out to evaluate the efficacy of the tested bioagents and the commercial preparations in controlling root rot diseases of date palm, as well as their effects on seedlings growth parameters.

The experimental design was a complete randomized block with 10 replicates. The experimental unit areas were $2m^2$ (1×2 m), each unit included 2-year-old date palm plants. The soils of date palm were drenched 3 times at 15-d intervals with inocula of each of the bioagents (30×10^6 cfu/ ml) and the bio-commercial preparations "Bio-zeid, *T. album* 10×10^6 cfu/ ml", and "Plant guard (*T. harzianum* 30×10^6 cfu/ ml)" separately, at the rate of 3 l per offshoot. Soils infested with each of the 4 mycopathogens only separately, act as positive control treatments. Untreated negative control soils were drenched three times at 15-d intervals with water. The recommended agricultural practices and irrigation dates for date palm were used.

The disease severity was assessed for each treatment after 6 months from the last application treatment as mentioned above according to Ilias, (2000). At the end of these field experiments the following plant growth parameters were estimated: Plant height (cm), number of leaves/ plant and leaflets numbers/ leaf.

2.6. Statistical analysis

Data were subjected to statistical analysis and compared according to the least significant difference (L.S.D.) as mentioned by Snedecor and Cochran, (1989).

3. Results and Discussion

3.1. Isolation, identification of the mycopathogens and rhizosphere mold fungi

Data in Table (1) indicated that, *R. solani* (31.58%) was the most frequently isolated fungus from the rotted samples of date palm trees. On the contrary, *M. phaseolina* (18.42%) recorded the lowest percentages of isolated pathogen. The isolated rhizosphere fungi were identified as; *Chaetomium globosum*, *Aspergillus niger*, *Gliocladium virens*, *T. album*, *T. harzianum*, *T. viride* and *T. hamatum*, recording frequencies of isolation of; 8%, 14%, 21%, 34%, 58%, 48% and 40%, respectively. *Trichoderma* spp. were selected for further study as being known to be effective antagonists, whereas, the remaining isolates were neglected.

3.2. Pathogenicity assay

Results demonstrated that the antagonistic potency of *R. solani*, *M. phaseolina*, *F. oxysporum* and *F. solani* were detected after 90 d from inoculation. *R. solani* recorded the highest disease rating scale and DSI of root rot of date palm (5- 64.67%), followed by *M. phaseolina* (4- 55.67%), respectively.

Similarly, *F. oxysporum* caused high incidence of root rot symptoms on the date palm plants (45.33%), and disease rating scale of (3). However, *F. solani* showed the lowest DSI of (36.33%) for root rot diseases and recorded disease rating scale of (3) (Table 2). These variations among pathogens might be due to different root exudates produced by date palm which cause pronounced increase in the number of saprophytes around roots, thus will protect these roots from infection by soilborne fungal pathogens (Mogle and Mane, 2010; Xue Jing *et al*., 2011). In addition to presence of different resistance genes in roots of date palm plants accordingly expressing different responses against these pathogens.

Table (1): Frequency of isolation of fungal pathogens from the rotten roots of date palm trees located in Beheira Governorate

Isolated fungal pathogens	Frequency of isolated fungi	
	no.	%
F. oxysporum (Schlecht)	9	23.68
F. solani (Mart.)	10	26.32
M. phaseolina (Tassi) Goid.	7	18.42
R. solani (Kuhn)	12	31.58
Total	38	100

Table (2): Pathogenicity assay of fungal pathogens on date palm plants under greenhouse conditions 90-d post inoculation

Tested fungal pathogens	Disease severity of root rot diseases		
	D	DSI	Plant survival %
F. oxysporum (Schlecht)	3	46.33	53.67
F. solani (Mart.)	3	36.33	63.67
M. phaseolina (Tassi) Goid.	4	55.67	44.54
R. solani (Kuhn)	5	64.67	35.33
Non-treated control	0	-	100
L.S.D. at 5%	-	1.82	1.92

-Results are averages of 10 plants for each treatment. Where; D: disease rating scale, DSI: disease severity index.

3.3. *In vitro* antagonistic potential of the bioagents on the radial growth of the mycopathogens

A variation in *in vitro* antagonism between *T. harzianum* and the mycopathogens in concern was observed. The highest % of decrease in radial growth of *F. solani*, *F. oxysporum*, *M. phaseolina* and *R. solani* on PDA was recorded by *T. harzianum*; 87.10, 81.55, 68.55, and 77.6 %, respectively. However, *T. hamatum* expressed least antagonistic potential compared to those produced by the remaining bioagents. Moderate antagonistic potency was demonstrated by Bio-zeid and Plant guard commercial preparations as clear in Table (3).

The growth inhibitory effects of antagonists are in agreement with Rudresh *et al*., (2005) who observed 72.1 and 77.0% *in vitro* growth inhibition in *R. solani* and *F. oxysporum*, respectively, by *T. harzianum* and *T. viride* which also exhibited strong mycoparasitic activity and completely overgrew the pathogens mycelia once in contact with them. In addition, these observed results might be because different pathogens have different own defense mechanisms against enzymes and toxic substances produced by the different bioagents (Ahmed, 2013). *Trichoderma* secretes some chemical metabolites such as phenols, steroids, flavonoids, quinines, terpenoids, xantones, peptides, cytocatalasins, alkaloids and phenyl propanoids which might be responsible for their *in vitro* inhibitory or antagonistic activity (Muthu *et al*., 2006). Moreover, *Trichoderma* spp. antagonise the mycopathogens through mycoparasitism as they degrade their cell wall by producing lytic enzymes such as; chitinases, peroxidase, and glucan 1-3 B-glucosidases as stated previously by Mausam *et. al*., (2007); Ahmed, (2013), or through production of viridian mycotoxin by *T. viride* (Mishra, 2010; Wahyudi *et al*., 2011).

Table (3): *In vitro* effects of the bioagents on reduction percentage of radial growth of the mycopathogens

Antagonists	% Reduction in radial growth of the mycopathogens				
	F. solani	***F. oxysporum***	***M. phaseolina***	***R. solani***	**Mean**
T. album	75.55	70.95	58.71	70.12	68.83
T. hamatum	74.82	66.67	56.67	66.33	66.12
T. harzianum	87.10	81.55	68.55	77.6	78.70
T. viride	85.30	78.80	63.33	75.55	75.75
Bio-zeid (*T. album*)	83.50	75.55	61.48	75.30	73.96
Plant guard *(T. harzianum)*	78.80	74.82	60.80	71.33	71.44
Mean %	80.85	74.72	61.59	72.71	-
Untreated control	00.0	00.0	00.0	00.0	-
L.S.D. at 5% for pathogenic fungi (P)= 0.85					
L.S.D. at 5% for antagonists (A)= 1.11					
L.S.D. at 5% for (A×P)= 1.32					

-Results are averages of 5 plants for each antagonistic treatment

3.4. Effects of bioagents on root rot diseases severity of date palm under field conditions

As observed in Table (4), all tested bioagents significantly reduced severity of root rot diseases, compared with positive control soil infested with the concerned mycopathogens only. Moreover, they increased the percentage of survived date palm plants in both seasons of 2016 and 2017. However, different bioagents varied in their potential against disease severity. *T. harzianum* was most effective bioagent in controlling root rot diseases of date palm causing reduction in disease severity percentages to be (3, 2%), followed by *T. viride* (4.5, 3%), during the two growing seasons, respectively, compared with the positive control soil (17.0, 15.0%). On the contrary, *T. hamatum* demonstrated the lowest efficacy in controlling both diseases during both seasons recording; 10, 8.4%, respectively. The two commercial preparations expressed moderate antifungal potential by reducing disease severity to (6, 5.4%) for Bio-zeid, and (5.5, 4.5%) for Plant guard. These results were in agreement with Mwangi *et al*., (2011) who reported the ability of an isolate of *T. harzianum* (P52) to enhance the growth and control root rot disease caused by *F. oxysporum* f.sp. *lycopersici* on tomato seedlings. Different *Trichoderma* spp. is well known to antagonise many pathogenic fungi recording success in a number of crop diseases (Omomowoa *et al*., 2018).

These results might be explained according to the dual effect of bioagents which produce plant growth regulators (Karlidag *et al*., 2010; Wahyudi *et al*., 2011; Al-Rajhi, 2013), in addition to the chemical effect of their antioxidants that play a key role in improving plant physiology and metabolism (Al-Taweil *et al*., 2009; Wahyudi *et al*., 2011).

Trichoderma spp. are known to act as effective bioagents through different mechanisms such as; mycoparasitic activity (Matei and Matei, 2008), through production of antifungal enzymes such as Endo-chitinase, β-glucosidase and α-1,3-glucanase; through production of mycotoxins such as trichodermin (Balode, 2010), however, *Trichoderma* isolates may also compete for space and nutrients (Baset *et al*., 2010).

Table (4): Effect of bioagents on root rot diseases severity under field conditions during the seasons of 2016 and 2017

Treatments	2016 growing season			2017 growing season		
	Disease severity (%)	Efficacy* %	% of plant survival	Disease severity (%)	Efficacy* %	% of plant survival
T. album	8.5	50.00	91.5	8.0	46.67	92.0
T. hamatum	10.0	41.18	90.0	8.4	44.00	91.6
T. harzianum	3.0	82.35	97.0	2.0	86.67	98.0
T. viride	4.5	73.53	95.5	3.0	80.00	97.0
Bio-zeid (*T. album*)	6	64.71	94.0	5.4	64.00	94.6
Plant guard *(T. harzianum)*	5.5	67.65	94.5	4.5	70.00	95.5
Positive control	17.0	0.00	83.00	15.0	0.00	85.0
L.S.D. at 5%	1.22			1.12		

-Results are averages of 10 plants for each treatment,

* % Efficacy of plant survival = (control-treatment/control) ×100

3.5. Effects of bioagents on the growth parameters of date palm plants under field conditions

All tested bioagents significantly increased the growth parameters of date palm plants *i.e.* plant height (cm), number of leaves/ plant and leaflet

number/ leaf. *T. harzianum*, *T. viride*, Plant guard and Bio-zeid, respectively, recorded the highest enhancement of all growth parameters compared with control treatment . On the other hand, *T. album* and *T. hamatum* gave the lowest increase in growth parameters during the two growing seasons of 2016 and 2017, respectively. These results were in agreement with those reported by (Khandelwal *et al*., 2012; Babu and Pallavi, 2013), who mentioned that *Trichoderma* spp. caused conspicuous improvement in the aforementioned crop growth parameters during the growing seasons. *Trichoderma* spp. are well known not only of being active bioagents for disease control, but act also as biofertilizers that enhance the growth of the plant through providing the soil with nutrients in utilizable forms that are absorbed by the plant roots, causing thus better plant nutrition (Pathak *et al*., 2007). In addition to the production of hormone like metabolites and release of nutrients from soil or organic matter thereby facilitate better plant growth (Yobo *et al*. 2010). Moreover, these results of enhancement of growth parameters might be due to variation in genetic pool of the date palm cultivars and/or the effects of climatic seasonal changes on their vegetative growth. Similar results were reported by Ahmed and Shaheen, (2016).

Table (5): Effect of bioagents on growth parameters of date palm plants grown under field conditions during the two growing seasons of 2016 and 2017

Treatments	Plant height (cm)		Number of leaves /plant		Number of leaflets/ leaf	
	2016	2017	2016	2017	2016	2017
T. album	166.4	160.9	5.17	5.05	90.3	87.5
T. hamatum	160.5	155.5	4.33	4.25	88.1	77.7
T. harzianum	234.7	232.6	6.36	6.05	125.4	121.4
T. viride	225.3	222.3	5.83	5.50	121.0	109.4
Bio-zeid	178.6	175.4	5.27	5.04	105.2	100.3
Plant guard	199.8	190.6	5.63	5.45	112.5	105.2
Control	110.6	105.0	1.27	1.16	28.4	26.2
L.S.D. at 5%	2.64	2.52	0.29	0.28	2.74	2.73

-Results are averages of 10 date palm plants for each treatment

-Conclusion

Several bioagents such as *T. album*, *T. hamatum*, *T. harzianum* and *T. viride* were isolated from the rhizosphere of date palm trees, and they showed high *in vitro* and *in vivo* efficacy in antagonising certain soilborne fungal pathogens causing root rot diseases of date palm. In addition, they increased the growth parameters of date palm plants under field conditions. However, *T. harzianum* and *T. viride* were the best effective bioagents. These two isolates could thus be formulated in an economic and utilizable form to be applied as biofungicides to biocontrol soilborne mycopathogens, and as biofertilizers to enhance the growth of date palm in the field. Thus we could displace using the non-ecofriendly synthetic fungicides.

4. References

Abdel-Monaim, M.F. (2010). Integrated management of damping- off, root and/or stem rot diseases of chickpea with sowing date, host resistance and bioagents. Egyptian Journal of Phytopathology. 38: 45-61.

Abdullah, F.; Ilias; G.N.M.; Nelson., M.; Nu Ain Izzati, M.Z. and Umi Kalsom, Y. (2003). Disease assessment and the efficacy of *Trichoderma* as a biocontrol agent of basal stem rot of oil palm. Res. Bull. Sci. Putra 11:31-33.

Ahmed, M.F.A. (2013). Studies on non-chemical methods to control some soil borne fungal diseases of bean plants *Phaseolus vulgaris* L. Ph.D. Thesis. Faculty of Agriculture, Cairo Univ., 137 pp.

Ahmed, M.F.A. (2005). Effect of adding some biocontrol agents on non-target microorganisms in root diseases infecting soybean and broad bean plants. M.Sc. Thesis. Faculty of Agriculture Moshtohor, Benha Univ. pp 142.

Ahmed, M.F.A and Shaheen, S.A. (2016). Evaluation of some *Trichoderma* isolates on controlling rust disease and enhance the yield of cowpea plants (*Vigna unguiculata* L.). Proceeding of 1st International Conference of Applied Microbiology, March 1-3, Agricultural Research Center (ARC), Egypt. pp. 250-260.

Al-Rajhi, A.M.H. (2013). Impact of biofertilizer *Trichoderma harzianum* Rifai and the biomarker changes in *Eruca sativa* L. plant grown in metal-polluted soils. World Applied Sciences Journal. 22: 171-180.

Al-Sa'di, A.M.; Drenth, A.; Deadman, M.; de Cock, A.W.A.M. and Aitken, E.A.B. (2007). Molecular characterization and pathogenicity of *Pythium* species associated with damping-off in greenhouse cucumber (*Cucumis sativus* L.) in Oman. Plant Pathology. 56: 140-149.

Al-Taweil, H.I.; Osman, M.B.; Hamid, A.A. and Wan Yussof, W.M. (2009). Development of microbial inoculants and the impact of soil application on rice seedlings growth. American Journal of Agricultural and Biological Sciences. 4: 79-82.

Arafat, K.H.; Mohamad, A.M. and Elsharabasy, S. (2012). Biological control of date palm root-rots diseases using Egyptian isolates of *Streptomyces*. Research Journal of Agriculture and Biological Sciences. 8(2): 224-230.

Babu, K.N. and Pallavi, P.N. (2013). Isolation, identification and mass multiplication of *Trichoderma* an important bio-control agent. International Journal of Pharmacy and Life Sciences. 4(1): 2320-2323.

Balode, A. (2010). Effect of tricodermin biological product against *Botrytis* in horticultural crops. Acta Horticulture. 877: 1583-1588.

Baraka, M.A.; Radwan F.A. and Arafat, K.H. (2011). Survey and identification of major fungi causing root rot on date palm and their relative importance in Egypt. Journal of Biological Chemistry and Environmental Science. 6(2): 319-337.

Barnett, H.J. and Hunter, B.B. (1987). Illustrated Genera of Imperfect Fungi. Burgess, Publ. Co., Minneapolis, USA, pp. 218.

Baset, M.M.A.; Shamsuddin, Z.H., Wahab, Z. and Marziah, M. (2010). Effect of plant growth promoting rhizobacteria (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Australian Journal of Crop Science. 4: 85-90.

Booth, C. (1985). The genus *Fusarium*. Common Wealth Mycological Institute, pp. 237.

Calistru, C.; Mclean, M. and Berjak, P. (1997). *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichodema* species. A study of the production of extracellular metabolites by *Trichoderma* species. Mycopathologia. 137: 115-124.

Eziashi, E.I.; Omamor, I.B. and Odigie, E.E. (2007). Antagonism of *Trichoderma viride* and effect of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. African Journal of Biotechnology. 6(4): 388- 392.

Hausbek, M.K. and Lamour, K.H. (2004). *Phytophthora capsici* on vegetable crops: Research progress and management challenges. Plant Disease. 88: 1292- 1303.

Ilias, G.N.M. (2000). *Trichoderma* pers. ex fr. and its efficacy as a biological control agent of basal stem rot of oil palm (*Elaeis guineensis jacq.*). Ph.D. thesis. Universiti Putra Malaysia, Selangor, Malaysia. pp. 283.

Johnson D.V.; Al-Khayri, J.M. and Jain, S.M. (2015). Introduction: Date Production Status and Prospects in Africa and the Americas. In: Al-Khayri J., Jain S., Johnson D. (eds) Date Palm Genetic Resources and Utilization. Springer, Dordrecht. pp. 3-18.

Johnson, L.F.; Curl, E.A.; Bond, J.H. and Fribourg, H.A. (1960). Methods for Studying Soil Microflora-Plant Disease Relationships. Burgess Publishing Company, Minneapolis, Minneapolis, USA. pp.178.

Kaewchai, S. and Soytong, K. (2010). Application of biofungicides against *Rigidoporus microporus* causing white root disease of rubber trees. Journal of Agricultural Technology. 6(2): 349-363.

Karlidag, H.; Esitken, A.; Yildirim, E.; Donmez, M.F. and Turan, M. (2010). Effects of plant growth promoting bacteria on yield, growth, leaf water content, membrane permeability, and ionic composition of strawberry under saline conditions. Journal of Plant Nutrition. 34: 34-45.

Khandelwal, M.; Datta, S.; Mehta, J.; Naruka, R.; Makhijani, K.; Sharma, G.; Kumar, R. and Chandra, S. (2012). Isolation, characterization & biomass production of *Trichoderma viride* using various agro products- as biocontrol agent. Advances in Applied Science Research. 3(6): 3950-3955.

Lugtenberg, B.J.J.; Bloemberg, G.V.; Woeng, C.A. and Thomas, F.C. (2003). Phenazines and their role in bio-control by *Pseudomonas* bacteria. New Phytologist. 153: 503-523.

Maciá-Vicente, J.G.; Jansson, H.B. and Lopez-Llorca, L.V. (2008). Colonization of barley roots by endophytic fungi and their reduction of take-all caused by *Gaeumannomyces graminis* var. *tritici*. Canadian Journal of Microbiology. 54: 600-609.

Maitlo W.A.; Markhand, G.S. Abul-Soad, A.; Lodhi, A.M. and Jatoi, M.A. (2013). Chemical control of Sudden decline disease of date palm (*Phoenix Dactylifera* L.) in Sindh, Pakistan. Pakistan Journal of Botany. 45: 7-11.

Matei, G.M. and Matei, S. (2008). Research on isolation, characterization and testing the interaction between *Trichoderma harzianum* and *Botrytis cinerea* for biological control of gray mold in strawberry. Horticulture. 51: 653-657.

Mausam, V., Brar, S.; Tyagi, R.; Surampalli, R. and Valero, J. (2007). Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochemical Engineering Journal. 37(1): 1-20.

Mishra, V.K. (2010). *In vitro* antagonism of *Trichoderma* species against *Pythium aphanidermatum*. Journal of Phytpathology. 2: 28-35.

Mogle, U.P. and Mane, R.Y. (2010). Antagonistic effect of bio-fertilizers against seed borne mycoflora of tomato (*Lycopersicon esculentum*). Research Journal of Agricultural Science. 1: 255-258.

Muthomi, J.W.; Otieno, P.E.; Chemining'wa, G.N.; Nderitu, J.H. and Wagacha, J.M. (2007). Effect of legume root rot pathogens and fungicide seed treatment on nodulation and biomass accumulation. Journal of Biological Sciences. 7: 1163-70.

Muthu, C.; Ayyanar, M.; Raja, N. and Ignacimuthu, S. (2006). Medicinal plants used by traditional healers in Kanchipuram district of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine. 2: 43.

Mwangi, M.W.; Monda, E.O.; Okoth, A.S. and Jefwa, J.M., (2011). Inoculation of tomato seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi and their effect on growth and

control of wilt in tomato seedlings. Brazilian Journal of Microbiology. 42: 508–513.

Nelson, E.P.; Toussoun, A.T. and Marasas, O.F.W. (1983). *Fusarium* species. An Illustrated Manual for Identification. The Pennsylvania state Univ. Press. pp. 191.

Omomowoa, I.O.; Fadijia, A.E. and Omomowob, O.I. (2018). Assessment of bio-efficacy of *Glomus versiforme* and *Trichoderma harzianum* in inhibiting powdery mildew disease and enhancing the growth of cowpea. Annals of Agricultural Sciences. (In press)

Pariona, A. (2017). Leading Countries Growing Dates (Fresh Date Palm Fruits). http://www.worldatlas.com/articles/worldleading-countries-growing-fresh-dates.html

Pathak, A.K.; Muralia, S. and Pathak, S. (2007). Biocontrol of Plant Diseases. Aavishkar Publisher, India, Jaipur, pp. 89–111.

Perveen, K. and Bokhar, N.A. (2012). Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. African Journal of Microbiology Research. 6: 3348-3353.

Rifai, W. A. (1969). A revision of the genus *Trichoderma*. Mycological paper No. 116. Fac. of Pure Science, Univ. of Sheffield, England, pp. 56.

Rudresh, D.L.; Shivaprakash, M.K. and Prasad, R.D. (2005). Effect of combined application of *Rhizobium*, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer arietinum* L.). Applied Soil Ecology. 28: 139-146.

Singh, R.S. (1982). Plant Pathogens "The Fungi". Oxford and IBH Publishing Co. New Delhi, Bombay, Calcuta. pp. 443.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State Univ. Press, Ames, Iowa USA. pp. 503.

Soytong, K.; Pongak, W. and Kasiolam, H. (2005). Biological of *Thielaviopsis* bud rot of *Hyophorbe lagenicaulis* in the field. Journal of Agricultural Technology. 1(2): 235-245.

Wahyudi, A.T.; Astuti, R.I. and Giyanto (2011). Screening of *Pseudomonas* sp. isolated from rhizosphere of soybean plant as plant growth promoter and biocontrol agent. American Journal of Agricultural and Biological Sciences. 6(1): 134-141.

Xue Jing, W.; Yong Sheng, J.; Wei, L.; BaoSheng, T. and You Nian, W. (2011). Identification and inhibitory effects of antagonistic bacteria against strawberry root rot (*Fusarium oxysporum*). Acta Horticulturae Sinica. 38: 1657-1666.

Yobo, K.S.; Laing, M.D. and Hunter, C.H. (2010). Application of selected biological control agents in conjunction with tolclofos-methyl for the control of damping-off caused by *Rhizoctonia solani*. African Journal of Biotechnology. 9: 1789-1796.

Ziedan, E.H.; Farrag, E.S.H. and Sahab, A.F. (2013). First record and preliminary evaluation of *Mucor hiemalis* as biocontrol agent on inflorescence brown rot incidence of date palm. Archives Phytopathology and Plant Protection. 46(5): 617-626.