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Evaluation of seed coating with certain bio-agents against damping-off and root rot diseases of fennel under organic farming system

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Abstract

Native isolates of certain antagonists *i.e. Trichoderma harzianum, T. viride, Bacillus subtilis* and *Pseudomonas fluorescens* and bio-commercial preparations (Bio Zeid "*T. album*" and Bio ARC "*B. megaterium*") were evaluated against fungi have been reported to attack fennel roots *i.e. Fusarium oxysporum, F. solani, Rhizoctonia solani, Macrophomina phaseolina, Sclerotium rolfsii* and *Pythium* spp. causing root rot and damping off diseases. These diseases cause economic losses in fennel yield and a wide range of other cultivated plants. The most dangerous effects of *R. solani* occurred due to pre- and post- emergence damping-off and root rot diseases. All tested antagonists which coating fennel seeds at the rate of 5g/kg seeds reduced the incidence of pre-, post-emergence damping off and root rot diseases. *Trichoderma harzianum*, *T. viride* and Bio Zeid "*T. album* were the most effective antagonists as shown by the highest plants survival and the best fennel yield under field conditions. Moreover application of these antagonists recorded the highest increase in oil amount and oil components as compared with the control. On the other hand, *P. fluorescens* showed the lowest effect. This trend was true during the two successive growing seasons 2015 and 2016.

Key words: *Foeniculum vulgare*, *Trichoderma* spp., *Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina.*

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Introduction

Fennel (*Foeniculum vulgare* L.) is a member of Umbellifera (*Apiceae*). Although the original homeland of fennel is the Mediterranean basin, however fennel now is very common in the most world countries. Fennel is considered one of the most popular medicinal crops of the world. Fennel is used as spices, beverage or for medical purposes (Gebily, 2015). *Rhizoctonia solani* Kuehn (*Thantephorus cucumeris* (Frank) Donk) causes serious losses in fennel in many parts of the world. Symptoms of rot have been attributed to the action of several enzymes that degrade cell walls (Wilhelm, 1998). The fungus is an economically important pathogen of many crops of worldwide. It occasionally causes serious root rot. Damping off in fennel and coriander seedlings is due to *R. solani*. This pathogen was isolated from different Egyptian soil samples (Saleh et al., 2013). Fennel is attacked by soilborne diseases such as *Fusarium oxysporum, F. solani, Rhizoctonia solani, Macrophomina phaseolina, Sclerotium rolfsii* and *Pythium* spp. which cause economic losses in yield. Different antagonists *i.e. Trichoderma* spp. and *Bacillus subtilis* used as seed coating reduced the incidence of pre-, post-emergence damping-off and root rot diseases. *Trichoderma harzianum, T. viride* and *B. subtilis* were the most effective antagonists as shown by the highest plants survival, the best seed yield and oil components under field conditions (Gebily, 2015). The antagonistic activity of *Trichoderma* spp. was tested *in vitro*, in pot and in field. Role of bio-agents in enhancing some enzymes (chitinase, peroxidase and polyphenoloxidase) related to disease resistance in plant was detected (Pieta & Pastucha, 2004). Using *T. harzianum* and *T. viride* as seed dressing for bean seeds at the rate of 4g/kg seeds, showed specific suppression of damping-off, root rot and improving fresh, dry weight of shoots, dry weight of roots, yield components number and weight of pods/plant and dry weight of 100 seeds (Ahmed et al., 2015; Ahmed, 2013; Ahmed, 2005; Sullivan, 2004). *Trichoderma* spp. as able to control various plant diseases, especially soil-borne diseases. It affects plant pathogens with different mechanisms such as competition, antibiosis and parasitism (Saksirirat et al., 2009). Also, *Trichoderma* spp. gave the best hyperparasitic behavior against *Macrophomina phaseolina* and can induce resistance in treated plants (Larralde-Corona et al., 2008). *Trichoderma harzianum* can be considered as ideal biocontrol agent for its good characteristic. This antagonist is very easy to be isolated and it grows rapidly on organic stuff. *Trichoderma harzianum* acts through different modes of action *i.e.* mycoparasitism (Ahmed, 2013; Lumsden et al., 1995), production of antifungal substances (Robinson et al., 2009), also it owns an enzymatic system that causes destruction of the pathogens (Ziedan et al., 2005). In addition to these modes of action, *Trichoderma* also acts as inducer for resistance in treated plants against certain pathogens (Harman, 2006; Ahmed, 2005; Homer, 1993) and can grow within wide range of temperature and other environmental conditions (Singh et al., 2010; Bailey et al., 2008). *Bacillus subtilis* BN1 proved it as a potent biocontrol agent, whereas

exhibited strong antagonistic activity against *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani*. *Bacillus subtilis* BN1 produced lytic enzymes, chitinase and beta-1, 3-glucanase which are known to cause hyphal degradation and digestion of the cell wall component of *M. phaseolina* (Singh et al., 2008). *Trichoderma album*, *T. hamatum*, *T. harzianum* and *T. viride* were reported to significantly reduce the mycelial growth of *F. solani* and *R. solani.* In addition, it gave the highest plant survival percentage and improved yield component. The results showed that the levels of chitinase, peroxidase and polyphenol oxidase activity were highly increased in treated strawberry plants compared to untreated ones (Latha et al., 2009; Prlak & Kose, 2009; Saksirirat et al., 2009; Cherif et al., 2007). The present work aimed to decrease fungicides use in agriculture to produce high quality food in sufficient quantity and to enhance biodiversity system. In addition, an attempt was tried to find out the most suitable bio agent that has the ability to protect fennel plants against some soilborne fungal diseases.

Materials and methods

Isolation of the causal pathogens: Samples of root fennel plants were collected from Sakran Farms, Abshoway, Fayoum Governorate, Egypt. The infected roots were washed in tap water, air dried, surface sterilized by dipping in 1% Sodium hypochlorite solution for 3 minutes, washed several times with sterilized distilled water and dried between two sterilized filter papers. The sterilized fragments were aseptically transferred to plates, each contained 15 ml potato dextrose agar (PDA) medium. Plates were incubated at 25±2°C and examined periodically. The developed mycelial growth of each emerged fungus was picked up and transferred onto PDA medium. Purification of each isolated fungus was carried out using the hyphal tip technique (Hawker, 1956; Brown, 1924). Identification of the isolated fungi was carried out according to their cultural and morphological characteristics described by Gilman, (1957), Barnett and Hunter, (1987) and Singh, (1982). Stock cultures were maintained on PDA slants and kept in a refrigerator at 5°C for further studies.

Isolation of the antagonistic microorganisms: Roots of apparently healthy fennel plants were collected from soil to isolate different antagonistic microorganisms using the method described by Ahmed, (2005) and Ahmed (2013). One gram of the soil was obtained from rhizosphere of fennel root plants, on dry basis, added aseptically to 99ml sterile distilled water (to make stock dilution of 1/100) and was shaked periodically for approximately 15 minutes. In similar way, the stock soil suspension was used to make serial dilutions of 10^{-2} to 10^{-6}. Autoclaved peptone dextrose agar + rose Bengal + streptomycin medium (Johnson et. al., 1960) and Soil extract agar medium (Lochhead, 1940) were used for isolating the antagonistic fungi and bacteria. Soil suspensions of dilutions 10^{-4} (Johnson et. al., 1960) and 10^{-6} (Lochhead, 1940) were used for isolating antagonistic fungi and bacteria, respectively. One ml of a

known dilution was aseptically transferred to sterilized Petri-dishes each containing about 10 ml of melted warm agar medium. Three plates were used for each dilution. All plates were incubated at 25±1ºC for 2 – 4 days. The isolated microorganisms, which grew in separate colonies on the dilution plates, were selected, sub-cultured and identified according to their morphological, cultural characters (Rifai, 1969; Comm, 1955). Identification was confirmed at the Mycology and Plant Disease Survey Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt.

Pathogenicity tests: Pathogenicity tests were carried out under greenhouse conditions located at Integrated Pest management Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt. Plastic pots 20 cm diameter were sterilized by dipping in 5% formalin solution for 5 min., then left in open air till dryness. Disinfected clay loam soil (with 5% formalin) was distributed in plastic pots (3.0 kg sterilized soil pots and infested by 10 g of corn sand meal (CSM) medium inoculated by *F. oxysporum, F. solani, R. solani, M. phaseolina, S. rolfsii* and *Pythium* spp. corn sand meal (CSM) medium enriched with 0.2% peptone solution (Ahmed, 2013; Ahmed, 2005; Abd El Moity, 1985). Soil infestation was performed 10 days before sowing tested crop. Seeds of fennel obtained from organic Sakran farm, Abshoway, Fayoum Governorate, Egypt, were used in this experiment. Seeds were sown in the infested pots or non-infested (control) at the rate of 5 seeds /pot and three pots were used as replicates for each particular treatment. Data were recorded as percentage of disease incidence in each treatment.

***In vitro* experiments:** The effect of different antagonists on the linear growth of the pathogenic fungi was conducted under laboratory conditions. *Trichoderma harzianum, T. viride, B. subtilis* isolated from rhizospher soil of fennel plants and commercial preparation "Bio Zeid (*T.album*) and Bio ARC (*B.megaterium*)" were evaluated during this study. Unless otherwise mentioned, autoclaved Gliotoxin Fermentation Agar (GFA) and the Nutrient Glucose Agar (NGA) media were used for growing the antagonistic fungi and bacteria, respectively. Plates 9 cm each contains 10 ml of GFA or NGA media were used for studying effects of antagonistic fungi and bacteria, respectively. The antagonistic fungi, bacteria and the commercial preparation suspensions were added to warm sterilized GFA and NGA medium, respectively at the rate of 10% and poured before solidification into Petri dishes (10 ml/plate). The treated plates were inoculated at the center with discs obtained from the periphery of 5 days old cultures of pathogenic fungi. Plates contained media without antagonists and inoculated with pathogenic fungi were served as control treatment. Three plates were used for each particular treatment. Inoculated plates were incubated at 25±1°C. The experiment was terminated when mycelial mats covered the medium surface in control treatment, all plates were examined and percentage of reduction in mycelial growth of pathogenic fungi means were calculated using the formula suggested by Abd El

Moity (1985), Ahmed (2005) and Ahmed (2013) as following:

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

Where, G1= growth of pathogenic fungi in plates inoculated with the pathogen alone, G2= growth of pathogen against antagonist.

Field experiments: All field experiments, unless otherwise indicated, were carried out at Sakran farm, Abshoway, Fayoum Governorate, Egypt on 15th October, 2015 and 2016 growing seasons, where soil is light loamy textured with natural infestation. Nile water is available in this area with surface irrigation system. In this experiment, suspensions containing propagules of the tested biocontrol agents, *i.e. T. harzianum*, *T. viride* and *B. subtilis* were prepared as described by Ahmed, (2005) and Ahmed, (2013). Fennel seeds were soaked in 5% Arabic gum, then mixed in the bioagent and commercial preparations "Bio Zeid (*T. album*) and Bio ARC (*B. megaterium*)" at the rate of 5g/kg" seeds of fennel with Tween 80 at concentration 0.3% for 12 hours before sowing. All the experiments were conducted in a complete randomized block design with three replicated plots, the area of the experimental plot was 9 m^2 and comprised of 3 rows (3m long × 50cm width) with about 50 cm apart. Each row was planted with 60 seeds of fennel in naturally infested soil. In all field experiments, percentages of pre-, post-emergence damping off and root rot diseases were determined after 10, 21 and 45 days, respectively from sowing according to the method described by El-Helaly et. al., (1970), Ahmed, (2005) and Ahmed, (2013). The survived plants were counted, uprooted and used for determining the yield components of fennel. Samples of treated plants were collected from different treatment to find out effect of all biological treatments on fennel seeds contents. The following experiments were carried at Central Laboratory of Biotechnology, Plant Pathology Research Institute, ARC, Giza, Egypt. Fifty gm of fennel seeds were extracted. These seeds were hydro distilled for 2.30 to 3.00 hours to extract oil content as mentioned by Anonmous, (1968). Percentage of active substance in fennel oil (Anethole, D-limonene and Estragole) were determined using HP 6890 Series Gas Chromatograph System to illustrate effect of different biagents on quality of oil in fennel seeds. In additional, percentage of oil in different samples was calculated according to the next formula:

$$\text{Oil (\%)} = \frac{\text{observed volume oil (ml)}}{\text{weight of sample (g)}} \times 100$$

Statistical analysis: Data were subjected to statistical analysis and compared according to the least significant difference (LSD) as mentioned by Snedecor and Cochran, (1989).

Results and Discussion

Isolation and identification of fennel root rot pathogens: Data in Table (1) indicate that *R. solani*, *F. solani* and *M. phaseolina* were the most frequently isolated fungi from the rotted samples of fennel collected from Abshoway,

Fayoum Governorate, Egypt. Identification was carried out according to the cultural and morphological characters described by Gilman, (1957), Barnett and Hunter, (1987) and Singh, (1982).

Table 1: Frequency (%) of fungi isolated from the rotten roots of fennel collected from Fayoum Governorate, Egypt.

Isolated fungi	Frequency of isolated fungi	
	No.	%
Fusarium oxysporum	3	10
F. solani	7	23.3
Rhizoctonia solani	9	30
Macrophomina phaseolina	6	20
Sclerotium rolfsii	3	10
Pythium spp.	2	6.7
Total	30	100

Pathogenicity tests: Data in Table (2) illustrate that the most dangerous effects of all soilborne diseases *i.e. F. oxysporum, F. solani, R. solani, M. phaseolina, S. rolfsii* and *Pythium* spp. have occurred at the stages of pre-, post-emergence damping-off and root rot diseases. *Rhizoctonia solani* was the most aggressive soilborne disease and caused the highest effect on pre-, post-emergence damping-off and root rot incidence (35, 30.0 and 15.0%), followed by *M. phaseolina* (30.0, 25.0 and 12%), respectively. The opposite trend was recognized for *Pythium* spp that showed the lowest records (15.0, 10.0 and 8.0%) for pre-, post-emergence damping-off and root rot diseases, respectively and showed the highest percentage 67% on standing plants. However, no significant variations were detected between *F. oxysporum* and *Sclerotium rolfsii* treatments particularly at the stages of pre-, post- emergence damping off and root rot diseases. These results are in agreement with those reported by Coly-Smith (1976), Sennoi et. al., (2010) and Ahmed et. al., (2015) who mentioned that the destruction on root caused by soilborne pathogens was due to the synergistic action between polygalacturonase and oxalic acid produced by these pathogenic fungi.

Effect of antagonists on the linear growth of the pathogenic fungi: Data in Table (3) indicate that the different antagonistic isolates were significantly varied in their inhibitory effects against the *in vitro* linear growth of both tested pathogenic fungi. In this respect, *T. harzianum* significantly caused the highest reduction of mycelial growth being 82.08 % followed by *T. viride* (79.87 %), Bio Zeid "*T.album*" (78.11 %), *B. subtilis* (75.72%) and Bio ARC "*B.megaterium*" (72.15%) on the average. On the other hand, *P. fluorescens* gave the least effect and the average recorded decrease in the pathogen growth was 66.06%. The average of reduction in one to the six antagonists of *F. solani* (79.34 %) was significantly higher than that of *R. solani* (74.72%) and of *M. phaseolina* (72.86%). This phenomenon might be explained in the light of fact that different pathogens with different

striations own different defense mechanisms against enzymes and toxic substances that produced by different antagonists (Ahmed et al., 2015; Ahmed, 2013; Ahmed, 2005; Tuner, 1971). *Trichoderma* spp. degraded the cell wall pathogen due to the production of lytic enzymes such as chitinases, peroxidase, polyphenoloxidase and glucan 1-3 B-glucosidases (Mausam et. al., 2007; Pieta & Pastucha, 2004). *Bacillus subtilis* occupied the second rank after *Trichoderma* spp., this might be due to that it produces a group of enzymes, which dissolve the cell wall of the pathogen (Ahmed, 2013; Ahmed, 2005), antibiotics such as bacterocin and subtilisin (Bender, et. al., 1999), volatile compounds and phytotoxic substances (Hoagland & Cutler, 2000; Glick, et. al., 1999). *Pseudomonas fluorescens* occupied the third rank after the previous antagonists, this might be due to the offensive plant growth-promoting bacteria "PGPB" colonization and defensive retention of rhizosphere niches to enable production of bacterial allelochemicals, including iron-chelating siderophores, antibiotics, biocidal volatiles, lytic enzymes and detoxification enzymes (Compant et. al., 2005; Hass & Defago, 2005), production of mycolytic enzymes namely protease, lipase and secondary metabolites such as hydrogen cyanide (HCN), salicylic acid (SA) and iron chelating siderophores using standard protocols (Anand & Kulothungan, 2010).

Table 2: Effect of artificial inculcation with the tested fungi on the incidence of pre- and post-emergence damping off of fennel under greenhouse conditions.

Tested fungi	Pre emergence (%)	Post emergence (%)	Root rotted plants (%)	Plant survival (%)
Fusarium oxysporum	20.0	15.0	10.0	55.0
F. solani	25.0	20.0	09.0	46.0
Rhizoctonia solani	35.0	30.0	15.0	20.0
Macrophomina phaseolina	30.0	25.0	12.0	33.0
Sclerotium rolfsii	20.0	15.0	09.0	56.0
Pythium spp.	15.0	10.0	08.0	67.0
Control "Untreated"	00.0	00.0	00.0	100.0
LSD at 5%	1.42	1.33	1.57	2.17

Table 3: Effect of the antagonistic fungi on the percentage of reduction in linear growth of the pathogenic fungi.

Antagonists	Reduction in liner growth of pathogenic fungi (%)			
	F. solani	*M. phaseolina*	*R. solani*	Mean
T. harzianum	87.10	77.6	81.55	82.08
T. viride	85.30	75.55	78.80	79.87
B. subtilis	78.80	73.55	74.82	75.72
P. fluorescens	68.20	63.33	66.67	66.06
Bio Zeid (*T.album*)	83.50	75.30	75.55	78.11
Bio ARC (*B.megaterium*)	73.70	71.80	70.95	72.15
Control "Untreated"	00.00	00.00	00.00	00.00
Mean	79.34	72.86	74.72	
LSD at 5% for Pathogenic fungi (P)	= 0.93	Antagonists (A)	= 1.12	
A x P	= 1.29			

Effect of certain antagonists on damping off disease incidence under field conditions: Data in Table (4) clearly demonstrate that all antagonist treatments significantly reduced disease incidence and increased the percentage of healthy plants compared to the control treatment during the two growing seasons 2015 and 2016. *Trichoderma harzianum* showed the highest efficacy (34.00 and 32.94%) followed by *T. viride* (32.14 and 30.00%) in controlling damping-off during the two successive growing seasons (2015 and 2016), respectively. On the other hand, *P. fluorescens* showed the lowest efficacy in controlling fennel disease during the two successive growing seasons, being 17.29 and 16.32%, respectively in comparison to control treatment. These results can be explained in the light of data obtained by Ahmed (2005) and Ahmed (2013) who stated that the efficacy of antagonists depends on their capacity to compete with other microorganisms which occupy rhizosphere area under different environmental conditions, as well as, directly through the production of phytohormones. The light effect of *Trichoderma* spp. in agriculture can provide numerous advantages such as colonization of the root and rhizosphere of the plant, control of the plant pathogens by different mechanisms such as parasitism, antibiosis production and inducing systemic résistance, improvement of the plant health by promote plant growth and stimulation of root growth (Harman, 2006; Harman et. al., 2004).

Table 4: Effect of treating fennel seeds with different antagonists at the rate of 5g/kg seeds on damping off disease incidence under field conditions during 2015 and 2016 growing seasons.

Antagonists	2015 growing season				2016 growing season			
	Damping-off (%)		Plant survival (%)	Efficacy * (%)	Damping-off (%)		Plant survival (%)	Efficacy* (%)
	Pre-	Post-			Pre-	Post-		
T. harzianum	4.9	1.3	93.8	34.00	6.5	3.1	90.4	32.94
T. viride	5.5	2.0	92.5	32.14	7.8	3.8	88.4	30.00
B. subtilis	7.4	2.2	90.4	29.14	11.5	6.4	82.1	20.74
P. fluorescens	12.6	5.3	82.1	17.29	14.5	6.4	79.1	16.32
Bio Zeid (*T.album*)	5.7	2.4	91.9	31.29	8.4	4.8	86.8	27.65
Bio ARC (*B.megaterium*)	9.0	3.1	87.9	25.57	13.5	5.5	81.0	19.12
Control "Untreated"	18.9	11.1	70.0	------	17.8	14.2	68.0	------
LSD at 5%	1.98	1.55	2.21	------	1.95	1.33	2.29	------

* % Efficacy of plant survival = ((Treatment/Control)×100)-100 according to Ahmed, (2005) and Ahmed, (2013).

Effect of certain antagonists on oil components under field conditions: Data obtained from these analysis are presented in Table (5) beside data previously mentioned about effect of these bioagents on damping-off just to correlate and understand the role of these bioagents in changes may be occurred in oil fennel seed components and reflection of these changes on degree of resistance or increase in yield. Data in Table (5) show that *T. harzianum* was the highest effective treatment led to the

highest amount of oil components compared with control treatment during the two successive growing seasons (2015 and 2016), respectively. On the contrary, *P. fluorescens* show the least effective treatment led to the least content in yield component rather than control treatment. No clear trend can be deduced when treatments with slight differences in efficacy correlated with any of used oil component analysis under test. These results are in harmony with those obtained by Gebily, (2015). This due to that potassium element is responsible for oil formation. Protecting fennel root system by adding different antagonists, this lead to improve potassium absorbing consequently increase percentage of oil (Mahfouz & Sharaf-Eldin, 2007).

Table 5: Effect of treating fennel seeds with different antagonists on oil components under field conditions during 2015 and 2016 growing seasons.

Antagonists	2015 growing season				2016 growing season			
	Oil components			Total Amount oil (%)	Oil components			Total Amount oil (%)
	Anethole (%)	Dlimonene (%)	Estragole (%)		Anethole (%)	Dlimonene (%)	Estragole (%)	
T. harzianum	2.98	23.15	85.88	1.55	2.96	22.62	82.88	1.52
T. viride	2.93	22.62	82.44	1.52	2.90	22.01	81.32	1.50
B. subtilis	2.54	22.01	77.35	1.32	2.50	19.88	75.88	1.30
P. fluorescens	1.96	19.88	72.44	1.24	1.95	17.81	71.35	1.18
Bio Zeid	2.90	22.50	80.00	1.50	2.88	21.29	76.15	1.48
Bio ARC	2.25	20.29	75.88	1.28	2.15	18.62	72.04	1.24
Control	0.90	16.61	65.00	0.55	0.88	14.49	62.65	0.50
LSD at 5%	0.84	1.21	2.79	0.08	0.77	1.45	2.59	0.07

Table 6: Effect of treating fennel seeds with different antagonists on yield under field conditions during 2015 and 2016 growing seasons.

Antagonists	2015 growing season			2016 growing season		
	Dry weight of 100 seeds (gm)	Dry seeds weight/plant (gm)	Total Yield of seeds (Kg/acre)	Dry weight of 100 seeds (gm)	Dry seeds weight/plant (gm)	Total Yield of seeds (Kg/acre)
T. harzianum	1.50	50.66	1300	1.48	49.00	1250
T. viride	1.30	46.33	1267	1.28	44.33	1107
B. subtilis	1.26	41.33	1150	1.25	38.00	950
P. fluorescens	1.10	38.00	950	1.07	33.000	850
Bio Zeid	1.28	44.33	1250	1.24	42.33	1058
Bio ARC	1.24	40.00	1100	1.22	36.66	900
Control	0.60	16.66	750	0.50	15.66	700
LSD at 5%	0.13	0.56	2.92	0.12	0.55	2.73

Effect of certain antagonists on yield components: Presented data in Table (6) show that applying any of the tested antagonists at the rate of , 5g/kg seeds) for treating fennel seeds increased in the assessed yield parameters in 2015 growing season than in 2016 growing season. *Trichoderma harzianum* significantly caused the highest increase in dry weight of 100 seeds, dry seeds weight/plant significantly and total yield of seeds, being 1.50gm, 50.66gm and 1300 Kg, respectively in the 2015 growing season and gave 1.48gm, 49.00gm and 1250 Kg, respectively in 2016 growing season in comparison with the control. On the other hand, *P. fluorescens* was the lowest effective one during the two growing seasons. In most cases there were significant differences in the estimated values in both growing season due to using *T. viride*, Bio Zeid (*T. album*), *B. subtilis* and Bio ARC (*B. megaterium*). These results are in harmony with those obtained by Sullivan, (2004) who reported that *R. solani*, *S. rolfsii* and *F. solani* soil-borne diseases result from a reduction of biodiversity of soil organisms. Restoring beneficial organisms that attack otherwise antagonize disease-causing pathogens will render a soil disease-suppressive. Plants growing in disease-suppressive soil resist diseases much better than in soils low in biological diversity. The increase of yield also may be due to either healthy root system that absorb and supply adequate amount of raw nutrient or the syntheses of these raw nutrient materials effectively in presence of high amount of chlorophyll and protein, that led to more fruit yield (Ahmed et al., 2015; Gebily, 2015).

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