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Use of biological control and bio-fertilization against Fusarium wilt disease and its effect on growth characteristics and tomato productivity

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Abstract

In this research had tried control by application of biological control and biofertilizer of the pathogenic fungus causing Fusarium wilt of tomato under greenhouse and field conditions. In this, results indicated to treatment with *Trichoderma harzianum*, alone or biofertilizer dual inoculation with *Azospirillum brasilense*, proved more powerful in decreasing disease severity % of foliar yellowing and wilt or vascular browning by about (16.25 and 16.67%) and (8.75 and 10.71%), respectively of tomato cv Super-strain B, plants than other treatments and compared with control. On the other hand, previous treatment gave the highest, observed in case of inoculation with *A. brasilense* with *T. harzianum*, records of growth characters, i.e. plant height (cm), number of branches per plant, plant fresh weight and plant dry weight g/plant, by about (77.6 cm, 5.75/plant, 552.9 and 87.13g.) respectively, yield and yield components of tomato plants, i.e. Number of fruits/plant, weight of one fruit (g), fruits yield/plant (kg) and fruits yield/plant (ton/fed) by about (14.8/plant, 113.4g, 2.07kg and 25.13 ton/fed) respectively compared with the other treatments and control. Also, in previous treatment gave the highest values observed in case of inoculation with *A. brasilense* with *T. harzianum*, of dehydrogenase activity (µg TPF/g dry soil/day), N_2-ase activity (nmoles C_6H_2/g dry soil/hr) at flowering stage and increased phenols mg/g fresh weight and total soluble solids (TSS) of tomato c.v Super-strain B, plants compared with the other treatments and control.

Key words – *Trichoderma harzianum* – vascular browning – biological control – pathogenic fungus – growth characteristics – tomato productivity

Introduction

Tomato an important crops over the world including Egypt it's used for food and industrial purpose (El-Mougy-Nehal 1995). Egypt ranks as fourth of the world regarding the production of unit area and number one vegetable cash crop (FAO 2013). Tomato plants are subjected to attack by several soil-born fungal pathogens, which cause serious diseases and important yield losses in Egypt such as root rot and wilt (Awad 1990). Fusarium wilt caused by *Fusarium oxysporum* (Schlecht) f.sp *lycopersici* (Sacc), is one of the most important diseases attack tomato crop under

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the Egyptian climate conditions (Saleh et al. 2016). Fusarium wilt on tomato sometimes became the main reason for restriction expanding tomato area and causing yield losses of tomato production. (Morsy et al. 2009).

Due to the environment need to more stringent regulations and the use of chemicals to control the plant diseases has always been an expensive remedy and may also reduce populations of beneficial microorganisms in soil, thus biological control has become more attractive (Cook 1993). Plant growth-promoting rhizobacteria (PGPR) suppress a variety of root and vascular disease caused by soilborne pathogens (Mahmoud et al. 2016). They have several key functions in plants, such as; biological control of pathogens by antagonistic effects or induction of systemic resistance, increment the bio availability of the mineral nutrients such as phosphate solubilization, nitrogen fixation or phytostimulation, antibiotic production, phytotoxins degradation and siderophores production (Mantilla 2007, Lugtenberg & Kamilova (2009) and Van Hulten et al. (2010). Kumar et al. (2012) reported that a large number of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium and Serratia* have to enhance plant growth by with their different plant growth promoting activities including phosphate solubilization. This work was carried out to study is the role of biological control and biofertilizers for controlling Fusarium wilt of tomato and its effect on growth characteristics and tomato productivity.

Materials & Methods

Effect of some bioagents isolates on wilt disease severity of tomato plants under greenhouse conditions

Isolation of pathogenic fungal, bioagents and identification

The most virulent isolate of *F. oxysporum* f.sp *lycopersici* was previously isolated and identified as well as confirmed their pathogenic capabilities by (Saleh et al. 2016). Different bioagent isolates were isolated from the native microflora in the rhizosphere of naturally wilted tomato plants that may antagonize the pathogenic fungi causing wilt disease of tomato. Plants were carefully removed from soil and the rhizosphere soil was collected by gently mechanical removal of the adhering soil. About 2 g soils were added to 200 ml sterile distilled water in conical flask. The contents of the flasks were then stirred vigorously for 5 min. Basic dilution were prepared to obtain10^{-3},10^{-4}and10^{-5}. Isolation of the bioagents was performed as described by Saleh (1997). The given identification revealed that the isolated bioagent fungi belong to *T. harazianum* and *T. viride* and bacterial isolates belong to *Bacllius ceris* and *Pseudmonas fluorescence*. *Trichoderma* isolates were identified according to their morphological and microscopic characteristic Domsch et al. (1980) and confirmed by Assiut University, Mycological Center (AUMC) Faculty of Science. Whereas bacteria was identified according to Burbage et al. (1982).

Preparation of the inocula of pathogenic fungal isolate and bioagents

The inoculum of the *F. oxysporium* isolate was prepared by mixing inoculated sorghum grains with sterile soil abovementioned that by (Saleh et al. 2016).

Firstly, Trichoderma isolates were inoculated individually into conical flasks 1000 ml containing 250 ml vermiculate, 250 ml Czapeks liquid medium (vol/vol) and autoclaved for 20 minutes at 121^{o}C, on two consecutive days with *T. harazianum* and *T. viride*, incubated at 25^{o}C for 25 days and used for inoculation. After 25 day's incubation period, contents of flasks were transferred to plastic plates under sterile conditions; left to dry then mixed in a blender to become powder and calculated the inoculum density in 1 gm formula by using serial dilutions *T. harazianum* and *T. viride*, individually mixtures contain 15.9×10^{7} CFU/gm formula and kept in polyethylene bags at room temperature until used. The formulated antagonists were added to previously infested soil with the most virulent isolate *F. oxysporum* f.sp *lycopersici*, (at the rate of 1 and 2% (w/w) after which they were transplanted directly in wet pot. (Chang & Kommedahl 1968).

Next, bacterial isolates of *B. ceris* and *P. fluorescence* consisted of aqueous solutions prepared from 3-day-old cultures of bacteria grown on PD broth media and inoculated individually into conical flasks 250 ml containing 100 ml PD broth, and incubated at 27°C for 2 days and used for inoculation. Root system of transplants were dipped in bacterial suspension (2.5×10^7 cfu/ml) for half hour after which they were transplanted directly in wet pot. Three replicates (pots) were used and 3 seedlings/pot. Two controls were used; the first control treatment was performed in similar manner but emerging root system of transplants in Trichoderma and bacterial suspension in infested soil with the pathogenic fungus *F. oxysporum* f.sp *lycopersici*. The second one was performed using sterile soil without inocula. Three replicate pots were used for each treatment and were irrigated directly after transplanting and subsequently as when necessary.

Preparation of fungicide

The fungicide Monceren 25%WP (Pencycuron) was tested against the pathogenic fungi causing wilt disease of tomato under greenhouse condition and used at 3 g/L for comparison. Root system of seedling of tomato cv. Super- Strain B dipped in the fungicide for half hour as above.

Disease assessment

At 30 days after transplanting the following assessments were calculated:

Disease index of foliar browning

Disease severity of foliar yellowing was determined by rating each leaf on the severity of wilt symptoms and yellowing according to 0:4 scale and computing the average grade for plant as a whole according to the following formula: % of foliar yellowing = (Sum of foliar yellowing value/ (4 × Total number of leaf) x 100 (El-Zawahry-Aida 1984, Fakhouri & Buchenaure 2003, Song et al. 2004). In the present study, the following numerical grades were used:

0 = Healthy plants.
1 = 1- less than 25% of plant leaflets are yellow (slight chlorosis, wilting or stunting).
2 = 25 - less than 50% of plant leaflets are yellow (moderate chlorosis, wilting or stunting).
3 = 50 - less than 75% of plant leaflets are yellow (severe chlorosis, wilting or stunting).
4 = 75 - less than 100% of plant leaflets are yellow (very severe chlorosis, complete wilting or dead plant).

Disease index of vascular browning

Disease index of vascular browning was determined by estimating the internal discoloration (browning) area of vascular bundle by making longitudinal and transverse section of root according to the scale described by (Gothoskar et al. 1953).

0 = no brown discoloration in vascular bundles of root and the crown and stem.
1 = 1- less than 25% of vascular root bundles are brown.
2 = 25 - less than 50% of vascular root bundles are brown.
3 = 50 - less than 75% of vascular root bundles are brown.
4 = 75 - less than 100% of vascular root bundles are brown.

The percentage of internal discoloration was calculated following the formula: % of vascular browning = (Sum of vascular browning values/ (4 × Total number of plants) × 100. Pathogenicity test revealed El-khatatba (Minofiya Gov.) isolate was the most virulent (Saleh et al. 2016).

Effect of combined treatment between different biocontrol agents and biofertilizer on wilt disease severity of tomato plants against natural soil infection with Fusarium wilt disease under field conditions

Combined treatments between the biofertilizer *i.e Azospirillum brasilense* and either *T. harzianum*, *T. viride*, *P. florescence* or *B. ceris*, as well as combined with the fungicide Monecern 25% (Pencycuron) were carried out for controlling Fusarium wilt under naturally infection in field during season 2015. On the other hand, individual treatments of the different tested bioagents and

biofertilizer and the fungicide were evaluated during this study. The experimental area was 21 m^2 (6m × 3.5 m). Plants were transplanted at 35 cm a part. Every plot consists of six rows; 3.5 m length and 1m width. This experiment included the following treatments: The experiment was designed as randomized block design with three replicates. Two controls were used; the first control treatment was performed in similar manner but emerging root system of transplants in bacterial suspension in natural soil infection with Fusarium wilt disease and fertilized by *A. brasilense*. The second one was performed using soil natural soil infection with Fusarium wilt disease and unfertilized.

Treatment of transplants

Except for control and bio-control agent treatments, tomato transplants (cv. Super-strain B) were washed with water and air dried, then transplants (7days-old) were inoculated by dipping the root system in cell suspension of *A. brasilense* (11×10^8 cell/ml) for 60 min before transplanting. Sucrose solution (30%) was added as an adhesive agent prior to inoculation. Also, in chemical control treatment, the transplants of tomato were immersed in the fungicide, Monecern (25% Pencycuron) for half hour and treated as above before transplanting. Regarding the bio-control agent treatments, transplants were inoculated by dipping in 2 days-old cell suspension of *B. ceris* and *P. florescence* (2.5×10^7 cfu/ml) for 60 minutes before transplanting. Regarding the *T. harzianum* and *T. viride* treatment, tomato was inoculated by dipping the root system in 7 days-old cell suspension of *T. harzianum* and *T. viride* (2.5×10^7cfu/ml) for 60 minutes before transplanting.

Cultivation process

Except for control-2 treatments soil was fertilized and natural infection of *F. oxysporum* f.sp *lycopersici*; all plots were supplemented with the recommended doses of nitrogen of 150 kg N/feddan as ammonium sulphate applied in three equal doses *i.e.* at vegetative, flowering and setting stages. Control (2) treatment soil was unfertilized and natural infection; all plots were supplemented with potassium sulphate at a rate of 150 k; potassium sulphate (48 % K_2O) in three equal doses as mentioned before. A control treatment was prepared where the soil was left without fertilization and transplants were soaked in N-deficient medium instead of Azospirillum inoculum. Another control was also prepared where transplants were kept without inoculation, but the soil was fertilized with recommended dose of NPK and chemical control by Monecern (25% Pencycuron) transplants soaking. Transplanting was performed on 2015 season.

The experimental studied in farm of Mallawy Agricultural Research Station, to investigate in summer season 2015, evaluate to the effect of treatments inoculation with, *Azospirllum brasilense* and the biocontrol agents on reduce the percentage of disease severity compared to tomato transplants or than individual inoculation after 60 days from sowing data percentage of infested plants was recorded under field conditions. The studied microorganisms were grown separately in nutrient agar medium for four days at 30°C (giving 11×10^8 cell/ml for *A. brasilense*). This culture wase used as bacterial inocula. Seed inoculated tomato seedlings were procedure was carried out by root inoculation (Gupta et al. 1995) as follows:

Seven days old inoculated seedlings were uprooted, washed carefully 2-3 times in sterilized distilled water and their roots were immersed in a bacterial cell suspension ($11x10^8$ cell/ml for *A. brasilense*) for 60 minutes. Such seedlings were considered as seed root inoculated seedlings. After inoculation plants repotted again. Seedlings without root inoculation were used as control.

Effect of biological control and biofertilizer on growth characters, yield and yield components, enzyme activity, total phenols compounds and total soluble solids in tomato fruits under field condition

In this trial, experiment was carried out in summer season 2015 in farm of the Mallawy Agricultural Research Station, to efficient antagonistic strains (*T. harzianum, T. viride, B. ceris* and *P. florescence*) in combination with (*A. brasilense*) evaluate their ability to protect tomato plants (cv.Super - Strain B) against natural infection of *F. oxysporum* f.sp *lycopersici* on growth

parameters, yield and yield components, enzyme activity, total phenols compounds and total soluble solids in tomato fruits under field conditions:

Growth characters:

Growth characters, *i.e*. plant height (cm), number of branches per plant, plant fresh weight and plant dry weight g/plant, were determined and reported by Barakat & Gabr (1998) at flowering stage for natural infection of tomato plants cv. Super- Strain B.

Yield and yield components:

Yield and yield components of tomato plants, *i.e*. Number of fruits/plant, weight of one fruit (g), fruits yield/plant (kg) and fruits yield/plant (ton/feddan) were estimated by Barakat & Gabr (1998).

Microbiological determinations:

Dehydrogenase activity was assayed according to Thalmann (1967). Nitrogenase activity (N2-ase) was measured by using the acetylene reduction technique given by Diloworth (1970).

Chemical analysis:

Phenolic compounds were determined using colorimetric method according to Snell & Snell (1953). Total soluble solids were assayed according to (A.O.A.C. 1980).

Statistical analysis:

Data were subjected to statistical analysis of variance. The experimental design (S) of all studies was a completely randomized with three or four replications, analysis of variance (ANOVA) of the data was performed with the MSTAT-C statistical package (A) micro-computer program for the design, management, and analysis of agronomic research experiments. Michigan State Univ, USA. Least significant difference (LSD) was used to compare treatment means (Gomez & Gomez 1984). Using the computer program (Costat). The differences between the mean values of various treatments were compared by Duncan s multiple range test (Duncan 1955).

Results

Effect of some bioagents isolates on disease severity of Fusarium wilt on tomato plants (cv. Super-strain B) under greenhouse conditions in artificially infested soil with *F. oxysporum* f.sp *lycopersici*

Results in Table 1 reveal that application of either, *T. harazianum, T. viride, P. fluorescence, B. ceris* and Moncern (25% Pencycuron) to artificially infested soil with the most virulent isolate were decreased the disease severity of *F. oxysporum* f.sp *lycopersici*. Moreover, *T. harazianum* proved more powerful in decreasing foliar yellowing and wilt and vascular browning disease severity by about (16.25 and 16.67%) compared with the other treatments and control. Similar followed by *T. viride, P. fluorescence, B. ceris* respectively, while Moncern was the least affected by about (38.65 and 52.65%). The interesting point is that the effect of *T. harazianum* in decreasing disease severity surpassed the effect of the Moncern fungicide. Regard to *P. fluorescence* and *B. ceris* surpassed the effect of Monecern when the latter was used at 3 gm /liter (W/V) as a dip procedure method of the root system compared with the other treatments and control.

Effect of biological control and biofertilizer on diseases severity of Fusarium wilt on tomato plants (cv. Super-strain B) under field condition

Data presented in Table 2 indicated that application of (*A. brasilense* + *T. harzianum*), (*A. brasilense* + *T. viride*), (*A. brasilense* + *florescence*), (*A. brasilense* + *B. ceris*), (*A. brasilense*) alone and) *A. brasilense* + Monecern 25%), decreased disease severity by different degrees, respectively. Data also proved that, *T. harazianum* gave more powerful in decreasing disease

severity of foliar yellowing and wilt or vascular browning by about (8.75 and 10.71%) respectively, which was significantly higher than any tested treatments followed by (*A. brasilense* + *T. viride*), (*A. brasilense* + *florescence*), (*A. brasilense* + *B. ceris*), (*A. brasilense*) alone and (*A. brasilense* + Monecern 25%), compared with other treatments and control.

Table 1 Effect of some bioagents isolates on diseases severity of Fusarium wilt on tomato plants (cv. Super-strain B) under greenhouse conditions in artificially infested soil with *F. oxysporum* f.sp *lycopersici*.

Treatments	Reduction diseases severity%	
	Foliar yellowing and wilt	**Vascular browning**
T. harzianum	16.25	16.67
T. viride	31.46	31.57
P. fluorescence	35.91	43.16
B. ceris	37.58	47.68
Monecern (25% Pencycuron)	38.65	52.65
F. oxysporum (control-1)	45.14	62.04
Control-2 (uninoculated),	0.00	0.00
Mean	29.28	33.84
L.S.D at 0.05%	3.45	3.89

Table 2 Effect of biological control and biofertilizer on diseases severity of Fusarium wilt on tomato plants (cv. Super-strain B) under field condition.

Treatments	Reduction diseases severity%	
	Foliar yellowing and wilt	**Vascular browning**
T. harzianum + *A. brasilense*	8.75	10.71
T. viride + *A. brasilense*	27.44	26.49
P. fluorescence+ *A. brasilense*	33.47	36.84
B. ceris+ *A. brasilense*	36.82	43.34
Control-1 (fertilized and natural infection)	38.11	51.39
Monecern (25% Pencycuron) + *A. brasilense*	40.91	56.02
Control-2 (unfertilized and natural infection)	0.00	0.00
Mean	27.00	32.11
L.S.D at 0.05%	5.43	5.97

Effect of biological control and biofertilizer on growth characters, yield and yield components, enzyme activity, total phenols compounds and total soluble solids in tomato (cv. Super-strain B) fruits under field conditions

Growth characters:

Data in Table 3 reveal that, growth parameters of tomato plants, *i.e.* plant height, number of branches, fresh and dry weight were better than (control-2). Results indicated also that, growth characters of tomato plants were significantly increased by treated with *T. harzianum* + *A. brasilense* compared to any other treatment. In this respect treated with *A. brasilense* + *T. harzianum*, gave higher records plant height (cm), number of branches per plant, plant fresh weight and plant dry weight g/plant, by about (77.6 cm, 5.75/plant, 552.9 and 87.13g) respectively.

Table 3 Effect of biological control and biofertilizer on growth characters of tomato plants (cv. Super-strain B) under field conditions.

Treatments	Measure			
	Plant height (cm)	**Number of branches per plant**	**Plant fresh weight g/plant**	**Plant dry weight g/plant**
T. harzianum + A. brasilense	77.6	5.75	552.9	87.13
T. viride + A. brasilense	70.0	5.57	535.4	81.2
P. fluorescence + A. brasilense	69.4	5.44	520.7	80.9
B. ceris + A. brasilense	66.7	5.38	517	78.0
Control-1 (fertilized and natural infection)	64.6	5.22	513.4	76.8
Monecern (25% Pencycuron) *+ A. brasilense*	64.2	5.09	509	76.5
T. harzianum	63.6	4.62	506	75.6
T. viride	63.1	4.44	489.2	75.4
P. fluorescence	61.9	4.13	485.6	75.3
B. ceris	60.8	3.97	468.5	74.9
Monecern (25% Pencycuron)	57.1	3.96	467.7	73.5
Control-2 (unfertilized and natural infection)	19.99	1.33	274.9	24.5
Mean	61.6	4.57	486.69	73.32
L.S.D at 0.05%	6.73	0.259	19.196	4.069

Yield and yield components:

Data in Table 4 indicated that yield components of tomato plants, *i.e*. Number of fruits/plant, weight of one fruit, fruits yield/plant (kg) and fruits yield/plant (ton/fed) were better than untreated soil control-2. The fungicide application and biofertilizer significantly increased yield and yield components of tomato plants. Tomato plants inoculation with *A. brasilense* combined with *T. harzianum*, individually showed remarkable increases in yield and yield components, compared to any other microorganisms as well as untreated (control-2). In this respect, the highest values of yield and yield components of tomato plants, *i.e*. Number of fruits/plant, weight of one fruit (g), fruits yield/plant (kg) and fruits yield/plant (ton/fed) were observed in the treatment of *A. brasilense + T. harzianum* by about (14.8/plant, 113.4g, 2.07kg and 25.13 ton/fed) respectively.

Microbiological determinations:

Data presented in Table 5 show that, the control-2 treatment recorded the lowest values of dehydrogenase (DHA) and N_2-ase activity compared to other treatments and control. Dual inoculation of tomato transplants with *A. brasilense + T. harzianum*, showed higher values of dehydrogenase (DHA) and nitrogenase (N_2-ase) activity in tomato rhizosphere vegetative and flowering stages activity than individual inoculation. This result could be attributed to the synergistic effect in case of dual inoculation. Data also illustrated that, the highest values of (DHA) and (N_2-ase) at flowering stage activity were observed by treatment with *A. brasilense + T. harzianum*.

Chemical analysis:

Data in Table 6 reveal that, untreated soil control-2 gave the lowest values of total phenols in shoots of tomato plants. Fungicide application and biofertilizer remarkably increased the total phenols content in tomato shoots compared to treatment with *A. brasilense* or biocontrol agents

either individually or together. Dual inoculation with *A. brasilense* and biocontrol agents significantly increased the total phenol content in comparison with individual inoculation. Treated with *A. brasilense* + *T. harzianum*, gave the higher value of total phenols content in tomato shoots.

Also, the presented data in Table 6 indicated that, untreated soil (control-2) gave the lowest values of total soluble solids (T.S.S.), in tomato fruits. Dual inoculation with *A. brasilense* and biocontrol agents significantly increased abovementioned parameters compared to individual inoculation. The highest value of total soluble solids (T.S.S.), in tomato fruits were observed in the treatment with *A. brasilense* + *T. harzianum*. In this respect, the present study submitted sufficient evidence to how important recommendation of use the mixture of antifungal strains of *T. harzianum, T. viride, P. fluorescence, B. ceris* and Monecern 25% in combination with dinitrogen fixers of *A. brasilense* is successful biocontrol agent against soil-borne pathogens of wilting diseases of tomato.

Table 4 Effect of biological control and biofertilizer on yield and yield components of tomato plants (cv. Super-strain B) under field conditions.

Treatments	Measure			
	Number of fruits/plant	**Weight of one fruit (g)**	**Fruits yield/plant (kg)**	**Fruits yield (ton/fed)**
T. harzianum + A. brasilense	14.8	113.4	2.07	25.13
T. viride + A. brasilense	14.13	106.07	2.0	24.33
P. fluorescence + A. brasilense	14.03	102.7	1.92	23.6
B. ceris + A. brasilense	13.42	101.4	1.91	23.5
Control-1 (fertilized and natural infection)	13.4	100.2	1.63	23.34
Monecern (25% Pencycuron) + *A. brasilense*	13.19	97.9	1.6	23.14
T. harzianum	13.12	97.3	1.58	23.0
T. viride	11.76	94.3	1.56	22.22
P. fluorescence	11.71	92.5	1.48	22.08
B. ceris	11.36	89.1	1.47	21.55
Monecern (25% Pencycuron)	11.11	86.4	1.48	21.29
Control-2 (unfertilized and natural infection)	7.29	31.2	1.46	12.5
Mean	12.44	92.7	1.63	22.15
L.S.D at 0.05%	2.66	9.44	0.343	0.866

Table 5 Effect of biological control and biofertilizer on dehydrogenase and nitrogenase activity of tomato plants (cv. Super-strain B) under field conditions.

Treatments	Enzyme activity			
	Dehydrogenase activity µg TPF/g dry soil/day		**N2-ase activity (nmoles C_6H_2/g dry soil/hr)**	
	Vegetative stage	**Flowering stage**	**Vegetative stage**	**Flowering stage**
T. harzianum + A. brasilense	35.17	94.3	90.7	365.3
T. viride + A. brasilense	34.2	85.87	88.9	363.2
P. fluorescence + A. brasilense	33.6	84.6	87.8	355.9
B. ceris + A. brasilense	32.67	83.2	87.2	352.2

Table 5 Continued.

Treatments	Enzyme activity			
	Dehydrogenase activity µg TPF/g dry soil/day		N2-ase activity (nmoles C_6H_2/g dry soil/hr)	
	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
Control-1 (fertilized and natural infection)	29.8	82.9	87.0	308.2
Monecern (25% Pencycuron) + *A. brasilense*	29.27	82.4	86.1	301.5
T. harzianum	26.4	80.2	85.8	298.2
T. viride	26.2	72.4	85.4	290.8
P. fluorescence	25.6	65.9	75.9	283.8
B. ceris	17.07	18.8	42.8	175.8
Monecern (25% Pencycuron)	13.47	16.3	40.7	155.6
Control-2 (unfertilized and natural infection)	12.13	15.4	35.2	145.8
Mean	26.3	65.19	74.46	283.0
L.S.D at 0.05%	0.720	10.12	0.307	10.2

Table 6 Effect of biological control and biofertilization on phenols and total soluble solids in tomato fruits (cv. Super-strain B) under field conditions.

Treatments	Phenols mg/g fresh weight and total soluble solids (TSS)			
	Total phenol	Free phenol	Conjugated phenol	total soluble solids (TSS)
T. harzianum + A. brasilense	15.7	6.4	9.16	5.63
T. viride + A. brasilense	15.3	6.3	8.98	5.6
P. fluorescence + A. brasilense	14.95	6.2	8.93	5.58
B. ceris + A. brasilense	14.92	6.16	8.89	5.55
Control-1 (fertilized and natural infection)	14.6	6.02	8.87	5.45
Monecern (25% Pencycuron) + *A. brasilense*	14.5	5.87	8.83	5.44
T. harzianum	14.3	5.84	8.74	5.35
T. viride	14.0	5.73	8.6	5.3
P. fluorescence	13.9	5.59	8.56	5.25
B. ceris	12.0	5.43	7.95	5.22
Monecern (25% Pencycuron)	11.8	5.31	7.72	5.1
Control-2 (unfertilized and natural infection)	9.6	3.42	4.53	4.5
Mean	13.80	5.69	8.31	5.33
L.S.D at 0.05%	0.327	0.333	0.356	0.436

Discussion

During the course of this investigation, eight fungal were isolated from the roots and stems under naturally infected wilted plants obtained from locations belonging to 8 Egyptian Governorates, all fungal isolates of *Fusarium oxysporum* had the potentiality to infect tomato plants although they were varied in their pathogenicity from weakly to highly pathogenic. In this respect,

isolate obtained from El-khatatba gave the most virulent of isolate. These results are in partial agreement with other investigator working on tomato Fusarium wilt (Kraft & Haglund 1978, Hart & Endo 1981, El-kazzaz et al. 2008). On the other hand, El-kazzaz et al. 2008 reported that, the high-pathogenic Fusarium isolates which caused Fusarium wilt in their differentiation belong to *F. oxysporum* f.sp *lycopersici*. Saleh et al. 2016 in their study reported that, eight different isolates of *F. oxysporum* were isolated from naturally infected tomato plants collected from some Egyptian Governorates *i.e*. Behera, Minofiya, Ismailia and Minia and investigated and identified as *F. oxysporum* f.sp *lycopersici*. Pathogenicity test revealed El-khatatba (Minofiya Gov.) isolate was the most virulent on tomato Super-strain B cultivar.

Under greenhouse conditions, evaluation capability of isolates *i.e*. (*T. harzianum, T. viride, P. florescence,* and *B. ceris*) and Monecern 25% (Pencycuron) fungicide on disease severity of tomato plants (Super-strain B cultivar) to infection with the most virulent of isolate the results indicated that, *T. harazianum* gave the best control of the disease and reduced disease severity compared with control. Previous results are in agreement with Datroff et al. (1995), Bowers & Parke (1993), Kim et al. (1997) and Mao et al. (1997) who's found *Trichoderma* spp. particularly *T. harzianum* have been shown to be effective of tomato. *Bacillus subtilis* has also been shown to suppress diseases caused by *Pythium* spp., *Rhizoctonia solani* and *Fusarium* spp. Numerous fungi and bacteria are known for their antagonistic activity to soil borne pathogens and could be utilized as biocontrol agents against *Fusarium* wilt disease (Cook 1993). A significant increase in plant growth and development has been noticed to be associated with utilizing biocontrol agents (Baker et al. 1984, Chang et al. 1986, Hassan 1992, Linderman 1994, Ousley et al. 1994). Moreover, Gupta et al. (1995) demonstrarted that, *Azotobacter chrococcum, Azospirillum* spp. and *Pseudomonas fluorescence* which isolated from rhizosphere of tomato plants and used to inoculate seeds and roots in greenhouse increased seedling emergence rate and reduced disease incidence and severity of damping-off of tomato seedling. In addition, results were obtained by Jacobsen et al. (2004) who found that *Trichoderma*, *Pseudomonas* and *Bacillus* species. *Bacillus*-based biocontrol agents are quite important in the management of pests and plant diseases. Zaghloul et al. (2008, 2015) found that *Trichoderma harzianum, Pseudomonas fluorescence* and *Bacillus subtilis* were the bioagent controlling *Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium oxysporium F*. spp. *lycopersici*. Khalifa (1991) showed that, *Trichoderma harzianum,* as a biological control against suppressed the growth of *Fusarium oxysporium F*. spp. *lycopersici* decreased the population of *F. oxysporium F*. spp. *lycopersici* up to the 4th week after transplanting of tomato. Ghonim (1999) reported that, treatment of tomato seeds with *B. subtilis* reduced tomato wilting disease severity caused by *F. oxysporum F*. spp. *lycopersici*. *B. subtilis* application improved some growth parameters. Also, biofertilizers are which were used these microorganisms such as *Azotobacter* spp., *Azospirillum* spp., *Bacillus polymyxa* successfully and for increasing the yield and improved the quality of many crops when applied (Aguilar et al. 1996, Saikia et al. 2013, Sahoo et al. 2014).

In this trial, application of *T. harzianum + A. brasilense***,** *T. viride***+** *A. brasilense***,** *P. fluorescence* **+** *A. brasilense, B. ceris* **+** *A. brasilense, A. brasilense* (control-1), Monecern (25% Pencycuron) + *A. brasilense*, *T. harzianum, T. viride, P. fluorescence, B. ceris* and Monecern (25% Pencycuron) to artificially infested soil of the most virulent of isolate decreased disease severity by different degrees, respectively compared with control. As for the tested other treatments + *A. brasilense*, to natural infection with *F. oxysporum* in field, were produced the lowest percentage of disease severity. Results, obtained by Larkin & Fravel (1998) who found that treatment tomato seedlings (using dip procedure method) with biocontrol agent including non-pathogenic *Fusarium* spp, *Trichoderma* spp., *Gliocldium virens*, *Pseudomonas florescence* and *Burkholderia cepacia* before transplanting in infested soil with *F. oxysporum* f.sp *lycopersici* reduced infection by 30 to 60%.Also results indicated that dual inoculation with *T. harzianum* alone or inoculation with *A. brasilense* with combined *T. harzianum*, gave lower percentage of disease severity. In the respect, *A. brasilense* inoculation in combination with *T. harzianum*, gave higher records of growth characters, *i.e*. plant height (cm), number of branches per plant, plant fresh weight and plant dry weight g/plant, these are in harmony with those reported by Barakat & Gabr (1998) who found that

inoculation of tomato transplants with *A.chroococcum, Azospirillum* sp. and *B. polymyxa* as single or mixed biofertilizers significantly increased the growth characters of tomato, Sanhita et al. 1995, Aguilar et al. (1996) and Kennedy et al. (2004) found that inoculation of tomato roots with *A. chroococcum, B. subtilis* and *P. fluorescence* significantly increased plant growth parameters and increased the total dry weight. Also, these findings are in agreement with Niknejad et al. (2000) and Tsahouridou & Thanassoulopoulos (2002) found that using the antagonist *T. harzianum* increased plant growth characters of tomato and Bashan et al. (2004) observed that *Azospirillum* inoculation can change the root morphology via producing plant growth regulating substance.

On the other hand, the highest values of yield and yield components of tomato plants, *i.e.* Number of fruits/plant, weight of one fruit (g), fruits yield/plant (kg) and fruits yield/plant (ton/fed) were observed in the treatment of inoculation with *A. brasilense* combined with *T. harzianum*. The authors concluded that *Azospirillum* was shown to exert beneficial effects on plant growth and crop yields both in greenhouse and in field trials this results obtained by Barakat & Gabr (1998) who indicated that, the application of N fertilizer combined with inoculation by *Azotobacter* sp, *Azospirillum* sp. and *Klebsiella* sp. alones as single biofertilizers or together increased number of fruits/ plant and the total yield/fed of tomato plants. While, Fang & Zhang (1990) and Niknejad et al. (2000) reported that, application of the selected antagonists (*B. subtilis, Pseudomonas* sp, *T. harzianum*) significantly increased number of fruits per plant, weight of fruits and total yield of tomato fruits. Also, Kennedy et al. (2004) found that, the application of biofertilizer (*Azospirillum, Azotobacter* and *Bacillus* sp.) significantly increased tomato fruits and total yield/fed compared with control, Saikia et al. (2013) and Sahoo et al. (2014) who's observed that *Azospirillum* including *A. lipoferum, A. brasilense, A. amazonense, A. halopraeferens* and *A. irakense* led to improve productivity of various crops.

In this study, results concluded that, the highest values of dehydrogenase (DHA) and nitrogenase (N_2-ase) at flowering stage activity were observed in case of inoculation with *A. brasilense* with combined *T. harzianum*. The higher activity of DHA and N_2-ase at flowering stage is likely be due to the difference in multiplication rate of different soil microorganisms which usually be maximum during flowering stage. Previous results are in agreement with the findings of Ravikumar et al. (2004) who found that non symbiotic N_2- fixers such as *A. chroococcum* and *Azospirillum* sp increased the nitrogenase and dehydrogenase activity over non-inoculated control. Also, Song (1990) and Kennedy et al. (2004) reported that inorganic N-fertilizers application decreased the dehydrogenase and nitrogenase activity compared to biofertilization with associative diazotrohps. Likewise, inoculation with *A. brasilense* and biocontrol agents gave higher records of total phenols content in tomato shoots. These results are in harmony with Ibrahim (2000) who found that positive correlation between level of phenols and root-rot and wilting infection caused by *S. rolfsii, R. solani* and *Fusarium* spp. in tomato. Many of these compounds exhibit antifungal properties. Therefore, phenols might play an important role in disease resistance and the highest records of total soluble solids (T.S.S.), in tomato fruits, these results are in harmony with George et al. (2004) who found that ascorbic acid change according to maturity of the fruits. Also, they found that fruit chemical content total soluble solids were affected by using biofertilizers, biological control agents and fungicides either individually or in combination.

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