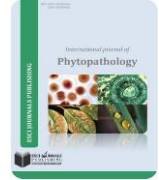




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ENHANCEMENT OF GROWTH PARAMETERS AND YIELD COMPONENTS IN EGGPLANT USING ANTAGONISM OF TRICHODERMA SPP. AGAINST FUSARIUM WILT DISEASE

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ABSTRACT

Eggplant is one of the important economic vegetable crop which is attacked by several serious diseases such as wilt. *Fusarium oxysporum* f. sp. *melongenae* was isolated from a naturally occurring epidemic of wilt in eggplant plants grown in New Valley governorate. In this study, the antagonistic activity of five *Trichoderma* species (*Trichoderma spirale*, *T. hamatum*, *T. polysporium*, *T. harzianum* and *T. viride*) against *F. oxysporum* f. sp. *melongenae* was evaluated using dual culture technique. *T. viride* (isolate TVM-5) and *T. hamatum* (isolate THM-2) showed the highest antagonistic activity, while *T. spirale* (TSM-1) was the lowest one. In pot experiment, the obtained data showed that all *Trichoderma* species reduced significantly area under wilt progress curve caused by *F. oxysporum* f. sp. *melongenae*. *Trichoderma viride* and *T. hamatum* recorded the highest reduction of area under wilt progress curve (AUWPC) (244.0 and 325.33 AUWPC as compared to 1125.33 in control treatment, respectively). Under field conditions results showed that, these treatments significantly reduced AUWPC and increased all tested plant growth parameters (Plant height, No. of branches plant⁻¹) and fruit yield components (number of fruits plant⁻¹, fruits yield plant⁻¹, fruit weight, No of fruit Kg⁻¹, fruit length, fruit diameters and fruits yield fed.⁻¹) compared with control during growing seasons (2011-2012 and 2012-2013). *Trichoderma viride* and *T. hamatum* were the best biocontrol agents as manifested by the significant reduction in both disease severity and increase plant growth parameters and fruit yield components.

Keywords: Eggplant, *Trichoderma* species, wilt disease, plant growth parameters, biological control.

INTRODUCTION

The Eggplant, Aubergine or Brinjal (*Solanum melongena* L.), of the family Solanaceae, is grown in the subtropical and tropical regions of the world. It is one of the most common, highly productive and popular vegetable crops grown in Egypt. It is quite popular as the poor man's crop (Gargi Chakravarty and Kalita, 2012). The unripe fruit of eggplant is primarily used as a cooking vegetable for the various dishes in many countries in the world. The eggplant is also reported to possess medicinal properties. Various plant parts are used for curing ailments such as diabetes, cholera, bronchitis, dysuria, dysentery, otitis, toothache, skin infections, asthenia and haemorrhoids. It is also ascribed narcotic, anti-asthmatic

and antirheumatic properties (Daunay *et al.*, 2003).

The major constraint, however, in the production of eggplant is the Fusarium wilt disease. *Fusarium oxysporum* f. sp. *melongenae* (Fomg) is the most destructive pathogen of Fusarium wilt of eggplant. The soil-borne fungus invades the vascular bundles, causes severe wilting and death of the above ground parts of plants by blocking the xylem transport system (Altınok, 2005). It is extremely difficult to control soil-borne fungi using conventional method such as the use of synthetic fungicides. Since their spores are able to survive for many years in the soil, biological control strategies for this pathogen should, therefore, be carefully selected and handled in an eco-friendly way instead of using chemical fungicides.

The application of microorganisms as biocontrol agents is important, since they may increase beneficial

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microbial activity which extends for a long period of time. *Trichoderma* spp. are considered as potential biocontrol and plant growth promoting agents for many crop plants (Verma *et al.*, 2007; Bai *et al.*, 2008; Savazzini *et al.*, 2009). The competition with pathogens, parasitism and the production of antifungal compounds are the most important mechanisms in biocontrol activity (Verma *et al.*, 2007; Savazzini *et al.*, 2009). *Trichoderma* populations can be established relatively easily in different types of soil and can continue to persist at detectable levels for months.

In the above context, the present study was undertaken to isolate the eggplant vascular wilt pathogen and evaluate the biocontrol potential of indigenous isolates of *Trichoderma* spp. applied by soil drench to control the disease under pots and field conditions. Also, the effect of *Trichoderma* spp. on growth parameters and fruit yield attributes were evaluated under field conditions.

MATERIALS AND METHODS

Pathogen Isolation: Eggplant plants showing wilt symptoms were collected from different fields at flowering stage growing in El-Kharga Oozes- New Valley, Egypt. Small pieces of diseased specimen were grown on *Fusarium* selective medium (Nash and Snyder, 1965). After purifications, isolates were identified according to morphological characteristics with the help of standard

$$\% \text{ Inhibition} = \frac{\text{Dia. of colony growth in control} - \text{Dia. of colony growth in treatment}}{\text{Dia. of colony growth in control}} \times 100$$

Evaluation of *Trichoderma* Isolates against *Fusarium* Wilt Disease in Greenhouse

Preparation of Inocula of *F. oxysporum* and *Trichoderma* Isolates: In order to prepare *F. oxysporum* f. sp. *melongenae* inocula, Erlenmeyer flasks containing 100 g of barley and 100 ml of water were autoclaved at 121 °C for 1 h on three successive days. After cooling, about 5-7 small plugs of seven- day- old culture of *F. oxysporum* f. sp. *melongenae* were dropped into each flask under sterilized condition. The flasks were kept at 25 °C for 4 weeks. Colonized barley grains were then transferred into paper pockets, and were dried and ground. Fourteen g of prepared powder was used to infest 1 Kg of soil (Frommel *et al.*, 1991). For preparation of *Trichoderma* inocula moistened wheat bran was poured into Erlenmeyer flasks which were autoclaved at 121 °C for 1 h on three successive days. The substrate mixture was then inoculated with a homogenized suspension of spore + mycelia of seven days old culture

key (Nelson *et al.*, 1983). Pathogenicity tests were conducted on potted plants and after reisolation, a pathogenic isolate of *Fusarium oxysporum* was selected for further studies.

Sources of *Trichoderma* Isolates: Isolation of *Trichoderma* spp. from soil was done following the technique used by Rifai (1969). For this purpose soil samples were collected from potato root rhizosphere (20 cm. deep) of different fields. Twenty g of each soil sample were gently mixed with 500 ml distilled water containing 0.2% citric acid, five ml of prepared solution were added to Petri plates containing 15 ml water agar at 50 °C and shaken to mix properly. After solidification, five mm plugs of these cultures were transferred into Petri plates containing Davet selective medium (Davet, 1979) and were incubated at 25 °C. After proper growth, isolates were purified and identified according to standard keys (Bissett, 1991).

Antagonistic activity: Five mm plugs of seven-day-old cultures of *F. oxysporum* f. sp. *melongenae* and *Trichoderma* were placed against each other on plates containing PDA. In case of control instead of *Fusarium oxysporum* plugs, PDA plugs were used each treatment was triplicated. Plates were incubated at 25 °C for 7 days and the% of radial growth rate inhibition were calculated by the following formula:

of *Trichoderma* isolates under aseptic conditions. Erlenmeyer flasks were incubated at 27 °C for 14 days. Ten g of this inoculum ($10^5 - 10^7$ CFU) was used to add to 1 Kg of pot soil (Ommati and Zaker, 2012). Surface sterilized eggplant transplanting (cv. Black Beauty) were grown in pots. All of the *Trichoderma* isolates performing well in lab. tests, were used in this experiment. Five seedlings per treatment were sown in plastic pot (30 cm in dim.), and four pots were used for each treatment as replicates. In control treatment, eggplant seedlings were planted in infested soil only and area under wilt progress curve was recorded.

Disease Assessments: Wilt severity was estimated at 10 days interval for 60 days after transplanting according to Abdou *et al.* (2001) using a rating scale of (0 - 5) based on leaf yellowing grading, viz., 0 = healthy, 1= one leaf yellowing 2= more than one leaf yellowing, 3= one wilted leaf, 4= more than one leaf wilted, and 5= completely dead plants. Disease severity index (DSI)

described by Liu *et al.* (1995) was adapted and calculated as follows:

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

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

The mean of area under disease progress curve (AUDPC) for each replicate was calculated as suggested by Pandey *et al.* (1989).

$$AUDPC = D [1/2 (Y_1 + Y_k) + (Y_2 + Y_3 + \dots + Y_{k-1})]$$

Where D= Time interval; Y₁= First disease severity; Y_k= Last disease severity; Y₂, Y₃,.....Y_{k-1}= Intermediate disease severity.

Field Experiments: Field experiments were carried out at New Valley Agric. Res. Station Farm, New Valley governorate during 2011-2012 and 2012-2013 seasons, to evaluate the efficiency of the tested *Trichoderma* spp. as Bio-control agents for controlling wilt disease of eggplant plants as well as its effect on growth parameters and fruit yield components. The chosen field test area was naturally infested with *F. oxysporum*. The experimental design was a complete randomized block with four replicates. The experimental unit area was 15 m² (5 × 3 m). Each unit included three rows; each row was 5 m in length and 1 m width. Soil treatments were done by applied 150 g of the prepared formulation/plot at the same time of planting. Eggplant seedlings (cv. Black Beauty) transplanted into the field in 1 October in both seasons at rate 10 seedlings per row; one seedling/hill was sown with 50 cm apart between hills. Untreated seedlings were used as control. The NPK mineral fertilizers were applied at the recommended dose of Ministry of Agriculture and Land Reclamation. Disease severity was recorded every 30 days for 4 months. The mean of area under disease progress curve (AUDPC) for each replicate was calculated as above. Plant height, number of branches, number of fruits plant⁻¹, fruits yield plant⁻¹ (Kg), fruit weight (gm), No of fruit Kg⁻¹, fruit length (cm), fruit diameter (cm) and the estimated fruits yield fed.⁻¹ (ton) were calculated at the end of the growing season.

Statistical Analysis: All experiments were performed twice. Analyses of variance were done using MSTAT-C program version 2.10 (1991). Least significant difference (LSD) was calculated at P ≤ 0.05 according to Gomez and Gomez (1984).

RESULTS

Isolation, Purification and Identification of the Fungi

Associated with Eggplant Diseased Plants: Nine fungal isolates were isolated from eggplant plants collected from different locations in New Valley, governorate that show wilt symptoms. Hyphal tip cultures of grown fungi were maintained on PDA medium. All fungi were purified using single spore technique cultures, then they were identified. Results indicate that all isolated fungi which identified as *F. oxysporum* f. sp. *Melongenae*.

Pathogenicity Test: The first sign of wilting on eggplant appeared around 40 days after inoculation and gradually intensified. Lower leaves developed the wilting first, then extended to the upper leaves. Vascular discoloration was evident from the early stages of infection, extending, upward throughout the plant. Data present in Fig (1) show that the most virulent isolates against eggplant plants were *F. oxysporum* f. sp. *melongenae* isolate F03 (77.23%) and isolate F07 (72.36%). While isolates F05 and F08 recoded the lowest virulent ones (25.45 and 36.36%, respectively).

In Vitro Evaluation of Antagonism of Trichoderma spp. Against F. oxysporum f. sp. melongenae: Under *in-vitro* conditions all the antagonists *Trichoderma* species i.e. *Trichoderma spirale*, *T. hamatum*, *T. polysporium*, *T. harzianum* and *T. viride* inhibited mycelial growth of *F. o. melongenae* ranged between 43.58 and 56.25 % as compared to control (Fig. 2). *T. viride* isolate TVM-5 and *T. harzianum* isolate THM-4 were found to be the most potent antagonistic *F. oxysporum* f. sp. *melongenae*, whereas inhibited the mycelia growth with 76.25 and 69.36%, respectively. On the other hand, *T. spirale* (TSM-1) recoded the lowest inhibition of *F. o. f. sp. melongenae* (43.58%).

Effect of Soil Treatment with Trichoderma species on Wilt Disease under Greenhouse Conditions: A pot experiment was carried out to examine the efficiency of *Trichoderma* spp. to antagonize *F. oxysporum* f. sp. *melongenae* under greenhouse conditions. Data presented in fig. 3 reveal that all *Trichoderma* spp. significantly decreased area under wilt progress curve (AUWPC) compared with control.

T. viride was the most effective one for reducing UDWPC from 1125.33 in control to 244.0 AUWPC followed by and *T. hamatum* (325.33 UDWPC). While *Trichoderma spirale* (TSM-1) and *T. polysporium* (TPM-3) recoded the lowest reduction for AUWPC caused by *F. oxysporum* f. sp. *melongenae* (685.36 and 845.74 AUWPC, respectively).

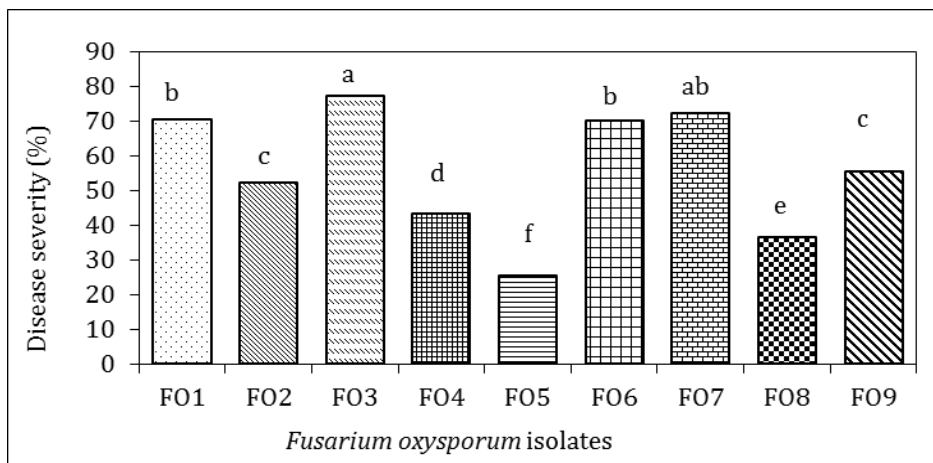


Figure 1. Pathogenicity tests of *Fusarium oxysporum* f. sp. *melongenae* isolates collected from different location to eggplant cv. Black Beauty. Different letters indicate significant differences between eggplant *Fusarium oxysporum* f. sp. *melongenae* isolates according to LSD test ($P \leq 0.05$).

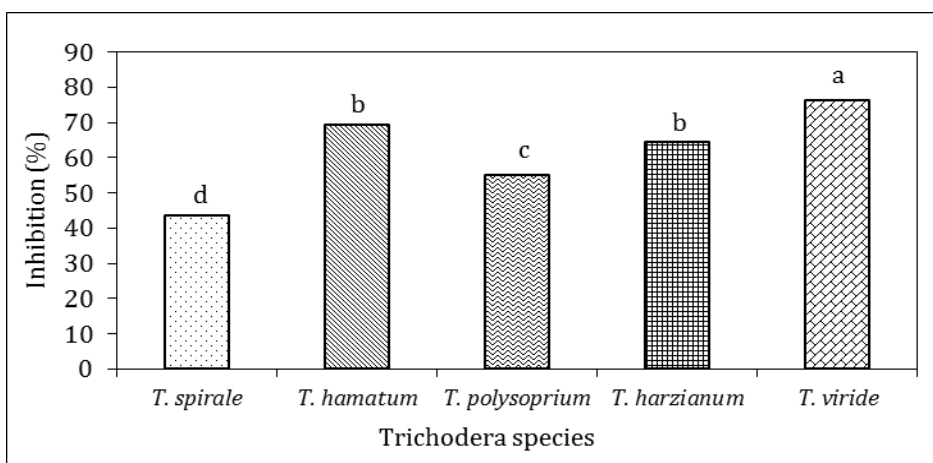


Figure 2. Antagonistic activity of *Trichoderma* spp. against growth of *Fusarium oxysporum* f. sp. *melongenae* *in vitro*. Values in the column followed by different letters indicate significant differences among treatments according to LSD test ($P \leq 0.05$).

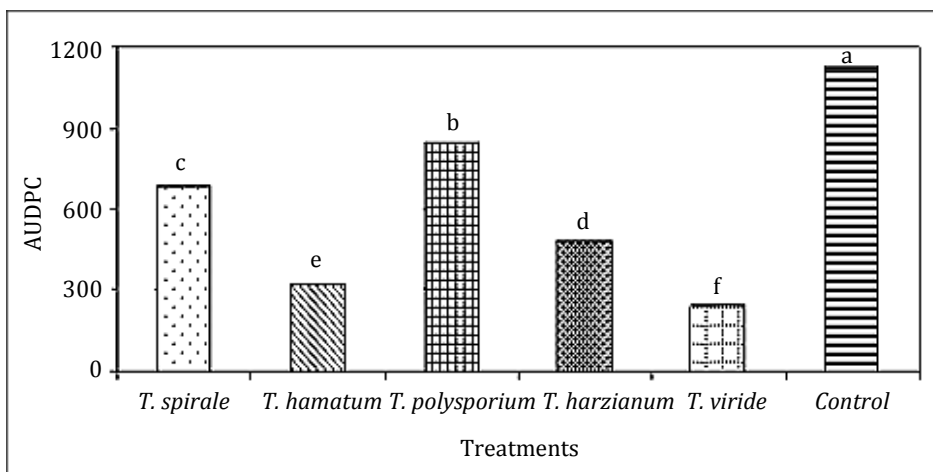


Figure 3. Effect of soil treatment with formulated *Trichoderma* species on Area under wilt progress curve of eggplant (cv. Black Beauty) under pot experiments. Values in the column followed by different letters indicate significant differences among treatments according to LSD test ($P \leq 0.05$).

Effect of Trichoderma species on Wilt Decease under Field Conditions: Effects of *Trichoderma* spp. on wilt disease incidence, some growth parameters, yield components of eggplant plants under field conditions in New Valley governorate was studied.

A) Effect of Trichoderma Species on Area under Disease Progress Curve: Data in Fig. 4 indicate that all *Trichoderma* species exhibit significant protection against wilt disease compared with control in both growing seasons (2011-2012 and 2012-2013). However, the most effective *Trichoderma* species were *T. viride* (159.36 and 163.58 AUWPC) followed by and *T. hamatum* (221.36 and 253.67 AUWPC) during both growing seasons, respectively. Conversely, *Trichoderma spirale* and *T. polysoprium* showed the lowest protection against wilt disease which recorded the AUWPC from 956.38 and 989.36 to 422.0, 489.36 and 658.74, 715.69 AUWPC in first and second growing seasons, respectively.

B) Effect on Growth Parameters: All the tested *Trichoderma* species significantly increased growth parameters i.e. plant height and branches number per plant compared with control treatment in both growing seasons (Table 1). *T. viride* was the most effective one for increasing plant height from 66.26 and 65.48 cm in control to 93.06 and 92.36 cm followed by *T. hamatum* (67.66, 67.67 cm in first and second growing seasons, respectively).

Conversely, effect of *T. polysoprium* for increasing of plant height was lower compared with the others. The same trend was also observed in case of number of branches plant⁻¹, while *T. viride* recorded the highest branches number plant⁻¹ (22.35 and 23.25 branch plant⁻¹) followed by *T. hamatum* (20.22 and 19.36), however *T.*

polysoprium recorded the lowest one in first and second growing seasons, respectively.

C) Effect on yield components: Data in Table (2) revealed a decrease in the yield components of eggplant plants, i.e. number of fruits plant⁻¹, fruits yield plant⁻¹, fruit weight, No of fruit Kg⁻¹, fruit length, fruit diameters and the estimated fruits yield fed.⁻¹ of control treatment. However, significant increase was determined with the *Trichoderma* species treatments. The most effective *Trichoderma* species were *T. viride* for all fruit parameters, number of fruit plant⁻¹ (14.73 and 14.89), fruit yield plant-1 (4.27 and 4.19 Kg), fruit weight (289.88 and 281.47 gm), No. of fruit Kg-1 (3.45 and 3.55), fruit length (13.44 and 13.24 cm), fruit diameter (12.67 and 11.97 cm) and total yield fed. -1 (26.59 and 25.49 ton) compared with 9.57 and 8.75, 2.29 and 2.08, 239.6 and 241.25, 4.17 and 4.15, 7.28 and 7.05, 7.1 and 6.80, 12.65 and 11.25 in control treatment in both seasons, respectively. *Trichoderma hamatum* came after of *T. viride* in increased all fruit parameters in both growing seasons. On the other hand, *T. harzianum* and *T. spirale* recoded the lowest increased of all fruit parameters in this respect during both growing seasons.

DISCUSSION

Many soil borne fungi play a major role in causing several diseases such as damping-off, root-rot, seed decay, collar rot, crown rot and wilt, etc. eggplant, an important vegetable crop is attacked by several diseases, mostly caused by fungi and bacteria leading to severe crop losses. Among the fungal diseases, the wilt incited by *Fusarium oxysporum* f. sp. *melongenae* (Fomg) is a major constraint in the production of eggplant under greenhouse and fields (Altınok, 2005 and Baysal *et al.*, 2013).

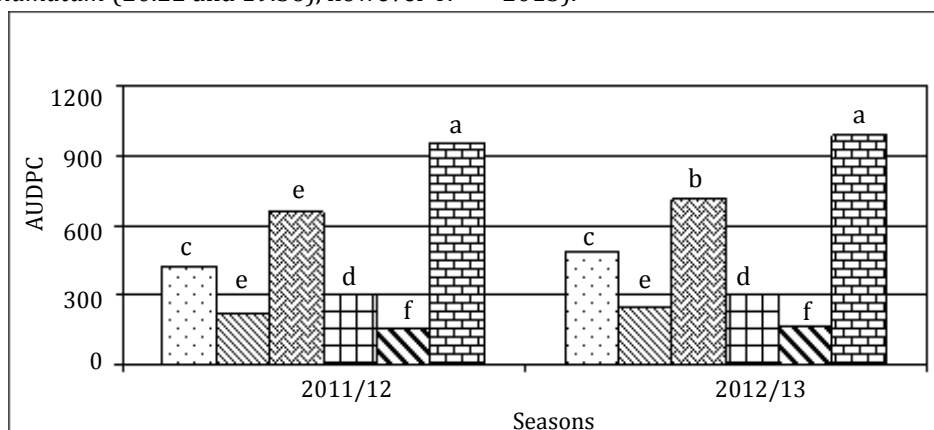


Figure 4. Effect of soil treatment with formulated *Trichoderma* species on area under wilt progress curve (AUWPC) of eggplant (cv. Black Beauty) under field conditions during seasons 2011-2013 and 2012-2013. Values in the column followed by different letters indicate significant differences among treatments according to LSD test ($P \leq 0.05$).

Table 1. Effect of soil treatment with formulated *Trichoderma* species on plant height and branch numbers plant⁻¹ of eggplant (cv. Black Beauty) under field conditions during 2011-12 and 2012-13 growing seasons.

Treatments	Season 2011-2012		Season 2012-2013	
	Plant height (cm)	Branch numbers plant ⁻¹	Plant height (cm)	Branch numbers plant ⁻¹
<i>T. spirale</i>	86.00 b	21.22 a	85.14 b	20.02 b
<i>T. hamatum</i>	91.33 a	20.22 ab	93.63 a	19.36 b
<i>T. polysoprium</i>	78.66 c	15.56 bc	75.14 c	13.25 c
<i>T. harzianum</i>	81.34 c	20.22 ab	82.36 b	18.24 b
<i>T. viride</i>	93.06 a	22.35 a	92.36 a	23.25 a
Control	66.26 d	15.22 c	65.48 d	13.18

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

Table 2. Effect of soil treatment with formulated *Trichoderma* species on fruit yield components of eggplant (cv. Black Beauty) under field conditions during 2011-2012 and 2012-2013 growing seasons.

Season 2012-2013							
Treatments	No. of fruits plant ⁻¹	Fruit yield plant ⁻¹	Fruit weight (gm)	No. of fruit Kg ⁻¹	Fruit length (cm)	Fruit diameter (cm)	Total fruit yield fed. ⁻¹ (Ton)
<i>T. spirale</i>	11.04 cd	2.89 d	262.60 bc	3.81 abc	9.86 c	9.33 bc	20.59 b
<i>T. hamatum</i>	14.73 b	4.27 b	289.90 a	3.45 c	13.44 a	12.67 a	26.59 a
<i>T. polysoprium</i>	12.60 bc	3.28 c	260.60 bc	3.84 abc	8.33 de	8.55 cd	17.51 bc
<i>T. harzianum</i>	10.08 d	2.53 e	251.30 cd	3.98 ab	9.15 cd	9.52 bc	14.87 cd
<i>T. viride</i>	18.21 a	5.01 a	275.10 ab	3.64 bc	11.62 b	10.90 ab	24.94 a
Control	9.57 d	2.29 e	239.60 d	4.17 a	7.28 e	7.10 d	12.65 d
Season 2012-2013							
<i>T. spirale</i>	10.08 bc	2.75 d	253.70 bc	3.94 ab	8.36 c	9.01 cd	19.36 bc
<i>T. hamatum</i>	14.89 a	4.19 b	281.50 a	3.55 c	13.24 a	11.97 a	25.49 a
<i>T. polysoprium</i>	11.48 b	3.19 c	251.00 bc	3.98 ab	8.08 cd	8.34 d	15.69 cd
<i>T. harzianum</i>	11.39 b	2.49 de	248.00 c	4.03 a	9.01 c	9.28 bc	14.05 de
<i>T. viride</i>	16.25 a	5.01 a	264.30 b	3.78 bc	10.99 b	9.89 b	22.47 ab
Control	8.75 c	2.08 e	241.30 c	4.15 a	7.05 d	6.80 e	11.25 e

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

During this investigation, nine *Fusarium oxysporum* f. sp. *melongenae* isolates were isolated from eggplant roots collected from different locations in New Valley governorate. In pathogenicity tests, all the isolates were pathogenic to eggplant with different degrees of disease severity. These results are in agreement with those reported by Altınok, 2005; Baysal *et al.*, 2013.

The management of this disease is difficult owing to long saprophytic survival ability of pathogen in soil (Dey, 2005). Control of the plant diseases by chemicals can be spectacular but this is relatively a short term and moreover, the accumulation of the harmful chemical residues sometimes causes serious ecological problem. In recent years, the increasing use of potentially hazardous pesticides and fungicides in agriculture has been the result of growing concern of both environmentalists and public health authorities.

Biological methods can be economical, long lasting and free from residual side effects and safe on human and animal health. The main purpose of the biological

control of the plant disease is to suppress the inoculum load of the target pathogen to a level, which would not cause potential economic loss in a crop. Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from the soil. The potential of *Trichoderma* species as biocontrol agents against various plant diseases has been reported by several workers (Verma *et al.*, 2007; El-Nagdi and Abdel-Khair, 2008; Bai *et al.*, 2008; Savazzini *et al.*, 2009; Joshi *et al.*, 2010 and Sundaramoorthy and Balabaskar, 2013). In this study, five *Trichoderma* species viz. *Trichoderma spirale*, *T. hamatum*, *T. polysoprium*, *T. harzianum* and *T. viride* were evaluated *in vitro* and *in vivo*. The obtained data indicated that all *Trichoderma* spp. suppressed mycelia growth of *F. oxysporum* f. sp. *melongenae* *in vitro*. *Trichoderma viride* (TVM-5) and *T. harzianum* (THM-4) were found to be the most potent antagonistic against the pathogen. The inhibition of *Fusarium oxysporum* f. sp. *melongenae* by *Trichoderma* species could probably be due to the secretion of extracellular cell degrading

enzymes such as chitinase β -1, 3- glucanase, which help mycoparasites in the colonization of their host. The inhibition of pathogen may be also being attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin. (Kamlesh and Gurjar, 2002; Muhammad and Amusa, 2003; Rehman *et al.*, 2013). The secondary metabolites of *Trichoderma* includes chitinase enzyme considered that most effective component against pathogenic fungi. Chitinase enzymes degrades the fungal cell walls which composed of chitin (Lorito *et al.*, 1996).

Also, all the tested *Trichoderma* species significantly reduced area under wilt progress curve (AUWPC) under pot and field conditions compared with control. *Trichoderma viride* and *T. hamatum* recorded the highest reduction of AUWPC compared with the other tested *Trichoderma* species. *Trichoderma* spp. is now the most common fungal biological control agents that have been extensively researched and deployed throughout the world. The primary mechanism of antagonism in *Trichoderma* is mycoparasitism. Lytic activity is the key feature responsible for the expression of mycoparasitism against several fungal pathogens (Chet, 1987). *Trichoderma* spp. is also good competitors in soil and producers of volatile and nonvolatile antibiotics to suppress target pathogens (Chet, 1987). Because of their effectiveness and ease of production for commercial application, at least nine commercial biological control products based on *Trichoderma* species are manufactured and marketed in Belgium, Sweden, Israel, USA, Denmark, India and New Zealand for use on several crops (Navi and Bandyopadhyay. 2002).

On the other hand, all *Trichoderma* species improved growth parameters (plant height, No. of branches plant⁻¹) and fruit yield components (number of fruits plant⁻¹, fruits yield plant⁻¹, fruit weight, No of fruit Kg⁻¹, fruit length, fruit diameters and fruits yield fed⁻¹). *Trichoderma viride* (TVM-5) and *T. hamatum* (THM-2) gave the highest increament in all tested plant growth parameters and yield components. Similar results on increased plant growth due to application of *Trichoderma gamsii* in tomato (Ozbay *et al.*, 2004 and Sundaramoorthy and Balabaskar 2013). The increase in plant growth might be associated with secretion of auxins, gibberellins and cytokinins. The increase in biomatter production may be due to the production of plant growth promoters or through indirect stimulation of nutrient uptake and by producing siderophore or

antibiotics to protect plants from deleterious rhizosphere organisms (Sundaramoorthy and Balabaskar, 2013).

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