Enhancement of growth parameters and yield components in eggplant using antagonism of Trichoderma spp. against fusarium wilt disease

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Accepted 20 May, 2014

Eggplant is one of the important economic vegetable crops which are 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 mycoparasitism inhibitory effects of five Trichoderma species (Trichoderma spirale, Trichoderma hamatum, Trichoderma polysporium, Trichoderma harzianum and Trichoderma viride) on the growth of the causal agent of eggplant Fusarium wilt were investigated by dual culture in laboratory condition. In this step, the maximum inhibitory effect was caused by T. viride (isolate TVM-5) and T. hamatum (isolate THM-2), while T. spirale (TSM-1) was the lowest ones. In pot experiment, the obtained data showed that all Trichoderma species reduced significantly the area under wilt progress curve caused by F. oxysporum f. sp. melongenae. T. viride and T. hamatum recorded the highest reduction of the area under wilt progress curve (AUWPC), where the AUWPC was reduced from 1125.33 in control to 244.0 and 325.33 AUWPC, respectively. Under field conditions, it showed that these treatments significantly reduced AUWPC and increased all tested 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 to the control during the growing seasons (2011-2012 and 2012-2013). T. viride and T. hamatum were the best treatments in both reducing disease severity or increase growth parameters and fruit yield components.

Key words: Eggplant, Trichoderma species, wilt disease, 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 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 in the production of eggplant is the Fusarium wilt disease. Fusarium oxysporum f. sp. melongenae (Fomg) is the most destructive disease agent 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

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xylem transport system (Altinok, 2005). It is extremely difficult to control soil-borne fungi conventional strategies such as the use of synthetic fungicides, etc. 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 microbial activity which extends for a long period of time. *Trichoderma* spp. are considered as potential biocontrol and 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 Fusarium wilt pathogen from the economically important eggplant crop and evaluate the potential of an isolated indigenous strain 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 was 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 key (Nelson et al., 1983). Pathogenicity tests were conducted on potted plants and after re-isolation, a pathogenic isolate of *F. 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 gram of each soil sample were gently mixed with 500 ml distilled water containing 0.2% citric acid, 5 ml of prepared solution were added to Petri plates containing 15 ml water agar at 50°C and shaken to mix properly. After solidification, 5 mm plugs of these cultures were transferred into Petri plates containing Davet selective medium (Davet, 1979) and were incubated at 25°C for 7 days. After proper growth, isolates were purified and identified according to standard keys (Bissett, 1991).

**Inhibitory mechanisms of Trichoderma species against F. oxysporum mycelial growth**

Five mm plugs of seven-day-old cultures of *F. oxysporum* and *Trichoderma* were placed against each other on plates containing PDA. In the case of control instead of *Fusarium oxysporum* plugs, PDA plugs were used. Plates were incubated at 25°C and checked daily for their reactions such as growth speed. Radial growth of the pathogen was measured daily and data were obtained. Data of laboratory tests were calculated by the following formula:

\[
\text{% Inhibition} = \frac{\text{Diameter of colony growth in control} - \text{Diameter of colony growth in treatment}}{\text{Diameter of colony growth in control}} \times 100
\]

**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* inocula, Erlenmeyer flasks containing 100 g of barley and 100 ml of sterilized 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* were dropped into each Erlenmeyer under sterilized condition. The flasks were kept at 25°C for 4 weeks. Colonized wheat grains were then transferred into paper pockets, and were dried and ground. Fourteen gram 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 of *Trichoderma* isolates under aseptic condition. Erlenmeyer flasks were incubated at 27°C for 14 days. Ten gram 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 laboratory tests were used in this experiment. Five seedlings per treatment were sown in plastic pot (30 cm in diameter) and four pots were used for each treatment as replicates. In control treatment, eggplant seedlings were planted in infested soil only and the area under wilt progress curve was recorded.

**Disease assessments**

Wilt severity was estimated at 10 days interval for 60 min after transplanting according to Abdou et al. (2001) using
a rating scale of $(0 - 5)$ based on leaf yellowing grading, where, $0 = \text{healthy}$, $1 = \text{one leaf yellowing}$, $2 = \text{more than one leaf yellowing}$, $3 = \text{one wilted leaf}$, $4 = \text{more than one leaf wilted}$, and $5 = \text{completely dead plants}$. 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, $d_{max}$ is the maximum disease rating, and $n$ is the total number of plants/samples examined in each replicate.

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

$$AUDPC= D \left[ \frac{1}{2} (Y_1 + Y_k) + (Y_2 + Y_3 + \ldots + Y_{k-1}) \right]$$

where $D =$ Time interval; $Y_1 =$ First disease severity; $Y_k =$ Last disease severity; $Y_2$, $Y_3$,$\ldots$,$Y_{k-1}$ = Intermediate disease severity.

### Field experiments

Field experiments were carried out at New Valley Agriculture Research 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 m$^2$ ($5 \times 3$ m). Each unit included three rows; each row was 5 m in length and 1 m in width. Soil treatments were done by application of 150 g of the prepared formulation/plot at the same time of planting. Eggplant seedlings (cv. Black Beauty) were transplanted into the field in 1 October in both seasons at a rate of 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. Plant height, number of branches, number of fruits plant$^{-1}$, fruits yield plant$^{-1}$ (Kg), fruit weight (gm), number 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 $P \leq 0.05$ 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, and were then identified. Results indicate that all isolated fungi were identified by *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 presented in Figure 1 showed that the most virulent isolates against eggplant plants were *F. oxysporum* f. sp. *melongenae* isolates FO3 (77.23%) and FO7 (72.36%), while isolates FO5 and FO8 recorded 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, that is, *T. spirale*, *T. hamatum*, *T. polysporium*, *T. harzianum* and *T. viride* inhibited mycelial growth of *F. o. melongenae* ranging between 43.58 and 56.25% as compared to the control (Figure 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 it inhibited the mycelia growth with 76.25 and 69.36%, respectively. While, *T. spirale* (TSM-1) recorded the lowest inhibition of *F. o. f. sp melongenae* (43.58%).

#### Effect of soil treatment with *Trichoderma* species on wilt disease under greenhouse condition

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 Figure 3 revealed that all *Trichoderma* spp. significantly decreased the area under wilt progress curve (AUWPC) as compared to the control. *T. viride* was the most effective one for reducing UDWPC from 1125.33 in the control to 244.0 AUWPC followed by and *T. hamatum* (325.33 UDWPC), while *Trichoderma*...
Figure 1. Pathogenicity tests of *Fusarium oxysporum* f. sp. *melongenae* isolates collected from different locations to eggplant cv. Black Beauty. Different letters indicate significant differences between eggplant *Fusarium oxysporum* f. sp. *melongenae* isolates according to LSD test (*P*≤0.05).

Figure 2. Antagonistic effect 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*≤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*≤0.05).
spiral (TSM-1) and T. polysoprium (TPM-3) recorded the lowest reduction for AUWPC caused by F. oxysporum f. sp melongenae (685.36 and 845.74 AUWPC, respectively).

Effect of Trichoderma species on wilt disease under field conditions

Effects of Trichoderma spp. on wilt disease incidence, some growth parameters, and yield components of eggplant plants under field conditions in New Valley governorate were studied.

Effect of Trichoderma species on area under disease progress curve

Data in Figure 4 indicate that all Trichoderma species exhibit significant protection against wilt disease compared with the 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 T. hamatum (221.36 and 253.67 AUWPC) during both growing seasons, respectively. Conversely, T. spirale and T. polysoprium showed the lowest protection against wilt disease while it recorded the UNWPC from 956.38 and 989.36 to 422.0, 489.36 and 658.74 AUWPC in the first and second growing seasons, respectively.

Effect of growth parameters

All the tested Trichoderma species significantly increased growth parameters, that is, 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 while it increased plant height from 66.26 and 65.48 cm in control to 93.06 and 92.36 cm in the first and second growing seasons, respectively followed by T. hamatum (67.66 and 67.67 cm for both seasons, respectively). Conversely, the effect of T. polysoprium for increasing plant height was lower compared with the others. The same trend was also observed in the 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 in the first and second growing seasons, respectively) followed by T. hamatum (20.22 and 19.36 in the first and second growing seasons, respectively); however, T. polysoprium recorded the lowest ones in both growing seasons.

Effect on yield components

Data in Table 2 revealed a decrease in the yield components of eggplant plants, that is, 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 was T. viride for all fruit parameters, which recorded high 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) when 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. T. hamatum came after T. viride in increasing all fruit parameters in this respect during both growing seasons.
### DISCUSSION

Many soils 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 (Altinok, 2005; Baysal et al., 2013). 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 Altinok (2005) and Baysal et al. (2013).

The management of the 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 in short term; 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 Abd-El-Khair, 2008; Bai et al., 2008; Savazzini et al., 2009; Joshi et al., 2010; Sundaramoorthy and Balabaskar, 2013). In this study, five *Trichoderma* species namely: *T. spirale*, *T. hamatum*, *T. polysporium*, *T. harzianum* and *T. viride* were evaluated in *in vitro* and *in vivo*. The obtained data indicated that all *Trichoderma* spp. suppressed mycelia growth of *F. oxysporum* f. sp. *melongenae* *in vitro*. *T. 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, cellulase and lectin, which help mycoparasites in the colonization of their host. The inhibition of pathogen may also be 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 which is considered as the most effective component against pathogenic fungi. Chitinolytic enzymes degrade the fungal cell walls which are composed of chitin (Lumore et al., 1996).

Also, all the tested *Trichoderma* species significantly reduced the area under wilt progress curve (AUWPC) under pot and field condition when compared with the control. *T. viride* and *T. hamatum* recorded the highest reduction of AUWPC when compared with the other *Trichoderma* species. *Trichoderma* spp. is now the most common fungal biological control agent that has 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. are also good competitors in soil and producers of volatile and non-volatile 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"). *T. viride* (TVM-5) and *T. hamatum* (THM-2) showed the highest increase of all growth parameters and yield components. Similar results on increased plant growth due to application of *Trichoderma gamsii* in cereals and legume crops were reported by 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 biomass 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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