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BIOLOGICAL CONTROL OF WILT OF EGGPLANT CAUSED BY *FUSARIUM SOLANI* F. SP. *MELONGENAE*

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ABSTRACT

Bhadra M, Khair A, Hossain MA, Shamoli FA, Sikder MM (2016) Biological control of wilt of eggplant caused by *Fusarium solani* f. sp. *melongenae*. *Int. J. Expt. Agric.* 6(2), 20-25.

An *in vitro* study was carried out to determine the potential of four selected isolates of *Trichoderma*; namely, *Trichoderma harzianum*, *T. koningii*, *T. viride* (green strain) and *T. viride* (yellow strain) against soil borne phytopathogenic fungi *Fusarium solani* f. sp. *melongenae*. All *Trichoderma* isolates showed inhibitory effect on mycelial growth of the pathogen in dual culture. The maximum inhibition was recorded in *F. solani* f. sp. *melongenae- T. viride* interactions. The pathogen was most susceptible to the volatile inhibitors produced by *T. koningii* and *T. viride* (yellow strain). Naturally untreated metabolites from *T. viride* (green strain) were found to be the most effective one. The fungicides Bavistin50 WP was found more effective than Dithane-M45. In pot culture experiment, the efficacy of *Trichoderma* spp. were measured by the growth parameters such as plant height, leaf number, internodal distance, branching number, leaf breadth, stem diameter at seedling stage in both solarized and non-solarized soil. Re-isolation of *Trichoderma* was also conducted from the experimental root samples.

Key words: *Trichoderma spp., biological control, fungicides, fusarium wilt, eggplant*

INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the most popular vegetables in Bangladesh and grows in an area of about 29,132 ha producing about 1,87,705 m tons (BAKB 2011). Among the different diseases that attack eggplant (*Solanum melongena* L.), wilt is one of them caused by *Fusarium solani* has become a major disease causing significant reduction in yield (Chakraborty *et al*. 2009). The wilt of eggplant is characterized by yellowing of foliage drooping of apical shoot to ultimate death of whole plant. This soil inhabiting fungus colonizes the senescing tissues of the diseased plant and may survive in the soil for many years (Joseph *et al*. 2008). *Fusarium* species, release extra-cellular enzymes which break down the pectin substances of the cell walls of many plants. Pectic enzymes of pathogens cause root rotting and wilting of plants. Indiscriminate use of pesticide has led to serious environmental threat. Hence, the present study was undertaken to evaluate the efficacy of bio-agents; *Trichoderma* spp. both in *in vitro* and *in vivo* trial. These beneficial fungi may be used to suppress the wilt pathogen and increase the yield of eggplant.

MATERIALS AND METHODS

Four species of *Trichoderma*; *Trichoderma harzianum*, *T. koningii*, *T. viride* (green strain), *T. viride* (yellow strain) were collected from spoiled (infected) mushroom spawn packets of *Pleurotus ostreatus* (Jacquin ex fr.) Kummer, at National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh. The fungal pathogen *Fusarium solani* f. sp. *melongenae* was isolated from a severely wilted eggplant. The morphological characterization of *Trichoderma* spp. isolated from oyster mushroom growing substrates was done before based on morphology such as colonies, hyphae, conidiophores, phialides and conidia. *Trichoderma harzianum* was characterized according to Choi (2009) and Barnett (1960). Others strain of *Trichoderma* in the present study were characterized as described by Barnett (1960). For isolation of *Fusarium solani* f. sp. *melongenae* PDA was used. The morphological and cultural characters of the pathogenic fungus *Fusarium solani* f. sp. *melongenae* which was isolated from infected plant roots and identified based on colony, mycelial characters, microconidia, macroconidia characters according to Joseph *et al*. (2008).

In vitro assay of antagonists

Trichoderma isolates were evaluated against *F. solani* f. sp. *melongenae* by dual culture technique as described by Kaur *et al*. (2006). Inoculated plates were incubated at 28±2ºC, 32 ± 2ºC and 35ºC for 7 days. Inhibition percent was calculated (Kaur *et al*. 2006) by the following formulae:

$$\text{Mycelial inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where, C = Radial growth of control plates, T = Radial growth of treated plates

The effect of released volatile metabolites of *Trichoderma* isolates on the mycelial growth of the pathogen was evaluated as methods described by Dennis and Webster (1971). Effects of non volatile metabolites on test fungi was studied as methods described by Kaur *et al*. (2006) at room temperature 28±2ºC. Effects of natural untreated metabolites by Dipping culture disc method was used as described by Ashrafuzzaman and Khan (1992). All plates were incubated at room temperature 28±2ºC and percent of inhibition was calculated.

In vitro assay of fungicides

The effect of fungicides, namely Bavistin50WP and Diathane-M45 on the radial growth of *Fusarium solani* f. sp. *melongenae* was determined on PDA medium. Requisite quantity of fungicides was added to the medium with a concentration of 30 ppm, 50 ppm, and 70 ppm. After mixing of fungicides, the medium was autoclaved. Three replicated PDA plates were used for each dose of respective fungicide. Three replicated PDA plate which received no fungicide as control. The inoculated plates were incubated at 28±2ºC and percent of inhibition was calculated.

Preparation of culture filtrates of antagonists and *F. solani* f. sp. *melongenae*

Antagonistic fungi were grown on PDA medium. Three mycelial agar blocks, each having 5mm diameter of an individual antagonist, were cut off from the advanced margins of 5 day old culture and inoculated into a 500 ml conical flask containing 250 ml potato dextrose broth medium. The inoculated flasks were allowed for 15 days incubation period at 28±2ºC. After incubation, the culture broth of each antagonist was filtered through a double ring filter paper (11 cm) and finally through a millipore filter paper under suction pump to obtain cell free and bacteria free extracts. Entire procedure was performed in aseptic condition.

Pot culture experiment

Soil samples were collected from the Botanical Garden, Jahangirnagar University and was moistened with sufficient water. Then soil samples were packed in transparent polythene bags and kept under direct sunlight up to 20 days**.**

Eggplant (BARI-6) seedlings were used in pot culture experiments having eleven treatments each for both in solarized and non-solarized soils with three replicates. Pots were filled with solarized and non-solarized soil. After transplanting, the seedlings were kept in shady place for 5 days to harden up. Then, four isolates of *Trichoderma* extracts and pathogen extracts were injected. The treatment was applied between 10-11 AM and 3-4 PM when translocation rate of plants were high.

Data collection and statistical analysis

All symptoms and impacts of extracts on plant growth parameters such as; plant height, leaf number, leaf breadth, inter-node distances, branching number, stem diameter were recorded during plant development. Root samples were collected randomly to assess root colonization.

The root samples were stained according to Ghahfarokhy *et al.* (2011). The data were statistically analyzed using SPSS program.

Re-isolation

Trichoderma sp. was reisolated from root samples. The roots samples were cut into 1-2 cm pieces, surface sterilized with 0.1% NaOCl for 5 minutes and rinsed with sterilized distilled water for 3-4 times under laminar air flow cabinet. Then placed this surface sterilized roots in PDA medium and plates were sealed with adhesive tape. Observation was done after 2, 4, 6 days respectively.

RESULTS AND DISCUSSION

Inhibition of *Fusarium solani* f. sp. *melongenae* in *in vitro* technique

All the four antagonistic fungi caused significant inhibition of mycelial growth of *F. solani* f. sp. *Melongenae* (Table 1). The inhibition of *F. solani* f. sp. *melongenae* varied from 69.2-86.7%. The maximum inhibition achieved with *T. viride* (yellow strain) followed by *T. viride* (green strain) and *T. harzianum*. The lowest mycelial inhibition was found with *T. koningii* at 28±2ºC temperature. At 32±2ºC temperature, the maximum inhibition (86.7%) was shown by both *T. viride* (green strain**)** and *T. viride* (yellow strain), and the least (73.3%) by *T. harzianum.* At 35ºC temperature, *F. solani* f. sp. *melongenae* did not show any radial mycelial growth both in control and treatment. Present findings are supported the results of earlier workers. Goswami and Islam (2002) observed that biocontrol agent such as *Trichoderma* spp. exhibited greater mycelial inhibition of tomato wilt pathogen -*F. oxysporum* f. sp. *lycopersici*. Bernal *et al.* (2001) observed the effective antagonism between *Trichoderma* spp. and *Fusarium oxysporum* f. sp. *cubense*. Abou-Zeid *et al*. (2007) found that biological agents (*T. viride* and *T. hamatum*) significantly inhibited the radial mycelial growth of the pathogenic fungi-*Fusarium oxysporum*. Chakraborty *et al*. (2009) reported that *T. harzianum* and *T. viride* were specifically found to have reduced the disease incidence of Fusarium wilt of eggplant caused by *Fusarium solani* up to 86% and 83%, respectively. Ahmed (2011) reported *T. koningii* the most effective as one in controlling soil borne pathogens. Afrin (2013) reported that *Trichoderma harzianum* showed more than 77.77% inhibition of the radial mycelia growth of the *Fusarium oxysporum* over control in dual culture technique.

Table 1. Mycelial growth inhibition (%) of *Fusarium solani* f. sp. *melongenae* by four *Trichoderma* spp. at different temperatures

Antagonists	Mycelial growth inhibition (%) of *Fusarium solani* f. sp. *melongenae*					
	Dual culture			Volatile	Non volatile	Naturally untreated
	28±2ºC	32±2ºC	35ºC	28±2ºC	28±2ºC	28±2ºC
T. harzianum	77 b	73.3 c	Nil	54.2 c	54.2 c	12.5 c
T. koningii	69.2 c	80 b	Nil	87.5 a	62.5 a	No effect
T. viride (greens train)	77 b	86.7 a	Nil	66.7 b	54.2 c	42.9 a
T. viride (yellow strain)	84.6 a	86.7 a	Nil	87.5a	58.3 b	33.3 b

Data recorded at 7 days of incubation, Data represents mean value of three replication, Column having the same letters do not differ significantly at 5% level of significance

In vitro effects of volatile metabolites on *Fusarium oxysporum* f. sp. *melongenae* revealed that *Trichoderma viride* (yellow strain) and *T. koningii* significantly inhibited (87.5%) the mycelial growth followed by *Trichoderma viride* (green strain) (66.7%) and the lowest due to *T. harzianum* (54.2%). The finding partially supported by Hajieghrari *et al.* (2008) who reported that volatile metabolites of *Trichoderma hamatum* gave the maximum mycelial inhibition (48.65%) of *Fusarium graminearum*. Mishra *et al*. (2004) also reported the secretion of volatile compounds from *Trichoderma virens* and found inhibitory effects on *F. oxysporum* f. sp. *gladioli.* Inhibitory effects of non-volatile metabolites on *Fusarium oxysporum* f. sp. *melongenae* varied between 54.2 to 62.5%. Effect of natural untreated metabolites of *Trichoderma* spp. showed variable inhibitory effects on studied organism. *T. viride* (green strain) showed maximum inhibition against test fungus. There are no literatures available in respect of the present findings.

Effect of Fungicides on *F. solani* f. sp. *melongenae*

Only 42% of mycelial growth inhibition of *Fusarium solani* f. sp. *melongenae* was obtained at 70 ppm by Dithane M-45 while others fungicide concentration gave unsatisfactory results (Table 2). Interestingly, Bavistin 50WP showed the maximum mycelial inhibition (99% to 100%) of fungi at each concentration (30 ppm, 50 ppm and 70 ppm) over Dithane M-45 (Table 2). Present findings are in conformity with the reports of previous workers. Mayur *et al.* (2001) found systemic fungicide-Bavistin was the most effective to inhibit the radial mycelial growth of chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* by 100 ppm concentration. Bhaskar *et al.* (2003) reported that Bavistin was found to reduce the disease incidence of dry corm rot disease in *Colocasia* caused by *Fusarium solani*. Chakraborty *et al*. (2009) cited that soil solarization integrated with applications of Bavistin (chemical name-carbendazim) was found to effective to control the Fusarium wilt of eggplant. Afrin (2013) cited that Bavistin 50 WP was found to inhibit the mycelial growth of *Fusarium oxysporum* completely even at 100 ppm.

Table 2. Effect of different concentration of Bavistin 50WP and Dithane M-45 on mycelial growth of *F. solani* f. sp. *melongenae* at 28±2ºC temperature

Concentration of fungicides	Radial mycelial growth inhibition (%) of *Fusarium solani* f. sp. *melongenae*	
	Bavistin	Diathane M-45
30 ppm	99.53±0.47 a	22.2±0.10 b
50 ppm	99.67±0.03 a	22.2±0.20 b
70 ppm	100±0.00 a	42.2±0.10 a

Data recorded at 7 days of incubation, Data represents mean ±SE, Column having the same letters do not differ significantly at 5% level of significance

Growth parameters of eggplant

Results on the growth parameters of eggplant in response to spent mushroom compost carrying *Trichoderma*, with or without *Trichoderma* extract inoculation have been presented in Table 3 and Table 4. Data were taken at 30 days after transplanting (DAT). There was no significant difference in plant height of eggplant in solarized soil treatment T_2, T_3 and T_7 but were significant over other treatments when non solarized soil was used as a potting media. At every stage *T. viride* (green strain) and *T. harzianum* added treatment showed better results. The highest numbers of leaves were recorded in treatment T_2 (6.00) as against only 4.67 in T_1 (control) at 30 DAT in solarized soil. In case of non-solarized soil, treatment T_4 showed significantly better performance over other treatments where *T. viride* (green strain) extract was applied at 30 days after transplanting (DAT). Treatment T_2 showed better performance in respect of stem diameter in solarized soil. In non-solarized soil, treatment T_3, T_6 to T_{11} were superior over others. In general, all growth parameters were found to increase more or less with treatments of *Trichoderma*. In case of leaf breadth, all the treatments showed better performance over control. Present finding are in conformity with previous reports. Singh *et al*. (2008) reported that *T. viride* and *T. harzianum* isolates were found to increase the germination percentages, radicle and plumule length of safflower significantly higher than those of untreated seeds in a control. Joshi *et al*. (2010) found the utmost germination (79.49%) enhanced the shoot and root length of chilli treated with *Trichoderma* isolates to be

significantly higher than in a control. Tancic *et al*. (2013) cited that there were significant positive effects on root length, germination, vigour index of soybean treated with four *Trichoderma* isolates under glasshouse condition. Abdel-Monaim *et al*. (2014) observed that *Trichoderma viride* and *T. hamatum* gave the highest increment of plant growth parameters (plant height, number of branches plant^{-1}) and fruit yield components (number of fruits plant^{-1}, fruits yield plant^{-1}, fruit weight, fruit length, and fruit diameters) of eggplant compared to control.

Table 3. Effect of various treatment combinations on growth parameters (plant height and number of leaves per plant) of eggplants at 30 days after transplantation

Treatments	Plant height (cm)		Number of leaves plant^{-1}	
	Solarized soil	Non solarized soil	Solarized soil	Non solarized soil
T_1: Control	17.73±0.82 a	13.00±1.15 b	4.67±0.33 b	3.00±0.00 d
T_2	16.60±1.42 a	16.27±0.88 a	6.00±0.00 a	4.67±0.33 ab
T_3	17.63±0.67 a	16.27±0.88 a	3.00±0.00 d	4.33±0.33 abc
T_4	17.93±0.33 a	14.00±0.58 b	4.00±0.00 c	5.00±0.00 a
T_5	16.07±1.16 a	13.00±0.00 b	3.33±0.33 d	4.33±0.33 abc
T_6	18.00±0.58 a	14.30±0.00 ab	3.00±0.00 d	3.33±0.33 cd
T_7	16.20±0.00 a	16.27±0.88 a	3.00±0.00 d	3.00±0.00 d
T_8	18.67±0.67 a	15.00±0.58 ab	4.00±0.00 c	3.67±0.33 cd
T_9	16.33±1.20 a	15.00±0.00 ab	3.00±0.00 d	4.00±0.00 bcd
T_{10}	17.07±0.52 a	15.00±0.00 b	3.00±0.00 d	3.33±0.33 cd
T_{11}	18.00±0.58 a	15.00±0.00 ab	4.00±0.00 c	4.00±0.58 bcd
Level of sig.	Non significant	Significant	Significant	Significant

Data represents mean ±SE of three replications, Column having the same letters do not differ significantly at 5% level of significance

Here, Ep= Eggplant, T_1 = only solarized or non-solarized soil, T_2 = Nss/Ss(143 gm) + Ep + Compost (7 gm), T_3 = Nss/Ss(143 gm) + Ep + Compost(7 gm) + inoculum *F. solani* extract (1ml), T_4 = Nss/Ss(150 gm)+ Ep + *T. viride*(green strain) extract (1ml), T_5 = Nss/Ss(150 gm) + Ep + *T. viride*(yellow strain) extract (1ml), T_6 = Nss/Ss(150 gm) + Ep + *T. koningii* extract(1ml),T_7 = Nss/Ss(150 gm) + Ep + *T. harzianum* extract(1ml),T_8 = Nss/Ss (150 gm) + Ep + *T. viride*(green strain) extract (1ml) + inoculum *F. solani* extract(1ml), T_9 = Nss/Ss(150 gm) + Ep + *T. viride*(yellow strain) extract (1ml) + inoculum *F. solani* extract(1ml), T_{10} = Nss/Ss(150 gm) + Ep + *T. koningii* extract (1ml) + inoculum *F. solani* extract(1ml), T_{11} = Nss/Ss(150 gm) + Ep + *T. harzianum* extract(1ml) + inoculum *F. solani* extract(1ml)

Table 4. Effect of various treatment combinations on growth parameters (leaf breadth and stem diameter per plant) of eggplants at 30 days after transplantation

Treatments	Leaf breadth (cm) plant^{-1}		Stem diameter (cm) plant^{-1}	
	Solarized soil	Non solarized soil	Solarized soil	Non solarized soil
T_1: Control	3.20±1.25 b	4.25±0.29 b	1.17±0.03 c	1.00±0.00 b
T_2	5.00±0.29 a	4.40±0.00 a	1.47±0.67 a	1.07±0.03 ab
T_3	5.40±0.20 a	4.40±0.00 a	1.17±0.03 c	1.00±0.00 a
T_4	5.13±0.07 a	4.40±0.00 a	1.30±0.00 b	1.10±0.00 ab
T_5	4.90±0.10 a	4.40±0.00 a	1.00±0.00 d	1.10±0.06 ab
T_6	4.80±0.00 a	4.40±0.00 a	1.10±0.00 cd	1.17±0.33 a
T_7	5.00±0.10 a	4.40±0.00 a	1.07±0.03 d	1.13±0.33 a
T_8	5.07±0.27 a	4.40±0.00 a	1.03±0.03 d	1.17±0.33 a
T_9	4.80±0.00 a	4.40±0.00 a	1.00±0.00 d	1.13±0.33 a
T_{10}	4.80±0.00 a	4.40±0.00 a	1.07±0.03 d	1.17±0.33 a
T_{11}	4.80±0.00 a	4.40±0.00 a	1.10±0.00 cd	1.13±0.33 a
Level of sig.	Significant	Significant	Significant	Significant

Data represents mean ±SE of three replications, Column having the same letters do not differ significantly at 5% level of significance

Here, Ep= Eggplant, T_1 = only solarized or non-solarized soil, T_2 = Nss/Ss (143 gm) + Ep + Compost (7 gm), T_3 = Nss/Ss(143 gm) + Ep + Compost (7 gm) + inoculum *F. solani* extract (1ml), T_4 = Nss/Ss (150 gm) + Ep + *T. viride*(green strain) extract (1ml), T_5 = Nss/Ss (150 gm) + Ep + *T. viride*(yellow strain) extract (1ml), T_6 = Nss/Ss (150 gm) + Ep + *T. koningii* extract (1ml), T_7 = Nss/Ss (150 gm) + Ep + *T. harzianum* extract (1ml), T_8 = Nss/Ss (150 gm) + Ep + *T. viride*(green strain) extract (1ml) + inoculum *F. solani* extract (1ml), T_9 = Nss/Ss (150 gm) + Ep + *T. viride*(yellow strain) extract (1ml) + inoculum *F. solani* extract (1ml), T_{10} = Nss/Ss (150 gm) + Ep + *T. koningii* extract (1ml) + inoculum *F. solani* extract (1ml), T_{11} = Nss/Ss (150 gm) + Ep + *T. harzianum* extract (1ml) + inoculum *F. solani* extract (1ml)

Trichoderma association with roots of eggplant

Possible association of *Trichoderma* isolates have been recorded when root samples were analyzed. In non-solarized soil, treatment T_2 (non-solarized soil + compost) root samples showed distinct association with *Trichoderma* spp., whereas others showed no association. A similar result was observed by Ghahfarokhy *et al*. (2011) in case of wheat root under greenhouse condition.

CONCLUSION

Based on the experimental results, it can be concluded that *Trichoderma* spp. is very effective to control the Fusarium wilt of eggplant. It can be used either singly or through integration with other non-chemical means of disease control.

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