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POSSIBILITY OF RECOVERING FUSARIUM WILT AFFECTED EGGPLANTS BY *TRICHODERMA*

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Abstract

The efficacy of *Trichoderma* suspension on the recovery of fusarium wilt infected eggplant was studied at Bangladesh Agricultural University Farm during July 2014 to April 2015. Suspension of *Trichoderma asperellum* CP (IPM 33) (5.8×10^7 CFU ml^{-1}) was applied to rhizosphere soils of eggplants @ 0, 20 and 40 ml $plant^{-1}$. *Fusarium oxysporum* f. sp*. melongenae* was inoculated to flowering and fruiting stages of healthy plants by soil drenching method 48 hrs before and after application of *Trichoderma* suspension. Observations were made on the severity and recovering of wilting. The eggplant variety BAU Begun 1 was found resistant to fusarium wilt while other two varieties BAU Begun 2 and Dohazari G showed susceptible reaction. Pre-inoculation application of *Trichoderma* @ 40 ml $plant^{-1}$ significantly reduced the severity of the disease. The vulnerability to fusarium wilt was higher at flowering stage of eggplant. Thus, the experimental results have opened up a possibility of using *Trichoderma* suspension over conventional farming inputs for sustainable and organic production of eggplant.

Keywords: *Fusarium* Wilting, *Trichoderma*, Infection Recovery

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Introduction

Among the vegetables grown in Bangladesh, eggplant (*Solanum melongena* L.) is most important in terms of round the year availability, food value, taste, farmer's income perspective and as an export item. In a subtropical country like Bangladesh, eggplant is grown all over the country on medium high land to high land in both *Rabi* and *Kharif* seasons. It is positioned the 2nd in acreage, production, yield, and in consumption next to potato. In the year 2014-15, total 76370 acres land were under eggplant cultivation, total production was 310,354 MT and yield was 4 t ha^{-1} (BBS, 2016).

Comparing with production and yield rate in other countries, the position of Bangladesh is very poor for the lack of appropriate knowledge of sound eggplant production, 20 and 30 tons ha^{-1}, respectively in India and Japan. The most devastating disruption in sound eggplant production, usually farmers suffer, is the management of fusarium wilt caused by *Fusarium oxysporum* f. sp. *melongenae.* Some 20-30% killing of eggplant in general due to *fusarium* wilting is a regular report from the farmers (Begum, 2007); it may turn into epidemic causing complete failure of the crop during November to December (Meah, 1997). The fungus chokes the vascular system of plant and plant dies from water and nutritional deficiency, the fungus attacks mainly when the plants have grown up (Agrios, 2006).

Trichoderma, a common and very important soil-borne fungus, has gained considerable recognition as biological agent against various soil-borne plant pathogenic fungi such as *Fusarium*, *Sclerotium*, *Rhizoctonia* etc. (Al-Chaabi and Matrod, 2002). *Trichoderma* produces chemicals called trichodermin, which is responsible for its antagonistic properties (Tverdyukov *et al*., 1994). Presence of *Trichoderma*, an antimicrobial bio-agent, in the soil is an indicative of the population status of plant pathogenic fungi. Its dominance indicates a pathogen-suppressive soil means a better crop growth (Elad *et al*., 1986; Papavizas and Lewis, 1981; Liu and Baker, 1980; Wells *et al*., 1972). Farmers use chemical fungicides for controlling wilting in eggplants. As a result, soil pollution arises; heavy indiscriminate use of chemical fungicides causes interference to the holistic plant health management strategies as well as to the environment and living being. So, there is a need for easy, economically feasible, eco-friendly and organically formulated biopesticide. As an alternative, IPM Lab biopesticide, an organic formulation of *Trichoderma* could replace or minimize the use of chemical fungicide for controlling wilting in eggplant. It is expected that application of *Trichoderma* suspension to control wilting in eggplant, the growth of the plant could be enhanced and crop environment becomes safe, eco-friendly and non-hazardous to the eggplant growers. The present work was undertaken to determine the efficacy of *Trichoderma* suspension in recovering *fusarium* wilt affected eggplants.

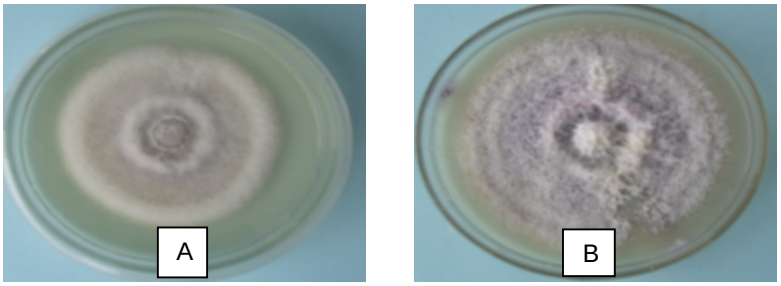
Materials and Methods

The laboratory experiments were conducted in the Plant Disease Diagnostic Clinic (PDDC) and IPM Lab, Bangladesh Agricultural University (BAU), Mymensingh. The field experiment was conducted in the Field Laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh during the period of July 2014- April 2015. The experiment was designed in a Randomized Complete Block Design (RCBD) with three replications. Seeds of the three eggplant varieties, *viz.* (1) BAU Begun 1 (2) BAU Begun 2 and (3) Dohazari G were obtained from IPM Lab, Department of Plant Pathology, BAU, Mymensingh. The isolation of *F. oxysporum* f. sp. *melongenae* was done by following tissue planting method as described by Ashrafuzzaman (1976). Culturing *F. oxysporum* f. sp. *melongenae* and *Trichoderma asperellum* CP (IPM 33) and preparation of spore suspension were done following the procedure of Ullah (2010). Whole field was inoculated at the flowering stage and again at the fruiting stage following the application of *Trichoderma* suspension to 50% of plants plot^{-1} at first and then just 48 hrs after inoculation of *F. oxysporum* f. sp. *melongenae* was done by soil drenching method and vice-versa. The treatments (T1= 20 and T2= 40 ml plant^{-1}) were applied either before pathogen inoculation i.e. pre-inoculation or after pathogen inoculation i.e. post-inoculation time. At the flowering and fruiting stages, observations were made on the number of wilting of lower leaves plant^{-1}, number of slightly drooping of leaves plant^{-1}, number of wilting of all leaves except terminal buds plant^{-1} and number of leaves regenerated plant^{-1}. The records on expression of symptoms on leaves were taken after inoculation at an interval of 3 days up to 21 days. Infection was expressed in percentage. To calculate the percent leaf wilted/disease incidence, total number of leaves plant^{-1} and number of leaves wilted plant^{-1} were counted. The disease severity was computed by adopting (0-4) scale for *F. oxysporum* f. sp. *melongenae* followed by Kapoor (1987).

Results and Discussion

Collection and Isolation of F. oxysporum f. sp. melongenae

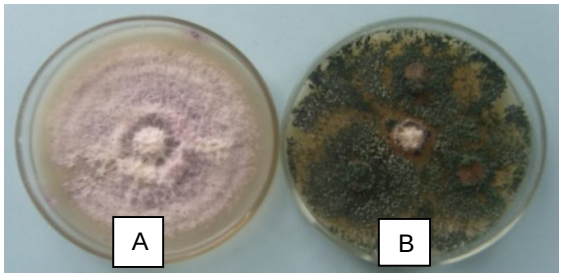
Inocula of the wilted plants of eggplant collected from the Field Laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), when planted on PDA grew out slowly. It was transferred, sub-cultured and purified. On PDA, the fungus grew slowly and with time produced pink pigmentation (Photograph 1). Microscopic slides were prepared and presence of macro- and micro-conidia was observed. Based on available literatures, the fungus was identified as *Fusarium oxysporum* f. sp. *melongenae* (Brooks, 1991; Agrios, 2006).



Photograph 1. Isolate of *F. oxysporum* f. sp. *melongenae*, (A) 6 days old and (B) 9 days old

Antagonistic effect of Trichoderma asperellum CP (IPM 33) in-vitro against F. oxysporum f. sp. melongenae

The antagonist *T. asperellum* CP (IPM 33) was tested against *F. oxysporum* f. sp. *melongenae* on PDA in dual culture method. *T. asperellum* CP (IPM 33) caused 100% growth inhibition of *F. oxysporum* f. sp. *melongenae* (Photograph 2).

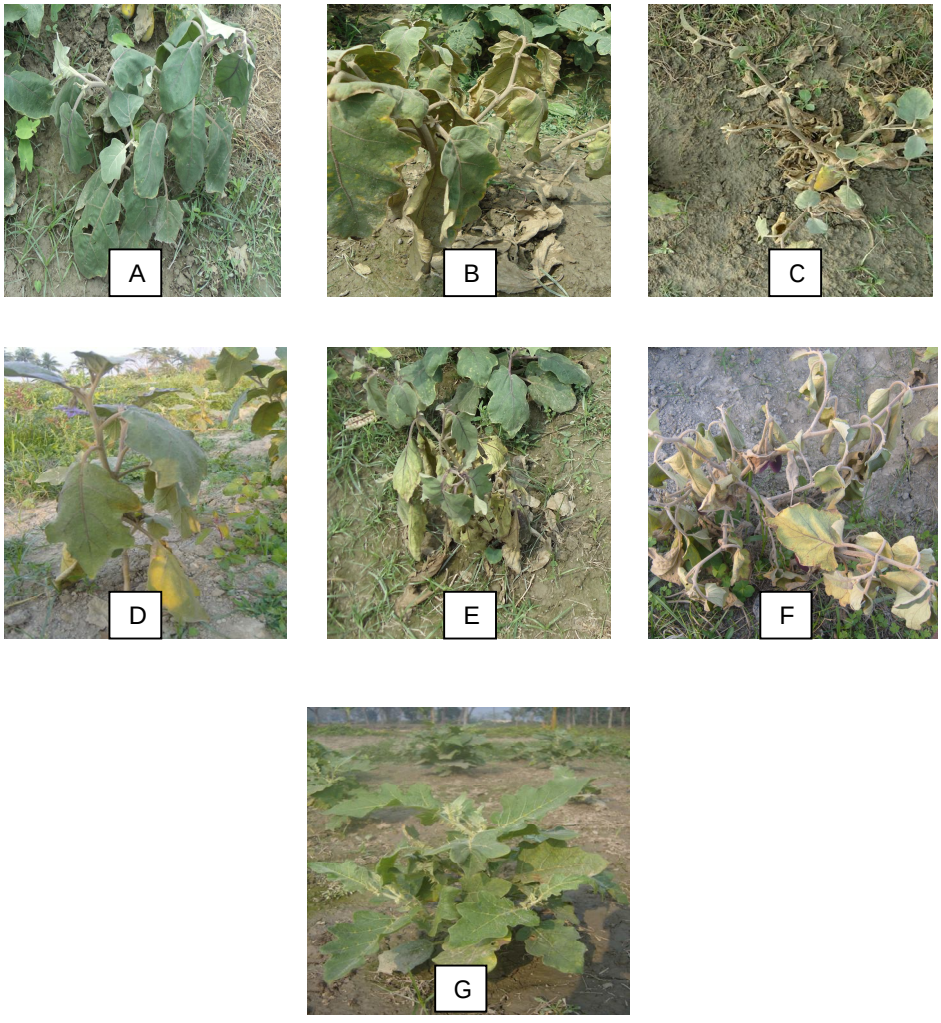


Photograph 2. Antagonistic effect of *Trichoderma asperellum* CP (IPM 33) *in- vitro* against *F. oxysporum* f. sp. *melongenae* A: Growth of *F. oxysporum* f. sp. *melongenae* in absence of antagonist, B: Growth of *F. oxysporum* f. sp. *melongenae* in presence of antagonist

Pathogenicity test of F. oxysporum f. sp. melongenae

The eggplants were inoculated with inocula of *F. oxysporum* f. sp*. melongenae* by soil drenching method to test the capability of the fungus producing disease symptoms in eggplants. The inoculated plant showed typical wilting symptoms. The result is supported by the findings of Begum (2007) who observed that *F. oxysporum* f. sp*. lycopersici* was able to produce wilting symptoms in tomato plants. The finding is also supported by the finding of Altinok (2005) who reported wilting in eggplants in Turkey by *F. oxysporum* f. sp*. melongenae.*

At flowering stage, eggplant var. BAU Begun 2 and Dohazari G inoculated with *F. oxysporum* f. sp*. melongenae* isolates showed typical wilting symptoms ranging from drooping of leaves to completely wilting (Photograph 3: A-F) but BAU Begun 1 did not show any symptom (Photograph 3: G). At fruiting stage, eggplant var. BAU Begun 1, BAU Begun 2 and Dohazari G inoculated with *F. oxysporum* f. sp*. melongenae* isolates did not show any symptom even in control plots.



Photograph 3. Development of fusarium wilt symptoms on the inoculated eggplants at flowering stage in the field, A: Drooping of leaves in BAU Begun 2, B: Yellowing of lower leaves in BAU Begun 2, C: Completely wilting in BAU Begun 2, D: Drooping of leaves in Dohazari G, E: Yellowing of lower leaves in Dohazari G, F: Completely wilting in Dohazari G, G: No wilting in BAU Begun 1

Varietal reactions of eggplant against fusarium wilt

The incidence and severity of the disease varied from 0.00 to 17.04 and 0.00 to 1.21%, respectively. The eggplant varieties BAU Begun 2 and Dohazari G had 12.09 and 17.04% wilted leaves, respectively.

Variation in the prevalence of *fusarium* wilt disease incidence and severity recorded in the present study is consistence with the observation of other workers (Rahman and Haque, 1986; Hossain *et al.,* 1991).

Effect of time of inoculation and treatment

The incidence was reduced greatly when the treatments were applied before inoculation of pathogen (Table 1). The percentage of recovery of wilted leaves was greatly enhanced up to 83.33% when the treatments were incorporated to plants by pre-inoculation application (Table 1). The incidence and severity of fusarium wilt disease varied due to variation of different inoculation and treatment application time. Pre-inoculation application of *Trichoderma* suspension showed the lowest percent leaves wilting as compared to post inoculation application. This result is supported by Begum (2007) who worked with fusarial wilting of tomato. This may be due to the extensive colonization of rhizosphere soil by *Trichoderma* prior to *F. oxysporum* f. sp. *melongenae* colonization. On the other hand, in post-inoculation application of *Trichoderma* suspension, some parts of the rhizosphere soil might already be colonized by the *F. oxysporum* f. sp. *melongenae* prior to *Trichoderma* colonization released from IPM Lab *Trichoderma* suspension (Begum, 2007).

Table 1. Effect of time of inoculation and treatment.

Time of treatment application	Number of Leaves plant^{-1}	% Leaves Exhibited Wilting	% Leaves Recovered
Pre-inoculation application	33.36	1.50c	83.33a
Post-inoculation application	39.67	12.54b	20.70b
Control	36.24	38.27a	0.00c
LSD (≤ 0.05)		1.32	0.26

Effect of doses of Trichoderma suspension against fusarium wilt of eggplant

The disease incidence was lower when the dose of *Trichoderma* suspension was 40 ml plant^{-1} as against 20 ml plant^{-1} (Table 2). There was no recovery of wilted leaves in case of control plots. The capability of recovering wilted leaves was considerably higher (85.47%) when the dose of *Trichoderma* suspension was 40 ml plant^{-1} and that of 16.58% in case of 20 ml plant^{-1}. (Table 2) The incidence and severity of fusarium wilt disease significantly varied with the variation of doses of *Trichoderma* suspension. Application of *Trichoderma* suspension reduced the growth rate of *F. oxysporum.* The findings are in agreement with that of Sivan and Chet (1989) who reported *T. harzianum* to reduce the chlamydospore germination rate of *F. oxysporum.* The present study revealed that the application of 40 ml per plant *Trichoderma* suspension showed the less percent wilting of eggplants as compared to 20 ml, which is supported by the work of Nabi *et al.* (2009) who applied IPM Lab biopesticide in controlling collar rot of eggplant, tomato and Indian spinach.

Table 2. Effect of time of inoculation and treatment.

Time of treatment application	Number of Leaves plant^{-1}	% Leaves Exhibited Wilting	% Leaves Recovered
Pre-inoculation application	33.36	1.50c	83.33a
Post-inoculation application	39.67	12.54b	20.70b
Control	36.24	38.27a	0.00c
LSD (≤ 0.05)		1.32	0.26

Effect of growth stages of eggplant for the development of fusarium wilt

The higher percentage of wilted leaves was recorded at flowering stage of eggplant whereas no wilting of leaves was recorded at fruiting stage (Table 3). The wilted leaves had the capability of recovery only at flowering stage and it was 26.47% of fusarium wilts disease at the fruiting stage whereas that occurred lower at flowering stage of eggplant. The result of the present investigation is supported by the findings of Nabi *et al.* (2009) who studied the efficacy of *Trichoderma* suspension in promoting both vegetative and reproductive growth of tomato, chili and Indian spinach and in reducing incidence and severity of wilt disease.

Table 3. Effect of growth stages of eggplant for the development of fusarium wilt.

Growth stage of eggplant	Number of Leaves plant^{-1}	% Leaves Exhibited Wilting	% Leaves Recovered
Flowering stage	36.42	7.52a	26.47a
Fruiting stage	47.50	0.00b	0.00b
LSD (≤ 0.05)		0.53	0.31

Interaction of eggplant varieties, crop growth stage, dose of Trichoderma suspension and application time on the severity of wilt and recovery of infection

In eggplant var. BAU Begun -1, no infection was observed for any single or combination of treatments. In the varieties BAU Begun 2 and Dohazari G, inoculation and treatments at fruiting stage did not produce any infection. In both the varieties BAU Begun 2 and Dohazari G, pre-inoculation application of treatment at flowering stage @ 40 ml plant^{-1} *Trichoderma* suspension yielded no infection. Post-inoculation application of treatment @ 20 and 40 ml plant^{-1} *Trichoderma* suspension at flowering stage produced 1.76-2.45% wilting while it resulted in a maximum of 95.24% recovery of infection.

Conclusion

The results obtained in the study indicate the efficacy of *Trichoderma* suspension in suppressing fusarium wilt disease of eggplant. It was better to apply the *Trichoderma* suspension prior to inoculation of the pathogen. Therefore, *Trichoderma* suspension should be applied before the appearance of the disease in the field. The results also indicated that the vulnerable stage of eggplant especially for fusarium wilt disease was flowering stage. In this concern, *Trichoderma* suspension can bring significant result in suppressing the wilt disease if it is applied before flowering stage of eggplant.

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