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Research Paper

Incidence of *Fusarium* wilt of tomato and control of pathogen by antagonistic fungi

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Abstract

Fusarium oxysporum Schlechttheis economically important wilting fungi of tomato. To know the incidence of the disease a survey was carried out during October 2012 to February 2013. The average percentage of wilt diseased tomato plant in different fields at Rajshahi was 45.84%. The highest percentage of infected plants was found at Shyampur moulovipara (55.76%) and the lowest was at Kismot kukhondi (37.64%). In an attempt to develop biocontrol system for management of fusarium wilt of tomato, *Penicillium* spp. *Trichoderma* spp. and *Aspergillus* spp. were evaluated for their antagonistic activity against *F. oxysporum in vitro* condition. The mycelia of *F. oxysporum* were found to be inhibited by all the three antagonistic microorganisms. Among them, the highest percentages of inhibition of radial mycelial growth were 82.0, 77.0 and 70.0, respectively. The present study indicates that *Penicillium* spp. was the best antagonist bioagent against *Fusarium* sp. which may be used in integrated management approach to control the wilt disease in future,

Keywords: *Fusarium* wilt, *Penicillium* spp. *Trichoderma* spp., *Aspergillus* spp., biocontrol, antagonistic effect

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important and popular vegetables in Bangladesh. It is affected by several pathogenic fungi, reflecting negatively on plant growth and yield (Jaiswal *et al*., 2015). Out of these, fusarium wilt caused by *Fusarium oxysporum*, is one of the most devastating diseases of tomato in Bangladesh. Species of the genus *Fusarium* are examples of phytopathogenic and toxine-producing fungi that have been reported to be widespread throughout the world (Harvey *et al*., 2001; Hussein and Brasel, 2001). *Fusarium* infections are responsible for destroying crops and dramatically reducing production yields. The wilt caused by species of *Fusarium* remains to be a challenging task in terms of management (Agrios, 2005).

The management of fusarium wilt disease is mainly through the synthetic fungicides and soil fumigation (Diehl and Fehrmann, 1999; Akkopru and Demir, 2005). Besides, chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material (Strider, 1985). In addition to target organism, pesticides also kill various beneficial organisms (Akhter *et al*., 2009). Their toxic forms persist in soil and contaminate the whole environment. Therefore, encouragement of beneficial biological agents to reduce pathogen inoculums is the only solution to maintain plant health. Many studied have been reported for antagonistic activities of *Trichoderma* species (Ramezani, 2010). Certain species of *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Gliocladium virens*, and other fungi have been identified as biocontrol agents contained in compost against *Fusarium* sp. (Boughalleb-M'Hamdi *et al.*, 2018). The antagonistic potential of *Trichoderma* sp. and *Gliocladium* sp. against several soil-borne fungal pathogens were well documented and led to their commercial use (Nagesh *et al*., 2006). However, there is very few information on the use of *Penicillium* sp. as an antagonistic potential to control the pathogen. Thus the present work has been carried out to give information on the incidence of fusarium wilt of tomato in Rajshahi

Metropolitan City and evaluate *in vitro* antagonistic effect of some bioagents against the growth of *F. oxysporum* causing fusarium wilt disease of tomato.

MATERIALS AND METHODS

The survey of fusarium wilt disease of tomato was conducted in Rajshahi Metropolitan City. The locations of survey area and sample collection of wilt disease of tomato are given below:

Table 1: List of location of survey and collection of wilt diseased tomato

Serial no.	location	Total no. of field
1	Shyampur moulovipara	6
2	Line budhpara	5
3	Kismot kukhondi	3
4	Meherchondi	5
5	Dohorompur	7
6	Modhobudhpara	4

Disease Incidence

The disease of tomato field percentage of infected plants and percentage of damaged plants was recorded by adopting the grading formula of Siddaramaiah *et al.* (1978):

$$\text{Disease incidence} = \frac{\text{Total No. of Infected Plants}}{\text{Total No. of Plants}} \times 100$$

Isolation and identification of pathogen

The wilt infected tomato plant's stems were collected in the polythene bags, which were made airtight. Collected materials were labeled properly and then brought to the Laboratory of Plant Pathology, Mycology and Microbiology, Department of Botany, University of Rajshahi, Bangladesh. Diseased parts of tomato plant were cut into small pieces. The pieces were then washed in running tap water, sterilized in 0.1% mercuric chloride solution and washed repeatedly for several times in sterilized distilled water to remove mercuric chloride solution. Three pieces of sterilized infected plant's parts were transferred to Potato Dextrose Agar (PDA) plate. Plates were incubated at 25±2°C for 15 days for recovery of pathogen. The pathogen was purified by single spore method and according to cultural and morphological characteristics the identification of pathogen was done with the help of standard keys (Booth, 1971; Nelson *et al.*, 1983). Pathogenicity tests were done on potted tomato plants according to Koch's postulate (Agrios, 2005).

Isolation and identification of antagonistic fungi from soil

For isolation of antagonistic fungi, soil samples were collected from rhizosphere of tomato plant from different tomato field of Botanical Garden, Rajshahi University. The samples were taken up to a depth of 20 cm after removing approximately 3 cm of the soil surface. The samples were placed in polyethylene bag, closed tightly and stored into the laboratory.

Sample suspension were prepared by adding 1.0 g sample to 10 ml of sterile distilled water and shaken for 15 minutes. Immediately afterwards, each suspension was serially diluted to 10^3 and 1 ml of this dilution was spread on a PDA plate. Then the culture plates were incubated at room temperature for one week and observed regularly. After one week there were many microorganisms were grown in the culture plates. From the culture plates the distinct colonies were observed by under microscope and the selected fungi were identified as *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp.

Antagonistic effect of selected fungi against *Fusarium* sp.

The antagonistic properties of three fungi were tested for their efficacy to inhibit growth of *F. oxysporum* in dual culture on PDA medium (R_2). Mycelial blocks were cut from the periphery of 7 days old culture of *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp. Different antagonistic agents and *Fusarium* sp. were placed on PDA plate facing opposite to each other and incubation in same at 27 °C for 7 days. Data were recorded on colony diameter after 7 days of incubation.

By measuring the radius of *Fusarium* sp. in the direction of the antagonistic colony in the control plate was considered as R_1. The two reading were transformed into percent inhibition radial growth (PIRG) using the formula of Skidmore and Dickinson (1976).

$$\text{PIRG} = \frac{R_1 - R_2}{R_1} \times 100$$

Where,

R_1 = Radius of colony of *Fusarium* sp. in control plate

R_2 = Radius of colony of *Fusarium* sp. in dual culture plate

Statistical analysis

Observation was recorded and data were analyzed statistically using one way ANOVA followed by Duncan's Multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Investigations of wilt disease in different tomato fields of Rajshahi Metropolitan City during October to February were conducted. It is evident from the table 2 that wilt disease of tomato in different fields at Rajshahi Metropolitan City were found in different percentages, the average percentage of infected plants was 45.84%. The highest percentage of infected plants was found at Shyampur moulovipara (55.76%) and the lowest was at Kismot kukhondi (37.64%).

Table 2: Disease incidence of fusarium wilt disease of tomato at different locations observed in Rajshahi

Serial no.	Locations	Total no. of field	Total no. of plants	Total no. infected plants	Disease incidence (%)	Average of Disease incidence (%)
1	Shyampur moulovipara	6	728	406	55.76	
2	Line budhpara	5	650	321	49.38	
3	Kismot kukhondi	3	425	160	37.64	**45.84**
4	Meherchondi	5	668	285	42.66	
5	Dohorompur	7	837	397	47.43	
6	Modho budhpara	4	521	220	42.22	

The occurrence of *Fusarium* sp. in association with tomato in all the surveyed areas in the Rajshahi Metropolitan City suggested their prevalence in soil in that region which influenced by prevailing environmental conditions of the area. It is also possible that apart from the ubiquitous nature of *Fusarium*, contentious cultivation of tomato on the same piece of land might have enhanced the rapid multiplication and spread of *Fusarium* in the region.

The symptoms of wilt disease appear on leaf, stem and root. The first indication of this disease is a yellowing and drooping of the lower leaves. This symptom often occurs on one side of the plant or on one shoot. Successive leaves yellowed, wilt and die, often before the plant reaches maturity. As the disease progresses, growth is typically stunted, and little or no fruit develops. If the main stem is cut, dark brown streaks may be seen running lengthwise through the stem. This discoloration often extends far up the stem and is especially noticeable in a petiole scar. The browning of the vascular system is characteristic of the disease. The above characteristics were considered for the identification of the wilt disease. The mycelia grew rapidly on the wilt infected tomato plant and also on PDA media with white, cottony to floccose colony hyphae. In culture, mycelial colony was circular (**fig 1, A**) to slightly irregular, pale colorless or rarely light brown, aerial mycelia were white on PDA media, this fungus changed its mycelialcolour from white to blackish brown. It was shown in cotton to floccose, generally abundant. Conidia formed under optimum conditions were 3-5 septate and measure 20-48 long, 6-19 thick (**fig 1, B)**.

A B

Figure 1: A. Culture of *Fusarium oxysporum;* B. Conidia of *Fusarium oxysporum*

Antagonistic effect of *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp. were presented in table 3. The value of Percent Inhibition Radial Growth (PIRG) of *Fusarium* sp. were recorded 82, 77 and 70 against *Penicillium* spp., *Trichoderma* spp. and *Aspergillus* spp., respectively after 7 days of incubation at 27°C on PDA plates. It was found that the highest (82%) PIRG was of *Penicillium* spp. and the lowest was (70%) of *Aspergillus* spp. against *Fusarium* sp.

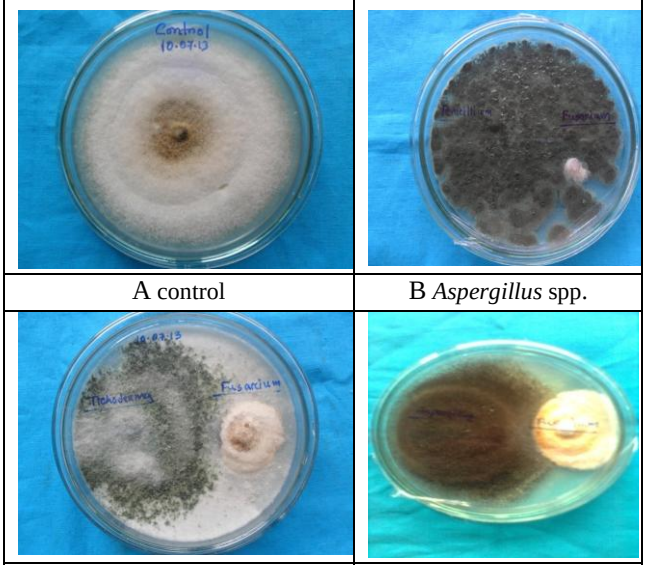
Table 3. Antagonistic effect of *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp. against *Fusarium* sp. after 7 days of incubation at 27°C

Antagonistic fungi	PIRG of mycelium after 7 days of incubation
*Penicillium*s pp.	82%
Trichoderma spp.	77%
Aspergillus spp.	70%

Plants are in continuous association with microbes which interact with them in positive, negative or neutral ways. The fusarium wilt of tomato (*Lycopersicon esculentum* Mill.) caused by *F. oxysporum* is recognized as one of the most devastating diseases in major tomato growing regions worldwide and also in Bangladesh. For eco-friendly and sustainable management of this disease,

three antagonistic fungi were evaluated against the pathogen. All the tested fungi (antagonistic) significantly reduced the growth of *Fusarium* sp.

Observation *in vitro* dual culture showed that the high antagonistic effect (over growth 82, 72 and 70%) was found against *F. oxysporum* f. sp. *lycopersici* in case of *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp. after 7 days of incubation at 27ºC on PDA plates has been shown in table 3. From this study the highest PIRG was 82% of *Penicillium* spp. and the lowest was 70% of *Aspergillus* spp. against *Fusarium* sp. (table 3 and fig. 2).



A control | B *Aspergillus* spp.

C *Trichoderma* spp. | D *Penicillium* spp.

Figure 2: Antagonistic effect against *Fusarium* sp.

The antagonistic properties of different species of *Pseudomonas* sp. and two *Trichoderma* spp. against *Fusarium* have also been reported (Rajeswari and Kannabiran, 2011). Among them, highest PIRG were 86.6, 84.0 and 60.0, respectively. This study also showed the antagonistic effect of *Trichoderma* spp. on *Fusarium.* The inhibitory effect of these bioagents tested pathogen was probably due to competition and/or antibiosis. Inhibition of germination and mycelial growth of pathogenic fungi *in vitro* was attributed to the antifungal properties of volatile compounds (alkyl pyrones) produced by *T. harzianum* (Claydon*et al*., 1987). The growth inhibition of *F. oxysporum* by antagonist fungi is possibly attributed to the secretion of antibiotics by the fungi or other inhibitory substances produced by the antagonists such as geodin, terricin, terric acid, aspergillic acid, dermadin, etc. (Upadhyay and Rai,1987). The degree of effectiveness varies according to the nature, quality and quantity of antibiotics/inhibitory substances secreted by the antagonists (Skidmore and Dickinson, 1976). Bashar and Rai (1994) observed that *A. flavus*, *A. niger*, and *T. viride* amended in soil suppressed the growth of *F. oxysporum* f. sp. *cicero* and exhibited strong fungistatic activity against germination of conidia of test pathogens. Mondal *et al*. (2000) reported that two metabolites (2-carboxymethyl 3-n-hexyl maleic acid and 2-methylene-3-hexylbutanedioic acid) isolated from *A. niger* AN27, had increased germination, shoot length, root length and biomass of cauliflower seedlings. It is well known that *Trichoderma* can parasitize fungal pathogens and produce antibiotics, besides the fungus have many positive effects on plants: increased growth and yield, increased nutrient uptake, increased fertilizer utilization efficiency, increased percentage and rate of seed germination and induced systemic resistance to plant diseases (Harman *et al*., 2004). Recently, Akrami *et al.* (2011) found that three isolates (*T. harzianum*, *T. asperellum*, *T. virens*) were effective against *Fusarium* rot of lentil. Ahmed (2011) studied the growth promoting ability of *T. koningii* and a white sterile fungus (which did not fructify) on tomato plants grown in soil inoculated with wilt pathogen *F. oxysporum* f. sp. *lycopersici.* He reported that antagonistic rhizosphere suppressed the deleterious soil microbes by competing at the active sites, reduced the intensity of disease development and subsequently, stimulated the growth/yield of plants. On the contrary, from this investigation, it is found that *Penicillium* spp. was the best antagonist against *Fusarium* sp. A number of *Penicillium* spp. have been reported as antagonists of plant pathogens with a mechanism of action based on the production of the antibiotic compounds and established of mycoparasitic interactions (Nicoletti and Stefano, 2012).

In conclusion, the present study indicates that *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp. can be explored further for the biological control of wilt disease of tomato which may help to obtain a higher yield and good health in agriculture.

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