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IN VITRO BIOLOGICAL CONTROL OF *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) SACC. AND *SCLEROTIUM ROLFSII* SACC., CAUSAL AGENT OF ANTHRACNOSE AND SOFT ROT OF *CORCHORUS CAPSULARIS* L.

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Abstract

Antagonistic potentials of two soil fungi *viz*., *Aspergillus niger* van Tieghem and *Trichoderma viride* Pers. ex Gray against two fungal pathogens of jute plants, namely *Colletotrichum gloeosporioides* (Penz.) Sacc. and *Sclerotium rolfsii* Sacc. were evaluated by dual culture technique and the efficacy of volatile and non-volatile metabolites released by them was evaluated by 'inverted plate method' and 'poisoned food technique'. In dual culture colony interaction, *A. niger* and *T. viride* showed 50 and 50.78% growth inhibiting effect of *C. gloeosporioides*. The same antagonistic fungi also showed 53.56 and 69.71% growth inhibition of *S. rolfsii*. Volatile metabolites from *T. viride* showed growth inhibiting effect of *S. rolfsii* (46%) and *C. gloeosporioides* (35.78%). Growth inhibiting effect of more than 50% was noted owing to the non-volatile metabolites of *A. niger* and *T. viride* on *C. gloeosporioides* at 20% concentration. These results suggest that *T. viride* may be exploited commercially as a biocontrol agent of anthracnose and soft rot pathogens of jute.

Introduction

Jute (*Corchorus* spp.) is the principal fiber crop of Bangladesh. It is obtained mainly from *Corchorus capsularis* L. and *C. olitorius* L. Among the jute growing countries of the world, Bangladesh ranks second in respect of production (Islam and Rahman 2008, Sadi 2007). Diseases play a major role for the severe yield loss of jute. According to Ahmed (1968) 24.5% loss in yield of fiber occurred due to different diseases. Major diseases of jute plants in Bangladesh are stem rot, anthracnose, soft rot, black band, die-back and powdery mildew. They are not only responsible for yield loss but also deteriorate the quality of fiber and seeds (Biswas *et al*. 1980). Among the various jute diseases anthracnose and soft rot diseases are very common and most destructive one. Anthracnose affected plants yield for poor quality fiber, mostly knotty in nature with adherent barks, which resists the retting. In mature stage the plants do not die but the disease badly affect the fiber quality. Soft rot of jute caused by *Sclerotium rolfsii* Sacc. is also one of the most destructive diseases in jute growing areas in the world.

In recent years scientists have begun to understand the consequences of widespread and repeated use of chemical fungicides that threaten human interest. Use of antagonist as biological control agent has now become one of the most exciting and rapidly developing areas in plant pathology. Presently, different species of *Trichoderma* are used to control different plant pathogens (Vinalea *et al.* 2008). Colony interaction between non-pathogenic and pathogenic fungi for determination of antagonism of fungi isolated from different habitats is studied (Skidmore and Dickinson 1976, Bashar and Rai 1994, Brozova 2002, Prince *et al.* 2011, Akter *et al.* 2014). Many

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microorganisms produce biologically active volatile substances which have been noticed inhibitory or stimulatory against the growth of fungi (Singhai *et al*. 1975, Skidmore and Dickinson 1976, Akter *et al.* 2014). The production of biologically active non-volatile toxic substances by fungi has also been discussed by several workers (Singh and Webster 1973, Skidmore and Dickinson 1976, Akter *et al.* 2014). However, in Bangladesh limited studies have been done in this regard. Hence, the present investigation was undertaken to find out the *in vitro* antagonistic effect of *Aspergillus niger* and *Trichoderma viride* on the growth of *C. gloeosporioides* and *S. rolfsii*.

Materials and Methods

Jute plants with anthracnose and soft rot symptoms were collected from selected fields of Manikganj (BJRI) and research field of the Botanical Garden, Dhaka University to record the prevalence of diseases during May, 2014 to November, 2014. Jute samples were collected in separate sterile polyethylene bags, labeled properly and then brought to the laboratory for isolating associated pathogenic fungi following “Tissue planting method” on PDA medium. From infected stems of jute showing anthracnose and soft rot symptoms 60 inocula, each measuring 2^2 mm were cut separately with a pair of sterilize scissors and kept in a separate sterilize Petri plate. The inocula were washed with sterile water and then surface sterilized by dipping in 10% chlorox solution for three minutes. The inocula were again washed with sterile water. In case of anthracnose and soft rot symptoms a total of 60 inocula were placed separately on 20 sterilized Petri plates containing 15 ml of PDA medium with an addition of 1 drop (ca 0.03 ml) of lactic acid to check the bacterial growth and incubated in an incubator (25 ± 2ºC) for 7 days.

Antagonistic fungi were isolated from rhizospheric soil of the host following serial dilution method (Brown 2004). Among the isolated soil fungi, *Aspergillus niger* and *Trichoderma viride* were selected to test their antagonistic potential against the pathogens following dual culture technique described by Bashar and Rai (1994). The parameter used for the assessment of the colony interaction were the width of inhibition zone, intermingled zone and per cent inhibition of radial growth was calculated by the formula of Fokkema (1976).

Effects of volatile and non-volatile metabolites of the selected soil fungi against the test pathogens were also studied following the methods described by Bashar and Rai (1994). The per cent growth inhibition of the test fungi was calculated by the following formula:

$$I = \frac{C - T}{C} \times 100$$

where, I = Per cent growth inhibition, C = Growth in control, T = Growth in treatment.

Results and Discussion

Four fungi were isolated from the anthracnose symptom of jute. The isolated fungi were *Colletotrichum gloeosporioides, Fusarium* sp., *Aspergillus niger* and *A. flavus. Sclerotium rolfsii* was exclusively isolated from the soft rot symptom of jute. Among the isolated fungi *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* were selected as test pathogens owing to their higher percentage of occurrence and previous reports as pathogenic organisms (Anon. 1997, Richardson 1979).

The results of colony interactions have been summarized in Table 1. Different antagonistic effects of the soil fungi were noted against the test fungi. Bii type (grade 2) was found to be the most commonly encountered type of colony interaction. In dual culture colony interaction *A. niger* and *T. viride* showed 50.00 and 50.78% growth inhibiting effect of *C. gloeosporioides* (Table 1). The same antagonistic fungi also showed 53.56 and 69.71% growth inhibition of *S. rolfsii*. The intermingled zone between *A. niger* and *C. gloeosporioides* and between *T. viride* and *C. gloeosporioides* were 0.73 and 0.45 cm, respectively (Table 1). The intermingled zone between *A. niger* and *S. rolfsii* was 1.13 cm. The maximum intermingled zone was observed in case of *A. niger* in both the cases (Table 1). In all the cases no inhibition zone was noticed between the antagonists and the jute pathogens.

Table 1. Effects of antagonists on the radial growth of *Colletotrichum gloeosporioides* and *Sclerotium rolfsii*.

Name of antagonists	Type	Per cent inhibition of radial growth and intermingled zone of test pathogens			
		Colletotrichum gloeosporioides		*Sclerotium rolfsii*	
		% inhibition of growth	Intermingled zone (cm)	% inhibition of growth	Intermingled zone (cm)
Aspergillus niger	Bii	50.00	0.73	53.56	1.13
Trichoderma viride	Bii	50.78	0.45	69.71	-

Bii = Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony. - = Absent.

Table 2. Per cent inhibition of radial growth of *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* owing to volatile metabolites of antagonistic fungi.

Name of antagonist	Per cent inhibition of radial growth of the test pathogens	
	Colletotrichum gloeosporioides	*Sclerotium rolfsii*
Aspergillus niger	25.00	19.36
Trichoderma viride	46.00	35.78

In contrast to the present study, Tapwal *et al.* (2015) reported that in dual culture colony interaction *T. viride* showed 12.50% growth inhibition of *C. gloeosporioides*. Dikshit *et al*. (2011) observed that *T. viride* reduced the growth of *S. rolfsii* by 68.24%. The differences between the present investigation and others might be due to the selection of separate strains of pathogens and antagonists.

The results of effect of volatile metabolites of antagonistic fungi against test pathogens are presented in Table 2. The maximum inhibition of radial growth of *C. gloeosporioides* was shown by *T. viride* (35.78%) followed by *A. niger* (19.36%) after 7 days of incubation at 25 ± 2ºC. Again the maximum inhibition of radial growth of *S. rolfsii* was also observed in case of *T. viride* (46%) followed by *A. niger* (25%) due to the volatile metabolites after 4 days of incubation at the same temperature. In contrast to the present study, Tapwal *et al.* (2015) reported that volatile substances produced by *T. viride* showed 34.37% growth inhibition of *C. gloeosporioides*. Thakur and Harsh (2014) reported that volatile metabolites produced from the culture of *Aspergillus niger* exhibited 42.43% inhibition of mycelial growth of *C. gloeosporioides.* Aktar *et al*. (2014) reported that volatile metabolites produced by an isolate of *T. viride* and *A. niger* inhibited mycelial growth of *Curvularia lunata* by 20.86 and 14.85%, respectively. Difference in per cent inhibition with the present study might be due to the difference in organisms involved in the interaction.

The results of effect of non-volatile metabolites of antagonistic fungi against test pathogens are presented in Figs 1-2. The maximum inhibition of radial growth of *S. rolfsii* was observed with the culture filtrates of *T. viride* (30%) followed by *A. niger* (28%) at 20% concentration (Figs 1-2) after 4 days of incubation at 25 ± 2ºC. But the inhibition of radial growth of *C. gloeosporioides* due the non-volatile metabolites showed more than 50% in case of *A. niger* (53.57%) and *T. viride* (55.35%) at 20% concentration (Figs 1-2).

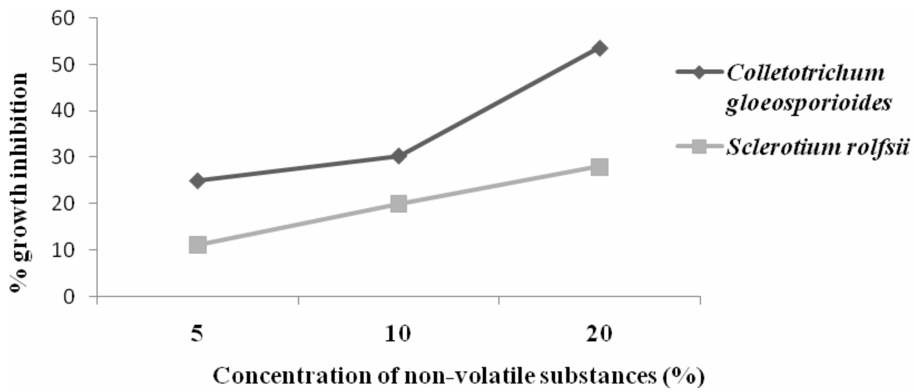

Fig. 1. Per cent growth inhibition of *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* by the non-volatile substances of *Aspergillus niger*.

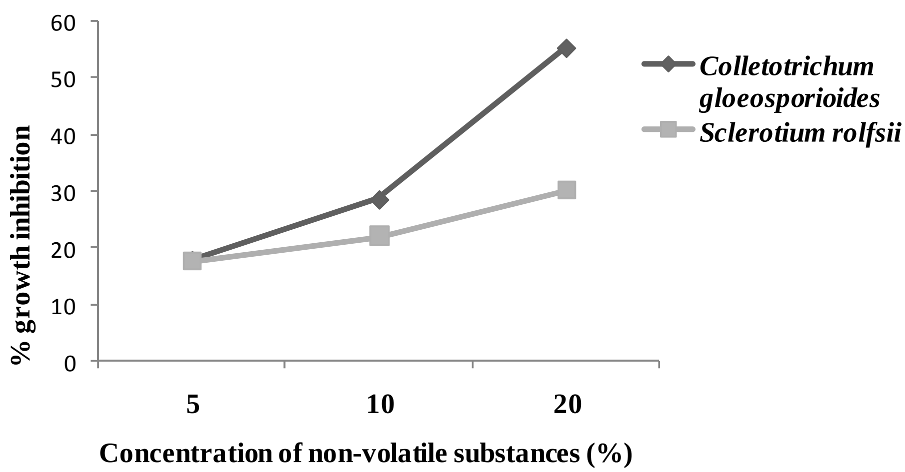

Fig. 2. Per cent growth inhibition of *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* by the non-volatile substances of *Trichoderma viride*.

Tapwal *et al.* (2015) reported that culture filtrates of *T. viride* showed 13.33% growth inhibition of *C. gloeosporioides*. Dikshit *et al*. (2011) reported that cell free culture filtrate of *T. viride* reduced 100% radial growth of *S. rolfsii* at 20% concentration, whereas 81.25% reduction of radial growth was observed at 15% concentration, 58.32% reduction at 10 and 31.45% reduction at 5% concentration were observed. Aktar *et al.* (2014) also reported that non-volatile metabolites produced by an isolate of *T. viride* and *A. niger* inhibited the mycelial growth of

Curvularia lunata by 60.07 and 52.50%, respectively. The per cent growth inhibition of *C. gloeosporioides* and *S. rolfsii* increased with increase of concentration of non-volatile substances in the culture medium.

Results of this experiment revealed that out of two soil fungi *Trichoderma viride* exhibited strong antagonistic effect against the two test pathogens. This effect might be due to its fast growing nature, rapid sporulation and toxin producing capacity (Skidmore and Dickinson 1976). *Trichoderma viride* is known to be capable of producing antibiotics which might have suppressed the growth of the test pathogens. The present investigation suggests that *Trichoderma viride* may be exploited commercially as a biocontrol agent of anthracnose and soft rot pathogens of jute.

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