

**ANTAGONISTIC POTENTIAL OF SOIL FUNGI AGAINST
COLLETOTRICHUM GLOEOSPORIODES (PENZ.) SACC., THE CAUSAL
AGENT OF ANTHRACNOSE OF RAUWOLFIA SERPENTINA (L.)
BENTH. EX KURZ**

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Abstract

Four antagonistic fungi *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tiegh. and *Trichoderma viride* Pers. were isolated from field soil of *Rauwolfia serpentina* by serial dilution method and selected to evaluate their antagonistic potentiality against *Colletotrichum gloeosporiodes* (Penz.) Sacc, the causal agent of anthracnose of *Rauwolfia serpentina* (L.) Benth. ex Kurz following dual culture colony interaction, volatile and non-volatile metabolites. In dual culture method *Trichoderma viride* showed maximum (84.28%) inhibition of test fungi followed by *Aspergillus niger* (77.39%), *A. fumigatus* (43.71%) and *A. flavus* (29.32%). Volatile metabolites of *T. viride* showed higher (77.64%) inhibition of test fungi followed by *A. flavus* (75.58%), *A. fumigatus* (60.88%) and *A. niger* (58.23%). Non-volatile metabolites of antagonistic fungi showed that *A. flavus* showed (94.42%) inhibition of test fungus followed by *T. viride* (90.90%), *A. niger* (86.13%) and *A. fumigatus* (73.73%). *Aspergillus flavus*, *A. niger* and *T. viride* may be exploited commercially as a biocontrol agent against anthracnose disease of *R. serpentina*.

Introduction

Rauwolfia serpentina (L.) Benth. ex Kurz is a medicinal shrub belongs to the family Apocynaceae. The shrub is locally known as "Sarpagandha" and also known as Indian snakeroot. It grows in India, Thailand and other parts of Asia, South America and Africa. It is widely distributed in the sub-Himalayan tract from Punjab to Nepal, Sikkim and Bhutan⁽¹⁾. In Bangladesh it grows in Chittagong, Dhaka, Sylhet and Mymensingh⁽²⁾. International Union for Conservation of Nature (IUCN) has placed this plant under endangered status⁽³⁾. Root of this shrub is mostly used as a good antidote for high blood pressure. Seventeen different alkaloids have been extracted from the bark of the root of this shrub⁽⁴⁾. Serpentine is one of those alkaloids.

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Diseases are a major source of leaf and plant damage that can be caused by a number of plant pathogenic organisms. Fungal diseases are the number one cause of plant loss worldwide. A fungicide is a specific type of pesticide that controls fungal disease by specifically inhibiting or killing the fungus causing the disease. Fungi often spoil stored fruits, vegetables, tubers, and seeds. A few infect grains to produce toxins (mycotoxins) capable of causing severe illness or even death in humans and animals when consumed. Fungicides are biocidal chemical compounds or biological organisms used to kill or inhibit fungi or fungal spores. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality, and profit. Fungicides are used both in agriculture and to fight fungal infections in animals.

Over the past few decades, farmers have increasingly relied on chemical pesticides for protecting plants against pathogens. Biological control of plant diseases including fungal pathogens has been considered a viable alternative method to chemical control. Biological control presents a better alternative with relative low cost, no side effects and reduced resistance development in the pathogen⁽⁵⁻⁶⁾.

Study of antagonist as biological control agent has now become one of the most exciting and rapidly developing areas in plant pathology because it has great potential to solve many agricultural and environmental problems. At present, *Trichoderma*-based products are considered as relatively novel biological control agents which can help farmers to reduce plant diseases and increase plant growth⁽⁷⁾.

In Bangladesh, research on biological control of fungal diseases of *R. serpentina* is very limited. So, for the sake of economy we need more information in this regard. Considering the importance of this endangered plant, present investigation was undertaken to find out the efficacy of antagonistic fungi against *C. gloeosporioides* the causal agent of anthracnose of *R. serpentina*.

Materials and Methods

An investigation was carried out to find out the fungi associated with leaves of *R. serpentina* during April, 2007 to August, 2013. Infected leaves of *R. serpentina* having characteristic samples were collected from four different locations namely, Curzon Hall Campus, Dhaka University, Lawachara Sylhet, Botanic Garden, Chittagong University Campus and Mymensingh Agricultural University campus properly and then brought to the laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka. The fungi associated with infected leaves were isolated following "Tissue planting method" on PDA medium⁽⁸⁾. A total of 15 species of fungi were isolated from infected leaves of *R. serpentina*. Pathogenecity test of the isolated fungi were done following 'Detached leaf technique' and 'Spraying of spore suspension' on healthy plants. Among the isolated fungi *C. gloeosporioides* was found to be pathogenic to *R. serpentina*⁽⁹⁾.

Serial dilution method was used to isolate antagonistic fungi from rhizosphere soil of the host plant. Among the isolated soil fungi, *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *T. viride* were selected to test their antagonistic potential against the following dual culture technique⁽¹⁰⁾. The parameter used for the assessment of the colony interaction and per cent inhibition of radial growth was calculated⁽¹¹⁾. Effects of volatile and non-volatile metabolites of the selected soil fungi against the test pathogens were also studied. Data on different parameters were analyzed following computer package MSTAT-C and means were compared using DMRT. The data were collected and evaluated by analysis of variance (ANOVA) by using STAR statistical program.

Results and Discussion

Fifteen species of fungi, namely *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link ex Fr. *Aspergillus niger* van Tieghme, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia lunata* (Wakker) Boedijn, *Fusarium* sp. 1, *Fusarium* sp. 2, *Macrophoma* sp., *Nigrospora sphaerica* (Sacc.) Mason, *Penicillium digitatum* Saccardo, *Penicillium italicum* Whemer, *Pestalotiopsis guepinii* (Desm.) Stay, *Pseudocercospora liebenbergii* (Syd.) Deighton, *Rhizopus stolonifer* Bull. Toney, *Trichoderma viride* Pers were found to be associated with diseased *R. serpentina*. Among the isolated fungi one was found to be pathogenic to *R. serpentina*. The pathogenic fungus was *C. gloeosporioides*. The pathogenic fungus was selected as test pathogen against selected four antagonistic fungi.

The results of colony interactions are summarized in Table 1 and Fig. 1. In this study, antagonistic relationships (Grade) among the soil fungi and test pathogens were 2 and 4. However, grade 2 was found to be the most commonly encountered type of colony interaction as 10 interactions were incorporated in this grade which was followed by grade 4 (2 out of 12) (Table 1).

Table 1. Colony interaction between *Colletotrichum gloeosporioides* and antagonists.

Name of antagonists	Grade*	Type**	% inhibition of colony of the pathogens	Intermingled zone (cm)	Inhibition zone (cm)
<i>Aspergillus flavus</i>	2	Bii	40.26	-	-
<i>A. fumigatus</i>	2	Bii	36.84	0.2	-
<i>A. niger</i>	4	C	43.48	-	0.2
<i>Trichoderma viride</i>	2	Bii	66.67	0.2	-

- = Absent, * = Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), based on Skidmore and Dickinson (1976). **Bii = Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony (2). C = Slight inhibition with a narrow demarcation line, 1 - 2 mm (4).

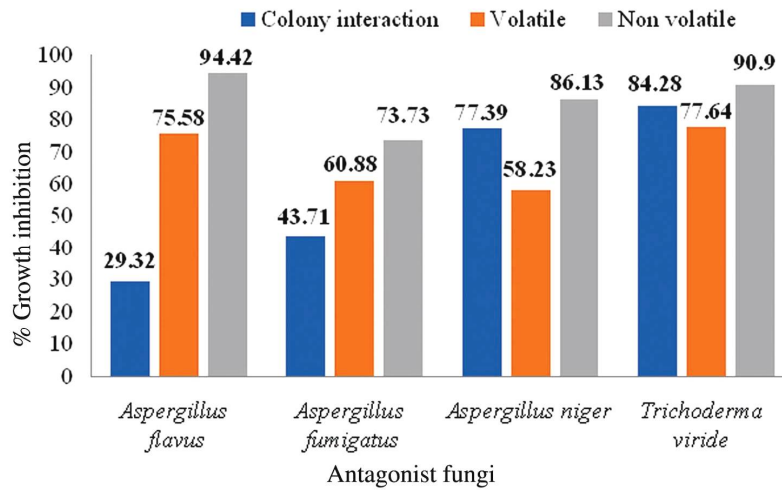


Fig 1. Evaluation of *in vitro* inhibition of *Colletotrichum gloeosporioides* using antagonistic fungi in different methods.

Figs 1 and 2 show that in dual culture colony interaction *T. viride* showed the highest growth inhibition on *C. gloeosporioides* (84.28%) which was followed by *Aspergillus niger* (77.39%), *A. flavus* (43.71%) and *A. fumigatus* (29.32%).

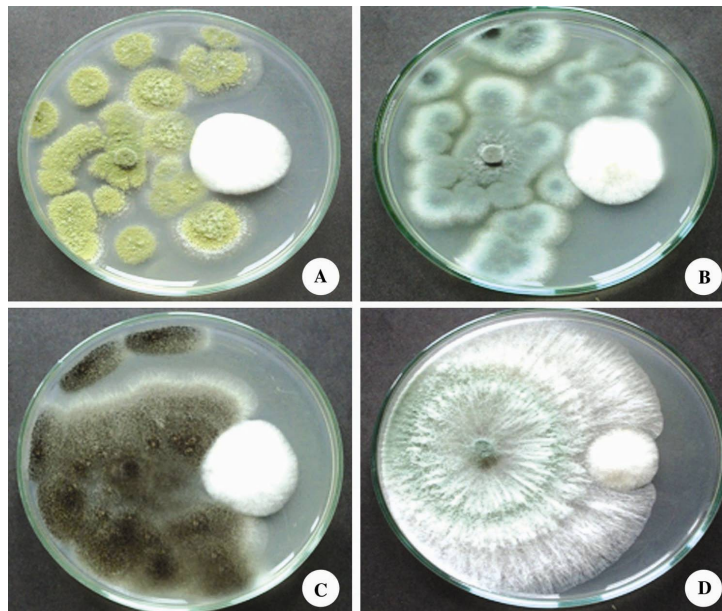


Fig. 2. Colony interaction between *Colletotrichum gloeosporioides* and antagonists. A. *Colletotrichum gloeosporioides* and *Aspergillus flavus*. B. *C. gloeosporioides* and *A. fumigatus*. C. *C. gloeosporioides* and *A. niger*. D. *C. gloeosporioides* and *Trichoderma viride*.

In contrast to the present study, Akter *et al.*⁽¹²⁾ reported that in dual culture colony interaction *A. niger*, *T. viride*, *A. flavus* and *A. fumigatus* showed 68.66, 57.24, 54.19 and 50.25% growth inhibition on *Colletotrichum* sp., respectively. Again *Aspergillus niger*, *Trichoderma viride*, *A. flavus* and *A. fumigatus* showed 75.87, 75.5, 51.78 and 45.52% growth inhibition on *Curvularia lunata*, respectively. Further *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* showed 56.52, 50.70, 47.36 and 46.15% growth inhibition on *Fusarium semitectum*, respectively. Bashar and Chakma⁽¹³⁾ reported that in dual culture colony interaction *A. niger*, *T. viride*, *A. flavus* and *A. fumigatus* showed 65.21, 64.24, 57.14 and 34.78% growth inhibition on *F. oxysporum*, respectively. This variation might be due to selection of different test pathogens. In dual culture technique, significantly maximum inhibition was recorded in *T. viride* (66.40%) according to Patel and Joshi⁽¹⁴⁾.

Tapwal *et al.*⁽¹⁵⁾ reported that in dual culture colony interaction *T. viride* showed 12.50% growth inhibition on *C. gloeosporioides*. The same antagonist also showed different effects on different fungi in the present investigation. This variation might be due to selection of different test pathogens. In dual culture technique, significantly maximum inhibition was recorded in *T. viride* (66.40%) according to Patel and Joshi⁽¹⁴⁾.

The results on the effect of volatile metabolites of antagonistic fungi against *R. serpentina* pathogens are presented in Figs 1 and 3. The maximum inhibition of radial growth of *C. gloeosporioides* was observed in *T. viride* (77.64%) which was followed by *A. flavus* (75.58%), *A. fumigatus* (60.88%) and *A. niger* (58.23%) due to the volatile metabolites after 6 days of incubation at $25 \pm 2^\circ\text{C}$.

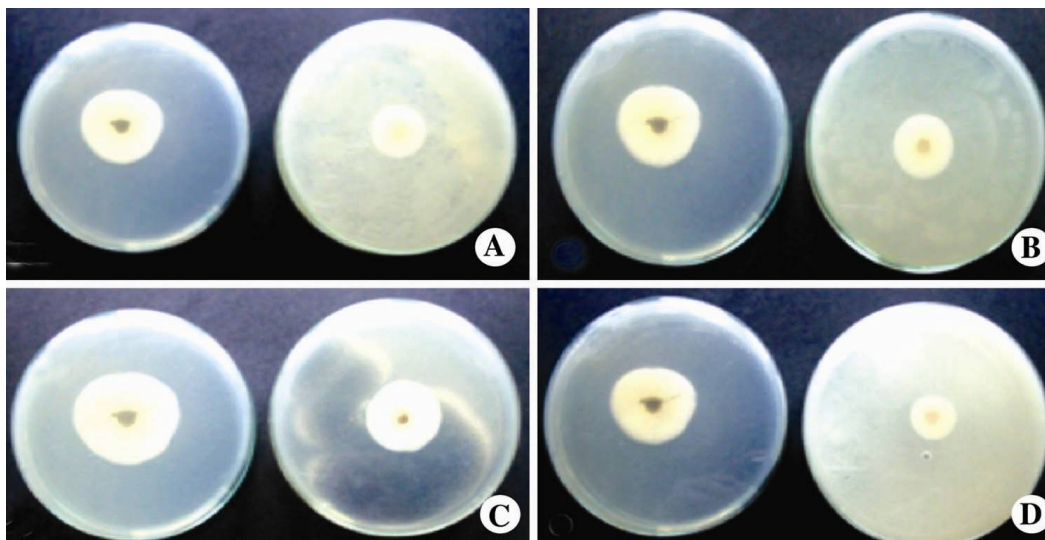


Fig. 3. Growth inhibition of *Colletotrichum gloeosporioides* owing to volatile metabolites of antagonists. A. *Colletotrichum gloeosporioides*: *Aspergillus flavus*. B. *C. gloeosporioides*: *A. fumigatus*. C. *C. gloeosporioides*: *A. niger* and D. *C. gloeosporioides*: *Trichoderma viride*.

In contrast to the result of present study, Aktar *et al.*⁽¹²⁾ reported that volatile metabolites produced by isolates of *A. niger*, *A. flavus*, *A. fumigatus* and *T. viride* inhibited the mycelial growth of *Colletotrichum* sp. by 14.68, 11.78, 11 and 11%, respectively. In addition the volatile metabolites produced by isolates of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *F. semitectum* by 13.5, 9.5, 8 and 7.75%, respectively. Differences in per cent inhibition with the present study might be due to the difference in organism involved in the interaction. Bashar and Chakma⁽¹³⁾ reported that volatile substances produced by *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* showed 29.75, 20.15, 15.78 and 12.25% growth inhibition on *F. oxysporum*, respectively. Thakur and Harsh⁽¹⁶⁾ reported that volatile metabolites produced from the culture of *A. niger* showed 42.43% inhibition of mycelial growth of *C. gloeosporioides*.

Figs 1 and 4 show the effect of non-volatile metabolites on the growth of *Colletotrichum* as was observed with the culture filtrates of *A. flavus* (94.42%), which was followed by *T. viride* (90.90%), *A. niger* (86.13%) and *A. fumigatus* (73.73%) at 20% concentration.

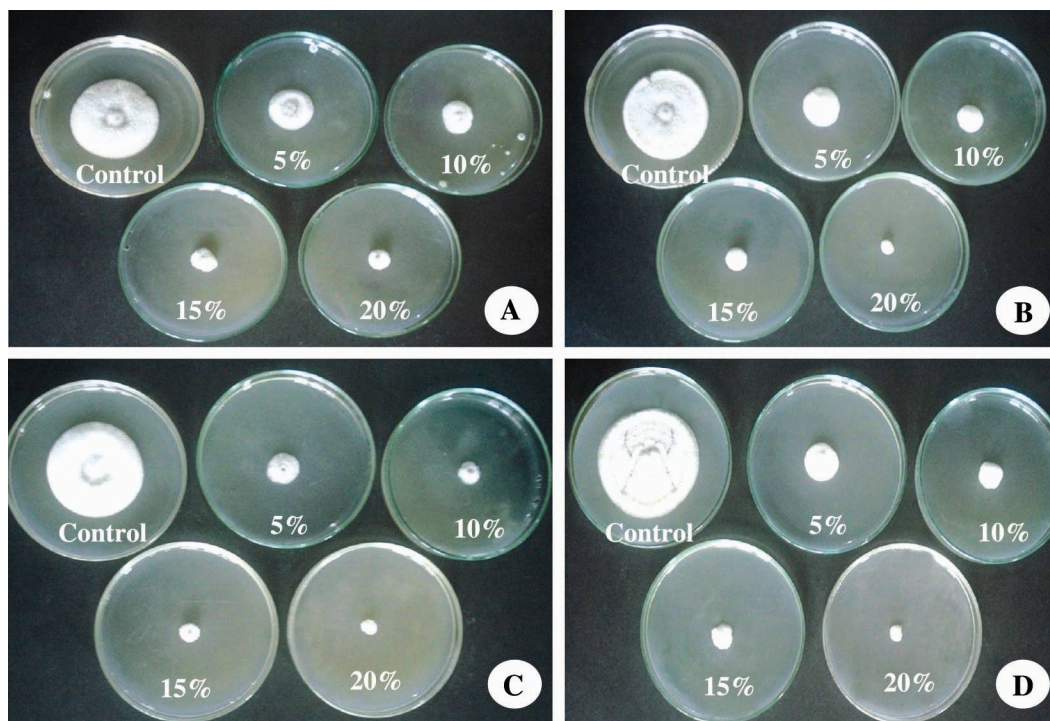


Fig. 4. Growth inhibition of *Colletotrichum gloeosporioides* owing to non-volatile metabolites of four antagonists at 5, 10, 15 and 20% concentrations. A. *Colletotrichum gloeosporioides*: *Aspergillus flavus*. B. *C. gloeosporioides*: *A. fumigatus*. C. *C. gloeosporioides*: *A. niger*. D. *C. gloeosporioides*: *Trichoderma viride*.

In contrast to the data obtained in the present study, Aktar *et al.*⁽¹²⁾ reported that non-volatile metabolites produced by isolates of *A. niger*, *T. viride*, *A. flavus* and *A. fumigatus* inhibited mycelial growth of *Colletotrichum* sp. by 52.56, 44.72, 40 and 37.2%, respectively. Further, the non-volatile metabolites produced by an isolate of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited mycelial growth of *F. semitectum* by 50, 45, 8 and 7.75%, respectively. Differences in per cent inhibition with the present study might be due to the difference in organism strains involved in the interaction. Bashar and Chakma⁽¹³⁾ reported that culture filtrates of *T. viride*, *A. fumigatus*, *A. niger* and *A. flavus* showed 82.05, 80.56, 72.22 and 66.66% growth inhibition of *F. oxysporum* at 20% concentration owing to non-volatile metabolites. Madhanraj *et al.*⁽¹⁷⁾ reported that culture filtrates of *T. viride* and *A. niger* inhibited the mycelial growth of *F. solani* by 85 and 70% at 20 per cent concentration, respectively. Tran⁽⁷⁾ used *T. viride* to control *S. rolfsii* and found effective result. Tapwal *et al.*⁽¹⁵⁾ reported that culture filtrates of *T. viride* showed 13.33% growth inhibition on *C. gloeosporioides*.

Two biocontrol agents *viz.*, *Aspergillus niger* and *Trichoderma viride* were tested against ten white rot and one brown rot wood decay fungi (WDF) by dual culture technique under laboratory conditions. The result showed that both *A. niger* and *T. viride* inhibit growth of all WDF tested. The percentage inhibition of radial growth values of *T. viride* and *A. niger* are almost the same (ranging from 29.2 to 66.7) and the average mean value of *T. viride* (51.7) is 13.3 more than that of *A. niger* (45.5).⁽¹⁸⁾

The present investigation suggests that *Aspergillus niger*, *A. fumigatus* and *Trichoderma viride* may be exploited commercially as a biocontrol agent to protect anthracnose disease of *R. serpentina* caused by *C. gloeosporioides*.

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