Screening of some antagonist soil fungi against white mold pathogen of mustard *in-vitro*

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1. Introduction

The stem rot fungus *Sclerotinia sclerotiorum* produces sclerotia which can survive a long time in the soil. These sclerotia give rise to fruiting body apothecia that produce a huge number of ascospores which create rot disease in different crops. This complicated pattern of life cycle and wide host range make it difficult to control the white mold disease of mustard. In general, plant diseases are controlled by using chemical fungicides and in certain cases by cultural practices. The widespread use of chemicals in agriculture has been a subject of public concern and scrutiny due to their potentially harmful effects on the environment and health. Some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Many species of fungi such as *Penicillium*, *Aspergillus*, *Trichoderma, Gliocladium* and bacteria such as *Bacillus*, *Enterobacter*, *Pseudomonas, Streptomyces* are used as bio-control agents (Suárez-Estrella et al. 2007). There are some studies on the biological control of *S. sclerotiorum* by using fungal bio-control agents either in the laboratory or in the fields (Hjeljord & Tronsmo, 1998, Tiwari et al. 2011, Jawadayn et al. 2015). In Bangladesh commercially cultivated varieties viz. BARI Sharisa 14, BARI Sharisa 17, local Tori, are highly susceptible to white mold (please check it) disease. Search for resistance is one of the disease management options, but it is time-consuming. Thus, the study on biological disease management is essential. Therefore, the investigation was undertaken to search for effective antagonist soil fungi against white mold pathogen.

2. Materials and Methods

Test pathogen

Sclerotinia sclerotiorum was isolated from severely white mold disease infected mustard stems following "Tissue planting" method. The pathogenicity of the isolated fungi was tested following "detached leaf and stem technique" (Shamsi et al. 2013).

Antagonists

The fungal antagonist used in this study **(***Aspergillus flavus, A. fumigatus, A. niger* 1, *A. niger* 2, *A. niger* 3, *Penicillium* sp. *Trichoderma harzianum, T. viride* and *T. virens*) were obtained from Plant pathology lab, Department of Botany, University of Dhaka, Bangladesh.

Dual culture method

Fifteen ml of sterilized potato dextrose agar medium was poured into sterile Petri plates and allowed to solidify. Fungal antagonists were inoculated at one side of the Petri plate and the test pathogen was inoculated at the opposite side of the same plate by leaving a 3 cm gap and incubated at 25±1^{o}C for 7 days. The percentage of inhibition over control was measured according to the formula given by Jeyaseelan et al. (2012).

Effect of volatile and non-volatile compounds

In volatile methods, the fungal antagonists were grown in Petri plates on PDA medium at 25±1^{o} C for 3 days. After the inoculation, the lid of each Petri plate was replaced by the same sized bottom plate, containing 15 ml PDA medium, centrally inoculated with a test pathogen. Then Petri plates were covered by Parafilm so that no volatile substances can be moved from the

inside of the Petri plates. The percentage of inhibition of the test fungi was calculated after the 7^{th} day of incubation.

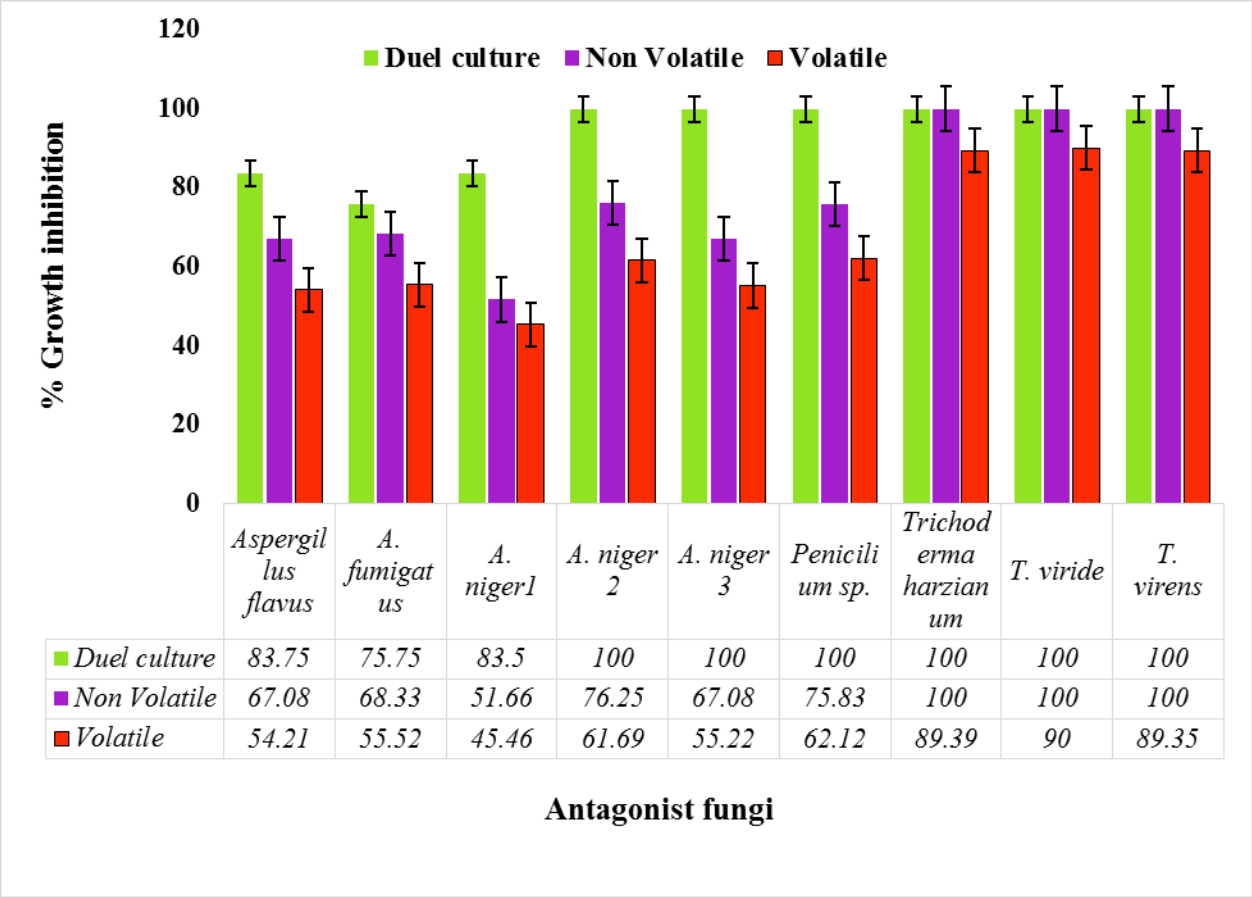
In non-volatile methods, nine fungal antagonists cultured in a conical flask on Potato Dextrose Broth and incubated at 25 ± 1° C for 15 days. Liquid culture filtrate was collected separately and filtered through filter paper; then, centrifuged at 3000 rpm for 20 minutes. Bio-efficacy of culture filtrates against test pathogen was tested by following "Poisoned Food Technique method". The percentage of growth inhibition was recorded by following the procedure mentioned earlier.

3. Results and Discussion

The result of colony interaction between the test fungi and the antagonist fungi has been summarized in Fig. 1. Out of nine soil fungi, six fungi *viz*. *Aspergillus niger* 2, *A. niger* 3, *Penicilium* sp, *Trichoderma harzianum, T. viride* and *T. virens* exhibited strong antagonistic effects against *Sclerotinia sclerotiorum* which were completely inhibited (100%) the radial growth of the test fungi in dual culture methods. The least inhibition of radial growth was noticed with *A. niger* 1 (45.46%).

The effect of volatile metabolites of antagonistic fungi against white mold pathogen is presented in Fig. 1. Volatile substances emanating from the cultures of the soil antagonist fungi inhibited the radial growth and sclerotia formation of pathogenic fungi. The maximum inhibition of radial growth of *Sclerotinia sclerotiorum* was observed in *Trichoderma viride* (90%) followed by *T. harzianum* (89.39%), and *T. virens* (89.35%) due to the volatile metabolites. *Penicillium* sp. showed an average mycelial inhibition (62.12%) followed by *Aspergillus niger* 2 (61.69%). The least radial growth inhibition was noted with *A. niger* 1 (45.46%).

The effect of non-volatile metabolites on the growth of *S. sclerotiorum* also has been showed in Fig. 1. The selected antagonists showed varying degrees of growth inhibition of the pathogen. The culture filtrates of three species of *Trichoderma viz*. *T. harzianum, T. viride* and *T. virens* equally inhibited the mycelial growth of *S. sclerotiorum* - 100% at 10% concentration after 5 days of incubation. Among the *Aspergillus* species tested, *Aspergillus niger* 2 inhibited the highest mycelial growth of the test fungi - 76.25%, followed by *A. fumigatus* (68.33%) and *A. flavus* (67.08%). The lowest inhibition of radial growth of *S. sclerotiorum* was observed in *A. niger* 1 (51.66%).



	Aspergillus flavus	*A. fumigatus*	*A. niger1*	*A. niger 2*	*A. niger 3*	*Penicilium sp.*	*Trichoderma harzianum*	*T. viride*	*T. virens*
Duel culture	83.75	75.75	83.5	100	100	100	100	100	100
Non Volatile	67.08	68.33	51.66	76.25	67.08	75.83	100	100	100
Volatile	54.21	55.52	45.46	61.69	55.22	62.12	89.39	90	89.35

Figure 1. Evaluation of *in vitro* inhibition of *Sclerotinia sclerotiorum* using antagonistic fungi in different methods. Vertical bars represent the standard error of the mean (*significantly different at 0.05% level)

Antagonistic level and grade of different antagonist fungi against stem rot pathogen

Maximum antagonist fungi showed common type of colony interaction (grade level 2). In dual culture method, eight fungi showed very high antagonistic level against test fungi. A low level of antagonistic reaction was found in *Aspergillus niger* 1 in volatile method. In the case of *Trichoderma harzianum*, *T. viride* and *T. virens*, very high level of antagonistic reaction was shown in dual culture, volatile and non- volatile methods.

The percentage of inhibition of the test pathogens against the soil fungi also varied due to differences in nature, quality and quantity of the inhibitory substances produced by the soil fungi. In the present investigation, five fungi (*Aspergillus niger* 2, *A. niger* 3, *T. harzianum*, *T. viride* and *T. virens*) were capable of stopping the sclerotia formation of *Sclerotinia sclerotiorum*. Hyphal interactions between *Trichoderma harzianum* and the pathogenic fungus *S. sclerotiorum* were examined in dual culture and found that *T. harzianum* hyphae grew towards and coiled around the *S. sclerotiorum* hyphae. *Trichoderma harzianum* and *T. virens* have also been capable to inhibit both sclerotia and mycelial growth of *Sclerotinia* spp (Jawadayn et al. 2015; Jacob et al. 1996).

4. Conclusions

The present observation suggests that there were qualitative and quantitative differences in the volatile substances and culture filtrate produced by various antagonist soil fungi, so they exhibited different degrees of growth inhibition against the test fungi. Among the nine antagonist soil fungi, *Aspergillus niger* 2, *Trichoderma harzianum*, *T. viride* and *T. virens* were

found to be effective which were capable to inhibit the mycelial growth and stop the sclerotia formation of *Sclerotinia sclerotiorum in vitro*.

5. References

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