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In vitro evaluation of *Trichoderma viride and Trichoderma harzianum* for its efficasy against *Alternaria alternata,* the leaf spot pathogen on Senna plant

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Abstract: *The antagonistic activity of Trichoderma viride and Trichoderma harzianum isolates were tested in vitro condition against Alternaria alternata, causal organism of leaf spot disease on Cassia angustifolia Vahl. Dual culture plate method revealed that the percentage growth inhibition of Alternaria alternata by Trichoderma viride and Trichoderma harzianum was 80.1% and 72.2% respectively. The results indicated that the growth inhibition of Alternaria alternata by Trichoderma viride and Trichoderma harzianum provides the use of excellent potential antagonists capable of controlling the leaf spot disease of Senna plant.*

Keywords: *Alternaria alternata, antagonistic activity, Cassia angustifolia, Trichoderma harzianum, Trichoderma viride.*

I. Introduction

Medicinal plants are valued for the content and chemical composition of their active components. The intensive use of fungicides for the control of diseases has resulted in the accumulation of toxic chemical which are hazardous to human beings and to the environment. The residual chemical pesticides on various parts also seriously affect the marketing potential of medicinal plants and their formulations. The increasing awareness of fungicide-related hazards has emphasized the need for adopting a safer biological method.

The genus, T*richoderma* is ubiquitous with high population density has been extensively studied for their biological control of plant diseases[1][2]. Strains of *Trichoderma* are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers [3]. The antagonistic activity of *Trichoderma* species depends on multiple synergistic mechanisms [4][5]. The species of *Trichoderma* have shown efficiency in the biological control of many foliar diseases. In Senna, the leaf production is severely affected by many foliar diseases. Among them, the leaf spot disease caused by *Alternaria alternata* results in serious yield loss by reducing the leaf biomass and 78% reduction in the sennoside yield. The disease incidence is inversely proportional to decrease in the sennoside content of the plant. Thus, the application of biological control agents seems to be one of the most promising approaches[6].

Cassia angustifolia Vahl., commonly known as tinnevelly senna is the important medicinal plant belonging to the family Caesalpinaceae. The dried leaves of Senna are widely employed in traditional Indian Ayurvedic and Unani systems of medicine. It is used in the treatment of skin diseases, leprosy, leucoderma, jaundice, helminthiasis, cough, bronchitis, typhoid fever, anemia and tumors. The major bioactive chemicals are sennosides. The senna leaf is an important drug used in the control of habitual constipation. Its use in commercial formulation is increasing day by day due to change in life style and urbanization of people.

Hence the present work is aimed to study the antagonistic capacity of *Trichoderma viride* and *Trichoderma harzianum* against *Alternaria alternata*, the leaf spot pathogen of Senna plant.

II. Materials and methods

The rhizosphere soil samples were colleted from the field, in which Senna plants were grown. Two species of *Trichoderma* were isolated by soil dilution plate technique [7] on modified Trichoderma Selective Medium (TSM) [8]. The green coloured colonies were identified by slide culture technique and compared with taxonomic key of Rifai (1969) into genus and species level [9]. The biocontrol agents, *Trichoderma viride* and *Trichoderma harzianum* were purified by single spore isolation technique. The cultures were maintained on PDA slants and stored in the refrigerator at 4°C for further use.

The leaf spot pathogen of Senna, *Alternaria alternata* strains (MTCC-2724 and MTCC-7202) were procured from Microbial Type Culture Collection, Chandigarh. This pathogen was maintained on PDA medium and tested for its pathogenecity. The positively tested pathogen was multiplied on PDA slants for experimental usage.

2.1 Antagonistic activity *in vitro*

The efficacy of *Trichoderma* species on the growth of the pathogen was evaluated by dual culture plate method [10]. A mycelial disc of 6mm was cut from the margins of actively growing regions of 7 day old cultures of *Trichoderma viride* and *Trichoderma harzianum* with sterilized cork borer [11]. The mycelial disc was inoculated on sterilized PDA medium at one end of Petri plate just 1cm away from the edge. Similarly, the mycelial disc of the pathogen, *Alternaria alternata* was inoculated onto other end of petriplate against biological control agent. Petriplates inoculated only with test pathogen was treated as control. Three replicates were maintained for each treatment and were incubated at 28±2°C. Percentage of growth inhibition was calculated by using the following formula.

$$I = C – T / C \times 100$$

Where I = Percentage of growth inhibition of pathogen.

C = Radial growth of the pathogen in control.

T = Radial growth of the pathogen in treatment

III. Results and discussion

The species of *Trichoderma* were isolated from the rhizosphere soils of Senna plant. They were identified on the basis of morphological features and micrometry observations. The morphological characterization of these antagonistic isolates was accomplished on the basis of colony color, texture, growth patterns, size of phialides and phialospores. To study the antagonistic activity, dual cultures of both biological control agents and pathogenic fungi were maintained on PDA medium. The fungal growth parameters were taken after 24 hrs of culture onwards. The growth initiation of both the biological control agents was observed after second day of inoculation. Whereas, the growth of the pathogen, *Alternaria* species is slow and appeared after 36 hrs of inoculation. On the 3rd day of dual culture , maximum growth was observed in both the species of *Trichoderma* and little growth was noticed in the pathogen in all the three replicates (Fig. I and II). The growth inhibition of pathogenic fungi was estimated by the radial growth of the pathogen. The percentage of growth inhibition of pathogenic fungi by biological control agents was calculated with standard formula and the results were presented in TABLE 1. Inhibition of growth in *Alternaria* by *Trichoderma viride* and *Trichoderma harzianum* were found to be 80.1% and 72.2% respectively. In 4th day of culture, the biological control agents showed maximum growth and contacted with the pathogen. At 5th and 6th day, the biological control agents were over grown the pathogen and severely inhibited the growth of the pathogen.

In all the treatments, the *Trichoderma* species grew much faster than the tested fungi and inhibited the growth of pathogen. But both the species of *Trichoderma* differed with each other in their ability to suppress the growth of the pathogen. Among two, *Trichoderma viride* showed high percentage growth inhibition of test pathogen when compared to *Trichoderma harzianum* (TABLE 2).

In dual culture screening, *Trichoderma viride* was found to be the most potent in reducing the growth and colonization of pathogen. So far there are very limited published research work on the leaf spot disease in different species of *Cassia* and more specifically in *Cassia angustifolia* . In this aspect this paper might be the pioneer work on biological control of leaf spot disease of Senna, caused by *Alternaria alternata*.

IV. Conclusion

This present study confirms the *in vitro* antagonistic activity of *Trichoderma viride* and *Trichoderma harzianum* towards *Alternaria alternata.* Among two, T. *viride* has shown strong inhibitory effect on the development of *Alternaria alternata* compared to *T. harzianum.* The *in vitro* activity can be exploited for further development of field formulation of biological control agents for the control of leaf spot disease of Senna, which is safe, natural without any residual chemical compounds.

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Table 1. Growth inhibition of *Alternaria alternata,* the leaf spot pathogen of Senna by biological control agents

S.No	Biological control agents	Treatment Replicate 1 (mm)	Treatment Replicate 2 (mm)	Treatment Replicate 3 (mm)	Average (mm)	Growth inhibition (%)
1.	*Trichoderma viride*	18	17	16	17	80.1
2.	*Trichoderma harzianum*	25.5	24.5	25.0	25	72.2

Table 2: Percentage of growth inhibition of the pathogenic fungi by *Trichoderma viride* and *Trichoderma harzianum* in the dual culture plate method.

S.No	Tested pathogenic fungi	Percentage of growth inhibition	
		Trichoderma viride	*Trichoderma harzianum*
1.	*Alternaria alternata*	80.1%	72.2%

Fig.1. *In vitro* antagonistic activity of *Trichoderma harzianum* against the *Alternaria alternata* in different replicates.

Replicate 1 of *T. harzianum.*

Replicate 2 of *T. harzianum.*

Replicate 3 of *T. harzianum.*

Fig.1. *In vitro* antagonistic activity of *Trichoderma viride* against the *Alternaria alternata* in different replicates.

Replicate 1 of *T. viride.*

Replicate 2 of *T. viride.*

Replicate 3 of *T. viride.*

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