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

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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 chilli. Dual culture plate method revealed that the percentage growth inhibition of *Alternaria alternata* by *Trichoderma viride* was 70.10% and *Trichoderma harzianum* 67.90% 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 Chilli.

Keywords: *Alternaria alternata*, antagonistic activity, Chilli, *Trichoderma viride*, *Trichoderma harzianum*

Introduction

Chilli (*Capsicum annum* L.) is an important spice cum vegetable crop in India. The severe use of fungicides for the control of diseases has resulted in the growth of toxic chemical which are harmful to human beings and to the location. The residual chemical pesticides on various parts also completely affect the marketing probable of medicinal plants and their formulations. The increasing attentiveness of fungicide-related hazards has emphasized the essential for adopting a safer biological method. The genus, *Trichoderma* is universal with high population density has been extensively studied for their biological control of plant diseases. Strains of *Trichoderma* are strong adaptable invaders, fast growing, creative producers of spores and powerful antibiotic producers. The antagonistic activity of *Trichoderma* species depends on multiple synergistic mechanisms. The species of *Trichoderma* have shown efficiency in the biological control of many foliar diseases. In Chilli, the leaf production is severely affected by many foliar diseases. Among them, the leaf spot disease caused by *Alternaria alternata* results in severe 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 appears to be one of the most promising approaches. 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 Chilli.

Material and Methods

The rhizosphere soil samples were collected from the field, in which Chilli plants were grown. Species of *Trichoderma* were isolated by soil dilution plate technique, on modified *Trichoderma* selective medium. The green colored colonies were identified by slide culture technique and compared with taxonomic key of Rifai (1969) [32] into genus and species level. The biocontrol agents, *Trichoderma viride* and *Trichoderma harzianum* were purified by single spore isolation technique. The cultures were maintained on PDA slants and deposited in the refrigerator at 4 °C for further use. This pathogen was maintained on PDA medium and tested for its pathogenicity. The positively tested pathogen was multiplied on PDA slants for experimental.

Antagonistic activity

In vitro the efficacy of *Trichoderma* species on the growth of the pathogen was estimated by dual culture plate method. A mycelial disc of 6mm was cut from the margins of aggressively growing regions of 7 day old cultures of *Trichoderma viride* and *Trichoderma harzianum* with sterilized cork borer. 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 27 ± 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.

Results and Discussion

The species of *Trichoderma viride* and *Trichoderma harzianum* were isolated from the rhizosphere soils of Chilli 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 colour, texture, growth patterns, and size. 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 viride* and *Trichoderma harzianum* and little growth was noticed in the pathogen. 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. Inhibition of growth in *Alternaria* by *Trichoderma viride* were found to be 70.10% and *Trichoderma harzianum* 67.90% respectively. At 7th day, *Trichoderma viride* and *Trichoderma harzianum* the biological control agents was over grown the pathogen and severely inhibited the growth of the pathogen. In all the treatments, the species developed much quicker than the tested fungi and inhibited the growth of pathogen. In dual culture screening, *Trichoderma viride* was found to be the most effective than *Trichoderma harzianum* reducing the growth and colonization of pathogen. In this aspect this paper might to be the discoverer work on biological control of leaf spot disease of Chilli, caused by *Alternaria alternata*.



Fig 1: Interaction between *Trichoderma viride* + *Alternaria alternata*



Fig 2: Interaction between *Trichoderma harzianum*+ *Alternaria alternata*

Table 1: *In vitro* evaluation of effect of bio-agent (*Trichoderma viride* and *Trichoderma harzianum*) on radial growth of *Alternaria alternata*

Biocontrol agent	Mean Colony dia 7 th DAI (mm)	Growth inhibition (%)
(<i>Trichoderma viride</i>)	70.10	56.91
(<i>Trichoderma harzianum</i>)	67.90	55.53
	S.E±	0.62
	C.D at 5%	1.86

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