

Evaluation of Bio-control Agents against *Colletotrichum capsici* in Doon Valley of Western Himalayas

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ABSTRACT

Anthraco disease caused by *Colletotrichum capsici* is a major disease in India, resulting a challenge for profitable cultivation of chilli in all most all the major chilli growing regions of India. An experiment was conducted in the department of plant pathology, Uttaranchal College of Agricultural and Sciences, Dehradun, Uttarakhand, to study effective Bio-agents were evaluate against *Colletotrichum capsici*. Three Bio-agents namely *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma asperellum* were evaluated *in-vitro* condition by using Dual culture techniques for their antagonist's activity against *Colletotrichum capsici*. Among the three antagonists, *Trichoderma viride* showed 56.34% inhibition followed by *Trichoderma harzianum* (50.67%). The least inhibition (34.0%) was recorded in *Trichoderma asperellum*.

Key words: *Colletotrichum capsici*, *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma asperellum*

Chilli (*Capsicum annum* L.) is almost non replaceable component of most of the cuisine of Indian subcontinent. Although it is a spice but some species are also used as vegetable delicacy in some part of India (Gaur *et al.* 2018). In almost all part of India chilli is grown but in some particular region chilli production and processing has become a major economic activity with great export potential. Although chilli could be successfully cultivated up to 2000 msl but it perform better in tropical and sub tropical climate (Saxena *et al.* 2016). Several biotic and abiotic factor effects the chilli production in India. According to Anamika *et al.* (2014) anthracnose disease caused by *Colletotrichum spp.* is a major problem in India and one of the significant limiting factor for sustainable chilli production, especially in tropical and subtropical regions of India. In India, primarily three important species, namely, *C. capsici*, *C. acutatum* and *C. gleosporoides* have been reported to be linked with the disease, but fruit rot or anthracnose of chilli caused by *Colletotrichum capsici* (Syd) Butler and Bisby is one of the most destructive among all the three (Saini *et al.* 2017).

The initial visible symptom of disease is reported to be on aerial parts of the plant such as stem, leaves and fruit. In ripened fruits light brown, sunken lesions containing salmon-colored masses of conidia and micro sclerotia are visible which reduces the quality and quantity of immature and mature fruits (Sharma *et al.* 2011). Due to this disease, more than 50 per cent crop loss has been reported from

different parts of India (Ramchandran *et al.* 2007). In recent years, the plant pathologists have focused their attention on developing disease management technique which are ecologically safe and have no adverse residual effect on environmental. Due to this bio control methods are gradually getting popular resulting in replacement and reduction of chemicals for the management of plant diseases. Bio control agents also have been reported to induce systemic resistance against several plant diseases which makes them more sustainable method of disease management (Radjacommar *et al.* 2002). With this background, in the present study antagonistic microorganisms were exploited for the effective management of *Colletotrichum capsici* in chilli.

MATERIALS AND METHODS

The present study was conducted in Laboratory of Uttaranchal College of Agricultural Sciences, Uttaranchal University, Dehradun and the protocol of the present study was as follows:

Fungal pathogen: Pure culture of *Colletotrichum capsici* has been obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi with the catalogue number 7389. The pathogen was transferred to PDA (Potato dextrose agar) and OMA (Oat meal agar) medium in Petri plates and the plates were incubated at $28 \pm 1^\circ\text{C}$ for growth. Sub-cultures were

made from the periphery of the mycelial growth, which appeared after 6 -7 days.

Pathogenicity tests: The pathogenicity of the isolated fungus *Colletotrichum capsici* causing chilli anthracnose was proved by Koch's postulate under *in-vitro* condition.

In-vitro condition: Healthy chilli fruits collected from the fields were washed under tap water and then surface sterilized with 70% ethyl alcohol. *Colletotrichum capsici* was cultured on PDA for 10 days. Then 0.7 cm agar plug containing mycelia of *Colletotrichum capsici* was placed on the pierced area on chilli fruit. Fruits inoculated with sterilized water served as control. Inoculated fruits were kept in moistened polythene bags to maintain humidity and incubated at $28 \pm 2^\circ\text{C}$ and observed daily for the disease symptoms (Intana et al. 2007). The pathogen was re-isolated from the infected fruits and compared with the original culture.

Effect of bio-control agents on the mycelial growth of *Colletotrichum capsici* – By dual culture technique

The three Bio-control agents namely *T. harzianum*, *T. viride* and *T. asperellum* were tested against fungal pathogen *Colletotrichum capsici* by Dual culture technique (Dennis and Webster 1971). The fungal pathogen was grown on PDA for 7 days. After 7 days with the help of a sterile cork borer, a disc of fungal growth from culture plate of *Colletotrichum capsici* was taken and placed at an equidistance of antagonists on fresh PDA plate, and then, similarly with the help of sterile cork borer, a disc of *Trichoderma* spp. was taken and placed the other side of PDA plate. The plates were kept for incubation at 30°C for 7 days in BOD incubator. Visual observations on the inhibition of growth of pathogen were recorded after 7 days of Incubation in comparison with the PDA plate simultaneously inoculated with only the fungal pathogen. Percentage of reduction in growth was calculated following the formula:

$$\% \text{ of inhibition (I)} = \frac{R_1 - R_2}{R_1} \times 100$$

Where,

R_1 : Radial growth of pathogen towards opposite side in control plate.

R_2 : Radial growth of pathogen towards the opponent antagonist in test plate.

Statistical analysis: The data were statistically analyzed with the help of analysis of variance (ANOVA). Three replications for each treatment were laid out in a completely randomized design.

RESULTS AND DISCUSSION

The phytopathogen *Colletotrichum capsici* was ordered from ITCC, IARI, New Delhi (catalogue number 7389). Showing typical growth on PDA and OMA media, the morphological characteristics of *Colletotrichum capsici* on OMA and PDA was recorded. The pathogen was little bit

slow growing and showed a radial growth of 74.16mm in PDA and 64.60mm in OMA. The pathogen produced dark black coloured mycelial growth on PDA and whitish growth formed on OMA (Plate 1).

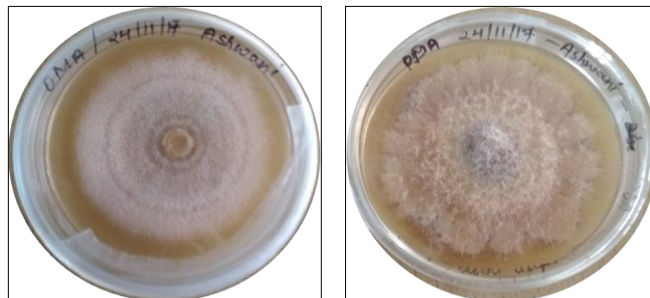


Plate 1 Growth of *Colletotrichum capsici* on PDA and OMA media

Pathogenicity test

The pathogenicity of the isolated pathogen was proved following Koch's postulates under *in-vitro* conditions. Healthy fruits inoculated with the phytopathogen showed mycelial growth and symptoms of anthracnose (Plate 2). The phytopathogen was re-isolated from the infected fruits and compared with the original culture and found to be the same. Fruits inoculated with sterile water which served as the control did not show the symptoms of the disease.



Plate 2 *In-vitro* pathogenicity test proving by Koch's postulates in chilli fruit

Management of disease

Dual culture technique: Antagonistic effect of the bacterial isolates was tested against the chilli anthracnose pathogen (*Colletotrichum capsici*) by the standard dual culture method. The results revealed that among the three bio-control agent *Trichoderma viride* showed maximum

inhibition of 56.34%, *Trichoderma harzianum* showed 50.67% whereas the least inhibition was 34.0% *Trichoderma asperellum* (Plate 3, Table 1). Similar result were showed by Bilal *et al.* (2010) where all the three antagonistic fungi caused significant inhibition of mycelial growth, maximum being with *T. viride* (69.21%) followed by *T. harzianum* (64.20%).

Table 1 Effect of bio-agents on radial growth of *Colletotrichum capsici*

Treatments	Mean colony diameter	Inhibition % of radial growth (mm)*
<i>Trichoderma viride</i>	1.68	56.34
<i>Trichoderma harzianum</i>	2.47	50.67
<i>Trichoderma asperellum</i>	2.53	34.0
Control	15.1	00.00
S.Em±		0.306
CD at 5% level		1.079

*Values are means of three replications ±SD

In this study, *Colletotrichum capsici* was a isolated and confirmed as the pathogen responsible for causing anthracnose of the chilli fruit which is the major causative agent of chilli anthracnose. The colony growth rate was determined which will be very helpful in developing control measure against pathogen *Colletotrichum capsici*. The

pathogenicity test was confirmed by using protocol for *in vitro* pathogenicity test. The two *Trichoderma* isolated showed significant level of antifungal activity against *Colletotrichum capsici*. These results could be used to manage chilli anthracnose at a significant level which could have a positive impact on chilli production in the region. It will increase the yield of chilli along with reduction in cost of production of chilli in this region.

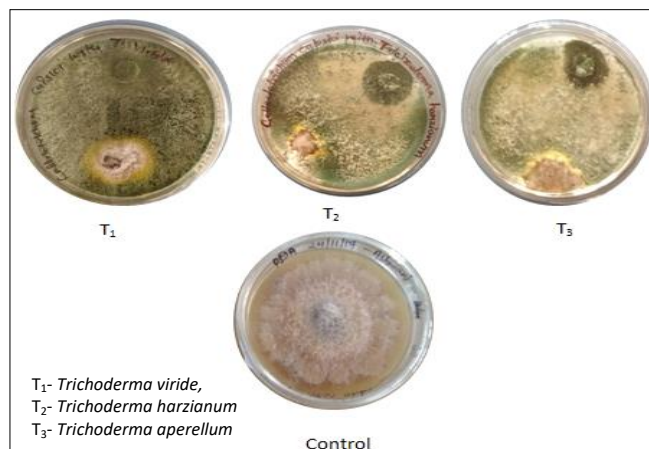


Plate 3 Antifungal activity of *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma asperellum* against *Colletotrichum capsici*

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