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ANTAGONISTIC EFFECT OF BIO-AGENT (*Trichoderma harzianum*) OVER PATHOGEN (*Colletotrichum capsici* Syd. Butler and Bisby) OF CHILLI (*CAPSICUM ANNUUM* L.) BY DUAL CULTURE TECHNIQUE

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ABSTRACT

Anthracnose is characterized by very dark sunken lesions, containing spores and the causal agent of chilli anthracnose is *Colletotrichum capsici*, *a* major constraint in chilli production leading to huge economic losses in the country. As a potent biocontrol agent, *Trichoderma harzianum* exhibits remarkable properties such as mycoparasitism, antibiosis, and aggressive competition against fungal pathogens. In the present study, an attempt was made to evaluate efficacy of *T. harzianum* (isolated from talc-based *Trichoderma* formulation) against *C. capsici* which promote the overall growth of chilli and to manage disease anthracnose under *in vitro* conditions. Under *in vitro* conditions, the results revealed that the *T. harzianum* inhibit the radial mycelial growth of the pathogen by dual culture (by 82.22 per cent).
Keywords **:** antagonistic, antibiosis, bioagents, *Colletotrichum capsici*, economic, formulation, mycoparasitism, pathogen, *Trichoderma harzianum*

Introduction

Chilli (*Capsicum annuum* L.) is one of the major spice crops grown throughout the world and belong to the family Solanaceae. It is a rich source of many antioxidant compounds such as phenolic compounds, vitamin C and carotenoid (Zhu et al. 2012). In 2020, India leads the world in chilli production, and has produced more than 1.7 million tonnes (Statista, 2022). Anthracnose is one of the most critical fungal diseases caused by various strains of *Colletotrichum* spp., including *C. acutatum*, *C. capsici*, *C. coccodes*, *C. gloeosporioides*, *C. scovillei*, and *C. truncatum*, which belong to the family Glomerellaceae (Ascomycota) (De Silva *et al*., 2019). The *C. capsici* will be fairly white to light grey colour with circular fluffy mycelia and black coloured acervuli scattered all over the colony (Gupta *et al*., 2017). Small, circular, sunken, necrotic greyish brown spots with dark margins appears firstly on leaves, typical anthracnose lesions leading to premature drying and dropping off of leaves (Robert *et al*., 2001). *C. capsici*, causing yield losses ranging from 10%–80% under favourable conditions, was formerly called *C. truncatum* (Gowda and Sriram 2023; Muhae-Ud-Din *et al*., 2025). Biological control is a promising tool to maintain the current scenario of the agricultural production to reduce the use of chemical fungicide which pollute the environment. *Trichoderma* is the most used bio-agents as most of the biological fungicide is being prepared from *Trichoderma* formulations. The main mechanism involved in bio-control like mycoparasitism, antibiosis, competition with fungal pathogen and act as an antagonist to the pathogen (Pani *et al*., 2021; Pavlovskaya *et al*., 2020).

T. harzianum is consider beneficial in the field agriculture because of their high level of antagonism against diverse phytopathogenic micro-organisms (Hermosa *et al.*, 2012). *Trichoderma* have been mostly were used as bio fungicides agents, they exhibit considerable activity against specific fungal diseases, their effects were equal or exceeded at, those which were of some chemical pesticides (Illipronti *et al.*, 1993; Hibar *et al*., 2005). Considerable success has been achieved by introducing antagonists to control

seed-borne fungal pathogens. *Trichoderma* was used against seed-borne fungal pathogen caused by *Rhizoctonia solani* and *Sclerotium rolfsii* both *in vitro* and pot culture with considerable results (Raihan *et al*. 2003; Haider 2005). Biocontrol is an effective alternative for disease management, offering disease control with minimal harm to humans, selective action, and a low risk of pathogen resistance development (Singh *et a*l., 2012).

Unnecessarily use of fungicide chemicals reduces the efficiency in cultivation and also have a negative impact to environment, chemical resistance and as well as the threat of poisoning to public health (Jallow *et al*., 2017; Damalas *et al*., 2011; Sutarman *et al.*, 2021). Considering the present situation, the present study has therefore been undertaken with the objective to evaluate the efficacy of *Trichoderma* against the major seed-borne pathogen *C. capsici* of chilli.

Materials and Methods

Isolation of fungal pathogen

The leaves of chilli showing typical symptoms were collected from central research farm of the Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh. The afflicted parts along with healthy portions of the leaves was washed with tap water and cut under aseptic conditions into small bits (2-3mm) into a sterile dish with the aid of scissors which was sterilized over a spirit lamp flame and surface sterilized in 1 % sodium hypochlorite (NaClO) for one minute, and washed three times in sterile distilled water under aseptic conditions using laminar air flow. The cut diseased was placed on Petri dishes (3-4 leaf bits per Petri plate) poured with solidified potato dextrose agar (PDA). The inoculated plates were incubated for five to seven days at room temperature (25±1˚C) and examined at frequent intervals until visible growths (fungal/spores) are seen on the plates. The fungal colonies growing in the incubated plates were sub-cultured into fresh medium until pure cultures were obtained. This pure culture was used for further study (Ahmad *et al*., 2013) (Plate-1).

Plate 1 : Pure culture of *Colletotrichum capsici*

Morphological studies of the pathogen

Identification of the fungus and examination of the fungal colony characteristics was done through microscopic examination. Morphological studies of the pathogen were conducted from pure culture. Spore suspension in sterilized distilled water was made from pure culture of the pathogen grown on PDA. One drop of spore suspension was placed on a slide and morphological characters were noted with the help of compound microscope. The pathogen *C. capsici* affecting chili was differentiated on the basis of morphological characters of mycelium, conidia, setae and conidiophores recorded. The fungus is localized on the skin of fruits and leaves and produces acervuli (Plate-2).

The acervuli are characterized by the hymenial layer, with short hyaline aseptate and unbranched conidiophores bearing the hyaline, falcate conidia, along with the dark coloured needle like setae. In general, conidia measured 25.27-26.15 x 3.14-3. 67 µm and contain centrally placed oil-globule, conidia are produced singly at the tip of unbranched conidiophore, single celled, curved with narrow ends and fusoid (Plate-3). They readily germinate producing thin hyaline germ tubes whose tips from appressoria one on each germ tube (Akhtar and Singh, 2007).

Plate 3 : Microscopic view of acervuli of *C. capsici* (10X)

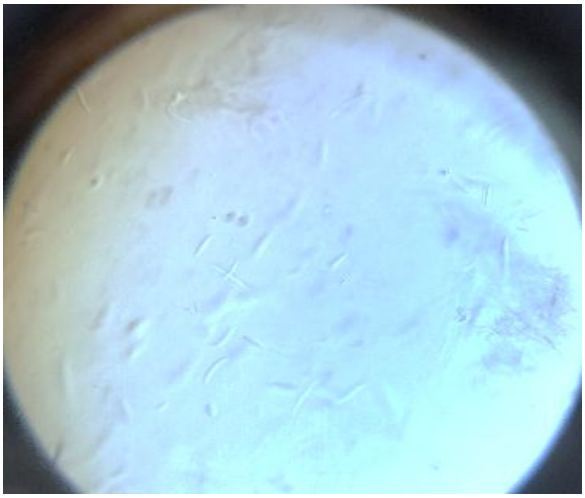

Plate 4 : Conidia (40X)

Source of material

Talc based formulation Katyayani *Trichoderma harzianum* 1% w/w (Packed and Marketed by Katyayani Organics Mx 175, E7 Extension, Area Colony, Bhopal 462016, M. P).

Isolation of *Trichoderma harzianum* from talc-based *Trichoderma* formulation

Isolation was done by employing serial dilution technique. The isolate was purified by single spore culture technique. Whitish to yellow pigmented fungal colonies identical to *Trichoderma harzianum* was maintained on PDA as pure culture and were used for further studies (Plate -5).

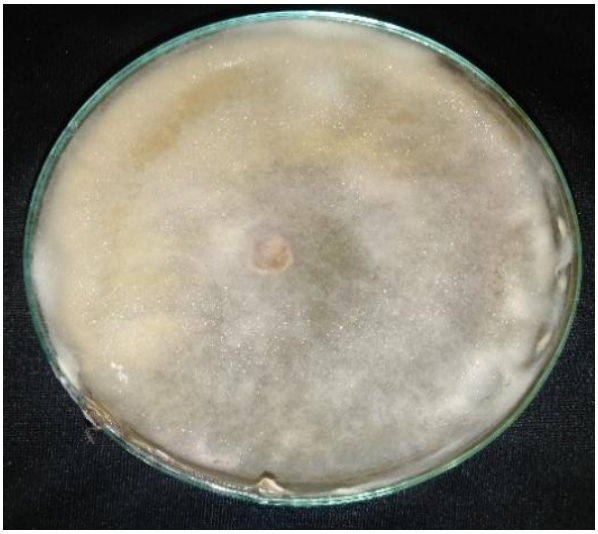

Plate 5 : Pure culture of *Trichoderma harzianum* in PDA

***In vitro* effect of *Trichoderma* antagonist against *C. capsici* / Dual culture technique**

Five mm diameter culture disc of *Colletotrichum capsici* were placed on PDA medium 25 mm away from the edge of the Petri plate. *Trichoderma harzianum* (5 mm dics) were placed at opposite side at 25 mm away from the edge of the Petri plate (9 cm in diameter) to check its antagonistic properties against *C. capsici* through dual culture technique (Faheem *et al*., 2010). And monoculture of pathogenic fungi (C. *capsici*) as controls accompanied by five mm propagule placed in the middle of a petri dish

(Sutarman *et al.*, 2021). Three replications were maintained. The plates were incubated at 25±1˚C for seven days and colony radius of pathogen and *Trichoderma* were recorded. Per cent inhibition over control was calculated using the formula (Vincent, 1947).

$$I = \frac{C-T}{C} \times 100$$

where,
I = Per cent inhibition,
C=Growth (mm) of test fungus in untreated control plates,
T=Growth (mm) of test fungus in treated plates.

Results and Discussion

Antagonistic effect of bio agent over pathogen by dual culture technique

The results revealed that the average mycelial growth of the pathogen (T_0) was 90 mm and in dual culture plate *Trichoderma harzianum* (T_1) radius colony was 16 mm. Percent growth inhibition was 0.00% (T_0) and 82.22% (T_1) as represented in table 1 and Plate-6 and 7).

Trichoderma harzianum produces a range of enzymes and metabolites that can inhibit the growth of various pathogenic fungi. These include chitinases, glucanases, and proteases, which degrade the cell walls of other fungi and disrupt their development. It can directly parasitize other fungi by attaching to their hyphae and eventually killing them. This is a result of the production of lytic enzymes that break down the cell walls of the target fungi. It competes for resources with pathogenic fungi. *T. harzianum* develops more rapidly than *C. capsici* in single as well as in double cultures. The intensive growth of *T. harzianum* gives it a significant advantage in competition with pathogens for nutrient elements and space (Barbosa *et al*., 2001; Gveroska and Ziberoski, 2011). By colonizing the rhizosphere or other substrates, *T. harzianum* can reduce the availability of nutrients and space for pathogenic fungi, thus suppressing their growth. *T. harzianum* produces secondary metabolites that have antifungal activity. The effect of seed treatment with *T. harzianum* were significantly effective against seed borne fungal pathogens as reported by Ngullie *et al*. (2010) Reddy *et al*. (2017) Tejaswi and Zacharia, (2022).

Hibar *et al*. (2005), found that the antagonism *in vitro* of *Trichoderma harzianum* showed an inhibition on the pathogenic fungus growth with a ratio more than 65%. *T. harzianum* suppressed the symptoms expression, conidial germination and 100% mycelial growth of *C. capsici* reported by Srinivas *et al*., 2006. Effect of different *T. harzianum* on two chilli cultivars reported by Sharma *et al*., 2005. Similarly, Inhibition percent of mycelial growth of each isolate of *Colletotrichum acutatum* by *T. harzianum* by direct confrontation resulted up to 86.64% (Es-Soufi *et al*., 2020).

According to Plate-6, the whole Petriplate is filled with *T. harzianum*, thus, it develops even over the culture of *C. capsici*. *In-vitro* antagonistic activity of *Trichoderma* sp. is expressed by suppressing test-fungi growth and its rapid growth (Mirkova, 1982; Gveroska and Ziberoski, 2011).

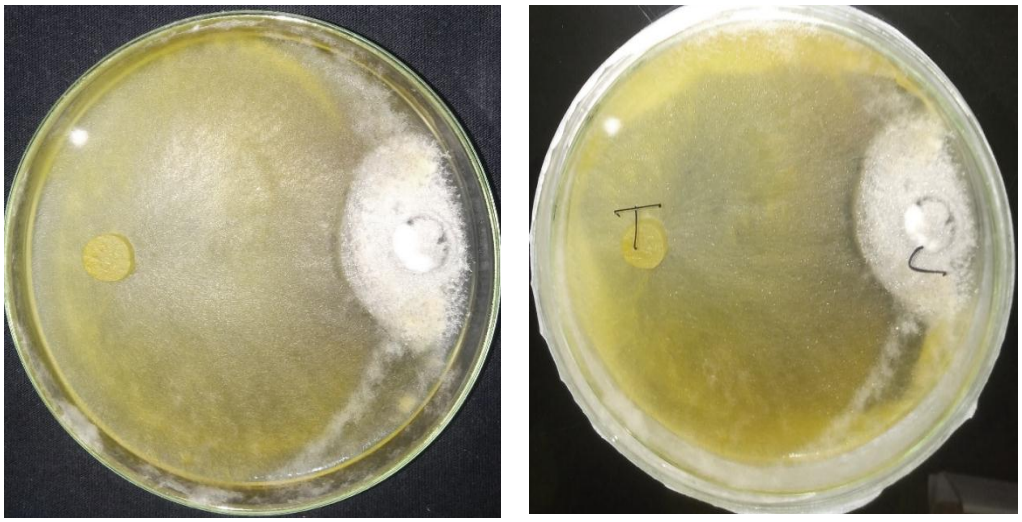

Plate 6 : The double culture at the end of incubation

Table 1: Antagonistic effect of bio agent over pathogen by dual culture technique

Tr.no	*Trichoderma*	Mycelial growth of the pathogen(mm)	Percent growth inhibition (%)
T_0	Control	90.00	0.00
T_1	*T. harzianum*	16.00	82.22

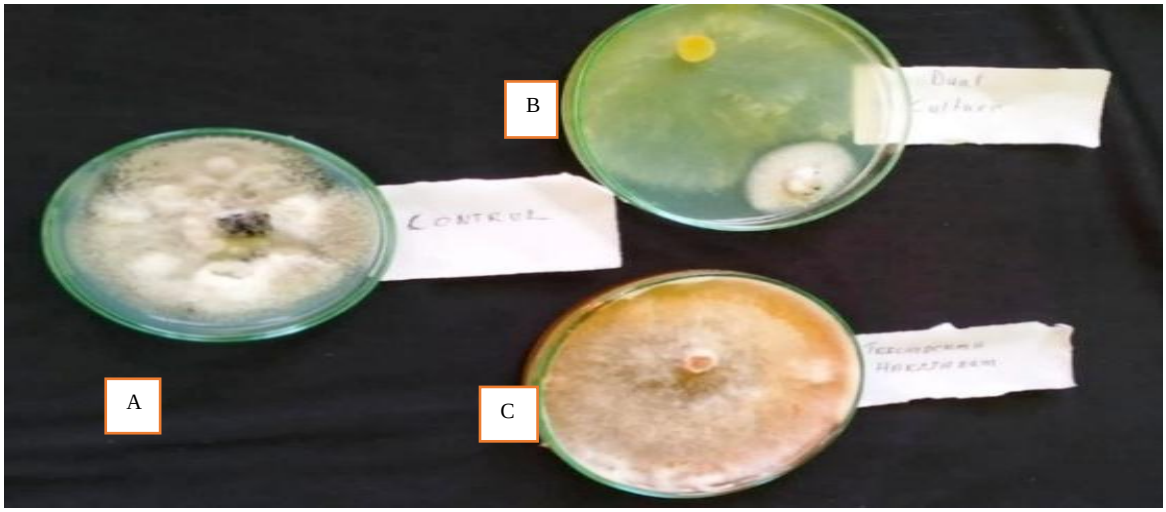

Plate 7 : Efficacy of *Trichoderma harzianum* against *Colletotrichum capsici* by dual culture technique. A-Control of pathogen, B-antagonistic effect of bio-agent over pathogen, C-control of bio-agent

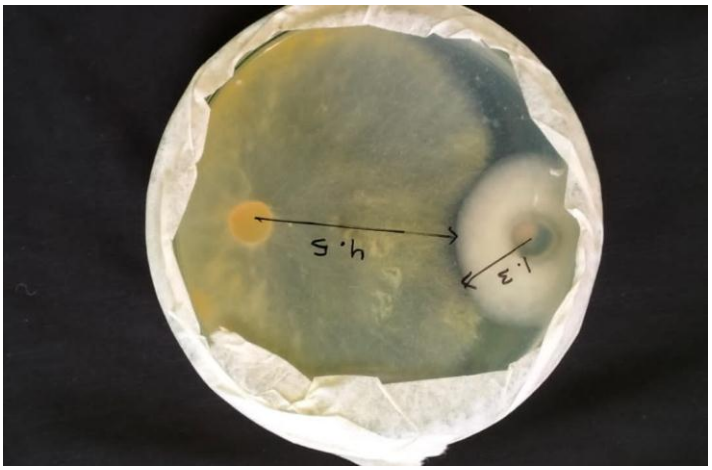

Plate 8 : Antagonistic effect of *Trichoderma harzianum* over pathogen.

$$I = \frac{(90-16)}{90} \text{x}100 = 82.22\%$$

Conclusion

The present studies clearly indicates that *T. harzianum* able to inhibit the growth of *C. capsici* colonies by 82.22 %. It appears that the hyphae of are damaged as a consequence of the influence of *T. harzianum* hyphae that are overlapping with the hyphal pathogen. *T. harzianum* shown strong reducing effect on the development of *C. capsici* of with various mechanisms of antagonistic influence. Therefore, it concluded that *T. harzianum* can be applied in biological control of *C. capsici* and can become a part in integrated management of chilli anthracnose.

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