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Bio-control ability of *Trichoderma* isolates on anthracnose disease (*Colletotrichum capsici*) of Chilli (*Capsicum annuum* L.)

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Abstract

Capsicum annuum (Chilli) is an important spice crop grown in tropical and subtropical areas of India. Chilli suffers from several diseases, out of which anthracnose of chilli is one of the important diseases. Keeping in view the negative impact of fungicides on the environment, an experiment was conducted to evaluate *Trichoderma* isolates against anthracnose disease of chilli. *Trichoderma* spp. are being used to control plant diseases in the sustainable disease management system. Nine isolates of *Trichoderma* spp., isolated from rhizosphere soil of chilli experimental field. Among these native isolates, *T. harzianum* was found as the most frequent five, while the *T. viride* was found as three, and *T. asperellum* as one. All nine isolates were screened for their antagonistic activity against *Colletotrichum capsici* of chilli. Results from present investigation revealed that all *Trichoderma* isolates significantly reduced the growth of the pathogen. Minimum radial growth of *Colletotrichum capsici* was recorded as 21.5 mm by *Trichoderma* isolate Tr-8, followed by Tr-3 and Tr-1 with maximum growth inhibition as 62.60, 61.74 and 59.13 percent, respectively, over control. Inhibition percent of growth by different isolates of *T. harzianum* ranged between 46.96-54.78 percent of *C. capsici*. Considering the frequency of *T. harzianum* among the isolates of native *Trichoderma* spp. in the experimental field and the growth inhibition percentage on *C. capsici*, it is concluded that *Trichoderma harzianum* can be part of integrated management of anthracnose disease of chilli.

Keywords: Chilli, Anthracnose, I.D.M., *Trichoderma* spp. and *T. harzianum*

Introduction

Chilli is the native of Southern America and was first cultivated in Peru at around 7500 BC (MacNeish, 1964) [12]. India is the world's largest producer, consumer and exporter of chillies in the world. India is the world leader in chilli production followed by China, Thailand, Ethiopia and Indonesia.

In India, total production of green chillies for 2017-18 was 3592000 MT with area under 309'000 Ha. (Horticultural Stat. 2018a) [8]. Chilli (*Capsicum annum* L.) is an important cash crop of East Nimar region of M.P (Divya *et al.*, 2020) [5]. Production Share of Uttar Pradesh in leading vegetable producing States for 2017-18 was 15.4% with total production of 28316450 MT. (Horticultural Stat. 2018b) [8]. In 2017-18, Uttar Pradesh has 10th place in leading green chilli Producing States of India, with 1.99% (71630 MT.), out of the ten, first nine are Karnataka, Madhya Pradesh, Bihar, Andhra Pradesh, Maharashtra, Jharkhand, Chhattisgarh, Telangana and Haryana, respectively (Horticultural Stat., 2018) [8].

Chilli suffers from a large number of viral, fungal, bacterial, nematode and phytoplasma diseases (Devi *et al.*, 2019) [5]. Chilli is affected by several diseases, out of which anthracnose of chilli is one of the important diseases. It is also termed as dieback or fruit rot. It is caused by *Colletotrichum capsici*. This devastating pathogen damages both immature and mature fruits reducing the nutritive and marketing value of chillies (Manda *et al.*, 2020) [14].

The control of anthracnose which is commonly done by farmers is using fungicide. However, if carried out continuously can cause resistance to the pathogen, therefore the fungicide does not work anymore, not to mention the negative impacts on the environment and humans as consumers.

Although a number of reports are there for controlling the pathogen *in vitro* and *in vivo* very few reports are there for large scale and successful application of biological control. Therefore, keeping in view the negative impact of fungicides on the environment, an experiment was conducted to evaluate *Trichoderma* isolates against anthracnose disease of chilli. *Trichoderma*

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spp. are soil-borne fungi have significant antagonistic potential against a wide range of phytopathogenic fungi (Elad *et al.*, 1982) [6]. *In vitro* studies were conducted to see the effect of different isolates of *Trichoderma* on conidial and mycelial growth of *C. capsici*.

Materials and Methods

Isolation and Pure culture of Pathogen: Pieces of infected Chilli leaves/stems/fruits having disease symptoms were surface sterilized with 0.1 percent Mercuric chloride and transferred to sterilized petri plates containing 10 ml. of culture media. The isolation was made inside of Laminar flow, which was pre-exposed to U.V. radiation. The petri plates were kept in B.O.D. incubator at 22 ± 1 °C for a suitable period for growth of pathogen. The isolates were revived at 7-10 days interval for further study.

Pure culture of the pathogen was obtained using single spore isolation method (Choi *et al.*, 1999) [4]. Briefly, a loopful of spore mass of 7-8 days old culture was transferred into a centrifuge tube which contained 10 ml of sterile distilled water, and the solution was gently shaken to disperse the spore mass. The solution was then poured onto PDA plates and left to stand for 5 minutes. The excessive solution on the plates was then poured off, and the plates were incubated slanting for 12 to 18 hours. Single spores of *Colletotrichum capsici* that germinated between 18 to 24 hours were transferred onto new PDA plates.

Isolation of Trichoderma: *Trichoderma* spp. was isolated from rhizosphere soil of experimental field. Nine isolates of *Trichoderma* were made and preparation of pure culture was established using single spore isolation (Choi *et al.*, 1999) [4], as described earlier for the establishment of *Colletotrichum* pure culture.

Effect of the Trichoderma sp. on Culture Plate: Each isolate of *Trichoderma* sp. was screened for its antagonistic effect against *Colletotrichum* sp. by using dual culture technique described by Begum *et al.*, 2008 [2]. Concisely, an agar disc containing mycelia of 7-day-old *Trichoderma* sp. culture was placed at one end of the agar plate while an agar disc containing mycelia of 7-day-old *Colletotrichum* sp. culture was placed at the other end on the same PDA plate (here in after referred to as treated plate). Plates, which served as controls, had agar disc of *Colletotrichum* sp. mycelia and a disc of PDA placing opposite each other on PDA plates.

A Complete Randomized Block design was used and each treatment (plate) was done in triplicate, and the plates were incubated for 15 days at room temperature. The plates were observed daily, and the average growth rate of *Colletotrichum* sp. and *Trichoderma* sp. was computed using the formula described by Rosli, 2017 [16] (Eq. 1) and the antagonistic activity of the *Trichoderma* sp. toward *Colletotrichum* sp., expressed as percentage of inhibition of radial growth was determined using the formula described by Begum *et al.*, 2008 [2] (Eq. 2) as below.

Average growth rate:

$$= \frac{(D_2 - D_1) + (D_3 - D_2) + (D_4 - D_3) + [DN - D(N-1)]}{N-1} \quad \text{Eq.1}$$

Where, D indicates the average colony radial of *Colletotrichum* sp. and N indicates the number of days after incubation.

$$\text{Percent inhibition of radial growth} = \frac{R_1 - R_2}{R_1} \times 100 \quad \text{Eq.2}$$

Where, R_1 indicates the radial growth of the fungal colony of the control set, and R_2 indicates the radial growth of the fungal colony of the treated set.

Results and Discussion

Biology of the Pathogen: Isolates of the causal agent on PDA resulted in a dark grey colour colony with cottony mycelium and concentric rings from the middle of the colony was the most predominant on the culture plate (Plate1), and therefore was selected to be sub-cultured for further studies. A pure culture of the predominant fungus had been successfully established.

Colletotrichum capsici is confirmed based observations and characteristic features described by Than *et al.*, 2008 [20]. Upper surface varied from white or grey to black colour with cottony mycelium. Growth rate 0.8 cm /day, presence of setae, size of conidia is 18-24µm, aseptate conidia with falcate or half-moon shape.

Isolation of Trichoderma isolates: A total 9 *Trichoderma* isolates were isolated from rhizospheric soil of chilli experimental field. Among them 3 isolates were identified as *T. viride*, 5 as *T. harzianum* and 1 as *T. asperellum* (Plate1). The present results are in agreement with Soesanto *et al.*, 2011 [17], who have isolated *Trichoderma* spp. and studied the colony colour as velvetiness with white and dark green floccose surface along with scattered green patches and yellow to green pigmentation on PDA medium.

Morphological characters of *Trichoderma* isolates with respect to radial growth and colony characters were studied on PDA. Culture was examined under microscope for structural conformation and morphological characteristic features.

Colour of colony of isolates Tr-1, Tr-3, and Tr-8 from upper surface varied from green to dark green and lower surface from yellow to amber. Surface mycelium was whitish and floccose in nature, average growth rate for 4 days ranged from 8-9.0 cms. Microscopic features conidia are round in shape with size of 4- 4.8 x 3.5- 4µm and green in colour. Phialides are flask shaped with 8-14 x 2.4-3µm in size. From the above observations isolates Tr-1, Tr-3, and Tr-8 were conformed to be *Trichoderma viride* (Plate: 1).

Colour of colony of the isolates Tr-2, Tr-4, Tr-5, Tr-7 and Tr-9 from upper surface varied from Dark green to greyish colour and lower surface from colourless to drab colour. And surface mycelium was whitish and compact in nature with concentric rings, average growth for 4 days ranged from 9.5 cms. Microscopic features conidia are smooth and sub-globose round in shape, 3.8-4x 3.1- 3.7µm in size and yellow to pale green in colour. Phialides are globose in shape with 6.3-15.6 x 2.7 -3.4µm in size. These isolates were conformed to *Trichoderma harzianum* (Plate1).

The Colony colour of isolate Tr-6 from upper surface varied from pale green to dark green colour and lower surface from colourless to drab colour, growth rate for 4 days ranged from 8.5 cms. Microscopic features of conidia are sub-globose in shape with size of 3-3.5 x 4 - 4.4µm and dark green in colour. Phialides are slightly globose (ampulliform) in shape with 10.2-11.8 x 2.3-2.8µm in size. This isolate was conformed to *Trichoderma asperellum* (Plate1).

The spores and phyllodes of all the isolates, typical structures correspond to *Trichoderma* species, present the typical form

and shape of *T. viride*, *T. harzianum* and *T. asperellum*, which was reported by different authors. (Barnett *et al.*, 1972; Romero-Arenas *et al.*, 2009; Chaverri *et al.*, 2015; Jaklitsch *et al.*, 2015) [1, 14, 3, 11].

Results from present investigation revealed that all *Trichoderma* isolates significantly reduced the growth of the pathogen. Minimum radial growth of *Colletotrichum capsici*

was recorded as 21.5 mm by *Trichoderma* isolate Tr-8, followed by Tr-3 and Tr-1 (*T. viride*) with maximum growth inhibition as 62.60, 61.74 and 59.13 percent, respectively, over control (Table 2). Similar findings were also seen Yelleti and Sunil (2022) [16]. Inhibition percent of growth by different isolates of *T. harzianum* ranged between 46.96–54.78 percent of *C. capsici* (Fig.2).

Table 1: Morphological and cultural characteristics of different isolates of *Trichoderma* spp.

Isolate No.	Colour of Colony on PDA	Growth Rate	Texture of Colony on PDA	Concentric Rings	Colour on the back Side of Plate	Shape of conidia	Size of conidia (µm)	Shape of Phialides	Size of phialides (µm)
Tr-1	Green to dark green	9.0 cms	Cotony	No Presence	Yellow to amber	Round	4-4.8 x 3.5-4 µm	Flask shaped	8-14 x 2.4-3 in
Tr-2	Dark green to greyish	9.4 cms.	Cotony	2 to 3 rings	Colourless to drab colour	Sub-globose round	3.8-4x 3.1-3.7 µm	Globose	6.3-15.6 x 2.7 - 3.4 µm
Tr-3	Green to dark green	8.8 cms	Cotony	No Presence	Yellow to amber	Round	4-4.8 x 3.5-4 µm	Flask shaped	8-14 x 2.4-3 in
Tr-4	Dark green to greyish	9.6 cms.	Cotony	2 to 3 rings	Colourless to drab colour	Sub-globose round	3.8-4x 3.1-3.7 µm	Globose	6.3-15.6 x 2.7 - 3.4 µm
Tr-5	Dark green to greyish	9.5 cms.	Cotony	2 to 3 rings	Colourless to drab colour	Sub-globose round	3.8-4x 3.1-3.7 µm	Globose	6.3-15.6 x 2.7 - 3.4 µm
Tr-6	Pale green to dark green	8.5 cms	Dusty	3 to 4 rings	Colourless to drab colour	sub-globose	3-3.5 x 4 - 4.4 µm	Slightly globose (ampulliform)	10.2-11.8 x 2.3-2.8 µm
Tr-7	Dark green to greyish	9.4 cms.	Cotony	2 to 3 rings	Colourless to drab colour	Sub-globose round	3.8-4x 3.1-3.7 µm	Globose	6.3-15.6 x 2.7 - 3.4 µm
Tr-8	Green to dark green	8.9 cms	Cotony	No Presence	Yellow to amber	Round	4-4.8 x 3.5-4 µm	Flask shaped	8-14 x 2.4-3 in
Tr-9	Dark green to greyish	9.6 cms.	Cotony	2 to 3 rings	Colourless to drab colour	Sub-globose round	3.8-4x 3.1-3.7 µm	Globose	6.3-15.6 x 2.7 - 3.4 µm

Table 2: Response of different isolates of *Trichoderma* spp. On growth of *C. capsici*.

Isolate No. or Treatment	Species of <i>Trichoderma</i> isolates	Effect on the growth of <i>Colletotrichum capsici</i>	
		Average growth (mm)	Percent inhibition on over control
Tr-1	<i>T. viride</i>	23.5	59.130
Tr-2	<i>T. harzianum</i>	27.5	52.174
Tr-3	<i>T. viride</i>	22	61.740
Tr-4	<i>T. harzianum</i>	31.5	45.218
Tr-5	<i>T. harzianum</i>	29	49.566
Tr-6	<i>T. asperellum</i>	36	37.391
Tr-7	<i>T. harzianum</i>	30.5	46.957
Tr-8	<i>T. viride</i>	21.5	62.609
Tr-9	<i>T. harzianum</i>	26	54.783
Control		57.5	0

C.D. = 2.615723906
C.V. % = 4.999328092
SE(d) = 1.24498996

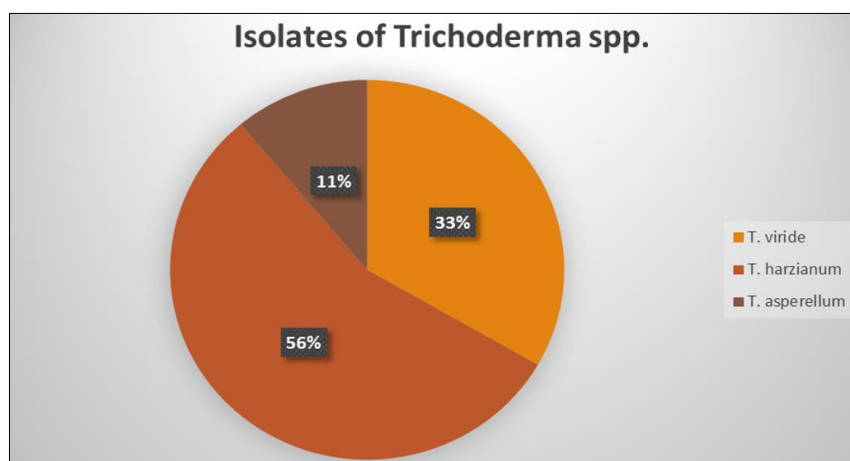


Fig 1: Frequency of native *Trichoderma* spp. among the different isolates

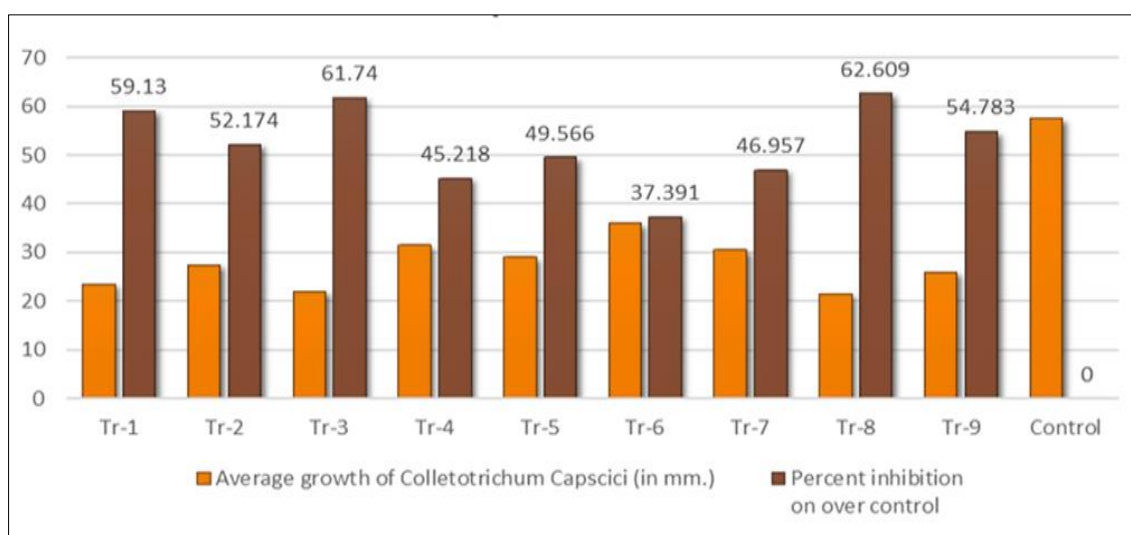
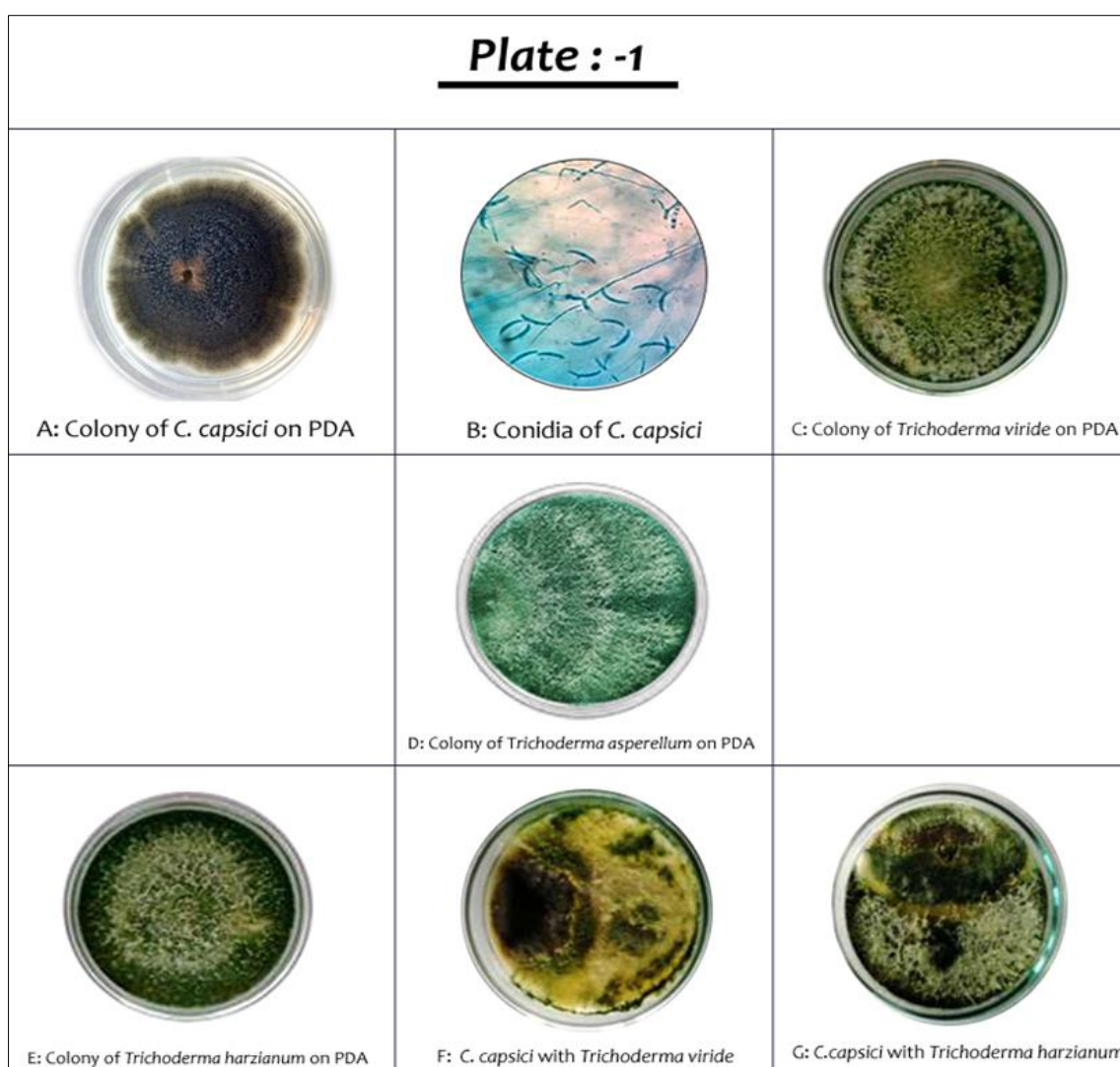


Fig 2: Effect of *Trichoderma* spp. on growth of *Colletotrichum capsici* in vitro



Conclusions

In the present study, it was observed that *T. harzianum* was most frequent among the isolates of the native *Trichoderma* spp. in the experimental field. And also, it was observed that *T. viride* and *T. harzianum* were proved to be most effective *in vitro* conditions when compared to the untreated control. *T. viride* isolates were most effective *in vitro* conditions followed

by the other different *Trichoderma* isolates. Therefore, it concluded that *Trichoderma harzianum* can become a part in integrated management of Anthracnose disease of chilli.

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