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ANTAGONISTIC POTENTIAL OF NATIVE ISOLATES OF *TRICHODERMA* SPP. FOR THE MANAGEMENT OF ANTHRACNOSE (*COLLECTOTRICHUM CAPSICI*) DISEASE OF CHILLI

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ABSTRACT

Capsicum annuum L., commonly known as chilli, is a highly significant vegetable and spice crop in the *Solanaceae* family. The term "Chilli" is derived from the Mexican word "chili" and has acquired the title of "wonder spice," Emphasizing emphasizing its importance as a staple crop. It is primarily grown for its ripe red, green fruit, which serves as a condiment, digestive aid, and flavoring and coloring agent for sauces, chutneys, pickles, and other foods. Its chromosome number is 2n= 24. In India, anthracnose, a seed-borne disease caused by *Colletotrichum capsici*, was first reported by Sydow (1928) from Coimbatore of Madras Presidency. Anthracnose symptoms on chilli appeared in the form of small circular spots appear on the leaves. On fruit typical symptoms were found as circular or angular sunken lesions with a slightly raised rim. The field experiment were conducted during 2024- 2025 at Agriculture research farm, School of Agriculture, Abhilashi University, Mandi (H.P.). The results revealed that in year 2023, seed treatment with *Trichoderma* spp. showed significant impact on *per cent* disease incidence at 90 DAT. The minimum disease incidence 22.22% was recorded in T_9 followed by T_8 - 27.78%, T_5 - 33.33%, T_7 - of 38.89%, T_1 - 44.44%, T_6 - 50.00%, T_4 -55.56%, T_2 -61.11%, and T_3- 66.67%, respectively. The seedling treatment with *Trichoderma* spp. showed significant impact on disease severity 60 DAT. The minimum disease severity 9.87% of chilli plants were recorded in T_9 (Seedling treatment with *Trichoderma* isolate-9@5gm/litre of water for 2 hours) followed by T_8 - 12.34%, T_5 - 13.58%, T_7 - 14.81%, T_1 - 16.04%, T_6- 17.28%, T_4 -19.75%, T_2 -20.98%, and T_3 -22.22%, respectively. On the basis of field experiment it was concluded that seedling treatment with native of *Trichoderma* isolates - 8 and 9 were found most effective in reducing the disease incidence and disease severity.

Keywords **:** Chilli, *Trichoderma* spp., growth parameters, disease and biological control.

Introduction

Chilli (*Capsicum annuum* L.) is a hot-testing tropical berry belonging to the *Solanaceae* family. Due to its wide spread usage, chillies are a highly significant vegetable found to have many medicinal properties. The term "Chilli" is derived from the Mexican word "chili" and has acquired the title of "wonder spice," Emphasizing emphasizing its importance as a staple crop. As green and ripe chilli fruits possess the alkaloid capsaicin, which gives food a distinctive spiciness and are used in a wide range of culinary applications, medicines, cosmetics etc. Globally, India is the leading chilli producer, consumer and exporter having maximum cultivable area. In India, anthracnose, a seed-borne disease caused by *Colletotrichum capsici*, was first reported by Sydow (1928) from Coimbatore of Madras Presidency, is an

emerging threat in chilli production that spreads under humid conditions, leaving very limited chances for growers to protect the crop (Srideepthi *et al*., 2017).

Green chilli provides vitamin-C while, the red chilli provides vitamin-A (Martin *et al.,* 2004) in addition to iron, potassium and magnesium. The alkaloid (Capsicinoid) present in chilli is responsible for pungency (Perez-Galvez *et al.,* 2003). Hottest pungent varieties reported are Carolina Reaper ‖ and Naga Jalokia. In India, chillies are cultivated in 1.69 lakh hectares with a production and productivity of 6.94 lakh tonnes and 4109 kg/ha, respectively (PJTSAU, Chilli Outlook. 2022) with the majority growing in Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra, Karnataka, Orissa and West Bengal. Anthracnose symptoms on chilli appeared in the form of small circular spots appear on the leaves. On fruit typical symptoms were found as circular or angular sunken lesions with a slightly raised rim. Plant disease known as anthracnose is characterized by very dark, sunken lesions that contain spores (Isaac, 1992). As they enlarge, they become irregular in shape, variable in size and give a scorched appearance. The disease results in dark spots, sunken necrotic tissue with concentric rings of acervuli, including die back in the stem, seedling blight, or damping off (Azad *et al*., 1991). Anthracnose caused marketable yield loss ranging from 50 to 80% in different parts of the world (Sariah, 1994).

Materials and Methods

The materials used and techniques adopted in the accomplishing the objectives of the present investigations were carried out on entitled "Antagonistic potential of native isolates of *Trichoderma* spp. for the management of anthracnose (*Colletotrichum capsici*) disease of chilli". The details of materials and methods used for techniques adopted for experimented purpose are given as under.

Experimental site

The present investigations were carried out in the premises of research farm Agriculture and laboratory studies were conducted in Plant Pathology laboratory, School of Agriculture, Abhilashi University Mandi (H.P.) during *Kharif* season 2024-2025.

Equipment's

The laboratory equipment's *viz.,* Autoclave, Laminar airflow cabinet, B.O.D. Incubator, Hot air oven, Refrigerator, Compound microscope, Weighing balance and etc. available in the Plant Pathology laboratory, Department of Plant Pathology, School of Agriculture, Abhilashi Uuniversity, Mandi (H.P.), were used as per requirements.

Cleaning and sterilization of glassware's

The glass wares used during experiments were cleaned by overnight dipping in the water and then washed with detergent under running tap water and air dried. The air dried glassware's were sterilized in hot air oven at temperature 180 ^{0}C for one hour (Aneja, 2004).

PDA preparation procedure

The required quantity of peeled potatoes were cut into small pieces and boiled in 500 ml of distilled water till the pieces become soft. The potato extract was filtered through muslin cloth and the filtrate was collected in the beaker. Rest of the 500 ml water was made warm. 20 gm agar and 20 gm dextrose was added properly by shaking through glass rod. Both solutions were mixed together in a beaker and volume was maintained up to 1000 ml by adding required amount of distilled water. Two hundred ml of this solution was dispensed in each conical flask of 250 ml. Flasks containing medium were sterilized at 121ºC at 15 lbs pressure/inch^2 for 15 minutes in an autoclave. Transferred medium was allowed to cool up to 45-50 ^{0}C before pouring into petri plates.

Collection of *Trichoderma* spp.

The culture of *Trichoderma* isolates were obtained from Plant Pathology Laboratory, School of Agriculture Abhilashi University, Mandi (H.P.).

Purification and maintenance of native isolates *Trichoderma* spp.

Three days old colonies of *Trichoderma* was picked up and purified by single hyphal tip method Tuite (1969). The purified cultures of *Trichoderma* isolate were maintained by periodical transfer on PDA by sub-culturing and the pure cultures in PDA cultures were preserved in refrigerator for further studies (Plate no.-1)

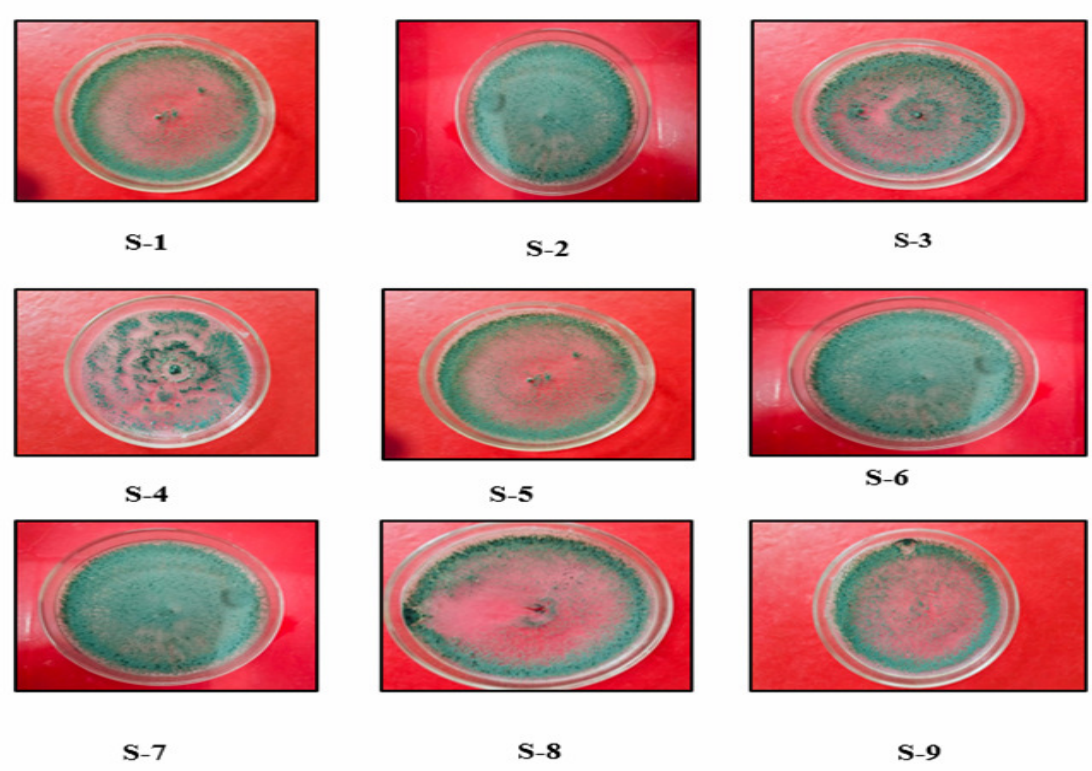

Plate 1 : Purification and maintenance of *Trichoderma* spp.

Mass multiplication of *Trichoderma* spp.

Wheat grains were used for mass multiplication of different *Trichoderma* isolate. The grains were soaked overnight in water for 12 hours and then spreaded on blotter paper to remove the excess water. Dextrose was added to the seeds @ 20 gm/kg of seed and then 150 gm of wheat grains were taken in each 250 ml conical flasks. Flasks filled with grains were plugged with non-absorbent cotton and wrapped in aluminum foil and sterilized in autoclave at 121 ^{0}C temperature at 15 lbs pressure/inch^2 for 15 minutes. Flasks were taken out from autoclave and were allowed to cool. After cooling, each conical flask which contained sterilized seeds were inoculated with 5 disks of *Trichoderma* punched from the cork borer (5 mm). The disks were punched from the periphery of actively growing 5 days old culture of *Trichoderma* isolate. All inoculated conical flasks were incubated in a BOD at 26±2°C. *Trichoderma* isolate were allowed to grow with periodic shaking of the flasks to ensure that the surface of all seeds were colonized with the growth of *Trichoderma* properly. When all the seeds were completely covered with mycelial growth, the colonized seeds were taken out from the flasks and shade dried in clean and sterilized place. After proper drying, powder of selected *Trichoderma* isolate was prepared with the help of mixer-cum grinder.

Experimental details

Design : Randomized Block Design (RBD)

Replications : 3

Plot size : 1 m x 1m =1m^2

Spacing : 30 cm x 15cm

Treatment Details:

T_1- Seedling treatment with *Trichoderma* isolate-1@5gm/litre of water for 2 hours

T_2- Seedling treatment with *Trichoderma* isolate2@5gm/litre of water for 2 hours

T_3- Seeding treatment with *Trichoderma* isolate-3@5gm/litre of water for 2 hours

T_4- Seedling treatment with *Trichoderma* isolate-4@5gm/litre of water for 2 hours

T_5- Seedling treatment with *Trichoderma* isolate-5@5gm/litre of water for 2 hours

T_6- Seedling treatment with *Trichoderma* isolate-6@5gm/litre of water for 2 hours

T_7- Seedling treatment with *Trichoderma* isolate-7@5gm/litre of water for 2 hours

T_8- Seedling treatment with *Trichoderma* isolate-8@5gm/litre of water for 2 hours

T_9- Seedling treatment with *Trichoderma* isolate-9@5gm/litre of water for 2 hours

T_{10}-Control

Field preparation and transplanting

Field preparation was done with the help of harrow mounted on a tractor. The weeds and crop residues were removed to get weed and crop residue free field. Chilli plants were transplanted with the help of randomized block design with three replication and plot size was of 1 x 1 m^2.

***Per cent* disease incidence (PDI)**

Observations of fungal disease incidence were noted on ten randomly selected chilli plants per field trial. The observations were recorded for the evaluation of disease incidence. Trial was conducted periodically in chilli-growing areas in various treatments to assess the disease incidence, calculated with the formula (Morris *et al*., 2017).

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Total number of infected plants}}{\text{Total numbers of plants}} \times 100$$

Disease Severity

Five plants were randomly selected. These plants were used the leaf disease severity scale system of 0-5, where 0 = no leaf spots and 5 = plants completely defoliated and killed by leaf spots (Table 1).

Table 1 : Scale (0-5) for the rating of the disease severity (Banerjee and Kalloo, 1987).

Grade	Disease severity	Disease Reaction	Description
0	0	Symptomless	No visual symptom
1	0-5%	Highly resistant (HR)	Curling and clearing of upper leaves
2	6-25%	Resistant (R)	Curling, clearing of leaves and swelling of veins
3	26-50%	Moderately resistant (MR)	Curling puckering and yellowing of leaves and swelling of veins
4	51–75%	Moderately susceptible (MS	Leaf curling and stunted plant growth and blistering of inter nodes.
5	More than 75%	Susceptible (S)	curling and deformed small leaves, stunted plant growth with small flowers and no or small fruit set

Per cent disease severity was calculated according to (Pramesh *et al*. 2017).

$$\text{Per cent disease severity (PDS)} = \frac{\text{Sum of total rating}}{\text{Total no of plants observed} \times \text{highest grade}} \times 100$$

Statistical analysis

Data were analyzed with the help of analysis of variance table wherever required. The F value was tested and critical difference (C.D.) was calculated at 5 *per cent* of significance for comparing treatment means (Gomez, 1996).

Results and Discussion

Effect of seedling treatment with *Trichoderma* spp. on *percent* disease incidence (PDI) anthracnose (*Colletotrichum capsici*) disease of chilli

The results presented in Table No. 2 and Figure No. 1 revealed that in year 2023, seed treatment with *Trichoderma* spp. showed significant impact on *per cent* disease incidence at 90 DAT. The minimum disease incidence 22.22% was recorded in T_9 followed by T_8 - 27.78%, T_5 - 33.33%, T_7 - of 38.89%, T_1 - 44.44%, T_6 - 50.00%, T_4 -55.56%, T_2 -61.11%, and T_3- 66.67%, respectively. Whereas, maximum disease incidence 72.22% was recorded in T_{10} control. Similarly, Patel *et al*. (2020) revealed that in field condition, among treatments with different *Trichoderma* isolates, it was observed that minimum PDI of 19.40% was recorded in seed treatment and three foliar sprays with T_2 isolate of *Trichoderma* with maximum yield of 69.55q/ha.

Table 2 : Effect of native isolates of *Trichoderma* spp. on *per cent* disease incidence (PDI) of anthracnose (*Colletotrichum capsici*) disease of chilli at 90 days after transplanting (DAT)

Sr. No.	Treatments	Disease incidence (%)
		90 DAT
T_1	Seedling treatment with *Trichoderma* isolate-1@5gm/litre of water for 2 hours	44.44
T_2	Seedling treatment with *Trichoderma* isolate-2@5gm/litre of water for 2 hours	61.11
T_3	Seedling treatment with *Trichoderma* isolate-3@5gm/litre of water for 2 hours	66.67
T_4	Seedling treatment with *Trichoderma* isolate-4@5gm/litre of water for 2 hours	55.56
T_5	Seedling treatment with *Trichoderma* isolate-5@5gm/litre of water for 2 hours	33.33

T_6	Seedling treatment with *Trichoderma* isolate-6@5gm/litre of water for 2 hours	50.00
T_7	Seedling treatment with *Trichoderma* isolate-7@5gm/litre of water for 2 hours	38.89
T_8	Seedling treatment with *Trichoderma* isolate-8@5gm/litre of water for 2 hours	27.78
T_9	Seedling treatment with *Trichoderma* isolate-9@5gm/litre of water for 2 hours	22.22
T_{10}	Control	72.22
	C.D. (at 5% level)	N/A
	SE(m)	2.270

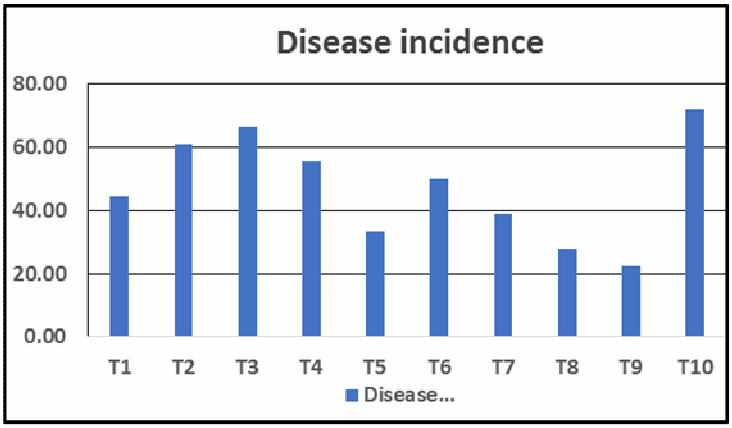

Fig. 1 : Effect of native isolates of *Trichoderma* spp. on *percent* disease incidence (PDI) of anthracnose (*Colletotrichum capsici*) disease of chilli at 90 days after transplanting (DAT)

Effect of native isolates of *Trichoderma* spp. on disease severity of anthracnose (*Colletotrichum capsici*) of chilli at 60 days

The results presented in Table No. 3 and Figure No. 2 revealed that in year 2023, seedling treatment with *Trichoderma* spp. showed significant impact on disease severity 60 DAT. The minimum disease severity 9.87% of chilli plants were recorded in T_9 (Seedling treatment with *Trichoderma* isolate-9@5gm/litre of water for 2 hours) followed by T_8 - 12.34%, T_5 - 13.58%, T_7 - 14.81%, T_1 - 16.04%, T_6- 17.28%, T_4 -19.75%, T_2 -20.98%, T_3 -22.22% respectively Whereas, maximum disease severity 23.45% was recorded in T_{10} control. Similarly, Karyan and Tuanh (2018) *Trichoderma* sp. decreased disease severity on chilli artificially inoculated fruits up to 64% when *Trichoderma* mycelial plug was used and 55% when culture filtrate was applied. Field trials are recommended to examine the antagonism of *Trichoderma* sp. in real production conditions.

Effect of native isolates of *Trichoderma* spp. on disease severity of anthracnose (*Colletotrichum capsici*) disease of chilli at 90 days

The results presented in Table No. 3 and Figure No. 2 revealed that in year 2023, seedlings treatment with *Trichoderma* spp. showed significant impact on disease severity 90 DAT. The minimum disease severity 20.98% of chilli plants were recorded in T_9 (Seedling treatment with *Trichoderma* isolate-9@5gm/litre of water for 2 hours) followed by T_8 - 23.45%, T_5 -24.69%, T_7 - 27.16%, T_1- 28.39%, T_6 - 29.62%, T_4 - 32.09%, T_2 -33.33%, T_3 (Seedling treatment with *Trichoderma* isolate-3@5gm/litre of water for 2 hours) 34.56% respectively. Whereas, maximum disease severity 37.03% was recorded in T_{10} control. Similarly, Chohan *et al.* (2024) reported, IC had a disease severity (DS) of 4.7, whereas, there were no disease symptoms in NIC. Moreover, all the treatments using *Trichoderma* showed significant reduction on DS in the chickpea plants and this reduction in DS was dose dependent. Treatments using combination of all these *Trichoderma*, all significantly decreased the DS in the chickpea plants. The reductions in DS were 21% and 23%, 29%, 31%, and 40%, and 47%, 71%, and 86% at concentration 1 and concentration 2, respectively, compared to the IC.

Table 3 : Effect of native isolates of *Trichoderma* spp. on disease severity anthracnose (*Colletotrichum capsici*) of chilli 60 and 90 days

Sr. no.	Treatments	Disease Severity (%)	
		60 DAT	90 DAT
T_1	Seedling treatment with *Trichoderma* isolate-1@5gm/litre of water for 2 hours	16.04	28.39
T_2	Seedling treatment with *Trichoderma* isolate--2@5gm/litre of water for 2 hours	20.98	33.33
T_3	Seedling treatment with *Trichoderma* isolate--3@5gm/litre of water for 2 hours	22.22	34.56
T_4	Seedling treatment with *Trichoderma* isolate--4@5gm/litre of water for 2 hours	19.75	32.09
T_5	Seedling treatment with *Trichoderma* isolate--5@5gm/litre of water for 2 hours	13.58	24.69
T_6	Seedling treatment with *Trichoderma* isolate--6@5gm/litre of water for 2 hours	17.28	29.62
T_7	Seedling treatment with *Trichoderma* isolate--7@5gm/litre of water for 2 hours	14.81	27.16
T_8	Seedling treatment with *Trichoderma* isolate--8@5gm/litre of water for 2 hours	12.34	23.45
T_9	Seedling treatment with *Trichoderma* isolate--9@5gm/litre of water for 2 hours	9.87	20.98
T_{10}	Control	23.45	37.03
	C.D.(at 5% level)	3.354	4.25
	SE(m)	1.118	1.458

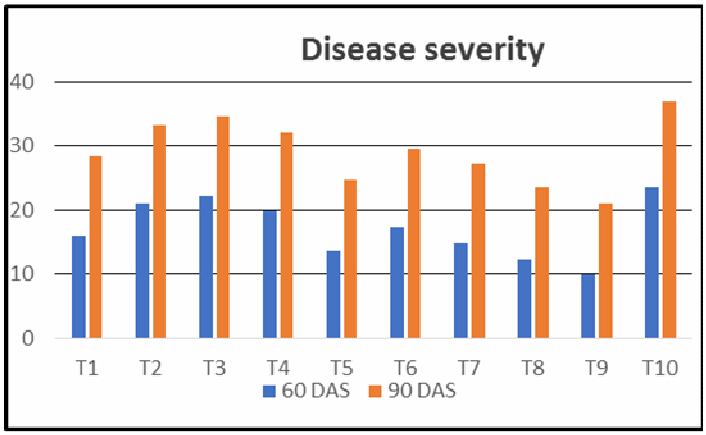

Fig. 2 : Effect of native isolates of *Trichoderma* spp. on disease severity anthracnose (*Colletotrichum capsici*) disease of chilli 60 and 90 days

Conclusion

On the basis of field experiment it was concluded that seedling treatment with native of *Trichoderma* isolates - 8 and 9 were found most effective in reducing the disease incidence and disease severity. Therefore, these treatments may be recommended for the management anthracnose (*Collectotrichum capsici*) disease of chilli.

Future work :- Further studies are required to be conducted under field conditions in multilocation trails to validate the effectiveness of *Trichoderma* isolates and more investigations are needed for morphological and molecular characterization of these *Trichoderma* isolates for accurate identification of potential of *Trichoderma* spp. isolates are the needed for better result.

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