

Original Research Article

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## Study of Antagonistic Capabilities of *Trichoderma* spp. against *Alternaria macrospora* Zimm. Causing Leaf Spot in Cotton

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### ABSTRACT

Biological agents provide economical and relatively non-polluting delivery systems for protective materials compared to other chemical methods and biocontrol measures against plant pathogens are widely recognised as a step towards ecofriendly farming. The present investigation carried out to know the interactions of *Trichoderma* spp. with *Alternaria macrospora* causing leaf spot of cotton. All the *Trichoderma* spp. significantly inhibited the growth of *A. macrospora* *in vitro* in dual culture experiments. Among the four *Trichoderma* strains evaluated, *T. harzianum* strain 3 inhibited the growth (45.29%) at 5 DAI (days after inoculation) with radial growth (0.93 cm). *T. harzianum* strain 1 isolate was the best isolate among the isolates tested as it grows faster and overlaps on the test pathogen. Interactions with all the *Trichoderma* strains in dual cultures revealed lysis of *A. macrospora* with evidence of protoplast aggregation, wrinkling of mycelium and germination of *Trichoderma* conidia on *Alternaria* mycelia. Thus *Trichoderma* is promising bioagent which can be explored in the IDM of *Alternaria* leaf spot of cotton.

#### Keywords

*Alternaria macrospora*,  
Cotton leaf spot,  
*Trichoderma* spp

#### Article Info

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### Introduction

*Alternaria macrospora* mainly affects the cotton leaf on which small, dull to dark brown, circular or irregular shaped spots appear, varying in diameter from 0.5 to 10 mm. They often develop concentric ridges with a target board appearance on the upper surface. Mature spots have dry, greyish centers which may crack and drop. The spots coalesce and occupy large areas of the leaf. Watkins (1981) observed dark chains of conidia on the spots. Veins of leaf may also be affected. Spots occur on the cotyledons which are severely

affected. Cankers occasionally develop on the stem. In India, circular lesions develop on the bolls resembling those on the leaves. The seeds may get infected and carry the infection (Padaganur, 1979).

The fungus produces dark mycelium in the culture. Conidiophores were dark brown, short or long, 1-8 septate and with solitary conidia at the apex. In the culture, the conidia measured on the average of 43.4 x 13.3 µm, in addition to the average length of the beak was 16.6 µm, long beak is the characteristic feature of *A. macrospora* (Vasudeva, 1960). In view

of *Trichoderma* spp. economic importance in cotton, an experiment was conducted to test the antagonistic capacity of *Trichoderma* strains and their interactions with *Alternaria macrospora* mycelia *in vitro*.

## Materials and Methods

### Isolation and identification of *Alternaria macrospora* Zimm.

*Alternaria macrospora* Zimm, was isolated from the infected cotton leaves collected from the field. Small bits of the infected leaves of 3 to 5 mm size were cut from the diseased area along with some healthy portion, surface sterilized with 1% sodium hypochlorite for 30 seconds and rinsed thrice with sterilized distilled water. The surface sterilized bits were transferred on to potato dextrose agar medium agar plate aseptically and incubated at  $27 \pm 1^{\circ}$  C for three days. Initial growth of the pathogen was sub-cultured in to agar slants.

The fungal culture was purified by single spore isolation. The fungus isolated during the study was identified based on the characteristics of the colony, hyphae, conidiophores and conidia. The measurements of the conidiophores and conidia were recorded and compared with the standard measurements of the species given in the "CMI Descriptions of Pathogenic Fungi and Bacteria (1952)" (Plate 1).

### Effect of bio-control agents on mycelial growth of *A. macrospora*

In order to find out the antagonistic effect of different microorganisms against radial growth of *A. macrospora*, three isolates of *Trichoderma harzianum* and one isolate of *T. viride* which were native to RARS, Lam were evaluated for their antagonistic activity against *A. macrospora* by employing dual culture technique (Dhingra and Sinclair, 1985).

Sterilized potato dextrose agar medium, melted and cooled at  $45^{\circ}$ C, was poured aseptically into sterilized Petri dishes. Mycelial discs of 5 mm diameter from the edge of actively growing culture of *A. macrospora* and isolates of *Trichoderma* spp. were separately cut with the help of a sterilized cork borer and the two discs were simultaneously placed on the periphery about one cm from the edge of the Petri dishes (9 cm diameter) on opposite sides.

Four replications were maintained for each treatment. The Petri dishes containing potato dextrose agar medium inoculated with the pathogen alone served as control. All the Petri dishes were incubated at room temperature of  $25 \pm 1^{\circ}$ C. The colony diameter of the pathogen was measured daily and the per cent inhibition of *A. macrospora* was calculated by following the formula given by Vincent (1927).

$$\text{Per cent inhibition} = \frac{\text{Radial growth in control (C)} - \text{Radial growth in treatment (T)}}{\text{Radial growth in control (C)}} \times 100$$

## Results and Discussion

### Effect of *Trichoderma* spp. on the growth of *A. macrospora*

All the *Trichoderma* spp. reduced the growth of *A. macrospora* compared to control (Table 1). Among the *Trichoderma* stains, *T. harzianum* strain 3 significantly reduced the growth of *A. macrospora* in all the incubation periods (2 DAI, 3 DAI, 4 DAI and 5 DAI). At 4 DAI, *T. harzianum* strain 3 recorded 0.83 cm radial growth with 40.71% inhibition while *T. harzianum* strain 2, *T. harzianum* strain 1 and *T. viride* strain were recorded 1.08 cm, 1.10 cm and 1.20 cm radial growth with 21.43%, 21.43% and 14.28% inhibition and those were statistically on a par (Plate 2).

**Table.1** Radial growth of *A. macrospora* dual cultured with *Trichoderma* spp. *in vitro*

S. No	Dual culture with	Radial growth of <i>A. macrospora</i> (cm)*				Inhibition (%)	
		2-DAI	3-DAI	4-DAI	5-DAI	4-DAI	5-DAI
1	<i>Trichoderma harzianum</i> strain 1	0.43 (0.66) <sup>ba</sup>	0.93 (0.97) <sup>ba</sup>	1.10 (1.05) <sup>cb</sup>	1.20 (1.10) <sup>cb</sup>	21.43	29.41
2	<i>T. harzianum</i> strain 2	0.50 (0.71) <sup>c</sup>	0.90 (0.95) <sup>ba</sup>	1.078 (1.03) <sup>b</sup>	1.13 (1.06) <sup>b</sup>	21.43	33.52
3	<i>T. harzianum</i> strain 3	0.40 (0.63) <sup>a</sup>	0.80 (0.92) <sup>a</sup>	0.83 (0.91) <sup>a</sup>	0.93 (0.96) <sup>a</sup>	40.71	45.29
4	<i>T. viride</i> strain	0.60 (0.77) <sup>d</sup>	0.97 (0.98) <sup>cb</sup>	1.20 (1.09) <sup>cb</sup>	1.38 (1.17) <sup>d</sup>	14.28	18.82
5	Monoculture ( <i>A. macrospora</i> )	0.40 (0.63) <sup>a</sup>	0.85 (0.92) <sup>a</sup>	1.40 (1.18) <sup>d</sup>	1.70 (1.30) <sup>e</sup>	..	..
	SEm ±	0.02	0.02	0.03			
	CD (P ≤ 0.05)	0.05	0.05	0.09			
	CV (%)	4.46	3.33	5.55			

DAI – Days after inoculation

\*Mean of four replications

Treatment means with same alphabet do not differ significantly

Figures in parentheses are square root transformed values

**Table.2** Radial growth of *Trichoderma* spp. dual cultured with *A. macrospora* *in vitro*

S. No.	Treatment	Radial growth of <i>Trichoderma</i> spp. (cm)*				Inhibition (%)
		2-DAI	3-DAI	4-DAI	5-DAI	5-DAI
1	<i>T. h</i> strain 1 - D	2	4	5.53	5.8	10.81a
	<i>T. h</i> strain 1 - C	2	4	5.5	6.5	
2	<i>T. h</i> strain 2 - D	1.8	3.53	5.13	5.4	12.95c
	<i>T. h</i> strain 2 - C	1.8	3.5	5	6.2	
3	<i>T. h</i> strain 3 - D	1.83	3.47	4.93	5.6	13.9d
	<i>T. h</i> strain 3 - C	1.7	3.3	4.6	6.5	
4	<i>T. v</i> strain 1 - D	1.63	3.2	4.7	5.5	11.34ba
	<i>T. v</i> strain 1 - C	1.6	3.2	4.7	6.2	
	SEm ±					0.21
	CD (P ≤ 0.05)					0.65
	CV (%)					3.43

*T. h* – *Trichoderma harzianum*

*T. v* – *Trichoderma viride*

D – Dual culture

C – Check

DAI – Days after Inoculation

\*Mean of four replications; Treatment means with same alphabet do not differ significantly

Fig.1 Effect of *Trichoderma* spp. on radial growth of *A. macrospora* in vitro

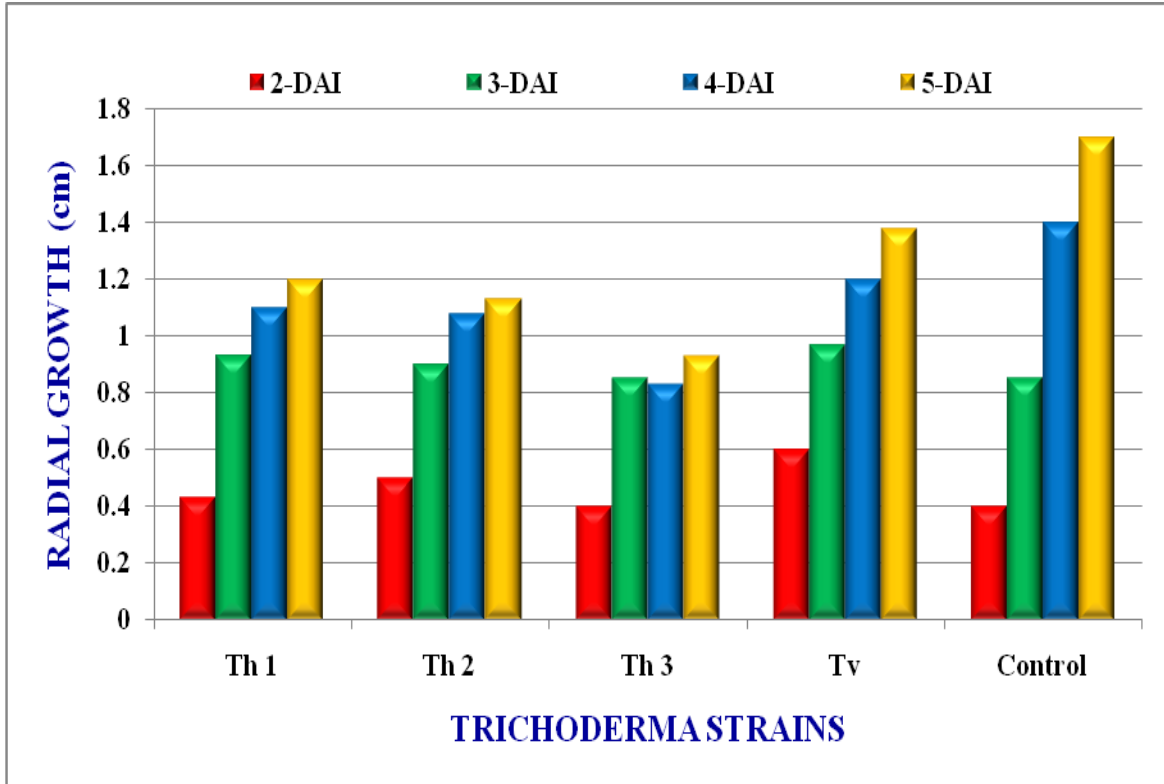
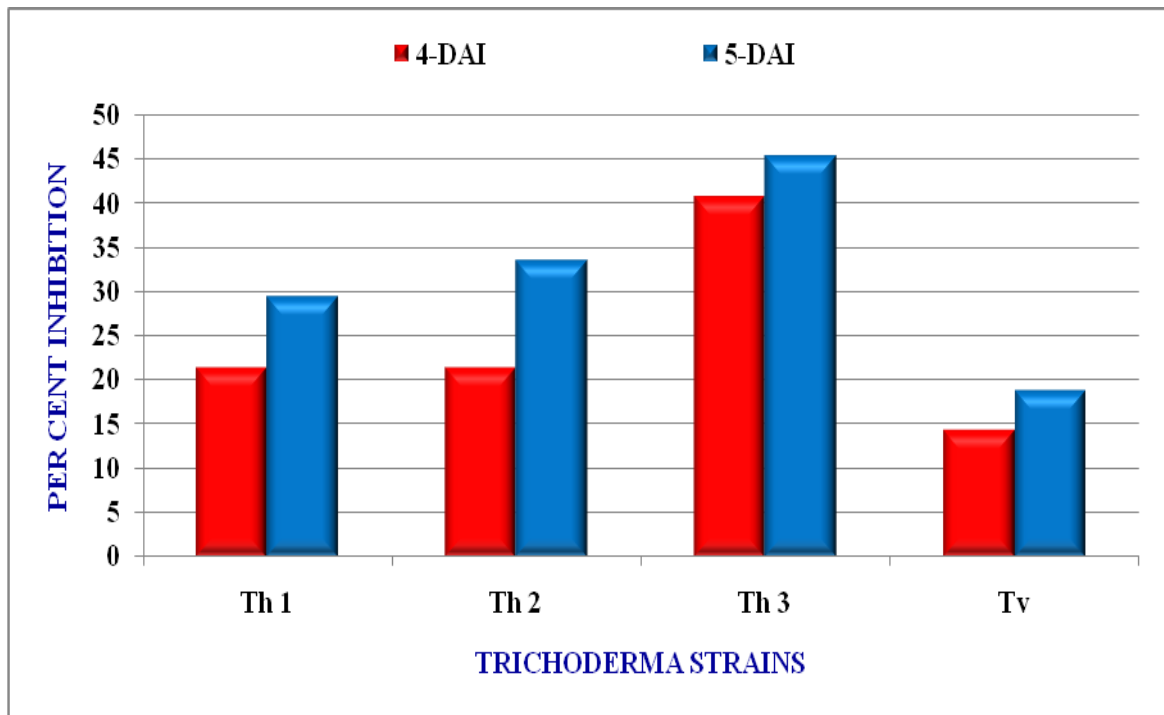


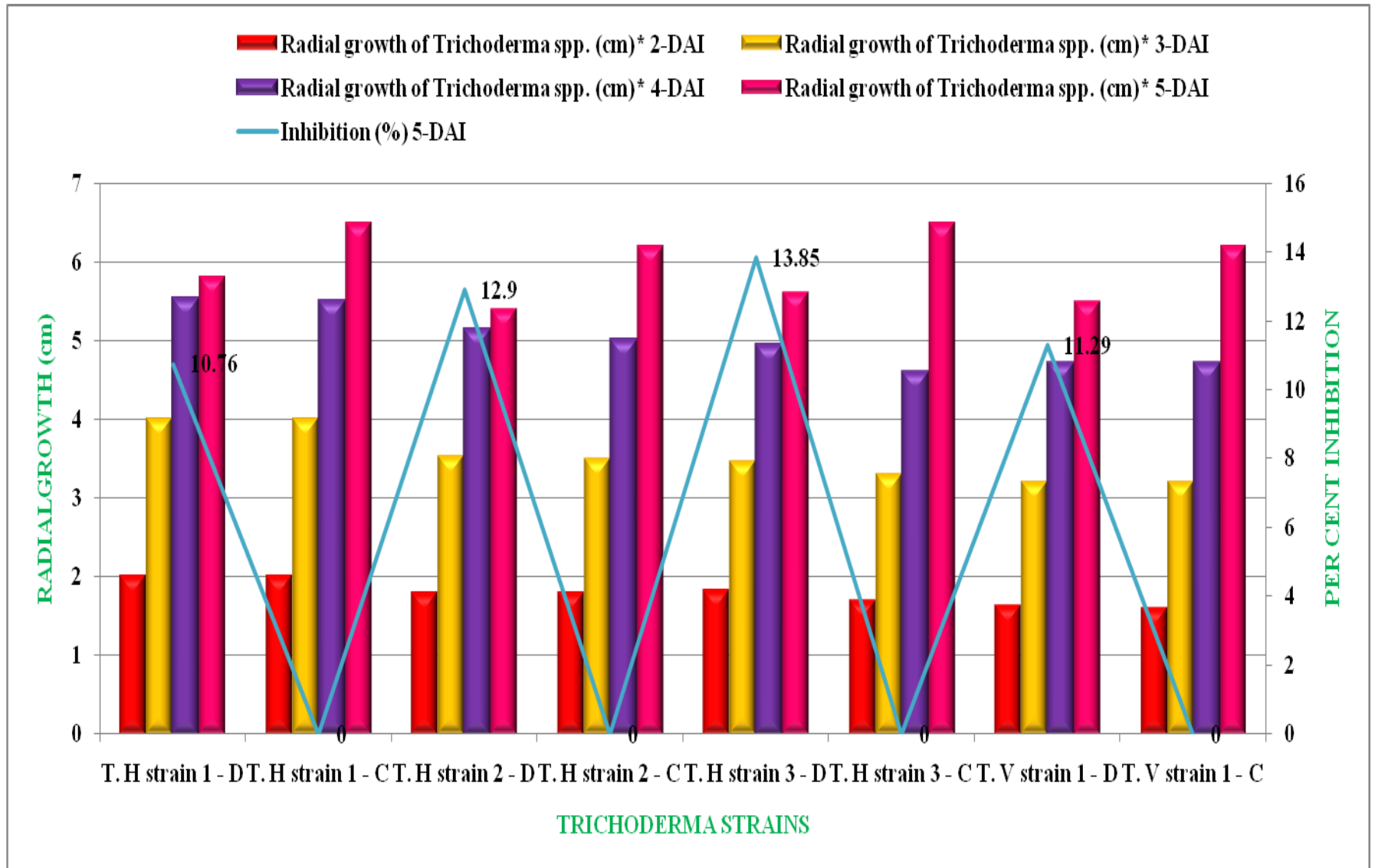
Fig.2 Inhibition in the growth of *A. macrospora* dual cultured with *Trichoderma* strains



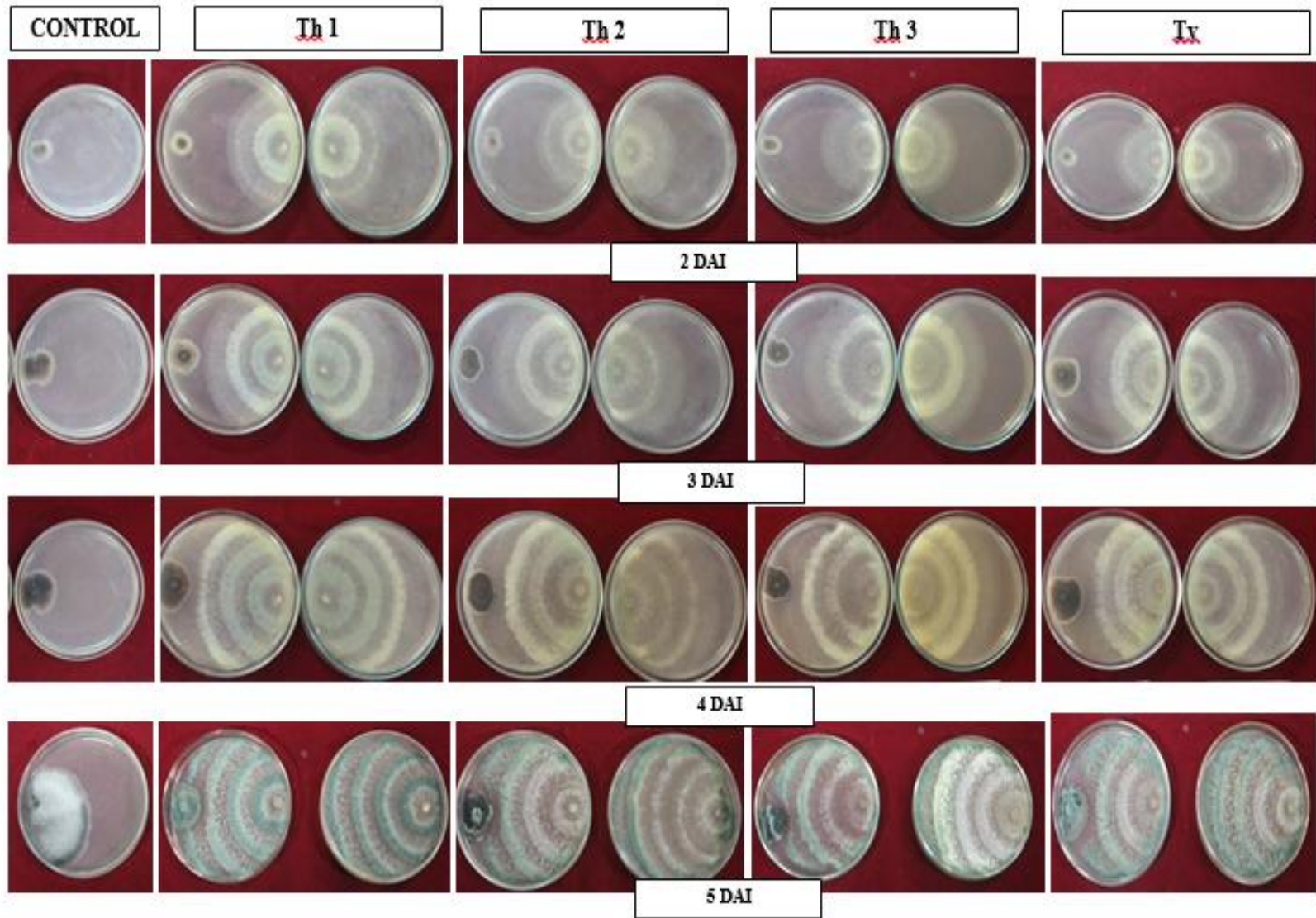
Th – *Trichoderma harzianum*

Tv – *T. viride*

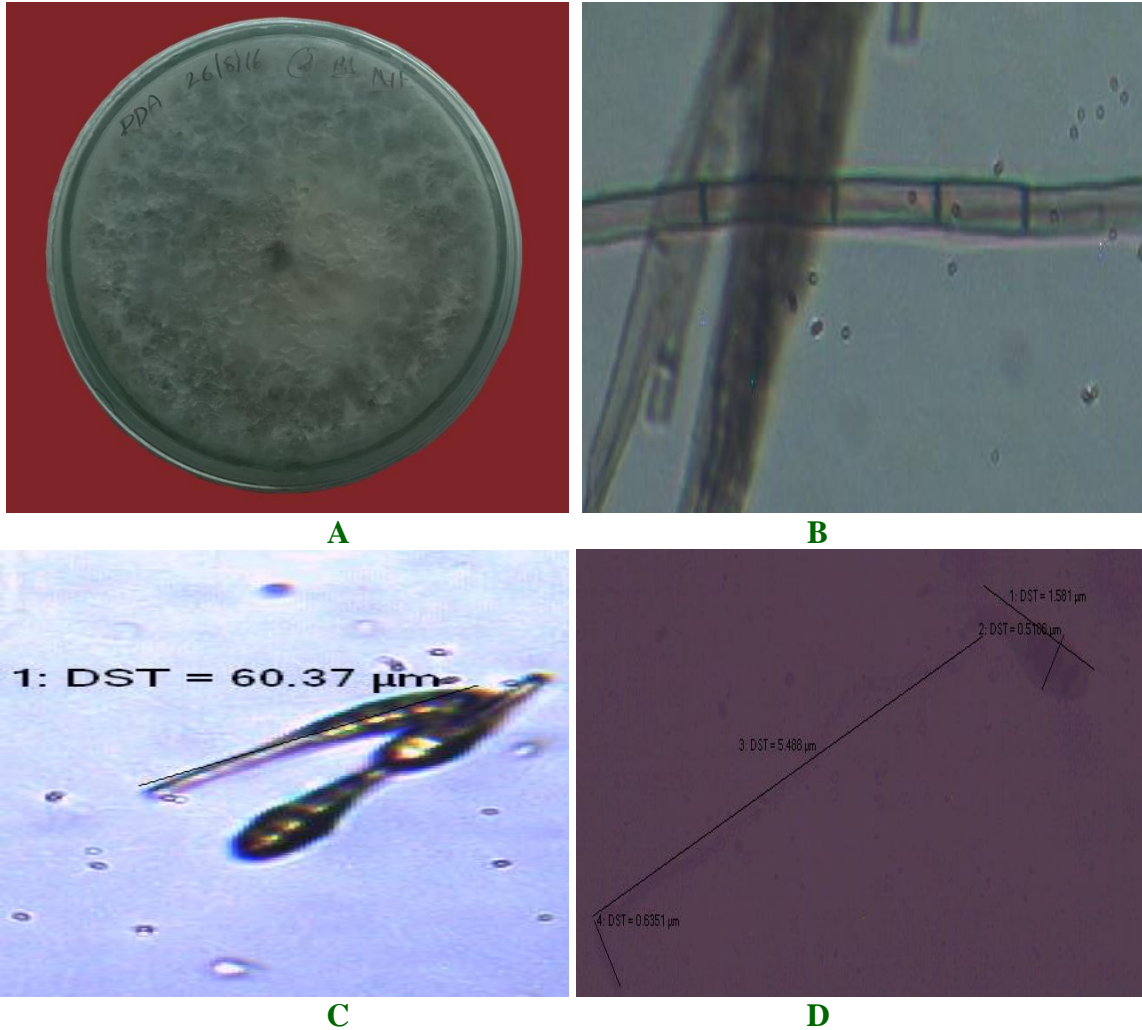
Fig.3 Radial growth of *Trichoderma* spp. dual cultured with *A. macrospora* *in vitro*



**Plate.2** Effect of *Trichoderma* spp. on radial growth of *A. macrospora*

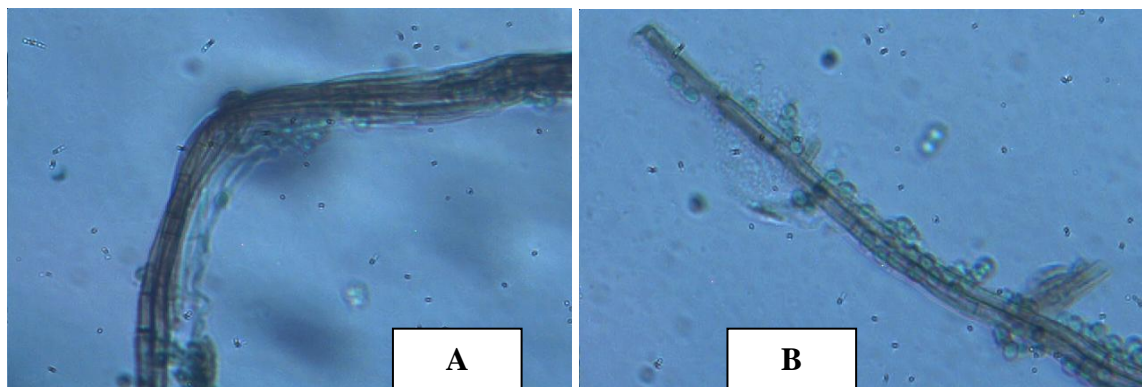


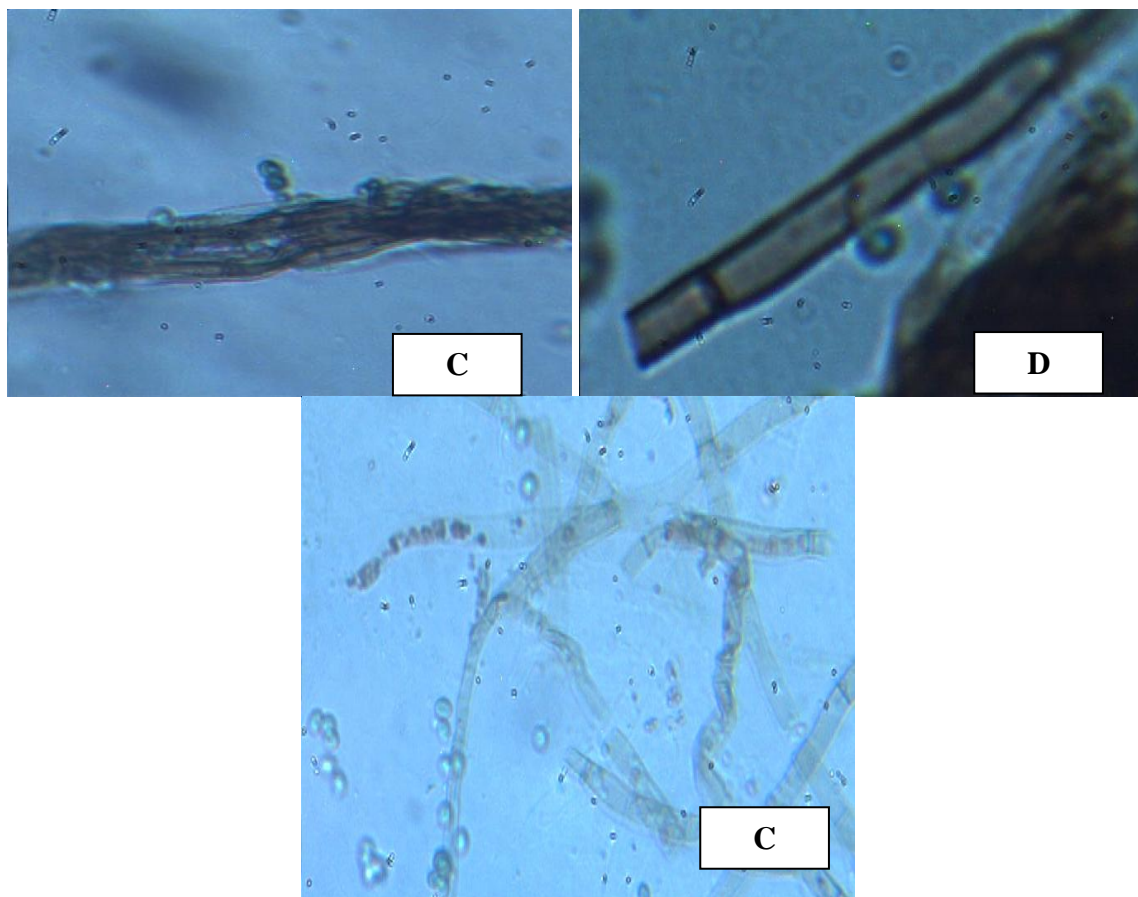
**Plate.1** Morphological characters of *A. macrospora*



A: Pure culture of *A. macrospora*  
B: Mycelium of *A. macrospora* (100 x)  
C: Conidia of *A. macrospora*  
D: Conidium and conidiophores of *A. macrospora*

**Plate.3** Interactions of *Trichoderma* spp. with *A. macrospora* in dual culture





- A: Abnormalities in *Alternaria* mycelium
- B: *Trichoderma* conidia on *Alternaria* mycelium
- C: Coiling of *Trichoderma* mycelium
- D: Conidial germination on *Alternaria* mycelia
- E: Protoplast aggregation in *Alternaria* mycelia

At 5 DAI, *T. harzianum* strain 3 recorded 0.93 cm radial growth with 45.29% inhibition; next best biocontrol agent, *T. harzianum* strain 2 recorded 1.13 cm radial growth with 33.52% inhibition which was on a par with *T. harzianum* strain 1 which recorded radial growth 1.20 cm with 29.41% inhibition.

*T. viride* showed lesser radial growth inhibition of 18.82% with 1.38 cm radial growth (Fig. 1 and 2).

Evaluation of efficacy of *Trichoderma* strains (Table 2) revealed that *T. harzianum* strain 1 was the best isolate, fast over grown on test pathogen and it was the best isolate among the test isolates of *Trichoderma* (Fig. 3).

### Interactions of *Trichoderma* spp. on *A. macrospora* in dual culture

Microscopic examination of dual culture plates revealed that all the four isolates of *Trichoderma* spp. inhibited the growth of *A. macrospora*.

Primarily *Trichoderma* hyphae coiled around the test pathogen. Later it caused wrinkling of hyphae.

*Trichoderma* spp. developed with their sporulation on the *Alternaria* mycelium. *Trichoderma* conidia penetrated into the *Alternaria* mycelium and finally induced lysis of the mycelium of *A. macrospora*. Protoplast aggregation in the *Alternaria* mycelium and



shortening the distance of septa was observed (Plate 3).

Adarsh Pandey (2010) reported that *T. viride* and *T. harzianum* had controlled the pathogen *A. alternata* by mechanism known as mycoparasitism. Mokhtar and Dehimat (2012) reported that antagonistic fungi *T. harzianum* prevented the spore formation in *Alternaria* spp.

Among the different bioagents tested, *Trichoderma viride* was found most effective against *A. macrospora*, with maximum growth inhibition (Gholve *et al.*, 2014). Zahra Ibrahim El- Gali (2015) reported that direct confrontation of the colonies of *T. harzianum* with *A. alternata* results in an inhibition and growth arrested. *Alternaria* mycelia showed abnormal morphology like haustoria formation, coiling and lysis of mycelia as a result of direct attack of *Trichoderma* spp. Sanjeev *et al.*, (2017) reported that among different biocontrol tested *T. harzianum*-21 showed significant reduction in mycelia growth of *A. alternata* *in vitro*.

Present studies confirms the antagonistic efficacy of *Trichoderma* against *A. macrospora* and suggests its application in the IDM of alternaria leaf spot of cotton.

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