

Original Research Article

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## Identification and Evaluation of Potential *Trichoderma* Strains against *Colletotrichum capsici* and *Fusarium oxysporum* f. sp. *capsici* Causing Anthracnose and Wilt Disease in Chilli

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### ABSTRACT

#### Keywords

*Trichoderma asperellum*,  
*T. harzianum*,  
*T. longibrachiatum*,  
*Colletotrichum capsici*, *Fusarium oxysporum* f. sp. *capsici*, Bioagents, Antagonistic.

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*Trichoderma* spp. is biocontrol agents extensively used in management of fungal diseases of crop plants exhibiting mycoparasitism against a wide range of plant pathogens. Twelve *Trichoderma* isolates *T. asperellum* (7), *T. harzianum* (4) and *T. longibrachiatum* collected from ten district of U.P from chilli ecosystem were characterized for their antagonistic activity against *Fusarium oxysporum* f. sp. *capsici* and *Colletotrichum capsici* of chilli. The isolates revealed differential reaction pattern against the test pathogens. However, the isolates CA-06 and CA-07, (*T. harzianum*) were most effective causing inhibition of mycelial growth in *Fusarium oxysporum* f. sp. *capsici* as 22- 77 % and *Colletotrichum capsici* as 31- 66 % respectively. From our investigation it was conclude that *T. harzianum* as a potent bio fungicide to control *Fusarium* wilt and Anthracnose disease in chilli.

### Introduction

India has been the major producer as well as exporter of the dried chillis in the world. About 42.2% (801,600 ha) cultivable land area under chilli production lies in India, producing approximately 21.4% (1.4 million t) of world's total chilli (FAOSTAT, 2012). In India, Uttar Pradesh has been one of the major producer and exporter of chilli crop. In Uttar Pradesh, Bareilly, Khurza, Faizabad and Mirzapur are the major chilli producing districts but recently due to a decline in chilli

production, it stands at third position in terms of its production. The decline in chilli production has been attributed to the diseases linked with crop. Among the large number of diseases affecting chilli cultivation, anthracnose and wilt have been most detrimental to its production.

Among the diseases, Anthracnose caused by *Colletotrichum capsici*, is one of the most important disease of chilli causing up to 50%

yield loss (Pakdevaraporn *et al.*, 2005). Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing blemishes have reduced marketability (Manandhar *et al.*, 1995).

*Fusarium* wilt is the second most important disease of chilli caused by soil borne fungal pathogen *Fusarium oxysporum* f. sp. *capsici* causing up to 20% yield loss annually. Transmission of disease occurs by means of chlamydospores which remains active in soil for several years.

Currently, the most widely used control measure for suppressing these diseases are the use of fungicides. However, problems encountered, such as development of pathogen resistance to fungicides, and inability of seed-treated fungicides to protect the roots of mature plants. Considering these limitations biological control is an important approach in this direction.

*Trichoderma* spp. are soil-borne fungi have significant antagonistic potential against a wide range of phytopathogenic fungi (Elad *et al.*, 1982). The choice of active *Trichoderma* isolate is important to design effective and safe bio-control mechanism, because many *Trichoderma* sp. have multiple activities for fungal antagonism and indirect effects on plant health or plant growth promotion.

Some species are potent antibiotic producer and their suitability for use in bio-control systems must be assessed carefully.

Therefore, an attempt has been made to cultivate 12 *Trichoderma* isolates. (7 *T. asperellum*, 4 *T. harzianum*, and 1 *T. longibrachiatum*) against *Colletotrichum capsici* and *Fusarium oxysporum* f. sp. *capsici* to identify potential biocontrol agent against these two pathogens.

## Materials and Methods

### Isolation and purification of pathogens

Diseased chilli plants with wilt and anthracnose symptoms were collected from different districts of U.P. Isolations from root and infected fruits were made on Potato Dextrose Agar medium incubated at 24–26°C, As soon as the growth of the pathogens occurred, hyphal bit from the periphery of the growing fungal colony was transferred and purified on potato dextrose agar slants.

### Soil samples and isolation of rhizospheric *Trichoderma*

Soil samples were collected from the rhizosphere soil of chilli ecosystem from Kanpur, Fatehpur, Pratapgarh, Allahabad, Lakhimpur, Sultanpur, Faizabad, Jaunpur, Amethi, Raibareli districts of Uttar Pradesh (India). Five-fold serial dilutions (Johnson and Curl, 1972) of each soil sample was prepared in sterilized distilled water and 0.5 ml diluted sample was poured on the surface of *Trichoderma* Specific Medium (TSM) (Elad *et al.*, 1981). Plates were incubated at  $28 \pm 2^\circ\text{C}$  for 96 h. Morphologically different colonies appearing on the plates were purified in Potato Dextrose Agar Medium (PDA) (HiMedia, India) and send to ITCC, New Delhi for identification.

### Antagonistic activity of *Trichoderma* isolates

The dual culture technique described by Morton and Stroube (1955) was used to test the antagonistic ability of 12 *Trichoderma* isolates against test pathogens. Pathogens and *Trichoderma* isolates were grown on PDA for a week at room temperature ( $28 \pm 2^\circ\text{C}$ ). 5 mm disc of the target fungi (*F. o. capsici* and *C. capsici*) cut from the periphery was transferred to the Petri

dish previously poured with PDA. *Trichoderma* isolates transferred aseptically in the same plate of opposite end and were incubated at room temperature with alternate light and darkness for 7 days and observed periodically. Control plates were maintained without *Trichoderma*. The experiment was replicated thrice and percent growth inhibition was calculated by the formula of  $I = (C-T)/C \times 100$ , where C is mycelial growth in control plate, T is mycelial growth in test organisms inoculated plate and I is inhibition of mycelial growth (Vincent *et al.*, 1999).

The effect of volatile compounds released by *Trichoderma* species was evaluated by 'inverse plate technique' (Dennis and Webster, 1971b). The antagonists and pathogens were inoculated in center of Petri plates poured with PDA. The Petri plates inoculated with pathogen was inverted over the Petri plate containing antagonist and two were sealed with the adhesive tape (parafilm) keeping antagonist in lower and pathogen in upper Petri plate.

In control, the Petri-plate containing pathogen was inverted over the Petri plate containing medium only and incubated at  $25 \pm 2^\circ\text{C}$ . The colony diameter of the pathogen was measured on the fifth day and compared with control.

Food Poison technique (Nene and Thapliyal, 1993) was followed to evaluate the effect of nonvolatile compounds/ metabolites on the growth of pathogens released by the *Trichoderma* spp. The *Trichoderma* spp. were grown in Potato Dextrose Broth (PDB) assuming that the antagonist will utilize the nutrients from broth and release some non-volatile metabolites the medium, which may affect the growth of pathogen. The *Trichoderma* spp. were incubated for two weeks and harvested at the interval of one

week. After the incubation, the broth was collected, filtered through Whatman-I filter paper and later through syringe filter (Ran Disc, PVD 0.45  $\mu\text{m}$ ) under aseptic conditions. PDA was amended with culture filtrate in three different concentrations (5, 10 and 15 percent) just before pouring and inoculated with pathogen. Colony diameter of pathogen was measured after fifth days and compared with the growth of pathogen maintained in control Petri plates amended with equal amount of distilled water. The colony diameter of the pathogen was measured on the fifth day and compared with control.

## Results and Discussion

### Collection of pathogens

A total 4 *Fusarium oxysporum* f. sp. *capsici* (*F.o. capsici*) isolates were obtained from infected roots of chilli plants collected from Etawah, Pratapgarh and Amethi district while, 2 isolates of *Colletotrichum capsici* (*C. capsici*) were isolated from infected fruits from Etawah and Kanpur district of U.P. Pathogenicity test was conducted for both pathogens on chilli susceptible cultivar G-4. Based on the disease incidence the isolates were characterized as highly pathogenic, moderately pathogenic and weakly pathogenic. Further studies were conducted with highly pathogenic isolates of *F. o. capsici* and *C. capsici*.

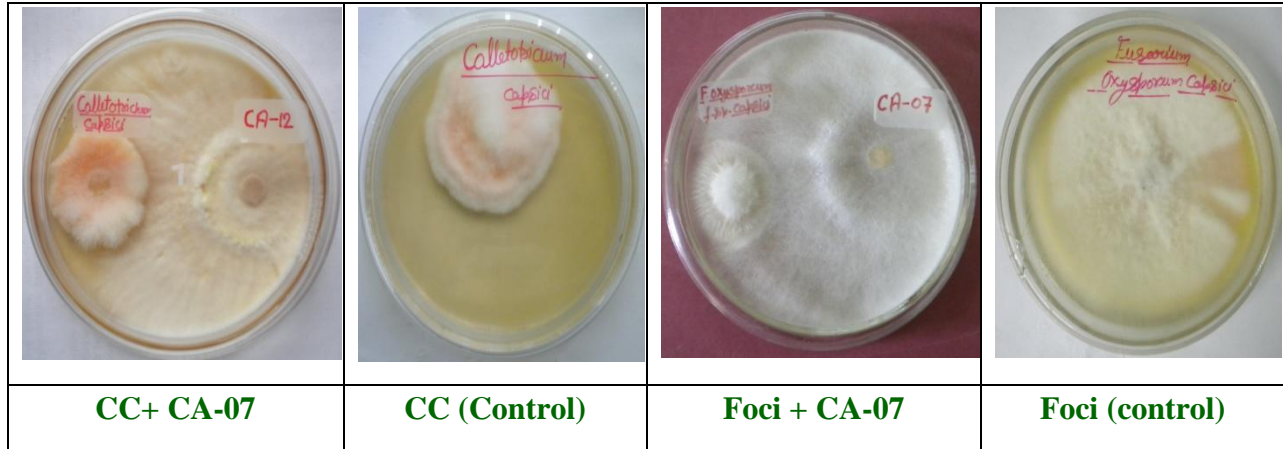
### Collection of bio-agents

A total 12 *Trichoderma* isolates were isolated from soil samples collected from Pratapgarh, Amethi, Sulatanpur, Kanpur, Etawah, Allahabad, Faizabad, Jaunpur, Lakhinpur and Raebareli district of U.P. and send to ITCC, New Delhi identification. Among them 7 isolates were identified as *T. asperellum* 4 as *T. harzianum* and 1 as *T. longibrachiatum* (Table 1).

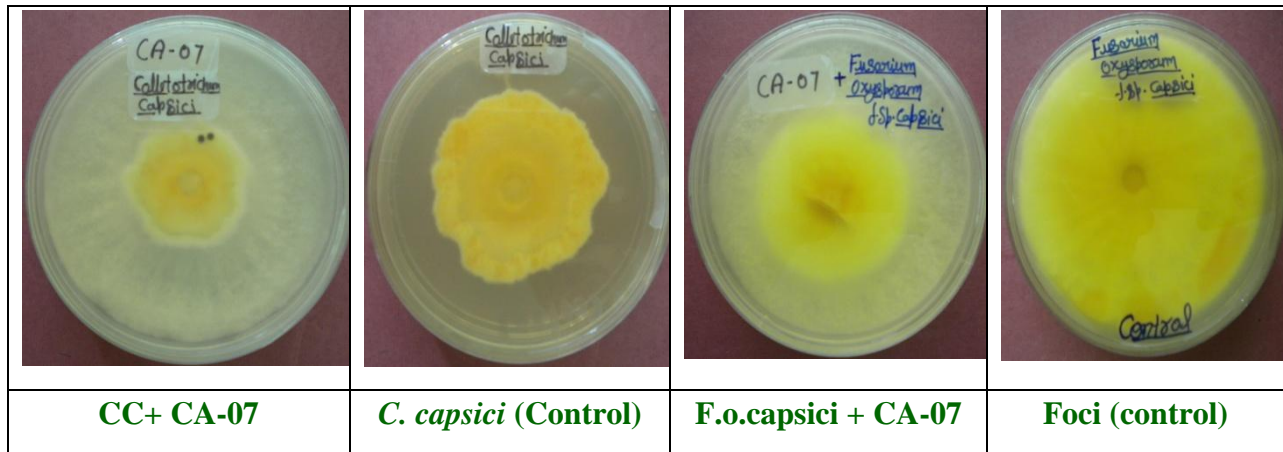
**Table.1** Effect of non-volatile metabolites on the growth of *Fusarium oxysporum* f.sp. *capsici* and *Colletotrichum capsici*

Treatment	<i>Fusarium oxysporum</i> f.sp. <i>capsici</i>						<i>Colletotrichum capsici</i>					
	5%		10%		15%		5%		10%		15%	
	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control	Average growth (mm)	Percent inhibition over control
CA-01	43.50	31.49	34.50	45.66	25.50	59.84	45.00	21.73	36.50	36.52	26.50	53.91
CA-02	42.50	33.07	31.50	50.39	23.00	63.77	46.50	19.13	33.50	41.73	24.00	58.26
CA-03	44.50	29.92	31.00	51.18	22.00	65.35	45.50	20.86	34.00	40.86	22.50	60.86
CA-04	47.50	25.19	38.50	39.37	27.50	56.69	52.50	8.69	35.50	38.26	27.50	52.17
CA-05	54.00	14.96	36.00	43.30	19.50	69.29	47.00	18.26	34.50	40.00	29.50	48.69
CA-06	40.50	<b>36.22</b>	29.50	<b>53.54</b>	17.00	<b>73.22</b>	44.50	<b>22.60</b>	29.50	<b>48.69</b>	21.50	<b>62.60</b>
CA-07	39.00	<b>38.58</b>	27.50	<b>56.69</b>	14.00	<b>77.95</b>	42.50	<b>26.08</b>	31.50	<b>45.21</b>	19.00	<b>66.95</b>
CA-08	46.00	27.55	31.50	50.39	28.00	55.90	48.50	15.65	33.00	42.60	29.00	49.56
CA-09	45.50	28.34	30.50	51.96	23.00	63.77	50.50	12.17	35.00	39.13	27.00	53.04
CA-10	44.00	30.70	34.50	45.66	22.50	64.56	45.00	21.73	36.50	36.52	26.00	54.78
CA-11	41.50	34.64	31.00	51.18	18.50	70.86	44.00	23.47	35.50	38.26	25.00	56.52
CA-12	48.50	23.62	37.50	40.94	30.00	52.75	45.00	21.73	37.00	35.65	26.50	53.91
CONTROL	63.50	0.0	63.50	0.0	63.50	0.0	57.50	0.0	57.50	0.0	57.50	0.0
<b>CD at 5%</b>	<b>5.82</b>		<b>2.99</b>		<b>3.11</b>		<b>4.64</b>		<b>3.08</b>		<b>3.85</b>	
<b>SE(d)</b>	<b>2.69</b>		<b>1.38</b>		<b>1.44</b>		<b>2.14</b>		<b>1.42</b>		<b>1.78</b>	

**Fig.1** Dual culture technique



**Fig.2** Invert-plate method



**Fig.3a** Food poison method

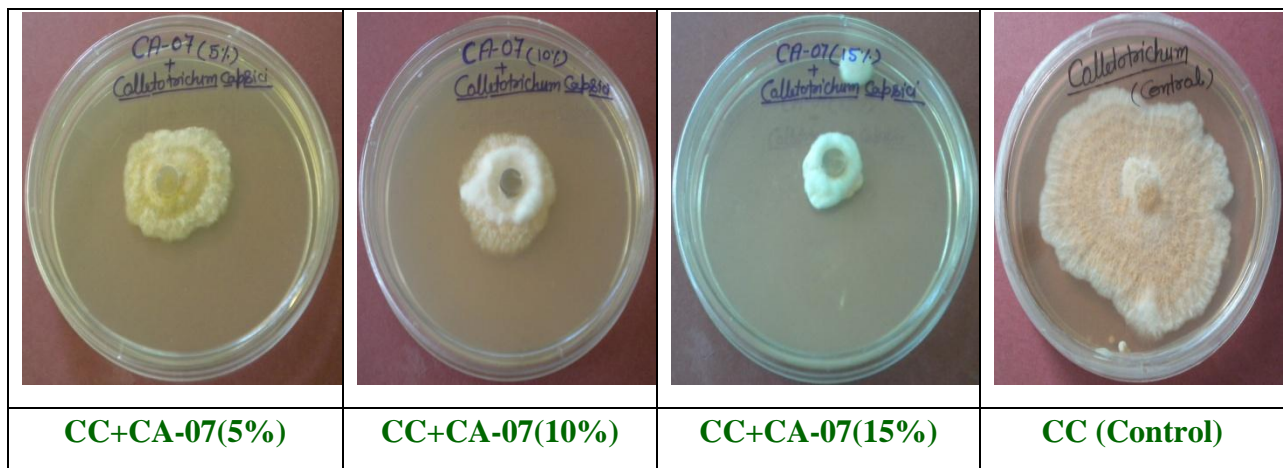
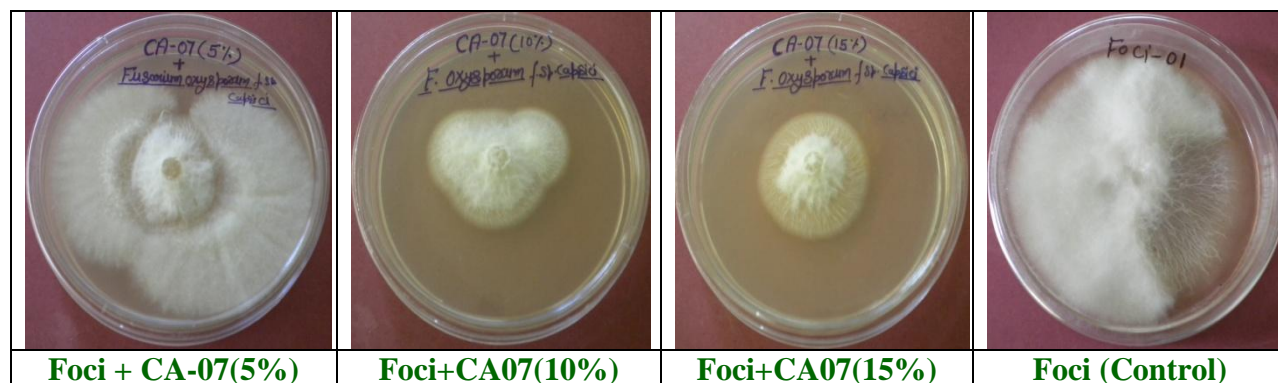


Fig.3b Food poison method



### Evaluation of antagonistic potential of *Trichoderma* isolates (Dual plate assay)

The results of dual culture technique revealed that all *Trichoderma* isolates significantly reduced the growth of the test pathogens. Minimum radial growth of *Fusarium oxysporum* f.sp. *capsici* and *Colletotrichum capsici* was recorded as 20.3 and 22.6 mm with *Trichoderma* isolates CA-07 and CA-06 with maximum growth inhibition as 60.6 and 52.1 percent respectively over control (Figure 1). Inhibition percent of growth by different *Trichoderma* isolates ranged between 45.1–60.6 percent and 35.9 – 52.1 in *F. o. capsici* and *C. capsici* respectively.

### Effect of volatile metabolites on the growth of *F. o. capsici* and *C. capsici* (Inverse plate technique)

The volatile compounds released by *Trichoderma* isolates also exerted inhibitory effect on the growth of the selected pathogens (Figure 2). Maximum growth inhibition in *Fusarium oxysporum* f.sp. *capsici* was recorded with isolates CA-09 (27.0%) followed by CA-07 (26.2%) while in case of *Colletotrichum capsici* maximum growth was inhibited by CA-07 (40.9%) followed by CA-05. The major advantage of antibiosis in case of volatile metabolites is that the toxic substances produced by the antagonists may

diffuse through air filled pores in soil and help in checking the root rot pathogen without establishing actual physical contact with pathogen. Amin *et al.*, (2010) studied effect of volatile metabolites released by *Trichoderma* species on fungal pathogens *F. oxysporum*, *R. solani* and *Alternaria* etc.

### Effect of non-volatile metabolites on the growth of *F. o. capsici* and *C. capsici* (Food poison technique)

Effect of non-volatile metabolites of 12 *Trichoderma* isolates on the growth of test pathogens was studied under in-vitro conditions (Figure 3). Observations revealed a significant inhibition in growth of test pathogens in comparison to respective control. Growth inhibition was increased with increasing concentration of culture filtrate. *Trichoderma* isolates CA-06 and CA-07 (*T. harzianum*), showed highest growth inhibition at all concentrations (5%, 10% and 15%) against test pathogens. Mishra *et al.*, (2011) studied the effect of non-volatile compounds of *Trichoderma* spp. on the growth of *Rhizoctonia solani*, *Fusarium oxysporum*, *Alternaria*, and *Colletotrichum sp.* concluded that growth inhibition was highest at 20% amendment. Similarly Trivedi *et al.*, (2013) studied the effect of volatile metabolites (1000 ppm) of *Trichoderma* sp. on growth of pigeonpea wilt pathogen *Fusarium udum* and

observed that the volatile compounds released by *Trichoderma* sp. significantly reduced the growth of the test pathogen.

The inhibition in growth of *Fusarium oxysporum* f. sp. *capsici* and *Colletotrichum capsici* in dual culture technique, inverse plate technique and food poison technique is attributed to inhibitory substance released by *Trichoderma* sp. Competition, mechanical obstruction and hyperparasitism are some of the main activities through which bioagents like *Trichoderma* sp. inhibits the growth of respective pathogen. In the present study, selected isolates of *Trichoderma* showed varied degree of inhibition against two soil and seed borne phytopathogens of chilli crop.

Isolates CA-06 and CA-07 (*T. harzianum*) showed high inhibitory effects in all *in-vitro* studies. This may be due to mycoparasitism and antibiosis caused by *T. harzianum*. These bioagents can be used for eco-friendly and effective management of Wilt and Anthracnose disease of chilli.

*Trichoderma* spp. are found to be effective across a range of plant crops, for example, lettuce, onion, cotton, grapes, peas, apples, tomato, carrots and others to control various pathogens like *Phytophthora*, *Pythium*, *Sclerotinia*, *Rhizoctonia*, *Colletotricum* and *Fusarium* etc., (Benitez *et al.*, 2004; Nirupama Devi *et al.*, 2013). Application of *T. viride* and *T. harzianum* have proved to be very effective in managing *Fusarium oxysporum* in different crops like tomato, cotton in terms of time taken to parasitize (Sharma, 2011) and inhibit mycelia growth as well.

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