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In-vitro Evaluation of Antagonistic Potential of the Trichoderma Antagonists against Some Important Chilli Fungal Pathogens

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ABSTRACT

The present study was conducted to know the biocontrol potential of Trichoderma harzianum, T. asperellum and T. longibracheatum under in vitro conditions against some important chilli fungal plant pathogens namely- Alternaria alternata, A. solani, Curvularia lunata, Corynespora cassicola, Colletotrichum capsici, Choanephora cucurbitarum and Lasiodiplodia theobromae. The results revealed that T. harzianum showed its maximum antagonist efficiency than T. longibracheatum and T. asperellum against all test pathogens. T. harzianum showed maximum inhibition against A. solani (84.12%) minimum inhibition was recorded against L. theobromae (52.94). Similarly T. asperellum showed Maximum and minimum inhibition was obtained in case of C. cucurbitarum (81.76%) and L. theobromae (40.59) and also T. longibrachiatum showed Maximum and minimum inhibition was obtained in case of Choanephora (74.71%) and L. theobromae (48.24).

Key words: *Trichoderma harzianum, T. asperellum, T. longibrachiatum, test pathogens, per cent inhibition etc.*

INTRODUCTION

Chilli, *Capsicum annum* L. cultivation has existed for several hundred years as a sustainable form of agriculture in India and in many other countries. It is an annual herbaceous spice/vegetable/cash crop grown in both tropical and sub-tropical regions and belongs to family Solanaceae. The crop is gaining popularity among farmers as a cash crop. The crop is grown all over West Bengal, but the major chilli growing districts are Nadia, South 24Parganas, Murshidabad and some parts of Midnapur. There are immense possibility for export of dry chilli and its derivatives especially that have low pungency and high colour[1]. Chilli (Capsicum sp.) is nowadays gaining popularity with an annual production of 52.3 tonnes of dry chillies, yielding 828 kg/ha in India[2].

Chilli suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also abiotic stresses. Among the fungal diseases anthracnose or fruit rot, Alternaria, Choanephora, powdery mildew and leaf spots are the most prevalent ones.

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During a survey 2014-2015, we isolated the prevalent fungal diseases like *Colletotrichum capsici*, *Alternaria alternata*, *Alternaria capsici*, *Choanephora cucurbitarum*, *Corynospora cassicola*, *Curvularia lunata* and *Lasiodiplodia theobromae* were noticed in major chilli growing areas of Nadia dist, West Bengal. Those diseases were associated with leaf and fruit infections of chilli,They cause considerable yield loss to the chilli crop. Keeping the increased incidence of these chilli fungal diseases in view, the present study was planned to conduct a systematic study on this disease with following objective.

Objective: *In-vitro* evaluation of antagonistic potential of the Trichoderma antagonists against some important chilli fungal pathogens.

MATERIALS AND METHODS

Isolation of the Trichoderma antagonists:

Isolation of Trichoderma from soil by serial dilution as described by[3]

Collection of soil samples: Soil was collected from chilli fields maintained at Jaguli Instructional farm of BCKV.

Isolation Technique: Trichoderma was isolated from the rhizosphere soil, using dilution plate method[4] on TSM. The collected soil was dried under shade and ground to powder with a mortar and pestle and passed through 2mm mesh sieve. Ten grams of powdered soil was mixed with 90 ml of sterile distilled water to prepare 10-1 dilution. This suspension was used for serial dilutions up to 10^{-4}. One ml of the suspension from 10-2, 10-3 and 10-4 were plated separately on 20 ml of solidified TSM in each of the sterilized petriplates. Five plates were inoculated for each dilution from a particular sample.

The suspension was then distributed uniformly on medium surface by horizontal shaking and was incubated at 28+ 10 C for seven days. The green colonies of the antagonists usually appeared at 4th or 5th day of incubation. Each colony was studied carefully under microscope, using 0.1 % lactophenol- cotton blue stain (0.1g cotton blue mixed in 100ml of standard lactophenol solution) and compared according to the monographs of Trichoderma[5] for identification at genus and species level. The shape, size and aggregation of phialospores and phialides were considered as main criteria for identification, besides cultural characters on TSM. The separated colonies were then transferred to PDA slants by using hyphal tip culture method and the slants were maintained for further use. Eight different strains of Trichoderma spp. were isolated. Out of them three were identified as *T. harzianum, T. asperellum,* and *T. longibrachiatum* which were finally selected for the study.

***In-vitro* evaluate on of antagonistic potential of the selected antagonists**

Antagonistic potential of Trichoderma spp. (*T. harzianum, T. asperellum,* and *T. longibrachiatum*) antagonistic activities of Trichoderma were measured through dual culture technique[6] against the test pathogens. In this experiment, 6 mm diameter blocks of the pathogens and Trichoderma were inoculated at the same time on the opposite sides of the PDA medium in petriplates (9 cm diameter). Both the pathogen and Trichoderma used were of same age. The plates containing paired cultures were incubated at 28+ 10 C for around 8 days. In each case, a control plate was also maintained. The antagonistic ability of each isolate was measured through modified Bell's scale[7] developed as follows:

S1 = Antagonist completely overgrew the pathogen (100 % overgrowth)

S2 = Antagonist overgrew at least 2/3 growth of the pathogen (75% overgrowth)

S3 = Antagonist colonized on half of the growth of the pathogen (50% overgrowth)

S4 = Antagonist and pathogen locked at the point of contact

S5 = Pathogen starts overgrowing the antagonist

RESULTS AND DISCUSSION

Identification of Trichoderma spp.

The five different isolates of Trichoderma were taken for characterisation for their species identification under light microscopy using a Zeiss- Axiostar Plus (Phase Contrast). The microscopic characters of those isolates were compared with Rifai's Monograph[5].

Among them one isolate was identified as *T. harzianum* characterised by the following characters The isolate produced ampuliform to sub-globose or lageniform phialides,measuring 3.5-7.5× 2.8-3.8 um in dimension, arising in crowded and diverse whorls of 2-6. The phialides were swollen in the middle. The phialospores were sub-globose toovoid, 1.7-3.2×1.3-2.5 um and appeared smooth under light microscope. Conidiophores were straight, sometimes seems to be flexuous, highly branched. The primary branches appeared at right angles usually in tufts. The colony of this isolate was seen to grow rapidly with white to yellowish floccose aerial mycelium. Reverse of the growth medium was dull yellowish.

Second isolate was identified as *T. asperellum* characterised by the following characters Phialides of this isolate varied from straight to irregularly bent, measuring 6.5 9×2.4-3.7 um in dimension. Phialospores were globose, green coloured and 4-4.5×3.6-4 um. Conidiophores were narrow, primary branching at regular intervals, short and not extensively branched. The mycelium was watery white becoming hairy from the formation of loose scanty aerial mycelium. The colonies become green to dark green with maturity and reverse remained uncoloured.

Other isolate was identified as *T. longibrachiatum* characterised by the following characters Colonies growing very rapidly, initially off-white, soon with greyish-green tufts of sporulation, first at the margin, later the entire colony. Hyphae hyaline, up to 10 µm wide. Conidiophores long, relatively poorly branched at right angles. Phialides mostly singly, flask-shaped with more or less cylindrical base and abruptly attenuated near the end, 6-14 x 2.5-3.0 µm, often curved, widely splaying out. Conidia broadly ellipsoidal, smooth-walled, green in mass, 3.5-7.0 x 2-3 µm. Chlamydospores terminal or intercalary, smooth-walled, hyaline, up to 10 µm wide.

Antagonistic potential of Trichoderma spp.

The *in-vitro* antagonistic potential of *T. harzianum and T.asperellum* were evaluated against eleven fungal pathogens viz. *C. capsici*, *A. alternata*, *A. capsici*, *C. cucurbitarum*, *C. cassicola*, *C. lunata* and *L. theobromae* by dual plate method. The antagonistic potential was rated on Bell's scale. Percentage inhibition was also calculated and the results have been presented in the following table.

Table 1: Antagonistic potential of *Trichoderma harzianum* determined by dual culture method

Sl. No.	Pathogen	Point of contact(DAI)	Distance covered (cm) at final day of observation by			Antagonistic potential on modified Bell's scale (at final day of observation)	Percent inhibition (%)
			Pathogen	Antagonist	Inhibition zone		
1	*Alternaria alternata*	2 days	0.7	5.05	-	S_2	83.53 (66.06)
2.	*Alternaria solani*	2 days	0.7	4.8	-	S_2	84.12 (66.52)
3	*Curvularia lunata*	2 days	0.72	4.40	-	S_2	82.94 (64.07)
4.	*Corynespora cassicola*	2 days	0.52	5.05	-	S_1	75.88 (60.60)
5	*Colletotrichum capsici*	2 day	1.02	4.40	0.15	S_2	71.50 (57.71)
6.	*Lasiodiplodia theobromae*	2 day	2.0	4.0	0.15	S_4 (Locked at one point)	52.94 (46.68)
7.	*Choanephora cucurbitarum*	2 day	1.15	5.0	-	S_2	72.94 (58.66)
SEm ±							0.796
CD (p=0.05)							2.437

Table 2: Antagonistic potential of *Trichoderma asperellum* determined by dual culture method

Sl. No.	Pathogen	Point of contact(DAI)	Distance covered (cm) at final day of observation by		Antagonistic potential on modified Bell's scale (at final day of observation)	Percent inhibition (%)
			Pathogen	Antagonist		
1.	*Alternaria alternata*	2 days	1.07	4.9	S_2	70.00 (56.77)
2.	*Alternaria solani*	2 days	1.27	5.10	S_2	74.71 (59.78)
3	*Curvularia lunata*	2 days	1.52	5.15	S_2	41.18 (39.89)
4.	*Corynespora cassicola*	2 days	0.62	5.35	S_2	65.49 (54.01)
5.	*Colletotrichumcapsici*	2 day	0.9	5.0	S_2	72.12 (58.11)
6.	*Lasiodiplodia theobromae*	2 day	2.52	3.55	S_2	40.59 (39.56)
7.	*Choanephora cucurbitarum*	2 day	1.55		S_2	81.76 (64.72)
SEm ±						0.654
CD (p=0.05)						2.002

Table 3: Antagonistic potential of *Trichoderma longibrachiatum* determined by dual culture method

Sl. No.	Pathogen	Point of contact(DAI)	Distance covered (cm) at final day of observation by		Antagonistic potential on modified Bell's scale (at final day of observation)	Percent inhibition (%)
			Pathogen	Antagonist		
2.	*Alternaria alternate*	2 days	1.15	5.7	S_1	72.94 (58.63)
2.	*Alternaria solani*	2 days	1.47	5.25	S_2	73.73 (59.15)
3	*Curvularia lunata*	2 days	1.07	5.05	S_1 (Over growth)	67.65 (67.04)
4.	*Corynespora cassicola*	2 days	0.65	5.55	S_1	71.76 (50.98)
5.	*Colletotrichum capsici*	2 day	1.67	4.95	S_2	60.41 (43.97)
6.	*Lasiodiplodia theobromae*	2 day	2.52	4.6	S_4	48.24 (46.68)
7.	*Choanephora cucurbitarum*	2 day	1.37	5.25	S_2	74.71 (59.79)
SEm ±						0.824
CD (p=0.05)						2.525

The data on dual culture test by Trichoderma spp., revealed that all the Trichoderma isolates significantly inhibited the radial growth of test pathogens, but there was a variation in their inhibition. All Trichoderma isolates exhibited more than 40.59% inhibition of mycelial radial growth against test pathogens. However, in three bio control agents *T. harzianum* was excellently effective against all pathogens than T. *longibracheatum* and *T. asperellum.*

From Table no.1 revealed that *T. harzianum* possess a significant antagonistic property against these seven pathogens. Percentage inhibition ranged from 52.94-84.12%, indicating that it is effective in controlling these pathogens. Maximum inhibition was obtained in case of *A. solani* (84.12% and S2) followed by *A. alternata* (83.53%) *C. lunata* (82.94%), *C. cassicola* (75.88) and minimum inhibition was recorded against *L. theobromae* (52.94 & S4 scale)(Figure no. 1 and Plate no.1). Growth of the pathogen was observed to be restricted by mycoparasitic activity of Trichoderma. Coiling of hyphae of pathogens was noticed under microscope. Colour change of the media (yellowish) was detected due to release of metabolites by *T. harzianum.* Studies of

comparative antagonistic properties of *T. harzianum* and *T. viride* against *A. alternata* under *in vitro* condition reported by Pandey[8]. Results indicated that *T. harzianum* reduced the growth of *A. alternata* by 67.07%.[9] reported antagonistic activities of different Trichoderma strains under *in vitro* condition against *C. capsici*, a causal agent of anthracnose fruit rot of chilli. They confirmed that *T. harzianum* IMI-392433 showed the highest inhibition (81.96 %) and mycelial overgrowth (78.98%) against *C. capsici*. and Suresh *et al.* reported that trichoderma isolates were effective against *L. theobromae* in mango[6].

The data pertaining Table no. 2 revealed that *T. asperellum* showed Maximum and minimum inhibition was obtained in case of *C. cucurbitarum* (81.76% and S2) followed by and *L. theobromae* (40.59& S2 scale) (Figure no. 2 and Plate No. 2). Similarly *T. longibrachiatum* possess a significant antagonistic property against these seven pathogens. They showed Maximum and minimum inhibition was obtained in case of *Choanephora* (74.71% and S2) followed by A. solani (73.73% and S2 scale)and *L. theobromae* (48.24& S2 scale) (Figure no. 3 and Plate No. 3). (7) reported that *T. viride* showed maximum inhibition against *A. alternata* (66.67%). So, these findings obtained from the present experiment of dual culture assay stands conformation with the previous results obtained by Pandey[8], Mukherjee[10] and Manibhushan[11] etc.

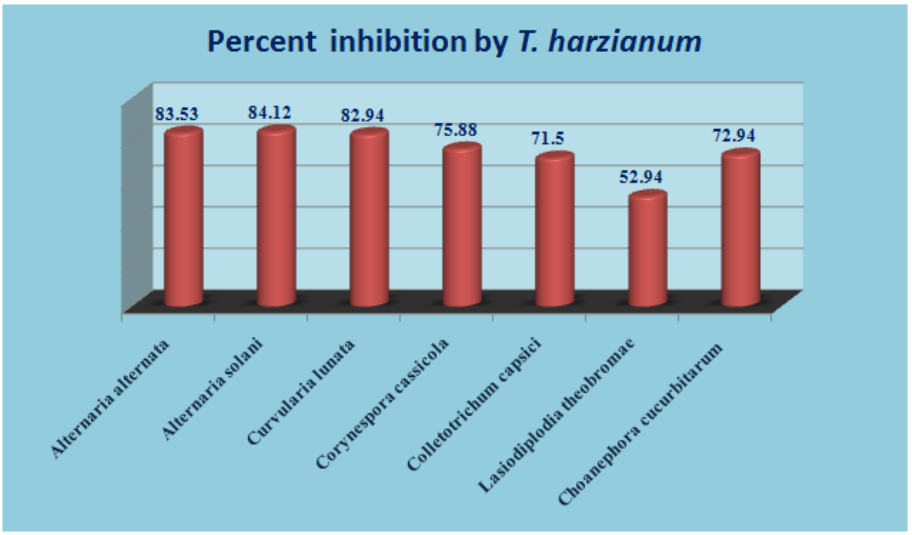

Fig. 1: Antagonistic potential of *Trichoderma harzianum* determined by dual culture method

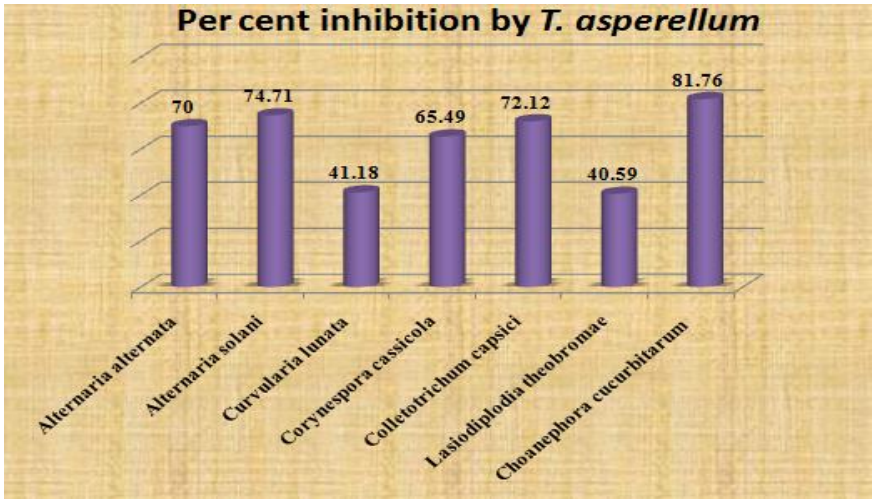

Fig. 2: Antagonistic potential of *Trichoderma asperellum* determined by dual culture method

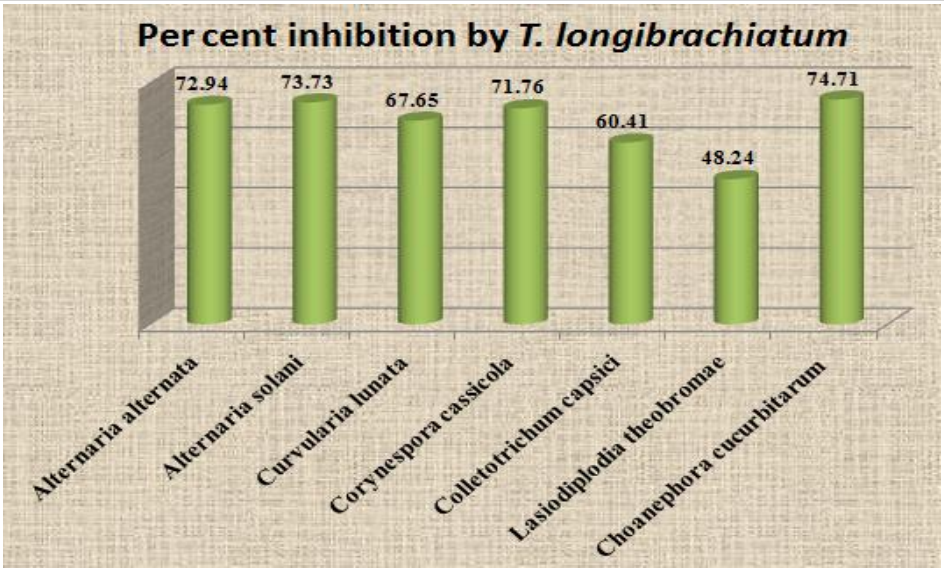

Fig. 3: Antagonistic potential of *Trichoderma longibrachiatum* determined by dual culture method

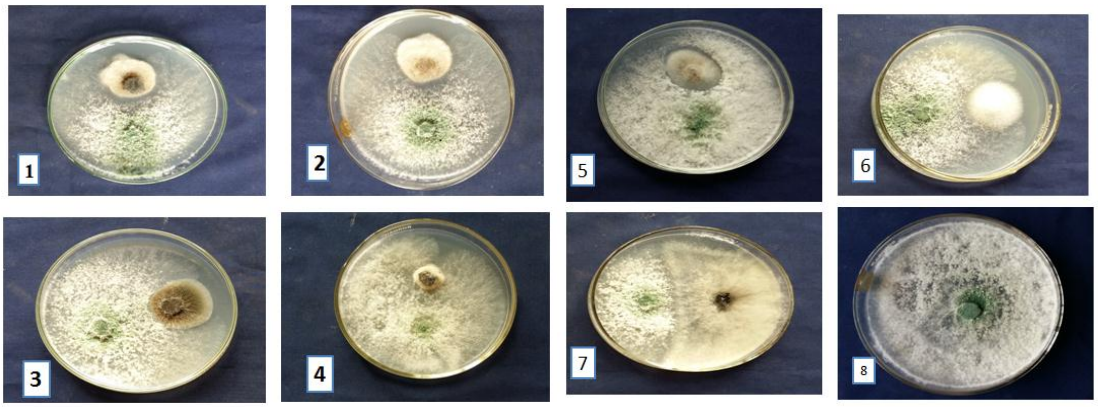

Plate no. 1. Antagonistic response of *T. harzianum* against (1) *A.alternata* (2) *A. solani* (3) *C. lunata* (4) *C. cassicola* (5) *C. capsici* (6) *L.theobromae* and (7) *C.cucurbitarum*

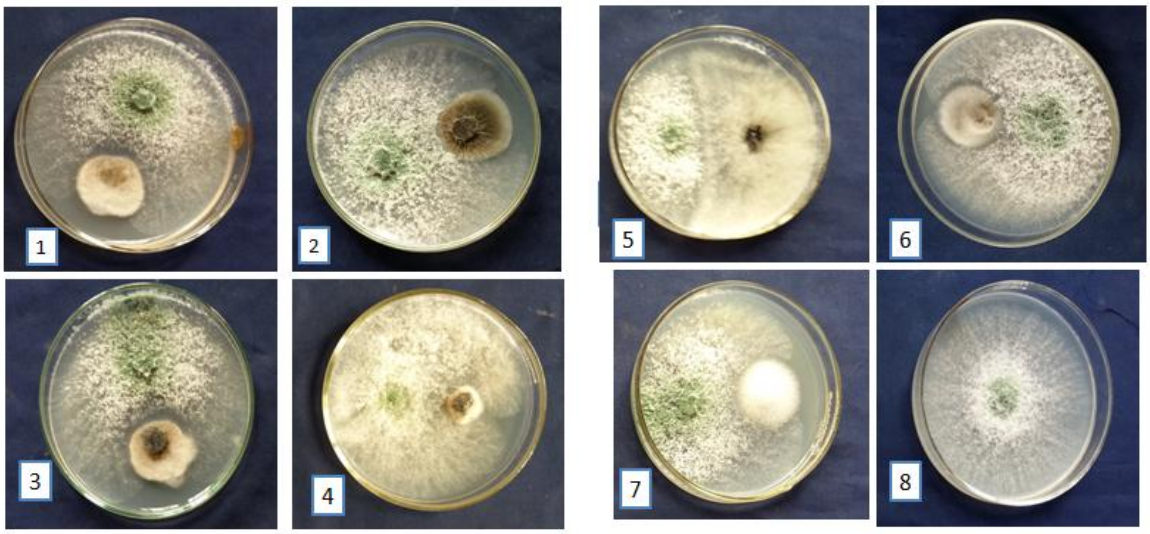

Plate no. 2. Antagonistic response of *T. asperellum* against (1) *A.alternata* (2) *A. solani* (3) *C. lunata* (4) *C. cassicola* (5) *C. capsici* (6) *L.theobromae* and (7) *C.cucurbitarum*

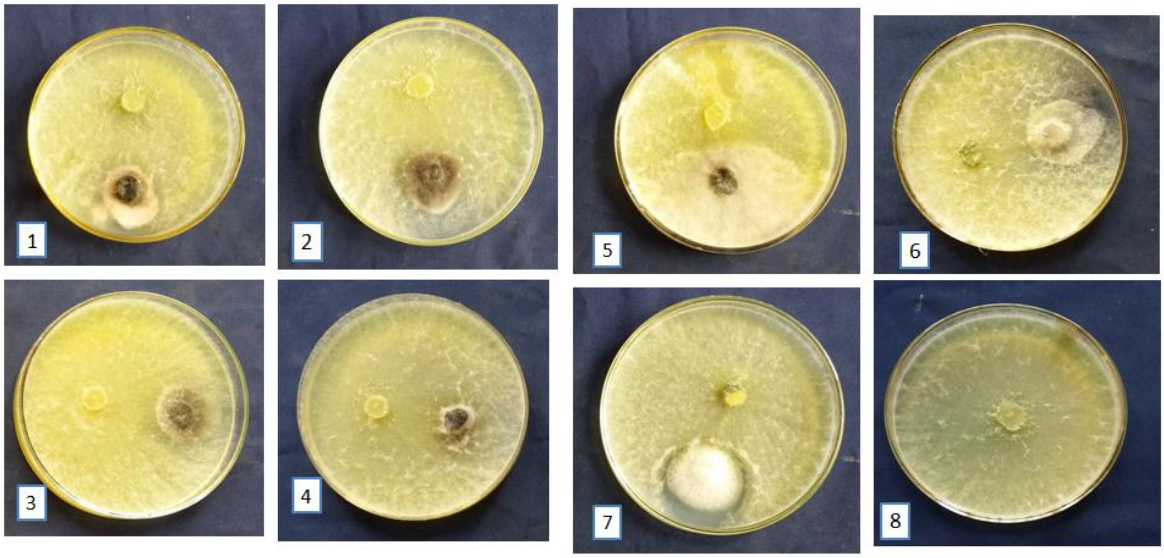

Plate no. 3. Antagonistic response of *T. longibrachiatum* against (1) *A.alternata* (2) *A. solani* (3) *C. lunata* (4) *C. cassicola* (5) *C. capsici* (6) *L.theobromae* and (7) *C.cucurbitarum*

CONCLUSION

The present study was conducted to know the Isolation of biocontrol agents and their potential of *Trichoderma sps.* under *in vitro* conditions. And their effect against some important chilli fungal plant pathogens. The isolated trichoderma agents were identified as *T. harzianum, T.longibracheatum and T. asperellum.* In which *T. harzianum* showed its maximum antagonist efficiency than *T. longibracheatum* and *T. asperellum* against all test pathogens. *T. harzianum* showed maximum inhibition against *A. solani* (84.12%) minimum inhibition was recorded against *L. theobromae* (52.94). Similarly *T. asperellum* showed Maximum and minimum inhibition was obtained in case of *C. cucurbitarum* (81.76%) and *L. theobromae* (40.59) and also *T. longibrachiatum* showed Maximum and minimum inhibition was obtained in case of *Choanephora* (74.71%) and *L. theobromae* (48.24).

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