In Vitro ANTAGONISTIC ACTIVITY OF *Trichoderma* SPECIES AGAINST AGRICULTURALLY IMPORTANT PLANT PATHOGENIC FUNGI

Surendra B.K.[1], Jharjhari Chakma[1], Rashila Maharjan[2] and Satyendra Pratap Singh[1]

[1]Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India
[2]Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India
Correspondence: bksurendra18@gmail.com

ABSTRACT

Various species of *Trichoderma* are widely recognized as prominent biocontrol agents, and they are known for their cost-effectiveness, efficacy, and environmentally friendly nature. However, conducting further experiments is essential to explore their full potential and validate their effectiveness in specific applications or under varying conditions. The present study aims at evaluating the antagonistic behavior of two most common *Trichoderma* species, *T. viride* and *T. harzianum*, against six highly problematic plant pathogenic fungi viz. *Alternaria solani, Bipolaris sorokiniana*, *Colletotrichum capsici*, *Curvularia oryzae*, *Fusarium oxysporum* f. sp. *lycopersici*, and *Phomopsis vexans* under *in vitro* conditions. The experiment was carried out in a completely randomized design with 3 replications for each treatment. Both the *Trichoderma* species displayed significant antagonistic activity against all the tested fungal pathogens in dual cultures, resulting in a 7-36% and 13-40% mycelial growth inhibition in tested pathogens by *T. viride* and *T. harzianum,* respectively. *T. viride* demonstrated greater efficacy against *C. capsici* and *C. oryzae*, while *T. harzianum* was more effective against *A. solani, B. sorokiniana, F. oxysporum* f. sp. *lycopersici,* and *P. vexans*.

Key words : Antagonist, dual culture, mycelial growth inhibition, phytopathogens, *Trichoderma*.

INTRODUCTION

Fungal pathogens play a significant role in the development of diseases on a wide range of agricultural crops, leading to significant yield losses. The increased use of fungicides has led to the buildup of toxic compounds that could be harmful to humans and the environment. The repeated application of a particular fungicide to combat a specific pathogen leads to the emergence of strains of the pathogen that are resistant to the fungicide (Shanmugam & Varma, 1998; Mamgain et al., 2013). To address these issues, effective alternatives to chemical control are being used. Biological control employs targeted microorganisms to disrupt plant pathogens, providing a nature-friendly alternative to chemical methods and addressing associated challenges. Fungi belonging to genus *Trichoderma* have been identified as highly promising biocontrol agents, effectively combating a wide range of plant pathogenic fungi (Anand and Reddy, 2009).

Trichoderma can serve as antagonists against a wide range of phytopathogenic fungi. The mechanisms of antagonism include competition for nutrient and space, mycoparasitism, antibiosis and the secretion of fungal cell wall degrading enzymes (Harman, 2006), enable the development of biocontrol strategies against filamentous fungi that harm plants (Benítez et al., 2004). Conducting *in vitro* screenings of various *Trichoderma* species with pathogens is an efficient and expedient approach to identify strains with potential antagonistic properties (Reddy et al., 2014). Therefore, the present study was conducted to evaluate the antagonistic potential of two different *Trichoderma* spp. viz. *T. viride* and *T. harzianum* in inhibiting the growth of six most widely occurring fungal pathogens viz. *Alternaria solani*, *Bipolaris sorokiniana, Colletotrichum capsici*, *Curvularia oryzae, Fusarium oxysporum* f. sp. *lycopersici*, and *Phomopsis vexans*.

MATERIALS AND METHODS

The disease samples were collected from crops grown on the research farm and brought into the Plant Health Clinic laboratory of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India, during June 2022.

Isolation of Pathogenic Fungi

Samples showing disease symptoms caused by target pathogens in different plant species were collected and brought to the laboratory for isolation, identification and preservation purposes. Diseased samples were cut into several small sections of 5-10 mm square, surface sterilized with sodium hypochlorite (1%) solution by dipping the cut samples for 30 seconds, washed three times in sterile water, blot-dried on clean sterile paper towels, placed on potato dextrose agar (PDA) plates, and incubated at 27 ± 1 ºC for 7-9 days. The fungal colonies that developed on PDA plates were sub-cultured, and a pure culture of the fungi was obtained using the single hyphal tip method (Sekar et al., 2017). These cultures were maintained on PDA slants and preserved at 4°C in a refrigerator for future studies. The pathogens isolated are given in Table 1.

Table 1. List of pathogens isolated and source of isolation

Pathogen isolated	Crop	Plant parts used for isolation
Curvularia oryzae	Paddy	Leaf
Bipolaris sorokiniana	Wheat	Leaf
Phomopsis vexans	Brinjal	Fruit
Fusarium oxysporum f. sp. *lycopersici*	Tomato	Fruit
Colletotrichum capsici	Chili	Fruit
Alternaria solani	Tomato	Leaf

Identification of pathogenic fungi

The pure cultures of each fungus were examined for mycelial color, texture, and growth characteristics. Microscopic slides were prepared from each culture to observe spores and mycelia under the microscope (Watanabe, 2002). Based on the colony characteristics and morphological features, the fungal isolates were identified.

Antagonists

Trichoderma viride and *T. harzianum*, previously isolated and its slants preserved in the refrigerator of Plant Health Clinic laboratory of the Department of Mycology and Plant Pathology, Banaras

Hindu University were collected, sub-cultured in the PDA medium and kept at 27 ± 1 °C in a BOD. These cultures were sub-cultured routinely and preserved at 4 °C in a refrigerator for further studies.

Dual Culture Assay

The PDA plates (90 mm) were inoculated with 5-mm mycelial discs of both isolates of *Trichoderma* spp. at one edge of the Petri plate and at the opposite edge 5 mm mycelial discs of test pathogens were inoculated. Both the discs were 20 mm away from the edge and 50 mm apart from each other. The control plates were maintained by inoculating the 5-mm mycelial disc of tested pathogens and *Trichoderma* spp. on the Petri plates separately. For each treatment, three replicates were maintained and all the plates were incubated at 27 ± 1 ºC for 4 days in the BOD incubator. Colony diameter of both the test pathogens and *Trichoderma* was measured by using a measuring scale from the lower view of the Petri plates up to 4 days. The percent inhibition caused by the *Trichoderma* isolates against tested pathogens was calculated by the following formula (Arora & Upadhyay, 1978):

$$I = \frac{(C - T)}{C} \times 100$$

Where,
I = % inhibition of mycelial growth
C = growth of pathogen in control Petri plates
T = growth of pathogen in dual Petri plates

Statistical Analysis

The recorded observations were analyzed statistically using online package softwares - WASP 2.0 developed by ICAR - Central Coastal Agricultural Research Institute, Goa and OPSTAT developed by Chaudhary Charan Singh Haryana Agricultural University, Haryana, India. The differences in means were separated by Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

The treatments (antagonists alone, pathogens alone and antagonists with pathogens) were significantly different for all incubation periods (1-4 days) (Table 2-7). The colony diameter of the antagonists (*T. viride* and *T. harzianum*) varied for 1 and 3 days of incubation and covered whole 90 mm plates on 4th day of incubation.

In dual cultures with the pathogen, *C. oryzae* (Table 2), the mean colony diameter of the pathogen, with both antagonists, was significantly lower than the pathogen alone on 4th day of incubation at 27 ± 1 ºC. Less (29.0 mm) mean colony diameter of the pathogen was measured when dual culture was done with *T. viride*, than when cultured with *T. harzianum* (34.0 mm). The mean colony diameter of the antagonist *T. viride* was higher than the diameter of *T. harzianum* in dual culture from the 2nd day of incubation. It was observed that the growth of *T. viride* was more robust in dual culture when compared with the control (*T. viride* alone). Conversely, for *T. harzianum*, its growth was more pronounced when it was cultured alone.

Table 2. Antagonistic effect of *Trichoderma viride* and *T. harzianum* against the growth of *Curvularia oryzae* in dual cultures at different incubation periods

Treatment	[†]Mean colony diameter (mm)			
	1st day	**2nd day**	**3rd day**	**4th day**
T. viride alone	20.33[c]	57.67[b]	67.00[a]	90.00[a]
T. harzianum alone	26.67[a]	57.33[b]	62.00[b]	89.67[a]
T. viride in dual culture with *C. oryzae*	24.00[b]	62.33[a]	69.00[a]	90.00[a]
T. harzianum in dual culture with *C. oryzae*	25.00[b]	46.00[c]	50.00[c]	78.00[b]
C. oryzae alone	14.67[e]	30.33[d]	36.33[d]	45.67[c]
C. oryzae in dual culture with *T. viride*	17.67[d]	25.33[e]	29.00[e]	29.00[e]
C. oryzae in dual culture with *T. harzianum*	16.00[e]	25.00[e]	28.33[e]	34.00[d]
CD (0.05)	1.391	3.585	4.365	2.261
SE(m)	0.454	1.182	1.425	0.745
SE(d)	0.642	1.671	2.016	1.054
CV%	3.816	4.766	5.174	1.980

[†]Means of 3 replications. Means in column with same superscript is not significantly differed by DMRT, ($P \leq 0.05$)

In dual cultures with the pathogen, *B. sorokiniana* (Table 3), the mean colony diameter of the pathogen, with both antagonists, was significantly lower than that of the pathogen alone on the 4th day of incubation at 27 ± 1 ºC. Less (20.33 mm) mean colony diameter of the pathogen was measured when dual culture was done with *T. harzianum*, than when cultured with *T. viride* (21.00 mm). The mean colony diameter of the antagonist *T. viride* was greater than that of *T. harzianum* in dual culture from the 2nd day of incubation. It was observed that the growth of *T. viride* was more vigorous in dual culture compared to the control (*T. viride* alone) up to 2nd day of incubation. Conversely, the growth of *T. harzianum* was more pronounced when it was cultured independently.

Table 3. Antagonistic effect of *Trichoderma viride* and *T. harzianum* against the growth of *Bipolaris sorokiniana* in dual cultures at different incubation periods

Treatment	Mean colony diameter (mm)			
	1st day	**2nd day**	**3rd day**	**4th day**
T. viride alone	[†]20.33[b]	57.67[b]	67.00[a]	90.00[a]
T. harzianum alone	26.67[a]	57.33[b]	62.00[b]	89.67[a]
T. viride in dual culture with *B. sorokiniana*	27.33[a]	65.00[a]	66.67[a]	90.00[a]
T. harzianum in dual culture with *B. sorokiniana*	28.00[a]	48.00[c]	50.67[c]	73.67[b]
B. sorokiniana alone	12.67[d]	21.00[d]	25.00[d]	31.00[c]
B. sorokiniana in dual culture with *T. viride*	14.67[c]	19.67[d]	20.33[e]	21.00[d]
B. sorokiniana in dual culture with *T. harzianum*	14.00[cd]	17.33[d]	18.33[e]	20.33[d]
CD (0.05)	1.591	4.083	4.244	3.781
SE(m)	0.519	1.333	1.386	1.234
SE(d)	0.735	1.886	1.960	1.746
CV%	4.384	5.652	5.420	3.601

[†]Means of 3 replications. Means in column with same superscript is not significantly differed by DMRT, ($P \leq 0.05$)

In dual cultures with the pathogen, *P. vexans* (Table 4), the mean colony diameter of the pathogen, with both antagonists, was significantly lower than the pathogen alone on 4th day of incubation at 27 ± 1 ºC. Less (20.67 mm) mean colony diameter of the pathogen was measured when dual culture was done with *T. harzianum*, than when cultured with *T. viride* (28.33 mm). The mean colony diameter of the *T. viride* was higher than the diameter of *T. harzianum* in dual culture from the 2nd day of incubation. It was observed that the growth of *T. viride* was more robust in dual culture, except on 2nd day of incubation, when compared with the control (*T. viride* alone). Conversely, for *T. harzianum*, its growth was more pronounced when it was cultured alone.

Table 4. Antagonistic effect of *Trichoderma viride* and *T. harzianum* against the growth of *Phomopsis vexans* in dual cultures at different incubation periods

Treatment	Mean colony diameter (mm)			
	1st day	2nd day	3rd day	4th day
T. viride alone	†20.33^{c}	57.67^{a}	67.00^{b}	90.00^{a}
T. harzianum alone	26.67^{a}	57.33^{a}	62.00^{c}	89.67^{a}
T. viride in dual culture with *P. vexans*	23.67^{b}	51.67^{b}	75.00^{a}	90.00^{a}
T. harzianum in dual culture with *P. vexans*	24.67^{b}	45.00^{c}	49.33^{d}	79.00^{b}
P. vexans alone	10.67^{e}	18.00^{e}	20.33^{f}	34.33^{c}
P. vexans in dual culture with *T. viride*	13.00^{d}	22.00^{d}	24.33^{e}	28.33^{d}
P. vexans in dual culture with *T. harzianum*	12.00^{d}	16.00^{e}	17.00^{f}	20.67^{e}
CD (0.05)	1.280	3.473	3.599	1.929
SE(m)	0.418	1.134	1.175	0.630
SE(d)	0.591	1.604	1.662	0.891
CV%	3.867	5.136	4.523	1.768s

†Means of 3 replications. Means in column with same superscript is not significantly differed by DMRT, (P≤0.05)

In dual cultures with the pathogen, *F. oxysporum* f. sp. *lycopersici* (Table 5), the mean colony diameter of the pathogen, with both antagonists, was significantly lower than the pathogen alone on 4th day of incubation at 27 ± 1 ºC. Less (29.33 mm) mean colony diameter of the pathogen was measured when dual culture was done with *T. harzianum*, than when cultured with *T. viride* (31.67 mm). The mean colony diameter of the *T. harzianum* was higher than the diameter of *T. viride* in dual culture for all the incubation days. It was observed that the growth of both antagonists were more robust in control (antagonists alone), when compared with the dual culture plates.

In dual cultures with the pathogen, *C. capsici* (Table 6), the mean colony diameter of the pathogen, with both antagonists, was significantly lower than the pathogen alone on 4th day of incubation at 27 ± 1 ºC. Less (22.00 mm) mean colony diameter of the pathogen was measured when dual culture was done with *T. viride*, than when cultured with *T. harzianum* (27.00 mm). The mean colony diameter of the *T. viride* was higher than the diameter of *T. harzianum* in dual culture from the 2nd day of incubation. It was observed that the growth of *T. viride* was more robust in dual culture, when compared with the control (*T. viride* alone). Conversely, for *T. harzianum*, its growth was more pronounced when it was cultured alone, except on day 1.

Table 5. Antagonistic effect of *Trichoderma viride* and *T. harzianum* against the growth of *Fusarium oxysporum* f. sp. *lycopersici* in dual cultures at different incubation periods

Treatment	Mean colony diameter (mm)			
	1st day	**2nd day**	**3rd day**	**4th day**
T. viride alone	†20.33^{c}	57.67^{a}	67.00^{a}	90.00^{a}
T. harzianum alone	26.67^{a}	57.33^{a}	62.00^{b}	89.67^{a}
T. viride in dual culture with *F. oxysporum* f. sp. *lycopersici*	17.33^{d}	40.67^{b}	57.00^{c}	78.33^{b}
T. harzianum in dual culture with *F. oxysporum* f. sp. *lycopersici*	23.33^{b}	52.67^{a}	65.67^{a}	78.67^{b}
F. oxysporum f. sp. *lycopersici* alone	17.67^{cd}	25.67^{c}	27.33^{d}	34.00^{c}
F. oxysporum f. sp. *lycopersici* in dual culture with *T. viride*	13.67^{e}	21.33^{c}	23.00^{e}	31.67^{cd}
F. oxysporum f. sp. *lycopersici* in dual culture with *T. harzianum*	15.33^{de}	25.00^{c}	28.00^{d}	29.33^{d}
CD (0.05)	2.782	9.090	1.850	4.102
SE(m)	0.909	2.968	0.604	1.339
SE(d)	1.285	4.198	0.854	1.894
CV%	8.200	12.837	2.220	3.762

†Means of 3 replications. Means in column with same superscript is not significantly differed by DMRT, (P≤0.05)

Table 6. Antagonistic effect of *Trichoderma viride* and *T. harzianum* against the growth of *Colletotrichum capsici* in different incubation periods

Treatment	Mean colony diameter (mm)			
	1st day	**2nd day**	**3rd day**	**4th day**
T. viride alone	†20.33^{c}	57.67^{b}	67.00^{a}	90.00^{a}
T. harzianum alone	26.67^{a}	57.33^{b}	62.00^{b}	89.67^{a}
T. viride in dual culture with *C. capsici*	24.00^{b}	64.67^{a}	69.67^{a}	90.00^{a}
T. harzianum in dual culture with *C. capsici*	27.00^{a}	49.67^{c}	52.00^{c}	73.67^{b}
C. capsici alone	10.33^{d}	16.67^{d}	18.67^{d}	31.00^{c}
C. capsici in dual culture with *T. viride*	10.33^{d}	14.67^{d}	18.67^{d}	22.00^{e}
C. capsici in dual culture with *T. harzianum*	11.67^{d}	17.33^{d}	20.33^{d}	27.00^{d}
CD (0.05)	1.591	3.158	3.014	1.850
SE(m)	0.519	1.031	0.984	0.604
SE(d)	0.735	1.458	1.392	0.854
CV%	4.832	4.498	3.869	1.730

†Means of 3 replications. Means in column with same superscript is not significantly differed by DMRT, (P≤0.05)

In dual cultures with the pathogen, *A. solani* (Table 7), the mean colony diameter of the pathogen, with both antagonists, was significantly lower than the pathogen alone on 4th day of incubation at 27

± 1 ºC. Less (18.67 mm) mean colony diameter of the pathogen was measured when dual culture was done with *T. harzianum*, than when cultured with *T. viride* (20.33 mm). The mean colony diameter of the *T. viride* was higher than the diameter of *T. harzianum* in dual culture from the 2nd day of incubation. It was observed that the growth of *T. viride* was more robust in dual culture, when compared with the control (*T. viride* alone). Conversely, for *T. harzianum*, its growth was more pronounced when it was cultured alone, except on day 1.

Table 7. Antagonistic effect of *Trichoderma viride* and *T. harzianum* against the growth of *Alternaria solani* in dual cultures at different incubation periods

Treatment	Mean colony diameter (mm)			
	1st day	**2nd day**	**3rd day**	**4th day**
T. viride alone	†20.33^{d}	57.67^{b}	67.00^{ab}	90.00^{a}
T. harzianum alone	26.67^{b}	57.33^{b}	62.00^{bc}	89.67^{a}
T. viride in dual culture with *A. solani*	24.00^{c}	62.67^{a}	68.33^{a}	89.67^{a}
T. harzianum in dual culture with *A. solani*	28.00^{a}	46.33^{c}	59.67^{c}	72.00^{b}
A. solani alone	13.33^{e}	15.67^{d}	22.00^{d}	25.27^{c}
A. solani in dual culture with *T. viride*	11.33^{f}	14.33^{d}	16.33^{d}	20.33^{d}
A. solani in dual culture with *T. harzianum*	11.00^{f}	15.00^{d}	17.00^{d}	18.67^{d}
CD (0.05)	1.220	1.768	5.902	1.850
SE(m)	0.398	0.577	1.927	0.604
SE(d)	0.563	0.816	2.726	0.854
CV%	3.587	2.602	7.481	1.806

†Means of 3 replications. Means in column with same superscript is not significantly differed by DMRT, ($P \leq 0.05$)

Both antagonists, *T. viride* and *T. harzianum*, showed the potentials to suppress the radial colony growth of tested pathogens under laboratory conditions. In the current *in vitro* study of biocontrol agents, *Trichoderma* isolates significantly inhibited the radial growth of tested pathogens on the 4th day of incubation. *T. harzianum* exhibited the greatest inhibition of mycelial growth (40%) when tested against *Phomopsis vexans*, while the lowest inhibition (14%) was seen when tested against *Fusarium. oxysporum* f. sp. *lycopersici*. Furthermore, *T. viride* displayed its highest mycelial growth inhibition (36%) when tested against *Curvularia oryzae*, whereas its least inhibition (7%) was observed opposed to *F. oxysporum* f. sp. *lycopersici* (Fig. 1). Competition and/or antibiosis were likely the causes of these bio-agents' inhibitory action.

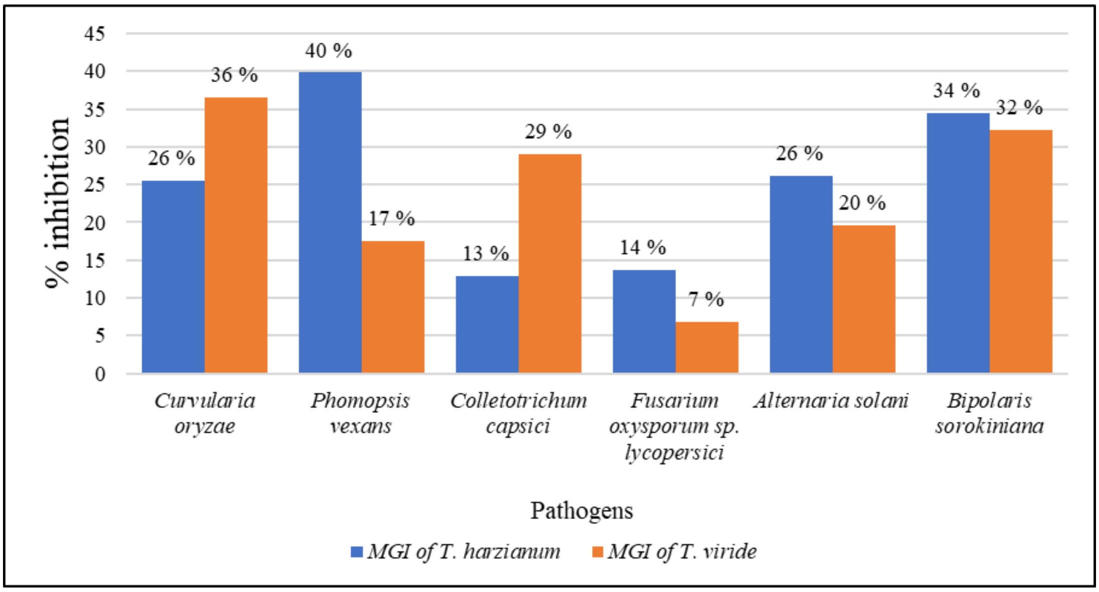

Fig. 1. Antagonistic effect of *Trichoderma viride* and *T. harzianum* on the mycelial growth inhibition (MGI) of different fungal pathogens on 4th day of co-incubation period at 27 ºC in BOD incubator.

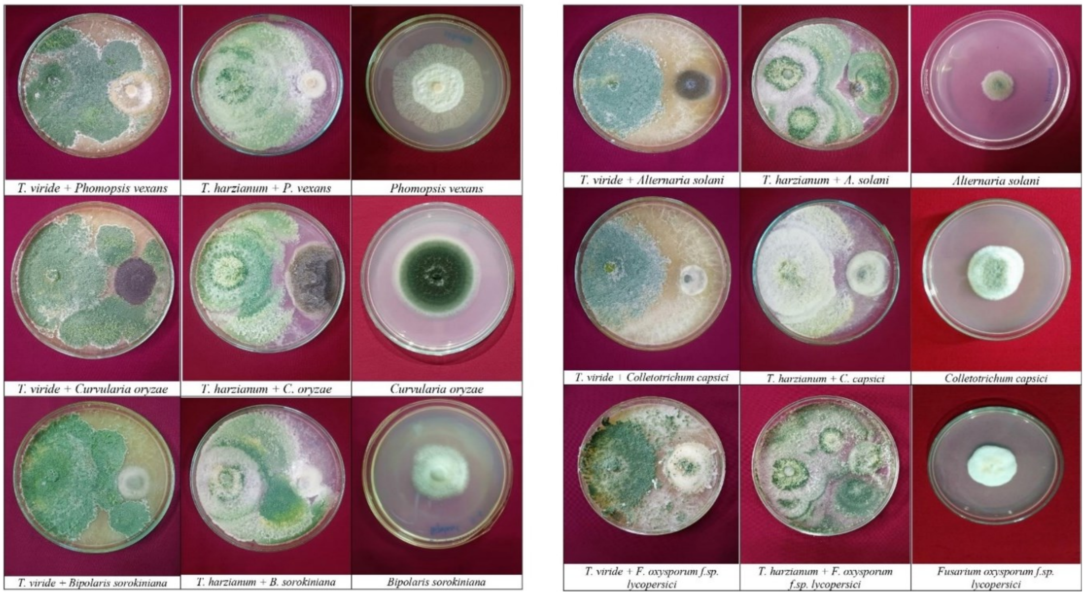

Fig. 2. Antagonistic effect of *Trichoderma viride* and *T. harzianum* against the mycelial growth inhibition (MGI) of different fungal pathogens on 4th day of co-incubation period at 27 ± 1 ºC in BOD incubator.

Both *Trichoderma* species displayed significant antagonistic capability against all the tested fungal pathogens in the present dual culture experiments. They are in line with many previous findings against *Curvularia oryzae* (Sunpapao et al., 2018), *Bipolaris sorokiniana* (Hasan et al., 2012; Yasmin et al., 2014; Bhandari, 2017; Singh et al., 2018), *Phomopsis vexans* (Das et al., 2014; Islam et al., 2016; Jakatimath et al., 2017), *Fusarium oxysporum* f. sp. *lycopersici* (Ramezani, 2010; Javaid et al., 2014; Suleiman et al., 2019), *Colletotrichum capsici* (Ekefan et al., 2009; Barhate et al., 2012; Aswini et al., 2016), and *Alternaria solani* (Naik et al., 2020; Rao et al., 2020).

Trichoderma species can employ various strategies against phytopathogenic fungi, including competition, mycoparasitism, antibiosis, induced resistance, and inactivation of the fungi's enzymes (Javaid et al., 2014). They hindered the growth of the targeted organisms by virtue of their faster growth rate, enabling them to efficiently compete for both space and nutrients with the pathogenic fungi. Competition for limited nutrients can result in nutrient scarcity, leading to starvation and ultimately leads to the natural degradation of fungal phytopathogens. *Trichoderma* spp. exhibited another important method of controlling pathogens i.e. mycoparasitism, involving hyphal interaction and parasitism. Siameto et al. (2010) reported that *T. harzianum*'s mycelia grew over *R. solani*, *Pythium* spp., and *T. harzianum*, coiled around their mycelia, directly penetrated their cell walls, and ultimately destroyed them.

CONCLUSIONS

Our study has demonstrated that two most common *Trichoderma* species viz. *T. viride* and *T. harzianum*, have the potential to control different agriculturally important plant pathogenic fungi *in vitro*. *T. viride* demonstrated greater efficacy against *Colletotrichum capsici* and *Cercospora oryzae*, while *T. harzianum* was more effective against *Alternaria solani, Bipolaris sorokiniana, Fusarium oxysporum* f. sp. *lycopersici,* and *Phomopsis vexans*. The potential use of these biocontrol agents can further be enhanced through advancement in the techniques such as isolation, formulation and application especially in field conditions.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India for providing necessary facilities to complete the research work.

REFERENCES

Anand, S. & Reddy, J. (2009). Biocontrol potential of *Trichoderma* sp. against plant pathogens. *International Journal of Agriculture Sciences*, 1(2), 30-39.

Arora, D.K., & Upadhyay, R.K. (1978). Effect of fungal staling growth substances on colony interaction. *Plant and soil*, 4(9), 685-690.

Aswini, A., Sharmila, T., Raaga, K., Sri Deepthi, R., & Krishna, M.S.R. (2016). *In vitro* antifungal activity of *Trichoderma* strains on pathogenic fungi inciting hot pepper (*Capsicum annuum* L.). *J. Chem. Pharm. Res*, 8(4), 425-430.

Barhate, B.G., Bidbag, M.B., Ilhe, B.M., & Shivagaje, A.J. (2012). *In vitro* evaluation of fungicides and bioagents against *Colletotrichum capsici* causing die-back of capsicum. *Journal of Plant Disease Sciences*, 7(1), 64-66.

Benítez, T., Rincón, A.M., Limón, M.C., & Codón, A.C. (2004). Mecanismos de biocontrol de cepas de *Trichoderma*. *International Microbiology*, 7(4), 249-260.

Bhandari, D. (2017). Bio-control ability of *Trichoderma* species against spot blotch disease (wheat) causing pathogen *Bipolaris sorokiniana* under *in vitro* condition. *Journal of Bioscience and Agriculture Research*, 14(02), 1194-1201.

Das, S.N., Sarma, T.C., & Tapadar, S.A. (2014). *In vitro* evaluation of fungicides and two species of *Trichoderma* against *Phomopsis vexans* causing fruit rot of brinjal (*Solanum melongena* L.). *International Journal of Scientific and Research Publications*, 4(9), 1-2.

Ekefan, E.J., Jama, A., & Gowen, S.R. (2009). Potential of *Trichoderma harzianum* isolates in biocontrol of *Colletotrichum capsici* causing anthracnose of pepper (*Capsicum* spp.) in Nigeria. *Journal of Applied Biosciences*, 20, 1138-1145.

Harman, G. E. (2006). Overview of Mechanisms and Uses of *Trichoderma* spp. *Phytopathology*, 96(2), 190-194.

Hasan, M.M., Rahman, S.M.E., Kim, G.H., Abdallah, E., & Oh, D.H. (2012). Antagonistic potentiality of *Trichoderma harzianum* towards seed-borne fungal pathogens of winter wheat cv. Protiva *in vitro* and *in vivo*. *Journal of Microbiology and Biotechnology*, 22(5), 585-591.

Islam, M.M., Hossain, D.M., Rahman, M.M.E., Suzuki, K., Narisawa, T., Hossain, I., Meah, B., Nonaka, M., & Harada, N. (2016). Native *Trichoderma* strains isolated from Bangladesh with broad spectrum antifungal action against fungal phytopathogens. *Archives of Phytopathology and Plant Protection*, 49(1-4), 75-93.

Jakatimath, S.P., Mesta, R.K., Mushrif, S.K., Biradar, I.B., & Ajjappalavar, P.S. (2017). *In vitro* evaluation of fungicides, botanicals and bio-agents against *Phomopsis vexans*, the causal agent of fruit rot of brinjal. *Journal of Pure and Applied Microbiology*, 11(1), 229-235.

Javaid, A., Afzal, L., Bashir, A., & Shoaib, A. (2014). *In vitro* screening of *Trichoderma* species against *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *lycopersici*. *Pakistan Journal of Phytopathology*, 26(1), 39-43.

Mamgain, A., Roychowdhury, R., & Tah, J. (2013). *Alternaria* pathogenicity and its strategic controls. *Research Journal of Biology*, 1, 1-9.

Naik, S.C., Narute, T.K., Narute, T.T., & Khaire, P.B. (2020). *In-vitro* efficacy of biocontrol agents against *Alternaria solani* (Early Blight of Tomato). *Journal of Pharmacognosy and Phytochemistry*, 9(5S), 550-552.

Ramezani, H. (2010). Antagonistic effects of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici* causal agent of tomato wilt. *Plant Protection Journal*, 2(1), 167-173.

Rao, Y.H., Devi, P.S., Vemavarapu, V.V., & Chowdary, K.R. (2020). Antagonistic potential of *Trichoderma* spp., botanicals and fungicides against *Alternaria solani* causing Early Blight of tomato *in-vitro* Conditions. *International Journal of Advances in Agricultural Science and Technology*, 7(9), 77-87.

Reddy, B.N., Saritha, K.V., & Hindumathi, A. (2014). *In vitro* screening for antagonistic potential of seven species of *Trichoderma* against different plant pathogenic fungi. *Research Journal of Biology*, 2, 29-36.

Sekar, G.R., Suriachandraselvan, M., & Patil, S.R. (2017). Epidemiology and cultural characterization of *Fusarium graminearum* causing head blight of wheat. *J. Soils Crops*, 27(1), 34-38.

Shanmugam, V. & Varma, A.S. (1998). *In vitro* evaluation of certain fungicides against selected antagonists of *Pythium aphanidermatum*, the incitant of rhizome rot of ginger. *Abs. J. Mycol. Pl. Pathol.*, 28, 70.

Siameto, E.N., Okoth, S., Amugune, N.O., & Chege, N.C. (2010). Antagonism of *Trichoderma harzianum* isolates on soil borne plant pathogenic fungi from Embu District, Kenya. *Journal of Yeast and Fungal Research*, 1(3), 47-54.

Singh, D., Pande, S.K., Kavita, J.K.Y., & Kumar, S. (2018). Bioefficacy of *Trichoderma* spp. against *Bipolaris sorokiniana* causing spot blotch disease of wheat and barley. *Int. J. Curr. Microbiol. App. Sci*, 7(3), 2322-2327.

Suleiman, A.S., Gambo, M.S., & Sunusi, M. (2019). An *in vitro* antagonistic effect of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici*. *Fudma Journal of Sciences*, 3(1), 369-374.

Sunpapao, A., Chairin, T., & Ito, S.I. (2018). The biocontrol by *Streptomyces* and *Trichoderma* of leaf spot disease caused by *Curvularia oryzae* in oil palm seedlings. *Biological Control*, 123, 36-42.

Watanabe, T. (2002). *Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species*. CRC Press.

Yasmin, S., Sultana, S., Adhikary, S.K., Jahan, N., Rahman, S., & Rahman, M.I. (2014). *In vitro* evaluation of *Trichoderma harzianum* (Rifai.) against some soil and seed borne fungi of economic importance. IOSR *Journal of Agriculture and Veterinary Science*, 7, 33-37.