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Compatibility of Fungicides and Antagonistic Activity of *Trichoderma* spp Against Plant Pathogens

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Abstract Compatibility of fungicides viz. Mancozeb and Captan were studied with *Trichoderma* spp (*T. viride, T. harzianum, T. koningii, T. pseudokoningii* and *T. virens*) *in vitro* at different concentrations. It was indicated that lower concentrations of Mancozeb and Captan did not affect the radial growth of *Trichoderma* spp. However, concentration of Mancozeb above 5000 µg/ml and of Captan above 500 µg/ml were significantly reduced the radial growth of *Trichoderma* species. *Trichoderma viride, T. harzianum, T. virens* and *T. koningii* showed resistance to Mancozeb and Captan. *Trichoderma pseudokoningii* is susceptible to these fungicides. However, when increased the concentration of Mancozeb and Captan decreased the radial growth. *Trichoderma* species are stronger antagonistic against number of plant pathogens and their mycoparasitic activity. All *Trichoderma* species inhibited the mycelial growth of *Alternaria alternata* while maximum inhibition performed by *T. viride*. *Trichoderma koningii* was highly antagonistic over *Rhizoctonia solani* followed by *T. viride*. Radial growth of *Aspergillus niger* was highly inhibited by *T. koningii* followed by *T. harzianum*. *Geotrichum candidum* was inhibited significantly by *T. viride* followed by *T. pseudokoningii* and *T. virens*. Significant antagonism was exhibited by *T. virens* against *Fusarium oxysporum* f. sp. *spinacae* followed by *T. harzianum* and *T. pseudokoningii*. Radial growth and sporulation of *Macrophomina phaseolina* was antagonized significantly by *T. viride* followed by *T. harzianum* and *T. pseudokoningii*.

Keywords *Trichoderma* spp, Compatibility Mancozeb, Captan, fungicides, Pathogenic fungi, Dual culture, Radial growth, Antagonism

Introduction

Trichoderma species have often been used in the management of crop plant diseases. *Trichoderma* is a genus of asexually reproducing fungi that is present in all types of soils. Some soil borne fungi are difficult to eradicate because they produce resting structures like sclerotia, chlamydospores or oospores for their survival for a longer period of time under adverse environmental conditions (Baker and Cooke, 1974). Use of fungicides for the control of soil borne diseases is costly and also produces environment and health hazards to human and adversely affects the beneficial microorganisms in soil (Dluzniewska, 2003). This was diverted the attention of plant pathologists towards alternate methods for the control of plant diseases. *Trichoderma* spp is a potential biocontrol agent for the management of various plant diseases like, sapota (Wagh and Bhale, 2011; Bhale, 2013), Leafy vegetables (Rajkonda et al., 2012), spinach (Bhale, 2012). *Trichoderma* species are known to suppress infections of soil borne pathogens like *Macrophomina phaseolina*, *Rizoctonia solani*, *Fusarium* species and *Pythium* species on various crops (Benitez et al., 2004, Adenkule et al., 2001, Ehtesham et al., 1990; Lutchmeah and Cooke, 1875; Howell, 1982). Species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Dubey et al., 2007; Benitez et al., 2004, Yedidia et al., 1998). The combined use of biocontrol agent and chemical pesticides has attracted much attention in order to obtain synergistic or additive effects in the control of soil borne diseases (Locke et al., 1985). Reduced amount of fungicide can stress and weaken the pathogen and render its propogules more susceptible to subsequent attack by the antagonist (Heiljord and Tronsmo, 1998). Srinivas and Ramkrishnan (2002) have reported that

integration of biocontrol agents and commonly used fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists' individually. Recently, biological control combined with chemical fungicide at lower concentration is applicable. It is being a part of IPM (Integrated Plant disease Management) strategy which was applied since chemical controls have used individually cause environmental pollution. Therefore the present study, *Trichoderma* species *viz., T. viride, T. harzianum, T. koningii, T. pseudokoningii* and *T. virens* were tested for compatibility with fungicides like Mancozeb and Captan.

1 Materials And Methods

1.1 Source of *Trichoderma* spp

Rhizospheric soils of irrigated and non irrigated plants were collected from different parts of Marathwada region of Maharashtra, India. From the rhizosphere soil samples, *Trichoderma* spp were isolated by using PDA and *Trichoderma* selective medium (TSM) by dilution plate technique (Johnson, 1957). The isolated species were identified up to species level based on colony characters, growth, structure of mycelium, conidiophores, phialides and conidia (Kubicek and Harman, 2002). All *Trichoderma* spp were purified by hyphal tip technique (Tuite, 1996). The isolated *Trichoderma* spp were maintained throughout the study by periodical transfers on PDA and TSM slants under aseptic conditions to keep the culture fresh and viable.

1.2 Fungicides

a) Mancozeb 75% WP (1000 – 8000 µg/ml) is a broad spectrum contact fungicide with a protective action which belongs to the dithiocarbamates (Manganese ethylene bisdithiocarbamte) family of chemicals, which also includes maneb.

b) Captan 50% WP (100-700 µg/ml) (N-trichlorome-thylthio-4-cyclohexene-1, 2-dicarboximide) were used for *in vitro*.

1.3 Compatibility with fungicides

Fungicides like Mancozeb and Captan was incorporated into the medium after sterilization. The fungicides in proportionate dosage were incorporated in to the molten Czapek Dox Agar (CZA) medium after sterilization and dispersed thoroughly by continuous shaking. This was poured in to 90 mm petridishes. Mycelial discs of 8 mm cut from the growing margin of 7 days old culture of *Trichoderma* species was inoculated at the centre of the petridish and incubated at 26 ± 2 C. The CZA plate without fungicide served as control. The diameter of the colony was measured after 7 days and compared with the control (Tronosmo, 1989).

1.4 Source of pathogenic fungi

The test fungi were isolated from naturally infected plants viz. leaf spot of brinjal (*Solanum melongana* L.) caused by *Alternaria alternata*, Fruit rots of sapota (*Manilkar zapota L.*) caused by *Rhizoctonia solani, Aspergillus niger* and *Geotrichum candidum*, leaf spot ofspinach (*Spinacea oleraea* L.) and fruit rot of ivy guard (*Coccinia indica* Wight & Arn.) caused by *Macrophomina phaseolina*.

1.5 Antagonistic Activity

Antagonistic efficacy of *Trichoderma* spp viz., *T. viride, T. harzianum, T. koningii, T. pseudokoningii* and *T. virens* were tested against the isolated pathogenic fungi by dual culture experiment (Morton and Stroube 1955). *Trichoderma* spp and test fungi were inoculated 6 cm apart. Three replicates were maintained for each treatment and incubated at 28 ± 2 ℃ for 7 days. Monoculture plates of both served as control. Seven days after incubation (DAI), radial growth of test fungi and *Trichoderma* spp were measured. Colony diameter of test fungi in dual culture plate was observed and compared with control. Percentage of radial growth inhibition (%RGI) was calculated by using the formula: 100 X [C - T / C], Where C = growth in control and T = growth in treatment (Vincent, 1947).

The degree of antagonism between each of the *Trichodema* species and test pathogens in dual culture was scored on scale of R_1-R_5 (Bell et al., 1982).

2 Statistical Analyses

Statistics recitation *in vitro* compatibility was statistically analysed using the main factor fungicide i.e. Mancozeb and Capton and pathogenic fungi i.e. *Alternaria alternate, Rhizoctonia solani, Aspergillus niger, Geotrichum candidum, Fusarium oxysporum* f. sp. *spinacae, Macrophomina phaseolina* and the sub-factors were the *Trichoderma* species. Arcsine transformation of biological control (*Trichoderma* species) percentage was calculated by using the following formula:

$$Y = \text{arcsine } \sqrt{p} = \sin^{-1}\sqrt{p}$$

Where, p is the percentage of inhibition and Y is the result of transformation

Statistical analysis of the experiments was performed using the Handbook of Biological Statistics (McDonald, 2008).

3 Results

3.1 Compatibility of *Trichoderma* species against fungicides

Species of *Trichoderma viz., T. viride, T. harzianum, T. koningii, T. pseudokoningii* and *T. virens* were isolated from the rhizosphere soil of different crop plants. These isolates were deposited in Research laboratory, Department of Botany, Arts Science and Commerce College Naldurg. These isolates were maintained on PDA (Potato Dextrose Agar) media slants. Species of *Trichoderma* were amended with fungicides and results obtained following (Table 1&2; Figure 1).

Table 1 Compatibility of *Trichoderma* spp with different concentrations of Mancozeb fungicide.

Sr. No.	*Trichoderma* spp	Control (mm)	Radial growth in different concentration (µg/ml)							
			1000	2000	3000	4000	5000	6000	7000	8000
1	*T. viride*	90	48	35	34	27	14	08	03*	00
			(55)	(37)	(36)	(29)	(15)	(09)	(05)	(00)
2	*T. harzianum*	89	72	63	57	49	40	32	19	00*
			(83)	(69)	(66)	(58)	(47)	(33)	(20)	(00)
3	*T. koningii*	90	47	41	36	33	27	21	12	00*
			(53)	(43)	(40)	(35)	(31)	(27)	(14)	(00)
4	*T. pseudokoningii*	90	31	24	18	09	00*	00	00	00
			(33)	(27)	(19)	(11)	(00)	(00)	(00)	(00)
5	*T. virens*	88	82	74	63	51	40	22	13	00*
			(86)	(83)	(72)	(56)	(45)	(23)	(15)	(00)

Note: *Significantly reduced the radial growth of mycelium.

Figures in parentheses are arcsine transformed value of radial growth of mycelium.

Table 2 Compatibility of *Trichoderma* spp with different concentrations of Captan fungicide.

Sr. No.	*Trichoderma* spp	Control (mm)	Radial growth in different concentration (µg/ml)						
			100	200	300	400	500	600	700
1	*T. viride*	90	53	38	28	13	04	02*	00
			(47)	(41)	(33)	(14)	(04)	(03)	(00)
2	*T. harzianum*	89	57	37	24	11	06	03*	00
			(62)	(38)	(25)	(12)	(06)	(04)	(00)
3	*T. koningii*	90	48	35	25	12	05	02*	00
			(51)	(37)	(29)	(14)	(07)	(02)	(00)
4	*T. pseudokoningii*	90	34	28	21	08	00*	00	00
			(35)	(30)	(24)	(11)	(00)	(00)	(00)
5	*T. virens*	89	62	47	28	09	00*	00	00
			(70)	(54)	(29)	(10)	(00)	(00)	(00)

Note: *Significantly reduced the radial growth of mycelium.

Figures in parentheses are arcsine transformed value of radial growth of mycelium.

a. *Trichoderma viride*: *Trichoderma. viride* showed growth on medium containing Mancozeb@1000, 2000, 3000, 4000 & 5000 µg/ml. The growth of *T. viride* is least on media containing Mancozeb@6000 and 7000 µg/ml, but no growth was observed when Mancozeb was used @8000 µg/ml. *Trichoderma viride* grow easily when Captan was used @ 100, 200, 300 and 400 µg/ml, the growth was minimum on Captan@500 and 600 µg/ml, but no growth were recorded on Captan@700 µg/ml.

b. *Trichoderma harzianum*: *Trichoderma harzianum* grow at lower concentration of Mancozeb. The growth was inhibited when concentration of Mancozeb was more than 8000 µg/ml. However, increased concentration of Mancozeb decreased the radial growth. In media containing Captan@700 µg/ml, no growth was recorded. The growth of *T. harzianum* was observed at lower concentration from 100 to 400µg/ml.

c. *Trichoderma koningii*: *Trichoderma koningii* showed growth in Mancozeb@1000 to 5000 µg/ml. The growth

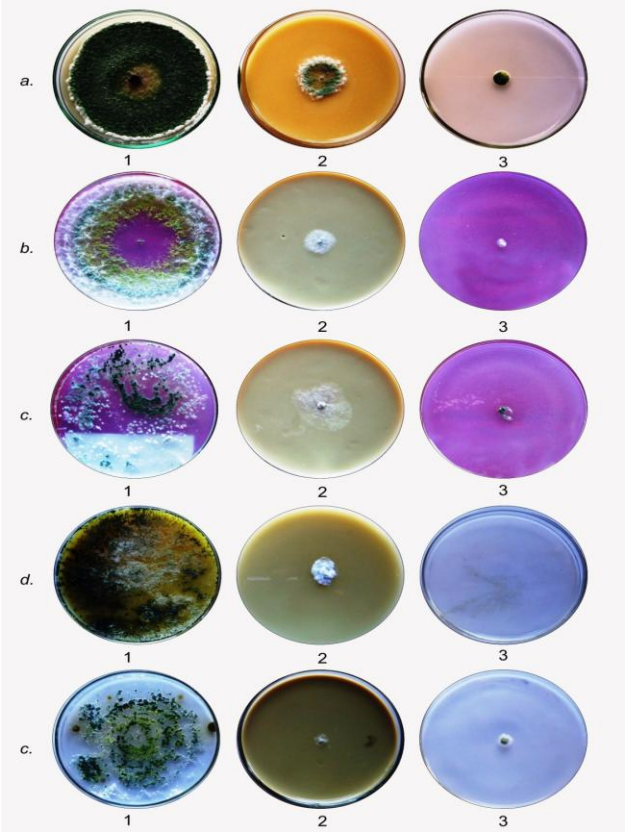

Figure 1 Compatibility of *Trichoderma* spp with Mancozeb & Captan fungicides.
Note: a) 1-*T. viride*(Control), 2-Mancozeb 8000 µg/ml, 3-Captan 700 µg/ml; b) 1-*T. harzianum*(Control), 2-Mancozeb 8000 µg/ml, 3-Captan 700 µg/ml; c) 1-*T. koningii* (Control), 2-Mancozeb 8000 µg/ml, 3-Captan 600 µg/ml; d) 1-*T. pseudokoningii*(Control), 2-Mancozeb 5000 µg/ml, 3-Captan 500 µg/ml; e) 1-*T. virens*(Control), 2-Mancozeb 8000 µg/ml , 3-Captan 500 µg/ml

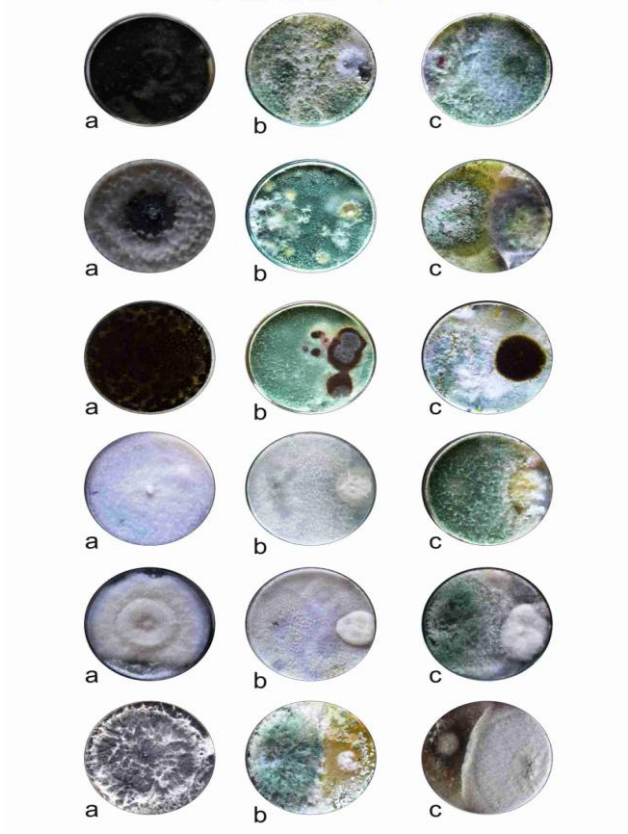

Figure 2 Antagonistic activity of *Trichoderma* spp on pathogenic fungi.
Note: Row 1: *Alternaria alternata* (a-control, b-*T. virens*, c-*T. koningii*)
Row 2: *Rhizoctonia solani* (a-control, b- *T. koningii*, c- *T. hrzianum*)
Row 3: *Aspergillus niger* (a-control, b-*T. koningii*, c-*T.viride*)
Row 4: *Geotrichum candidum* (a-control, b-*T. virens*, c-*T.koningii*)
Row 5: *Fusarium oxysporum* f. sp.spinaciae (a-control, b-*T. virens*, c-*T.viride*)
Row 6: *Macrophomina phaseolina* (a-control, b-*T. viride*, c-*T. virens*)

was very low in concentration of 7000 µg/ml, no growth occurred when Mancozeb was used above the @8000 µg/ml. *T. koningii* showed growth at Captan @100, 200, 300 µg/ml. The growth was slow at Captan @ 500 and 600µg/ml. No growth was observed on Captan@700µg/ml.

d. *Trichoderma pseudokoningii*: *Trichoderma pseudokoningii* grow slow under media containing Mancozeb@1000 2000, 3000 and 4000 µg/ml, but it was inhibited completely at Mancozeb@5000µg/ml. The radial growth of *T. pseudokoningii* was also low at Captan@100, 200, 300 and 400µg/ml. But no growth was observed on Captan@500 µg/ml.

e. ***Trichoderma virens*** : *Trichoderma virens* grow readily under low concentration of Mancozeb(@1000 – 5000 µg/ml). The radial growth was gradually decreased at Mancozeb@5000 to 7000 µg/ml and no growth was recorded at 8000 µg/ml. Treatment of Captan @100 to 300 µg/ml, the radial growth of *T. virens* was recorded. However, growth was stopped on Captan @ 500 µg/ml.

Only, *T. pseudokoningii* was found susceptible at lower tested concentrations on both fungicides. The obtained results indicated that low concentration of Mancozeb and Captan does not affect the radial growth of mycelium of *Trichoderma* spp. However, increased concentration of fungicides were decreased the radial growth of mycelium.

3.2 Antagonistic activity of *Trichoderma* species against pathogenic fungi

Isolated plant pathogens i.e. *Alternaria alternata, Rhizoctonia solani, Aspergillus niger, Geotrichum candidum, Fusarium oxysporum* f. sp. *spinacae* and *Macrophomina phaseolina* were evaluated their antagonistic nature against *Trichoderma* species under

in vitro condition. It was observed that growth of pathogenic fungi was reduced with respect to radial growth and sporulation. The mycelium of *Trichoderma* species when comes in contact with the test fungi it became fungistatic and the growth of test fungi were retarded (Table 3 & Figure 2).

Table 3: Antagonistic activity of *Trichoderma* species against pathogenic fungi.

Sr. No.	Treatments	% Antagonism					
		At	***Rs***	***An***	***Gc***	***Fo***	***Mp***
1	*T. viride*	73.3(86.7)	51.1(59.1)	44.4(50.2)	74.4(87.8)	55.5(64.2)	84.4(97.4)
2	*T. harzianum*	90.4(97.5)	38.8(42.6)	54.4(62.9)	61.1(71.5)	66.6(78.7)	83.3(96.4)
3	*T. konongii*	77.7(91.1)	67.7(79.5)	57.7(66.8)	66.7(78.6)	61.1(71.5)	55.5(64.2)
4	*T. pseudokoningii*	71.1(84.4)	44.4(50.2)	42.2(47.2)	72.2(85.5)	62.2(72.8)	81.1(94.7)
5	*T. virens*	74.4(87.8)	46.6(52.7)	44.4(50.2)	68.9(81.1)	70.0(82.7)	57.7(66.8)
6	Control (mm)	90					

Note: At-*Alternaria alternata*, Rs- *Rhizoctonia solani*, An-*Aspergillus niger*, Gc-*Geotrichum candidum*, Fo-*Fusarium oxysporum* f. sp. *spinacae*, Mp-*Macrophomina phaseolina*

a. *Alternaria alternata:* Results indicated that *Trichoderma* species significantly inhibited the radial growth of *Alternaria alternata* incitant of leaf spot of brinjal (*Solanum melangona*). Maximum inhibition of *A. alternata* was observed with *T. harzianum* (90.4%) followed by *T. koningii* (77.7%), *T. virens* (74.4%) and *T. viride* (73.3%). However, minimum inhibition showed under the treatment of *T. psudokoningii* (71.1%).

b. *Rhizoctonia solani:* Antagonisitic nature of *Trichoderma* species against *Rhizoctonia solani* causing fruit rot of sapota (*Manilkar zapota*) was recorded. The radial growth of *R. solani* was inhibited more by *T. koningii* (67.7%). Antagonism of *R. solani* was also showed by *T. viride* (51.1%), *T. virens* (46.6%) and *T. pseudokoningii* (44.4%). *Trichoderma harzianum* showed minimum antagonism against *R. solani* (38.8%).

c. *Aspergillus niger:* Antagonistic activity of isolated *Trichoderma* species against *Aspergillus niger* were evaluated under *in vitro* condition and results were recorded. *Trichoderma koningii* exhibited maximum antagonism against *A. niger* (57.7%). However, *T. harzianum* also showed antagonism against *A. niger* (54.4%). Radial growth of *A. niger* was also inhibited by *T. viride* (44.4%), *T. virens* (44.4%) and *T. pseudokoningii* (42.2%). It was observed that the isolated *Trichoderma* species showed moderate inhibitory effect over *A. niger.*

d. *Geotrichum candidum:* Results revealed that antagonistic effect of *Trichoderma* species over *Geotrichum candidum* causal agent of fruit rot of sapota (*Manilkar zapota*). Maximum inhibition against *G. candidum* was recorded by *T. viride* (74.4%), followed by *T. pseudokoningii* (72.2%), *T. virens* (68.9%), *T. koningii* (66.7%) and *T. harzianum* (61.1%). The isolated *Trichoderma* species exhibited significant antagonism against *G. candidum*.

e. *Fusarium oxysporum* f. sp. *spinacae: In vitro* antagonistic nature of *Trichoderma* species against *Fusarium oxysporum* f. sp. *spinacae* causal agent of wilt of spinach (*Spinacea oleracea*) was tested. It was cleared that *Trichoderma* species inhibited the mycelial growth of *F. oxysporum* f. sp. *spinacae*. Among *Trichoderma* species, maximum inhibition showed by *T. virens* (70.0%) followed by *T. harzianum* (66.6%). *T. pseudokoningii* (62.2%) and *T. viride* (55.5%).

f. *Macrophomina phaseolina:* Antagonistic effect of *Trichoderma* species against *Macrophomina phaseolina* incited fruit rot of ivy guard (*Coccinia indica*) was recorded and the results showed that *Trichoderma* species were significant in reducing the radial growth of mycelium of the test fungus. The inhibitory effect of *T. viride* (84.4%) found maximum followed by *T. harzianum* (83.3%) and *T. pseudokoningii* (81.1%). The antagonistic effects

by *T. virens* (57.7%) and *T. koningii* (55.5%) over *M. phaseolina* were recorded minimum but these were also significant.

3.3 Bell's Scale

According to modified Bell's scale, *T. harzianum* overgrew beyond 90 percent (R_1 scale). In case of *R.solani*, only *T. koningii* overgrew beyond 60 percent (R_3 scale).All *Trichoderma* species were failed to progress beyond 60 percent (R_3 scale) in *A.niger*. In *G.candidum, T. pseudokoningii* and *T. viride* overgrew at least two third of pathogen (R_2 scale) but others were beyond 60 percent (R_3 scale). In case of *F.oxysprum f. sp.spinaceae, T. virens* overgrew at least two-third pathogens (70% over growth). Except *T. koningii* and *T. virens* other *Trichoderma* species were overgrew at least two third of pathogen (R_2 scale) in *M. phaseolina* (Table 4).

Table 4 Evaluation of *Trichoderma* spp. against pathogenic fungi by dual culture using Bell's scale*(R).

***Trichoderma* spp**	**Test Pathogens**					
	A.alternata	*R.solani*	*A.niger*	*G.candidum*	*F.oxysporum*	*M. phaseolina*
T. viride	R_2	R_4	R_4	R_2	R_3	R_2
T. harzianum	R_1	R_4	R_3	R_3	R_3	R_2
T. koningii	R_2	R_3	R_3	R_3	R_3	R_3
T. pseudokoiningii	R_2	R_4	R_4	R_2	R_3	R_2
T. virens	R_2	R_4	R_4	R_3	R_2	R_3

Note: *Degree of antagonism

R_1=*Trichoderma* completely overgrew pathogens (100% over growth);

R_2=*Trichoderma* overgrew at least two-third pathogens (75% over growth);

R_3=*Trichoderma* colonizes on one half of the pathogens (50% over growth);

R_4=*Trichoderma* and the pathogens contact point after inoculation;

R_5= Pathogens overgrow bioagent – *Trichoderma*.

4 Discussion

Experiments were also conducted to determine the compatibility of biocontrol agents with commercially effective chemicals against white rot of apple (*Dematophora necatrix*) in India (Gupta and Sharma, 2004). They concluded that Carbendazim was inhibitory to all fungal antagonists whereas Mancozeb and Phorate were least inhibitory at 200ppm. Radial growth of mycelium of *Trichoderma* spp was inhibited at Carbendazim @ 1ppm and Triphonate Methyl @ 10ppm (Malathi et al., 2002). Inhibitory action of Isofenphos – methyl on *T. harzianum* was strong and fungistatic rate was 64.37% under treatment with 200µg/ml (Chinh et al., 2008). The fuingicide Iprodione and *T. harzianum* were not found to be compatible (Sumitra and Madhuban, 2006). The *T. harzianum* was least sensitive to Procymidone and Captan and most sensitive to Mancozeb, Tebuconazole and Thiram (Mclean et al., 2001). Inhibitory effect of Carbendazim and Thiophanate Methyl on *T. harzianum* while Captan and Thiram recorded least inhibitory effect on *T. harzianum* (Gowdar et al., 2006). Recently, the fungicide Bordeaux mixture 1% found highly inhibitory to *Trichoderma* as compared to Copper oxychloride and Mancozeb (Suseela and Joseph, 2010). Some strains of *Trichoderma* show compatibility with fungicides as they are tolerant of fungicides and successfully used in IPM strategy (Dutta and Chatterjee, 2004; Hetong et al., 2008). *Trichoderma viride* was not compatible with Dithane, Bavistin and Ridimil in any level of selected concentration (Tapwal et al., 2012)

Many workers in the discipline of plant pathology suggested that growth of plant pathogenic fungi were inhibited by the *Trichoderma* species because of some factors produced and these substances may be volatile and non volatile (Reusser, 1967).The growth inhibition of pathogenic fungi may be due to antibiotic secretion like trichodermin, trichoviridin, dermadin and sesquiterpene heptalic acid (Nakkeeran et al., 2002), nutrient impoverishment and pH alteration in the medium (Maheshwari et al., 2001). Such type of variability in antagonistic potential of *Trichoderma* species against plant pathogens has been also reported (Saha and Pan, 1996; Bell et al., 1982). Mathew and Gupta (1998) also reported that *T. harzianum* also exhibited maximum antagonistic activity causing 58% inhibition followed by *T. viride* (46%) and *T. virens* (45%). Isolates of *T. viride, T. harzianum* and *T. virens* were evaluated for their antagonism against *Pythium aphanidermatium* causing damping off of tomato and found that many isolates inhibited the growth of *P.*

aphanidermatum (Kumar and Hooda, 2007).*Trichoderma viride* is economically important because of their mycoparasitic ability which makes them suitable for the application as biocontrol agent against soil borne plant pathogenic fungi (Manczinger et al., 2000). The effective *in vitro* screening test of *T. viride* was carried out against *Rhizopus oryzae* and *Aspergillus flavus* pathoges of post harvest cassava (*Manihot esculents* Crantz.), root rot and reported that *T. viride* was most promoting candidate for the biocontrol (Ubalua and Oti, 2007). Growth of *Rhizoctonia solani*, a pathogen involved in cotton seedling disease was inhibited by the strains of *T. harzianum* and *T. longibracheatum* (Arsan-Amal et al., 2005). Strains of *T. koningii* were used for their antagonistic nature against *Rhizoctonia solani* under *in vitro* condition and inhibited mycelial growth by producing toxic metabolites (Melo and Faull, 2000). In the dual culture experiment evaluated by Hajieghrari et al. (2008), *T. virens* and *T. harzianum* inhibited the growth of soil borne pathogenic fungi such as *R. solani, M. phaseolina, Phytophthora cactorum* and *Fusarium graminearum* forming inhibition zone without physical contact between them. *In vitro* antagonistic potential of *T. viride* against *Alternaria alternata, Ulocladium botrytis, Cladosporium harbarum, Cephalosporium madurae, Penicillium chrysogonum, Fusarium oxysporum* and *Humicola grisa* were tested and found that significant inhibition of radial growth of these fungi in dual culture experiment (Abou-Zeid et al., 2008).

Rajendiran et al. (2010) evaluated antagonistic effects of *T. viride* on post harvest pathogens of fruit and vegetables such as *Aspergillus niger, A. flavus, A. fumigatus, Fusarium* sp and *Penicillium* sp. *Trichoderma viride* inhibited the radial growth of *A. niger* (55%), *A. flavus* (51%), *A. fumigates* (52%), *Fusarium* sp (64%) and *Penicillium* sp in dual culture. The impact of isolates of *T. viride, T. harzianum* and *T. virens* on soil borne fungal pathogens such as *R. solani, S. rolfsii* and *Sclerotinia sclerotiorum* were evaluated and inhibitory effects were reported (Amin et al., 2010). Reports on antagonistic potential of *T. harzianum* over *Fusarium oxysporum* f. sp. *vanilla* the stem rot pathogen of vanilla was showed by Naik et al. (2010).The isolates were found fully overgrown on all corm rot pathogens of saffron (Hassan et al., 2011). *Trichoderma viride* was found to exhibit effective antagonistic potentiality against *R. solani* (Giagole et al., 2011).

5 Conclusion

Trichoderma species can be used together with compatible fungicides in the integrated disease management towards the control of crop plants and soil borne pathogens. It is possible to develop *Trichoderma* tolerant of chemical fungicides without lack of antagonistic activity. The antagonistic nature of *Trichoderma* species against pathogenic fungi were evaluated under *in vitro* condition. It was observed that growth of pathogenic fungi was reduced with respect to radial growth and sporulation. The mycelium of *Trichoderma* species when comes in contact with the test fungi it became fungistatic and the growth of test fungi were retarded. The antagonism was exhibited with respect to secretion of extra cellular enzymes, antibiotics and competition related food and space. Pathogenic fungi and *Trichoderma* species created competition and the latter found to be dominant over the pathogenic fungi. Mycoparasitic properties of *Trichoderma* species was found to be the main reason responsible for their antagonistic nature.

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References

Abou-Zeid A. M., Altahi A D., and Abd El-Fattah R. I., 2008, Fungal control of pathogenic fungi isolated from some wild plants in Taif Governorate, Saudi Arabia. Mal. J. Microbiol., 4(1):30-39.

Adenkule A.T.,Cardwell D.A., Florini and Ikotun T., 2001. Seed treatment with *Trichoderma* species for control of damping off of cowpea caused by *Macrophomina phaseolina*, Biocontrol Science and Technology, 11: 449-457.
http://dx.doi.org/10.1080/09583150120067481

Amin F., Razdan V. K., Mohoddin F. K., Bhat K. A. and Bandey S., 2010, Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules, Journal of Phytology, 2(10): 38-41.

Arsan-Amal A., Abd-Elsalam K.A., Omar M. R., and Aly A. A., 2005, Antagonistic potential of *Trichoderma* spp against *Rhizoctonia solani* and use of M13 microsatellite-primed PCR to evaluate the antagonist genetic variation, Journal of Plant Diseases and Protection, 112(6): 550-561.

Baker K.F., and Cooke R.J., 1974, Biological control of plant pathogens, W.H. Freeman Press, San Fransisco.

Bell D.K., Wells H.D. and Markham C.R., 1982, *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens, Phytopathology, 72, 379-382.
http://dx.doi.org/10.1094/Phyto-72-379

Benitez T., Rincon A.M., Limon M.C., and Codon A.C., 2004, Biocontrol mechanism of *Trichoderma* strains, Intl. Microbiol., 7(4):249-260.

Bhale U.N., Ambuse M.G., Chatage V.S. and Rajkonda J.N., 2012, Bioefficacy of *Trichoderma* isolates against pathogenic fungi inciting spinach (*Spinacea oleracea* L.), JBiopest., 5(2): 222-227.

Bhale U.N., Wagh P.M. and Rajkonda J.N., 2013, Antagonistic confrontation of *Trichoderma* spp against fruit rots pathogens on Sapodilla (*Manilkara zapota* L.), Journal of Yeast and Fungal Research, 4(1): 5-11.

Ching D.M., 2008, Compatibility of mycelial growth and conidia germination of *T. harzianum* with several soil applied insecticides, Journal of Anhui Agricultural Sciences, pp.25 -32.

Dluzneiwska J.,2003, Reaction of fungi of *Trichoderma* genus to selected abiotic factors,Elec. J. Polish Agr. Uni. Agron, 6 (2): 239-242.

Dubey C.S., Suresh M. and Singh B., 2007, Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp.*ciceris* for integrated management of chickpea wilt, Biological Control, 40(1):118-127. http://dx.doi.org/10.1094/Phyto-72-379

Dutta S. and Chatterjee N.C., 2004,.Raising of carbendazim-tolerant mutants of *Trichoderma* and variations in their hydrolytic enzyme activity in relation to mycoparasitic action againts *Rhizopus stolonifer*, Journal of Plant Diseases and Protection,111(6):557-565.

Ehteshamul H.S., Zaki M.J., and Gaffar A., 1990, Biological control of root rot disease of okra, sunflower, soybean and mungbean. Pak. J. Bot., 22: 121-124.

Giagole A.H., Wagh G.N., and Khadse A.C., 2011, Antifungal activity of *Trichoderma* species against soil borne pathogens, Asiatic J. Biotech. Res., 2(4): 461-465.

Gowdar S.B., Babu-Ramesh H.N., Nargund V.B., and Krishnappa M., 2006, Compatibility of fungicides with *T. harzianum*, Agricultural sciences Digest, 26(4): 203-209.

Gupta V.K., and Sharma K., 2004, Integration of chemicals and biocontrol agents for managing white root rot of apple ISHS Acta Horticulturae Eds. A. Vanacter) XXVI International Horticultural Congress: Managing soil borne pathogens: A sound Rhizosphere to improve productivity in intensive horticultural systems, Acta horticulturae, pp. 307-313.

Hajieghrari B., Torabi-Giglou M., Mohammadi M.R., and Davari M., 2008, Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi, African Journal of Biotechnology, 7(8): 967-972.

Hassan M.G., Devi L.S., Ahmad S., Manojkumar V., and Williams P., 2010, The biological control of paddy disease brown spot (*Bipolaris oryzae*) by using *Trichoderma viride in vitro* condition, Journal of Biopesticides, 3(1): 93-95.

Hetong Y., Ryder M. and Wenghua T., 2008,Toxicity of Fungicides and Selective Medium Development for Isolatiom and Enumeration of *Trichoderma spp*. In: Agricultural soils. International Subcommision on *Trichoderma* and *Hypocrea* Taxonomy, China 2008.

Hjeljord I. and Tronsmo A., 1998, *Trichoderma* and *Gliocladium* in biological control: an overview. In: *Trichoderma* and *Gliocladium*-Enzymes, Biological control and Commercial Applications (Eds.): G.E. Herma and C.P. Kubick. Taylor & Frasncis Ltd London, Great Britain, pp.74-106.

Howell C.R., 1982, Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizoctonia solani* and damping off of cotton seedlings, Phytopathology, 74:106.

Johnson L.A., 1957, Effect of antibiotics on the number of bacteria and fungi isolated and fungi isolated from soil by dilution plate method, Phytopathology,47: 21-22.

Kubicek C.P. and Harman G.E., 2002, *Trichoderma* and *Gliocladium* (vol. I). Basic Biology,Taxonomy and Genetics. pp. 14-24.

Kumar R. and Hooda I., 2007, Evaluation of antagonistic properties of *Trichoderma* species against *Pythium aphanidermatum* causing damping off of tomato, J. Mycol. Pl. Pathol., 37(2): 240-243.

Locke J.C., Moris J.J. and Papavizas G.C., 1985, Biological control of *Fusarium* wilt of greenhouse grown Chrysanthemums. Plant Dis, 69: 167-169. http://dx.doi.org/10.1094/PD-69-167

Lutchmeah R.S. and Cooke R.I., 1985, Pelleting of seeds with the antagonist *Pythium oligandrum* for biological control of damping off, Plant Pathology, 34:528 – 531. http://dx.doi.org/10.1111/j.1365-3059.1985.tb01403.x

Maheshwari D.K., Dubey R.C., and Sharma V.K., 2001, Biocontrol effects of *Trichoderma virens* on *Macrophomina phaseolina* causing charcoal rot of peanut, Indian J. Microbiol., 41:251-256.

Malathi P., Vishwanathan R., Padmanabhan P., Mohanraj D., and Ramesh Sunder A., 2002,Compatibility of biocontrol agent with fungicides against red rot of sugarcane, Suger Tech., 4 (3-4):131-136. http://dx.doi.org/10.1007/BF02942694

Manczinger L., Antal Z. and Kredics L., 2000, Ecophysiology and breeding of mycoparasitic *Trichoderma* strains, Acta. Microbiol. Immunol., 49: 1-14.

Mathew K.A., and Gupta S.K., 1998, Biological control of root rot of frenchbean caused by *Rhizoctonia solani*, J. Mycol. Pl. Pathol., 28: 202-205.

McDonald J.H., 2008, Handbook of Biological statistics. Sparky House Publishing, Baltimore,Maryland, pp.160-164.

Mclean K.L., Hunt J., and Stewrat A., 2001, Compatibility of *Trichoderma harzianum* with selected fungicides, New Zealand Plant Protection, 54: 84-88.

Melo I.S., and Faull J.L., 2000, Parasitism of *Rhizoctonia solani* by strains of *Trichoderma* spp, Sci. Agric., 57(1): 73-77. http://dx.doi.org/10.1590/S0103-90162000000100010

Naik G., Saifulla B., Nagaraja R ,and Basavraja M.K., 2010, Biological control of *Fusarium oxysporum* f. sp. *vanillae*, the causal agent of stem rot of vanilla *in vitro*, International Journal of Science and Nature., 1(2):259-261.

Nakkeeran S., Krishnamoorthy A.S., Ramamoorthy V., and Renukadevi S**.,** 2002, Microbial inoculants in plant disease control, J. Ecobiol., 14:83-94.

Rajendiran R., Jagdeeshkumar D., Sureshkumar B.T. and Nisha T., 2010, *In vitro* assessment of antagonistic activity of *Trichoderma viride* against post harvest pathogens, Journal of Agricultural Technology, 6(1): 31-35.

Rajkonda J.N., Jadhav D.S., and Bhale U.N., 2012, Efficacy of *Trichoderma* species on leafy vegetable crops. Shodhankan (International Multidiciplinary referred & reviewed Research Journal, Special Issue. pp.62-67.

Reusser F., 1967, Biosynthesis of antibiotics. U-22, 324, a cyclic polypeptide, J. Biol. Chem., :242-243.

Saha D.K. and Pan S., 1996, *In vitro* antagonistic potential of different isolates of *Gleocladium virens* of West Bengal, J. Natl. Bot. Soc., 50: 13-18.

Srinivas P., and Ramkrishnan G., 2002, Use of native microorganism and commonly recommended fungicides in integrated management of rice seed borne pathogens, Annl Pl Protect. Sci., 10(2): 260-264.

Sumitra A. and Madhuban G., 2006, Bioefficacy of Iprodione against two diseases, its compatibility with *T. harzianum* and residues on cabbage crops, Journal of Environmental Science and Health Part B., 41(6): 949-963.
http://dx.doi.org/10.1080/03601230600806152

Suseela B.R. and Joseph T., 2010, Compatibility of *Trichoderma harzianum* (Refai) with fungicides, insecticides and fertilizers. Indian Phytopath,63(2): 145-148.

Tapwal A.R. Kumar N.G. and Pandey S., 2012, Compatibility of *Trichoderma viride* for selected fungicides and botanicals, International Journal of Plant Pathology, 3(2):89-94.
http://dx.doi.org/10.3923/ijpp.2012.89.94

Tronosmo A., 1989, Effect of fungicides and insecticides on growth of *Botrytis cinera, Trichoderma viride* and *T. harzianum*, Biol.Control, 1:59-62.

Tuite J., 1996, Plant Pathological Methods. Fungi and Bacteria,Burgess Pub. Co. Minneapolis, Minn. USA. p. 293.

Ubalua A.O. and Oti E., 2007, Antagonistic properties of *Trichoderma viride* on post harvest cassava root rot pathogens, Afr. J. Biotechnol., 6(21): 2447-2450.

Wagh P.M. and Bhale U.N., 2011, In *vitro* biocontrol activity of *Trichoderma* species against fungal phytopathogen *Rhizoctonia solani* from post-harvest sapota, Proceeding of UGC sponsored National Conference on "Role of non agricultural Institution in the important of Agricultural technology" held at Dept of Botany, Shrikrishna Mahavidyalaya gunjoti on 23-24th Jan.2011. Bionanofrontier, Special issue. pp. 36-38.

Yedidia I.N., Benhamou N. and Chet, I., 1999, Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by biocontrol agent *Trichoderma harzianum*, Appl. Environ. Microbiol., 65: 1061 – 1070.