

Original Research Article

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Compatibility of *Trichoderma viride* and *Trichoderma harzianum* with Fungicides against Soil Borne Diseases of Tomato and Cabbage

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ABSTRACT

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A study was undertaken to evaluate the compatibility of commonly used fungicides at recommended dosages with *T. viride* and *T. harzianum* being used as a biocontrol agents against soil borne diseases of tomato and cabbage under *in vitro* and *in vivo* conditions. Results indicated that among systemic fungicides, Azoxystrobin was found highly compatible with *T. viride* and *T. harzianum* at all the three tested concentrations (0.05, 0.10 and 0.15 %) followed by Metalaxyl. With respect to non-systemic fungicides, Mancozeb recorded least inhibitory effect on *Trichoderma* sp. Further compatible combinations were evaluated *in vivo* to know the survival population of *Trichoderma* in soil media cropped with tomato and cabbage. Compatible combinations at their recommended dosage can be recommended for integrated management of soil borne pathogens of these crops.

Introduction

Biological control of soil borne plant pathogens by species of *Trichoderma* is a vital area of plant pathological research all over the world in these days (Mukhopadhyay, 1987). Most of the soil borne diseases is not amenable for management through chemicals. Use of several antagonistic species of *Trichoderma* (*T. viride*, *T. harzianum*) against a range of economically important soil borne plant pathogens have been well documented (Cook and Baker, 1983). In recent years, the search of biological control agents for the

management of dreaded soil borne diseases has been advocated widely. Since the biocontrol agents are applied either to seed or soil or both, there is every possibility of interaction and interference that would arise with the commonly used fungicides. The full expression of potential biocontrol is considered in terms of rhizosphere competence, suppression of pathogens, tolerance to pesticides, competitive saprophytic ability, adaptability to environment etc. Combined application of biocontrol agents with commonly used fungicides may result either in synergism /

antagonism between these two. Various fungicides are recommended for the management of major soil borne pathogens. Looking for the broad spectrum performance of *T. viride* and *T. harzianum* against most of the soil borne fungi and foliar diseases, its compatibility with the other recommended chemical fungicides are found to be essential so that synergistic effect can be obtained for the management of diseases. Though few studies about the sensitivity of biocontrol agents with certain fungicides are available, studies with special reference to commercially available biocontrol agents, species of *Trichoderma* are meagre compatibility of living organisms with modern inputs in plant protection like fungicides is pre-requisite for disease management. Hence, there is need to test the compatibility studies of fungicides with *T. viride* and *T. harzianum*, their influence on rhizosphere mycoflora of various crops. Tomato and cabbage are very important crops grown in Karnataka and use of biocontrol agents as well as chemical fungicides are essential for managing disease of these crops especially soil borne and seed borne diseases. Therefore, it is very much essential to know the compatibility between these various bio agents and fungicides.

Materials and Methods

Collection of test organism

The culture of antagonistic microorganism *Trichoderma harzianum* was obtained from department of Plant Pathology, KRCCH, Arabhavi. Cultures of *Trichoderma viride*, *Bacillus subtilis*, *Lecaenicilium lecanii*, *Pseudomonas fluorescens*, *Metarrhizium anisopliae*, *Paecilomyces lilacinus* were obtained from department of Plant Pathology, UAS Dharwad.

Maintenance of culture

The fungus was sub cultured on potato dextrose agar slants and allowed to grow at

26±1⁰C for 7 days and such slants were preserved in a refrigerator at 4⁰C and sub cultured once in 30 days.

Compatibility of *Trichoderma* sp. with fungicides under *in vitro* conditions

Efficacy of six fungicides was evaluated against the *T. viride* and *T. harzianum* by poisoned food technique as described by Dhingra and Sinclair (1995). Using poisoned food technique in PDA media, for each treatment, 60 ml PDA was taken in 100 ml conical flask and sterilized. To this medium specified quantity of the test chemical was added to get desired concentration at lukewarm temperature and mixed thoroughly by shaking the flasks. The poisoned medium was poured aseptically into sterilized petri plates and allowed to solidify. For each treatment, three replications were maintained. Mycelial discs (5 mm diameter) of the test isolates of *T. viride* and *T. harzianum* culture (one week old) were cut from periphery of colony with sterile cork borer and transferred into the centre of poisoned medium in each of the petri plate. Suitable controls were maintained by placing discs (5mm) of isolates of *Trichoderma* sp. in petri plates containing untreated medium (i.e., without chemical). All the inoculated petri plates were incubated at 28±2⁰C in a BOD. The colony diameter of various isolates of *Trichoderma* sp. was measured in two directions and average was recorded in each treated petri plates when the colony growth in the control plate was full. Per cent inhibitions of mycelia growth of the fungus were calculated by using following formula (Vincent, 1927).

$$PI = \frac{C - T}{C} \times 100$$

PI = Percentage inhibition

C = Radial growth of the *Trichoderma* in control plate (cm)

T = Radial growth of the *Trichoderma* in treatments (cm)

Evaluation of compatible fungicides under *in vivo* condition

The combined effect of the *Trichoderma* sp. and the effective fungicides were tested in pot culture. Sterilized pot containing sterilized red soil and farm yard manure in the ratio of 2:1 were used in the study. Three replications for each treatment were tried. The compatible treatments were evaluated in pot culture experiment to know the population density of *Trichoderma* sp. Tomato and cabbage seedlings were raised in protrays, 30 days old seedling was transplanted into pots. At the time of transplanting, *Trichoderma* at the concentration of 5 g/kg soil (2×10^8 cfu/ g) were treated with potting mixture. The compatible fungicides were applied to tomato and cabbage at 20 days after transplanting and again same treatments were taken up at 45 days after planting whereas other compatible bio agents were applied to soil at 5 g/kg soil at the time of transplanting. Soil samples were collected from rhizosphere of treated tomato and cabbage crops for estimation of *Trichoderma* sp. population. Re isolation of *Trichoderma* sp. was done at 30 days and 60 days after transplanting (DAT). The population of *Trichoderma* sp. were estimated by dilution plate method using *Trichoderma* specific media (TSM) (Elad and Chet, 1983). Control was not applied with any of the fungicides.

Re isolation of *Trichoderma* sp. from tomato and cabbage rhizosphere

Collected rhizosphere soil samples were air dried for 4 hr and used for the isolation of *Trichoderma* sp. by dilution plate technique (Elad and Chet, 1983). Serial dilution was done by pour plate method. One gram of the air dried soil was taken in 9 ml of sterile distilled water and serially diluted. Serial dilution up to 10^{-4} was made and 1 ml from each dilution was poured into Petri dishes and 20 ml of TSM was added. The plates were

incubated at $26 \pm 2^\circ\text{C}$ for 7 days. After seven days colony forming units were recorded.

Results and Discussion

Soil borne plant pathogens are very difficult to control by using any single method of control. Therefore, Integrated Disease Management (IDM) is an acceptable approach in control of the diseases caused by various soil borne pathogens. As biological control has become one of the important components in IDM, it is inevitable that biocontrol agents are often applied along with fungicides either to plant or soil or both which may result in either synergism or antagonism between them. As most of the commonly used fungicides exert either positive, negative or neutral effects on biocontrol agents, the present study on compatibility of *Trichoderma* sp. with various fungicides commonly used in tomato and cabbage crops were evaluated under *in vitro* and *in vivo* condition. Results obtained in the investigation are discussed here under.

Compatibility of *Trichoderma* sp. with fungicides

Compatibility of *Trichoderma* sp. was evaluated against systemic and non-systemic fungicides. Among systemic fungicides Azoxystrobin was found highly compatible with *T. harzianum* and *T. viride* (0.00 % inhibition) at three tested concentration followed by Metalaxyl. Highest inhibition was recorded in Carbendazim. Compatibility of *Trichoderma* sp. with Azoxystrobin was also reported by Ranganathswamy *et al.*, (2012). Similarly Sawant and Mukhopadyay (1990) observed that the radial growth of *T. harzianum* was not inhibited by Metalaxyl even upto 1000 ppm concentration and non-compatibility of *Trichoderma* sp. with Carbendazim was also reported by Ramarethinam *et al.*, (2001) and Bheemaraya *et al.*, (2012).

Table.1A List of fungicides

Sl. No.	Common name	Trade name	Chemical name	Formulation	Concentration (%)		
1.	Azoxystrobin	Amistar	Methyl(E)-2-{2-[6-(2cyanophenoxy)pyrimidin-4-yl oxy]phenyl}-3- methoxyacrylate	23% SC	0.05	0.10	0.15
2.	Metalaxyl	Mask	Methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate	35% WS	0.15	0.20	0.25
3.	Carbendazim	Teja	methylbenzimidazol-2-ylcarbamate	50% WP	0.05	0.10	0.15
4.	Chlorothalonil	Kavach	2,4,5,6- tetrachloroisophthalonitrile	75% WP	0.15	0.20	0.25
5.	Copper oxy chloride	Blitox	Copper oxychloride	50% WP	0.25	0.30	0.35
6.	Mancozeb	Dithane M-45	Manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt	75% WP	0.15	0.20	0.25

Table.1B Compatibility of *Trichoderma* sp. with fungicides at different concentrations

Sl. No.	Chemical name	Concentration (%)	<i>T. viride</i>		<i>T. harzianum</i>	
			Mycelial growth (cm)	Percent inhibition (%)	Mycelial growth (cm)	Percent inhibition (%)
1.	Metalaxyl (35% WS)	0.15	8.27	8.15 (16.56)*	8.00	11.11 (19.45)
		0.20	7.87	12.59 (20.77)	7.83	12.96 (21.09)
		0.25	7.00	22.22 (28.12)	6.30	30.00 (33.20)
2.	Azoxystrobin (23% SC)	0.05	9.00	0.00 (0.33)	9.00	0.00 (0.33)
		0.10	9.00	0.00 (0.33)	9.00	0.00 (0.33)
		0.15	9.00	0.00 (0.33)	9.00	0.00 (0.33)
3.	Mancozeb (75% WP)	0.15	8.90	1.11 (5.43)	8.87	1.48 (6.67)
		0.20	8.40	6.67 (14.94)	8.33	7.41 (15.77)
		0.25	8.33	7.41 (15.77)	8.23	8.52 (16.95)
4.	Copper Oxy Chloride (50% WP)	0.25	3.63	59.63 (50.55)	3.50	61.11 (51.42)
		0.30	3.03	66.30 (54.51)	2.90	67.78 (55.41)
		0.35	2.27	74.81 (59.87)	2.17	75.93 (60.62)
5.	Carbendazim (50% WP)	0.05	0.73	91.85 (73.43)	0.70	92.22 (72.78)
		0.10	0.53	94.07 (75.93)	0.53	94.07 (75.93)
		0.15	0.50	94.44 (76.39)	0.50	94.44 (76.39)
6.	Chlorothalonil (75% WP)	0.15	2.50	72.22 (58.19)	1.40	84.44 (66.77)
		0.20	2.23	75.19 (60.12)	1.13	87.41 (69.22)
		0.25	1.67	81.48 (64.51)	0.83	90.74 (72.29)
7.	Control	-	9.00	--	9.00	--
S. Em±			0.12	1.58	0.13	1.59
CD @ 1%			0.36	4.72	0.38	4.77

*Figures in the parentheses are arc sin transformed values

Table.2 Evaluation of compatible fungicides with *Trichoderma* sp. in tomato crop under in vivo conditions

Sl. No.	Treatments	Concentration (%)	Population density of <i>T. viride</i> (Cfu g ⁻¹ soil)		Population density of <i>T. harzianum</i> (Cfu g ⁻¹ soil)	
			30 DAT	60 DAT	30 DAT	60 DAT
1.	Mancozeb (75% WP)	0.15	4.44	4.70	4.33	4.78
		0.20	4.22	4.52	4.22	4.55
		0.25	3.89	4.19	3.89	4.33
2.	Azoxystrobin (23% SC)	0.05	4.78	4.93	4.33	5.00
		0.10	4.67	4.89	4.22	4.89
		0.15	4.00	4.33	3.89	4.78
3.	Metalaxyl (35% WS)	0.15	3.89	4.30	3.67	4.45
		0.20	3.67	4.00	3.56	4.22
		0.25	3.22	3.63	3.33	3.89
4.	Control	-	5.33	5.44	5.22	5.44
S. Em±			0.24	0.20	0.28	0.27
CD @ 5%			0.71	0.58	0.82	0.80

Cfu – Colony forming unit

DAT – Days After Transplanting

Table.3 Evaluation of compatible fungicides with *Trichoderma* sp. in cabbage crop under in vivo conditions

Sl. No.	Treatments	Concentration (%)	Population density of <i>T. viride</i> (Cfu g ⁻¹ soil)		Population density of <i>T. harzianum</i> (Cfu g ⁻¹ soil)	
			30 DAT	60 DAT	30 DAT	60 DAT
1.	Mancozeb (75% WP)	0.15	4.11	4.44	4.11	4.45
		0.20	3.78	4.22	3.89	4.33
		0.25	3.44	3.89	3.78	4.22
2.	Azoxystrobin (23% SC)	0.05	4.22	4.78	4.33	4.89
		0.10	4.22	4.67	4.20	4.56
		0.15	3.56	4.00	3.68	4.44
3.	Metalaxyl (35% WS)	0.15	3.44	3.89	3.56	4.00
		0.20	3.33	3.67	3.40	3.89
		0.25	2.78	3.22	3.11	3.44
4.	Control	-	5.11	5.33	5.22	5.33
S. Em±			0.24	0.16	0.23	0.15
CD @ 5%			0.72	0.48	0.68	0.44

Cfu – Colony forming unit

DAT – Days After Transplanting

In our study, non-systemic fungicide Mancozeb recorded least inhibitory effect on *Trichoderma* sp. (@ 0.25% conc.). Similarly

Mancozeb recorded least inhibitory effect of radial growth of *Trichoderma* sp. which was reported by earlier scientists Malathi *et al.*,

(2002) and Nandeeshha *et al.*, (2013). Other non-systemic fungicides *viz.*, Copper Oxy Chloride, Chlorothalonil were recorded incompatibility with *Trichoderma* sp. Incompatibility of these chemicals with *Trichoderma* bio agents were reported by Nishanth and Smita (2012).

In the present study, a progressive increase in per cent inhibition of radial growth of *Trichoderma* sp. was observed as the concentration of fungicides increased. These findings were agreement with the works of Durai (2004) and Srinivasulu *et al.*, (2005) who also found good growth of *Trichoderma* isolates at low and medium concentrations of various fungicides.

Among two *Trichoderma* sp. tested for their compatibility against fungicides, *T. viride* showed more compatible reaction than *T. harzianum* at different concentrations. The differential response of *Trichoderma* sp. to various fungicides in the present study might be due to their inherent resistance to the fungicides and their ability to degrade these chemicals. Some strains of *Trichoderma* show compatibility with fungicides as they are tolerant of fungicides and can successfully use in IPM strategy (Dutta and Chatterjee, 2004). Besides having great antagonistic potential, *Trichoderma* has the capability of degrading xenobiotic compound and can survive in environment with remnant of fungicide molecules (Hetong *et al.*, 2008). Results were displayed in Table 1.

Evaluation of compatible fungicides with *Trichoderma* sp. in tomato and cabbage crop under *in vivo* conditions

Different compatible combinations of fungicides were co-inoculated with *Trichoderma* sp. into the pot and *Trichoderma* population level were estimated at 30 and 60 days after inoculation. Among fungicides,

significantly maximum population of *Trichoderma* sp. was recorded with Azoxystrobin. Bhai and Joseph (2010) were reported compatibility of *T. harzianum* with agrochemicals. In addition, they were found supportive to increase the population of *T. harzianum* under *in vivo* condition. Population level of *Trichoderma* increased from 30 to 60 days after *Trichoderma* application. The increase in the mycoflora may be due to increased senescence of leaves resulting in more saprophytic colonization. This observation was in agreement with Sudha Mall (1979) who reported that considering the age of the plant, the mycoflora of root zone increased with advancement of growth and after attainment of peak there was a progressive decline and again a flaring up of flora at senescent obviously because of saprophytic colonization. Results were presented in the Table 2 and 3.

Increased concentration of fungicides reduced the population level of *Trichoderma* observed in both tomato and cabbage crops. Similarly Agarwal *et al.*, (2005) reported that as fungicides concentration increased, the growth has been decreased and minimum growth was observed with 1% concentration of bavistin as well as with dithane M-45. Both fungicides had deleterious effect on mycorrhizal spore number and percentage mycorrhizal root colonization. Similar effect may be operating under the present study condition also.

Summary and Conclusion are as follows:

✓ Among systemic fungicides, Azoxystrobin was found highly compatible with *T. harzianum* and *T. viride* at all the three tested concentrations (i.e., 0.05, 0.10 and 0.15 %) followed by Metalaxyl. The highest inhibition was recorded in Carbendazim.

✓ Among non-systemic fungicides, Mancozeb recorded least inhibitory effect on *Trichoderma* sp. and incompatibility was found with Copper Oxy Chloride and Chlorothalonil under *in vitro* conditions.

✓ Further compatible combinations were evaluated to know the survival population of *Trichoderma* in soil media cropped with tomato and cabbage crops. No significant difference in population of *Trichoderma* was noticed between treatments and control.

✓ The present study indicates that application of *Trichoderma* would be compatible with fungicides (*viz.*, Mancozeb, Azoxystrobin and Metalaxyl) at recommended dosage and can be recommended for integrated management of soil borne pathogens of these crops.

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