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Research Article
Compatibility *Trichoderma harzianum* with Systemic and Two non Systemic Fungicides of *in vitro*

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Abstract

Background and Objectives: *Trichoderma* spp. is a soil inhabitant fungus with an ability of inhibiting plant pathogens and immunity and growth enhancer in plants. Based on the available literatures, the objective of this investigation was planned to study the compatibility of *Trichoderma harzianum* along with two systemic and two non-systemic fungicides *in vitro*. **Materials and Methods:** To isolate the biocontrol agent i.e., *T. harzianum* from soil, soil samples were collected from crop fields of crop research centre (CRC) of SVPUA and T Meerut, India. Four fungicides viz Mancozeb, Thiram, Carboxin and Propiconazole at 25, 50, 75 and 100 ppm were tested for their compatibility with *Trichoderma harzianum* by poisoned food technique and inhibition (%) by individual fungicides were noticed and recorded at an interval of each 24 h. Data were subjected to analysis using appropriate statistical methods, analysis of variance and treatment means were differentiated using Fischer's t-test in Completely Randomized Design (CRD) in laboratory. **Results:** It was evident that all the four concentrations of Mancozeb were highly compatible with almost negligible toxic effect against *Trichoderma harzianum in vitro*. As there was no or very little (0.00, 0.00, 5.19 and 7.03) inhibition of radial growth of *Trichoderma harzianum* due to Mancozeb at 25, 50, 75 and 100 ppm concentrations, respectively. Thiram was less compatible than Mancozeb. Carboxin and Propiconazole were toxic and incompatible with *Trichoderma harzianum*. **Conclusion:** Two non-systemic fungicides i.e., Mancozeb and Thiram were found to be compatible, as compared to systemic fungicides viz. Carboxin and Propiconazole which exhibited acute toxicity for growth of *Trichoderma harzianum in vitro*.

Key words: Biocontrol agent, radial growth, plant pathogens, *Trichoderma harzianum,* Mancozeb, Thiram, non-systemic fungicides, carboxin and propiconazole

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Agriculture in modern era depends largely on the use of agrochemicals, for managing plant diseases and to enhance crop productivity. Agrochemicals are capable of minimizing the threats from diseases and enhancing crop yields, however at the same time pose serious threats to human health along with environmental hazards. This has resulted in an increasing interest in biological control as a promising alternative or a supplemental way of reducing the use of agro-chemicals. Some naturally occurring soil bacteria and fungi have shown great potential to inhibit plant pathogens, hence, biological control involving the use of such beneficial micro-organisms for plant protection is being considered as a viable substitute to reduce the use of agrochemicals in general and pesticides in particular[1].

Management of plant diseases by the use of antagonist micro-organisms might be an effective means[2]. A large number of plant diseases have been successfully managed through fungal and bacterial antagonists[2-5]. *Trichoderma* sp. have been used in the management of plant diseases. The duration and degree of active disease control can be extended by using chemicals and biological control agents (antagonists) together as a mixed formulation in integrated disease management system. In a mixed formulation even reduced amount of the fungicide may weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonists[6]. Chemical protectants may be effective even under such climatic conditions where antagonists are less effective, while an active biological control agent can prophylactically colonize wounds or senescent plant tissue and ultimately protect them against pathogenic infection[7]. Usually fungicidal resistant or tolerant isolates of bio-agents are readily screened and obtained through selection on pesticide containing media[8]. *Trichoderma* is being used as a biological component in the integrated disease management of soil borne pathogen of cardamom (*Elettaria cardamomum* Maton.) viz. capsule rot and rhizome rot[9,10]. There is considerable pressure from environment active groups and scientists to give lesser emphasis on use of chemicals and more emphasis on promotion of biological methods for management of crop pests and diseases. Though the use of fungicides is necessary at present and it will continue to be in near future too, however their use can be minimized as a long term solution to the crop health problem because they are hazardous and also eliminate natural enemies and beneficial micro flora. In addition, several pesticides are quite expensive and several of them are inducing pesticide resistance and thereby posing high risk of pest resurgence. Thus, today's need is to use eco-friendly compounds that can be fitted well into the holistic management strategy of the disease and compatible with commonly used antagonists viz. *Trichoderma* spp. and *Pseudomonas* spp. However, meager information's are available on the compatibility of these commonly used plant protection chemicals with *Trichoderma harzianum*, the bio-control agent. Hence, present study was undertaken to test the compatibility of *T. harzianum* with commonly used and recommended dosages of such fungicides *in vitro* so that such chemicals can be used in a compatible manner as a mixture with bio-control agents.

MATERIALS AND METHODS

Determination of *Trichoderma harzianum* compatibility with different fungicides *in vitro*: Compatibility of four fungicides (two non-systemic) viz. Mancozeb and Thiram, (two systemic) Carboxin and Propiconazole were tested. Each fungicides were tested at 25, 50, 75 and 100 ppm concentrations against *Trichoderma harzianum* by poisoned food technique *in vitro*. Initial experimental work was started on 7 November, 2014. The PDA plates were inoculated with *Trichoderma harzianum* to prepare at least 7 day old culture/ inoculums to be used for cutting 3 mm mycelial discs of test fungus. Required amount of all the test fungicides i.e., 25, 50, 75 and 100 mg L^{-1} of basal medium in case of Mancozeb, Thiram and Carboxin and 25, 50, 75 and 100 mL L^{-1} of basal medium in case of Propiconazole were added in conical flasks (250 mL capacity), containing 100 mL pre-sterilized basal media (PDA) to obtain 25, 50, 75 and 100 ppm concentration of each fungi toxicants and mixed thoroughly by shaking the flask prior to pouring in sterilized Petri plates. After pouring in Petri plates, the medium was allowed to be cool and solidified over night. After solidification, 3 mm mycelial discs from 7 days old culture of *Trichoderma harzianum* grown on PDA plates was placed in center of each Petri plate containing PDA with different concentration of test fungi toxicants. The PDA medium mixed with Sterilized distilled water only served as check. Inoculation of plates with mycelial bits of *Trichoderma harzianum* was done on 15 November, 2014. Three replications were maintained for each treatment. After inoculation with mycelial disc of *Trichoderma harzianum*, Petri plates were incubated at 28±2°C in BOD incubator and arranged in a fashion of Completely Randomized Block Design. Observations were recorded on radial growth of *Trichoderma harzianum* at an interval of each 24 h upto 10 days. Final observations on radial growth of *Trichoderma harzianum* were recorded on the evening of 25th November, 2014.

Experiment was continued only upto 10 days (25 November, 2014) because radial growth of *Trichoderma harzianum* in the Petri plates maintained as check (Containing basal medium without any fungicide) occupied full growth upto the periphery of Petri plate. On the basis of radial growth in respective treatments, inhibition (%) in the radial growth of *Trichoderma harzianum* was calculated by using the following equation:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where:

C = Growth of fungus in control (PDA mixed with sterilized distilled water)

T = Growth of fungus in respective treatments (PDA mixed with respective concentration of test fungicide)

Statistical analysis: This experiment was conducted following two factorial completely randomized design. Two factors consisted fungicides concentrations (17) and incubation periods (8). Three replications were maintained for each treatment. Average of data was analyzed using two way analysis of variance using OPSTAT1.EXE software . The data given in percentage were first transformed into angular value and then analyzed for test of significance[11,12].

RESULTS

The data presented in Table 1 indicated that all the four concentrations of Mancozeb i.e., 25, 50, 75 and 100 ppm (mL L^{-1}) were highly compatible with *Trichoderma harzianum in vitro.* There was absolutely no inhibition in radial growth of *T. harzianum* at 25 and 50 ppm concentration of Mancozeb after 192 and 240 h of incubation respectively on basal media containing these concentrations of Mancozeb. However a minimal inhibition in radial growth at 75 and 100 ppm concentrations of Mancozeb, respectively was noticed, after 240 h (10 days) of incubation on basal media i.e., PDA containing these concentrations of Mancozeb. Thiram, was comparatively more toxic to *Trichoderma harzianum* than Mancozeb and next in the order of compatibility/toxicity. In the initial period of incubation i.e., upto 48 h, there was absolute inhibition (%) in the radial growth of *Trichoderma harzianum* but with increasing in the days of incubation on Thiram containing basal media, mycelial growth of antagonist (*Trichoderma harzianum*) get started and quite visible also. At 72 h of incubation on basal media containing 25, 50, 75 and 100 ppm concentration of Thiram, there was

Table 1: Effect of different concentrations of Mancozeb, Thiram, carboxin and propiconazole on radial growth of *Trichoderma harzianum in vitro*

Fungicides	Concentration (ppm)	Radial growth (mm) 24 h	Inhibition (%)	Radial growth (mm) 48 h	Inhibition (%)	Radial growth (mm) 72 h	Inhibition (%)	Radial growth (mm) 96 h	Inhibition (%)	Radial growth (mm) 120 h	Inhibition (%)	Radial growth (mm) 168 h	Inhibition (%)	Radial growth (mm) 192 h	Inhibition (%)	Radial growth (mm) 240 h	Inhibition (%)
Mancozeb	25	3.00	55.02	15.00	30.78	27.00	34.15	48.00	15.79	61.00	6.15	87.00	3.33	90.00	0.00	90.00	0.00
	50	2.00	70.01	13.00	40.01	25.00	39.02	44.00	22.81	58.00	10.77	84.00	6.67	90.00	0.00	90.00	0.00
	75	0.47	92.95	9.00	58.47	22.00	46.34	39.00	31.58	53.00	18.46	80.00	11.11	85.00	5.56	85.33	5.19
	100	0.23	96.55	7.00	67.70	18.00	56.10	35.00	38.60	50.00	23.08	76.00	15.56	83.00	7.78	83.67	7.03
Carboxin 37.5%	25	0.00	100.00	0.67	96.91	3.33	91.88	6.67	88.30	10.33	84.11	12.00	86.67	12.00	86.67	12.33	86.30
	50	0.00	100.00	0.23	98.94	2.33	94.32	4.33	92.40	6.67	89.74	8.33	90.74	8.67	90.37	9.33	89.63
	75	0.00	100.00	0.17	99.22	1.33	96.76	3.33	94.16	5.00	92.31	5.67	93.70	6.00	93.33	6.33	92.97
	100	0.00	100.00	0.10	99.54	0.83	97.98	2.67	95.32	2.00	96.92	5.00	94.44	5.33	94.08	5.67	93.70
Propiconazole (25EC)	25	0.00	100.00	0.07	99.68	0.50	98.78	2.00	96.49	1.67	97.43	3.33	96.30	4.33	95.19	4.67	94.81
	50	0.00	100.00	0.00	100.00	1.00	97.56	1.67	97.07	1.50	97.69	2.33	97.41	3.33	96.30	3.67	95.92
	75	0.00	100.00	0.00	100.00	0.50	98.78	1.33	97.67	1.33	97.95	1.50	98.33	2.33	97.41	2.67	97.03
	100	0.00	100.00	0.00	100.00	0.00	100.00	0.67	98.82	0.67	98.97	0.83	99.08	1.33	98.52	1.67	98.14
Thiram	25	0.00	100.00	0.30	98.62	1.33	96.76	7.33	87.14	9.00	86.15	11.33	87.41	13.00	85.56	14.00	84.44
	50	0.00	100.00	0.13	99.40	0.83	97.98	5.00	91.23	6.00	90.77	9.00	90.00	11.00	87.78	12.00	86.67
	75	0.00	100.00	0.07	99.68	0.33	99.20	2.67	95.32	6.00	90.77	7.00	92.22	9.33	89.63	10.00	88.89
	100	0.00	100.00	0.00	100.00	0.00	100.00	1.33	97.66	3.67	95.92	4.67	94.81	7.00	92.22	7.33	91.86
Control		6.67		21.67		41.00		57.00		65.00		90.00		90.00		90.00	

CD at 5 % = Concentration (A) = 0.2800, Incubation periods (B) = 0.4081, CXH = 1.154

quite high level or absolute inhibition in radial growth of *T. harzianum*, whereas at 96 h, inhibition in radial growth was further reduced than those noticed at 72 h. At the end of experiment i.e., at 240 h (10 days) of incubation on basal media containing 25, 50, 75 and 100 ppm concentration of Thiram, the level of inhibition in radial growth of *T. harzianum* was 84.44, 86.67, 88.89 and 91.86% which was quite less than the inhibition noticed at 72 and 96 h.

Two systemic fungicides i.e., Carboxin and Propiconazole were found to be more toxic than the two non-systemic fungicides (Mancozeb and Thiram) at all the four concentrations (25, 50, 75 and 100 ppm) tested. However, among two systemic fungicides, the Propiconazole was more toxic for *Trichoderma harzianum* than Carboxin. At 24 h of incubation on basal media, containing at 25, 50, 75 and 100 ppm concentration of carboxin, there was absolute inhibition (%) in radial growth of *Trichoderma harzianum.* Level of inhibition at 48 h of incubation was comparatively less than those noticed at 24 h. From 72 h onward upto 192 h, the inhibition in radial growth ranged between 84.11-97.98% at 25, 50, 75 and 100 ppm concentration of Carboxin. After 240 h, the inhibition (%) in radial growth were lowest. There was gradual decrease in the level of inhibition with increasing the period of incubation. In case of Propiconazole, there was absolute inhibition in radial growth of *Trichoderma harzianum* at 25, 50, 75 and 100 ppm concentration, respectively. After 72 h onward up to 192 h, the inhibition in radial growth ranged between 95.19-99.08% at 25, 50, 75 and 100 ppm concentration, respectively of Propiconazole. After 240 h, the inhibition (%) in radial growth of *Trichoderma harzianum* were lowest but still it was above 90% at all the concentrations (25, 50, 75 and 100 ppm) of Propiconazole. Overall, it was noticed that the two non-systemic fungicides i.e., Mancozeb and Thiram were found safer. Two systemic fungicides viz. Carboxin and Propiconazole exhibited acute toxicity for growth of *Trichoderma harzianum in vitro*. Compatibility couldn't be measured beyond 10 days as the Petri plates containing basal medium without any fungicides filled completely with radial growth of antagonist within 10 days.

DISCUSSION

During this study, two systemic fungicides i.e., Carboxin and Propiconazole were found to be comparatively more toxic and incompatible at all the concentrations tested i.e., 25, 50, 75 and 100 ppm, respectively, against *Trichoderma harzianum.* Two non-systemic fungicides i.e., Mancozeb and Thiram were found to be compatible with the antagonist at all the concentrations tested. However, among the two systemic fungicides, Propiconazole was quite higher toxic than Carboxin at each concentration. Two systemic fungicides viz Carboxin and Propiconazole remained highly toxic after 10 days also, whereas one non-systemic fungicides i.e., Mancozeb was found to be compatible and safer even at starting period also i.e., 24 h. Thiram, an universally accepted and widely used seed dressing fungicide was found to be less compatible with *Trichoderma harzianum* than Mancozeb. However if go on with the level of inhibition in radial growth, Thiram can be considered as more compatible than Carboxin, as it's toxicity lesser against *Trichoderma harzianum* than that of Carboxin.

Thus, non-systemic fungicides i.e., Mancozeb and Thiram and one systemic fungicide i.e., Carboxin may be considered safer as compared to Propiconazole which exhibited acute toxicity for growth of *Trichoderma harzianum*. Bagwan[13] reported that thiram (0.2%), copper oxychloride (0.2%) and mancozeb (0.2%) were found comparatively safer against *Trichoderma harzianum* and *Trichoderma viride* as compared to other fungicides. However some other fungicides like captan, tebuconazole, vitavax, propiconazole and chlorothalonil were toxic to both the species of *Trichoderma.* These findings are in conformity with the findings of present study. Madhavi *et al.*[14], also evaluated the compatibility of *Trichoderma viride* with 25 different pesticides *in vitro* where they tested six chemicals as seed-treatment. *T. viride* showed a high compatibility with the insecticide, imidacloprid (7.6 cm mycelial growth), followed by Mancozeb (6.3 cm) and Tebuconazole (3.7 cm). Contact fungicides, viz., Pencycuron and Propineb were found to be fully compatible with *T. viride*. In addition, 10 herbicides were also tested by this group and it was noticed that, the fungus *Trichoderma viride* was highly compatible with Imazethapyr (9.0 cm) followed by 2,4-D Sodium salt (8.9 cm) and Oxyfluorfen (6.5 cm) while it was totally incompatible with systemic fungicides like Carbendazim, Hexaconazole, Tebuconazole and Propiconazole. This report is also in accordance with the present study. Correa and Soria[1], also observed that out of four systemic fungicides and two non-systemic fungicides tested *in vitro* for compatibility with potential bio-agent, Mancozeb was found highly compatible with *Trichoderma harzianum*. Thus the findings of present study is supported by the findings of Olga and Marcelo also. Ranganathswamy *et al.*[15], also tested the compatibility of fungicides with biological control agents, i.e., *Aspergillus niger*, *Trichoderma viride*, *T. koningii*, *T. harzianum* and *T. virens*. Among the fungicides, Azoxystrobin was less toxic and compatible up to 400 ppm. Captan, Propineb and Azoxystrobin can be used for mixed formulation of chemical and bioagents at 200-400 ppm depending upon the *Trichoderma* species. Pencycuron can

be incorporated with *Trichoderma* spp. even at a concentration of more than 400 ppm for seed treatment in the integrated management system. Rubayet and Bhuiyan[16] conducted an experiment to test the compatibility of three fungicides namely Provax-200 (Carboxin), Rovral 50 WP (Iprodione) and Bavistin 50 WP (Carbendazim) against T 10 isolate of *Trichoderma harzianum in vitro* and reported that it was compatible with Provax-200 and Rovral 50 WP only at lower concentration which are in accordance with current findings with respect to compatibility of carboxin with *Trichoderma harzianum*, Tapwal *et al*.[17] reported that among five fungicides viz., dithane M-45, ridomil, captaf, blue copper and bavistin, only captaf and blue copper were compatible to some extent with *T. viride*. Nandeesha *et al*.[18] also observed that out of four systemic fungicides and two non-systemic fungicides tested *in vitro* for compatibility with potential bioagent, mancozeb was found highly compatible with *Trichoderma harzianum*. An integrated management strategy was developed for collar rot of groundnut under glass house conditions. Thus the findings of present study is supported by the findings of Nandeesha *et al*.[18]. Kumar and Singh[19] conducted an experiment to determine *in vitro* and *in vivo* sensitivity of *T. viride* to chemical fungicides (hexaconazole, propiconazole, crossman, carbendazim and mancozeb) which are usually applied in cultivation of crops to reduce the severity of a number of plant pathogens. They reported that hexaconazole, propiconazole, crossman and carbendazim were not compatible with the *T. viride* at recommended dose or even at lower dosages. Whereas, mancozeb was found moderately compatible with *T. viride* at recommended dose (2000 ppm). Present findings also suggest mancozeb as compatible and propiconazole as incompatible with *Trichoderma harzianum*. Meena *et al*.[20] conducted an experiment to test the compatibility of 5 fungicides viz. Carbendazim Mancozeb Carboxin+Thiram Hexaconazole and Propiconazole I with *Trichoderma* spp. and found that mancozeb and carboxin+thiram were compatible. These findings also support the findings of present studies.

CONCLUSION

This study observed that Mancozeb was compatible with *Trichoderma harzianum* hence these two can be mixed together for seed treatment or spraying also. Thiram and Carboxin were also compatible with *Trichoderma harzianum* but Mancozeb should be preferred over these two. In the situation where Mancozeb is not required, then Thiram and Carboxin can also be mixed but Mancozeb should be preferred. Previously, carboxin have been in use for mixing with *Trichoderma harzianum* but this study suggested that Thiram is comparatively safer than carboxin for mixing with antagonist. Propiconazole should never be used for mixing with *Trichoderma harzianum*.

SIGNIFICANCE STATEMENT

This study discovers that Mancozeb was compatible with *Trichoderma harzianum* hence these two can be mixed together for seed treatment or spraying also. These findings can be beneficial for the agro industries involved in manufacturing of fungi toxicants and production of bio-control agents. This will also help the farmers by providing them an alternate method for minimizing chemical's use in agriculture. This study will help the researcher to uncover the critical areas of developing consortia of chemicals and micro-organists for plant disease management. Thus a new theory on integrated use of fungi toxicants and antagonist may be arrived at.

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