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Narender Kumar
C. S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

SK Singh
N. D. University of Agriculture and Technology, Faizabad Uttar Pradesh, India

Correspondence
Narender Kumar
C. S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Screening of tolerance and compatibility of *Trichoderma viride* against common fertilizers and fungicides

Narender Kumar and SK Singh

Abstract
In vitro toxicity and compatibility of commonly used fungicides like 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). Common fertilizers *i.e.*, urea, DAP, $ZnSO_4$, KCl and NH_4Cl are highly compatible with *T. viride*. Maximum population (5.33 cfu g^{-1} soil) of *T. viride* was recorded in untreated control followed by mancozeb (5.0 cfu g^{-1} soil), crossman (4.66 cfu g^{-1} soil), hexaconazole (4.33 cfu g^{-1} soil) and carbendazim (3.33 cfu g^{-1} soil) in fungicides treated pots at recommended doses after 30 days of inoculation. All these fungicides were found highly compatible with *T. viride*. Whereas, carbendazim was moderately compatible with *T. viride* at 30 and 60 days after inoculation. All above tested pesticides were found highly compatible with the *T. viride* at recommended dose after 90 days of inoculation. In present study is to determine *in vitro* and *in vivo* sensitivity of *T. viride* to chemical fungicides which are usually applied in cultivation of crops to reduce the severity of a number of plant pathogens.

Keywords: *Trichoderma*, fungicides, fertilizers

1. Introduction
Trichoderma are cosmopolitan in distribution and frequently present in all types of soil, manure and decaying plant materials (Alexander, 1961) [1]. *Trichoderma* spp. is strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers (Singh *et al.*, 2009) [17].

Trichoderma spp. is important in designing effective and safe management strategies. Many species of *Trichoderma* have multiple strategies for fungal antagonism and indirect effects on plant health (such as plant growth promotion and fertility improvements). Some strains are potent antibiotic producers and their suitability for use in biocontrol systems must be carefully assessed. *Trichoderma as* bioagent have evolved numerous mechanisms for both attacking other fungi and enhancing plant growth.

Trichoderma is used for better management of various foliar and soil borne plant pathogens. Rhizotonia blight of different plants is successfully managed by *T. harzianum* and *T. viride* with seed treatment and soil amendment (Mohanan, 1996) [14]. Seed treatment with *T. pseudokoningii* and *T. harzianum* reduce the seed mycoflora, enhance the germination and vigour in forest trees such as *Dendrocalamus striuctus, Phyllanthus embtica, Hardwickia binate* and *Dalbrgia latifolia.* These bio control agents (*T. pseudokoningii* and *T. harzianum*) have been shown to be superior over other treatments like chemical, physical and plant extract (Mamatha *et al.*, 2000) [13]. Some species of *Trichoderma* have been used widely as bio-control agents against root-knot nematode (*Meloidogyne javanica*).

Many potential bio control agents can't be moved from the experimental phase to a commercialization phase due to incompatibility with current production methods. Any bio control agent must be effective and compatible with modern agricultural practices so that its use can be integrated into the production system. The success of bio control agent depends on the clever blending of bio control agent with as fungicides. *Trichoderma* spp. is an eco-friendly and cheap fungal bio control agents used for suitable management of various foliar and soil borne plant pathogens (Khandelwal *et al.*, 2012) [11].

However, the success of bio control agents is dependent on its compatibility with other diseases management systems (Desai *et al.*, 2002) [6].

The integration of sub-lethal doses of compatible chemical fungicides with bio control agents which are resistant to relatively high doses of chemicals, is one of the most adoptive way to reducing the amount of fungicides as well as suitable management strategy for near future (Chet, 1987) [4].

The ability of *Trichoderma* spp. to control plant diseases by mycoparasitism and through production of wide range of antagonistic substances and its role in growth promoters has been known for many years (Harman *et al.*, 2004) [8].

Keeping above point in view for study of under taken as "Screening of tolerance and compatibility of *T. viride* against common fertilizers and fungicides" in the present investigation.

Materials and methods

Isolation of *Trichoderma* spp.

Trichoderma strain was isolated by serial dilution plate technique as described by Johnson and Curl (1972) [9]. *Trichoderma* species, the most commonly used isolation approach are direct techniques like soil dilutions and the growth of colonies on selective media (Askew and Laing, 1993; Davet and Rouxel, 2000; Papavizas and Lumsden, 1982) [2, 5, 15]. Soil sample (10g) from well pulverized, air dried soil was added into 90ml sterile water in a flask to make 1:10 dilution (10^{-1}). The mixture was vigorously shaken on a magnetic shaker for 20-30 minutes to obtain uniform suspension. One ml of suspension from flask was transferred into a test tube containing 9ml sterile water under aseptic condition to make 1:100 (10^{-2}) dilution. Further dilution of 10^{-3} was prepared by pipetting 1ml suspension in additional water. The Petri plates were incubated at 26±2^{0}C for 6 days in an incubator. As soon as the mycelial growth were visible in the culture medium, the hyphal tips from the advancing mycelium were cut and transferred into the culture slants containing PDA medium for further purification and identification of the isolates.

Purification of *T. viride*

The pure culture of *Trichoderma* spp. was obtained by adopting single spore technique. Individual colonies were picked up and transferred onto other slant containing PDA medium for further purification and identification of *Trichoderma*.

Identification of *Trichoderma* spp

The identity of the *Trichoderma* isolates was confirmed by both morphological and cultural characters. Colonies have key characteristics that can be used to identify them as Trichoderma, which includes growth pattern, growth rate, odour and colour (Gams and Bissett, 1998) [7]. These purified cultures were confirmed as *Trichoderma viride* on the basis of the following morphological and growth habit characters described by Rifai (1969) [16].

In vitro Compatibility of fungicides and fertilizers with *T. viride*

Tolerance to fungicides and fertilizers of *T. viride* was evaluated using poisoned food method. Fungicides *viz.*, mancozeb 75% WP (1500, 2000 and 2500 ppm); hexaconazole 5EC and propiconazole 25% EC (400, 500 and 600 ppm.); Crossman (500, 700 and 1000 ppm.) and carbendazim 50%WP (900, 1000 and 1100 ppm.) and fertilizers *viz.* Urea, DAP, ZnSo4 and KCl (50 and 100 ppm) were added to PDA medium. The concentrations of fungicides were selected as one lower and one upper doses of recommended dose. PDA medium without fungicides and fertilizers served as control. A five mm inoculum disc of *T. viride* was cut from the margin of actively growing colony and placed in centre of each Petri plate. Petri plates were incubated at room temperature. Five Petri plates were used for each treatment. Radial growth of *T. viride* was observed daily.

Compatibility of fungicides with *T. viride* in soil

The recommended dosages of fungicides *viz.*, Mancozeb (0.2%), Crossman (0.075%), Hexaconazole (0.05%) and Carbendazim (0.1%) were mixed in soil filled pots alongwith *T. viride.* Soil filled pots without fungicides served as control. Five Pots were used for each treatment. Population of *T. viride* was notice between treatments and control at 30, 60 and 90 days after inoculation.

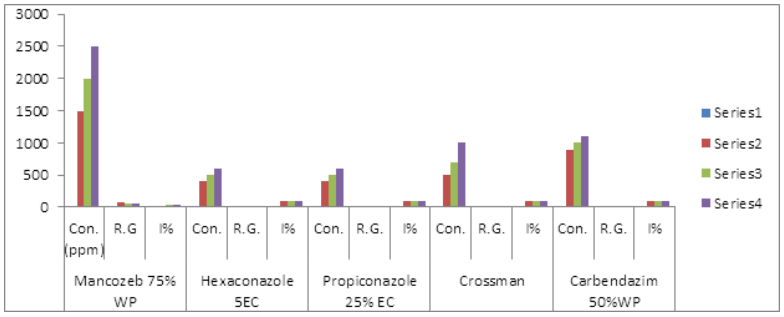
Results and discussion

In vitro toxicity study of commonly used fungicides showed that fungicides like hexaconazole, propiconazole, crossman and carbendazim were completely inhibited the mycileal growth of *T. viride* at recommended doses.

Table 1: *In vitro* effect of fungicides on radial growth of *T. viride* at 72 hrs

Mancozeb 75% WP			Hexaconazole 5EC			Propiconazole 25% EC			Crossman			Carbendazim 50%WP		
Con. (ppm)	R.G	I%	Con.	R.G.	I%	Con.	R.G.	I%	Con. (ppm)	R.G.	I%	Con.(ppm)	R.G.	I%
1500	71.66	20.37	400	00.00	100.00	400	00.00	100.00	500	00.00	100.00	900	00.00	100.00
2000	61.66	31.48	500	00.00	100.00	500	00.00	100.00	700	00.00	100.00	1000	00.00	100.00
2500	50.33	44.07	600	00.00	100.00	600	00.00	100.00	1000	00.00	100.00	1100	00.00	100.00
Check	90.00	-	Check	90.0	-	Check	90.00	-	Check	90.00	-	Check	90.00	-
SEM±	0.77	-	SEM ±	0.47	-	SEM±	0.50	-	SEM±	0.39	-	SEM±	0.29	
CD at 5%	2.37	-	CD at 5%	1.41	-	CD at 5%	1.50	-	CD at 5%	1.24	-	CD at 5%	0.85	

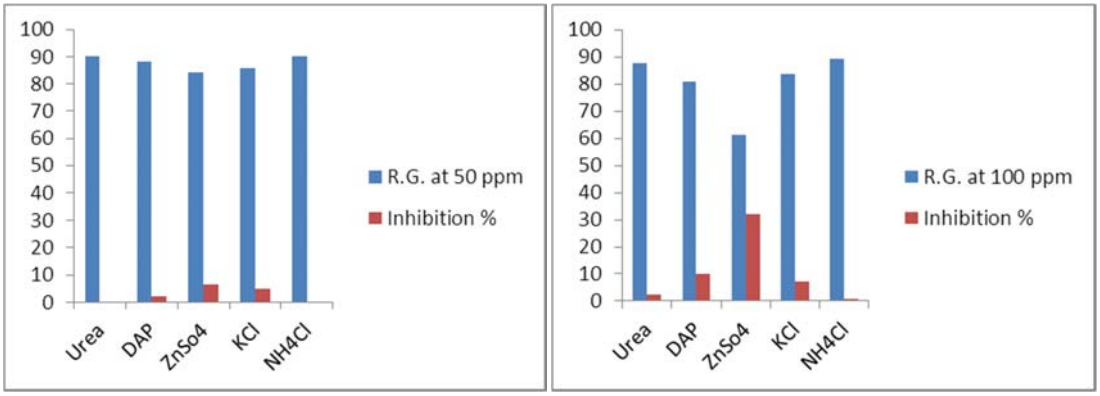
Con. = Concentrations, R.G. = Radial Growth, I% = Inhibition per cent



These fungicides are not compatible with the *T. viride* at recommended dose or even at lower dosages. Whereas, mancozeb was inhibited 31.48% mycelial growth at 2000 ppm concentration (recommended dose). Hence, it is moderately compatible with *T. viride*. Madhavi *et al.* (2011) [12] evaluated *in vitro* compatibility of *T. viride* with 25 pesticides. *T. viride* showed a high compatibility
the mancozeb followed by tebuconazole. *T. viride* is totally incompatible with systemic fungicides like carbendazim, hexaconazole, tebuconazole and propiconazole. *T. viride* was fully compatible with mancozeb at the concentrations of 0.025, 0.05, 0.1 and 0.2 per cent (Singh *et al.*, 2012) [18].
At the end of results is revealed that minimum radial growth (61.33 mm) and maximum inhibition per cent (31.86%) was recorded at 100 ppm concentration of $ZnSo_4$ followed by DAP (81.00 mm, 10.00%), KCl (83.67 mm, 07.03%), urea (87.67 mm, 02.59%) and NH_4Cl (89.33 mm, 00.74%), respectively as compared to control. All fertilizers are highly compatible with *T. viride*. Bhai and Thomas (2010) [3] also found the compatibility with *T. harzianum.*

Table 2: *In vitro* effect of fertilizers on radial growth of *T. viride* at 72 hrs.

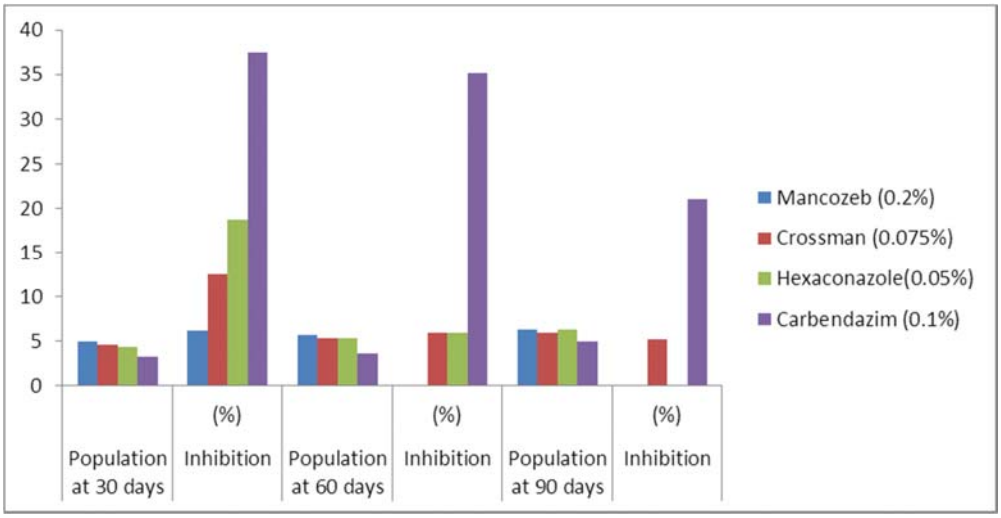
Fertilizers	Radial Growth (mm)			
	50ppm		100ppm	
	R. G.	Inhibition%	R. G.	Inhibition%
Urea	90.00	00.00	87.67	02.59
DAP	88.00	02.22	81.00	10.00
$ZnSo_4$	84.00	06.67	61.33	31.86
KCl	85.67	04.81	83.67	07.03
NH_4Cl	90.00	00.00	89.33	00.74
Check	90.00	-	90.00	-
SE(m)	-	0.040	-	0.038
CD at 5%	-	0.124	-	0.119



Maximum inhibition (37.5%) in population of *T. viride* was recorded in carbendazim treated pots followed by hexaconazole, Crossman, mancozeb and acetamiprid which were 18.7%, 12.5%, 06.91%, 06.19% and 06.1% respectively at 30 days after inoculation. The same trends in population of *T. viride* were also recorded at 60 and 90 days after inoculation.

Table 3: Effect of fungicides on population (g^{-1} soil) of *T. viride* at 30, 60 and 90 days after inoculation

Fungicides	Population at 30 days	Inhibition (%)	Population at 60 days	Inhibition (%)	Population at 90 days	Inhibition(%)
Mancozeb (0.2%)	5.00	6.19	5.67	00.0	6.33	00.00
Crossman (0.075%)	4.66	12.5	5.33	06.0	6.00	05.21
Hexaconazole (0.05%)	4.33	18.7	5.33	06.0	6.33	00.00
Carbendazim (0.1%)	3.33	37.5	3.67	35.2	5.00	21.01
Check	5.33	-	5.67	-	6.33	-
SEM±	0.49	-	0.43	-	0.39	-
CD at 5%	1.46	-	1.28	-	1.16	-



The overall results of pot experiment showed that there are no significant differences in population level of *T. viride* between fungicides treated soil at recommended doses and control except carbendazim treatment. All treatments were significantly at par to each other.

Similar trends were also recorded after 60 and 90 days after inoculation. It was interesting to note that population of *T. viride* in pesticides treated soil. No effects of fungicides were recorded in population of *T. viride* after 90 days in all the treatments except carbendazim. Thus, it is very clear that the population of *T. viride* increases in fungicides treated pots as well as in control with an increase in time.

Toxicity and compatibility study of mancozeb, hexaconazole and Crossman are highly compatible with *T. viride*. Whereas, carbendazim was moderately compatible with *T. viride* at 30 and 60 days after inoculation. All these fungicides are highly compatible with the *T. viride* at recommended dose after 90 days of inoculation. Bhai and Thomas (2010) [3] also recorded compatibility of *T. harzianum* with chemicals. The same results were also reported by Khan (2007) [10].

The combined use of bio-control agents and fungicides has attracted much attention in successful crop production to combat the pest. The success of bio agents is dependent on its compatibility with other disease management system (Desai *et al*., 2002) [6].

Conclusion

Chemical based strategies among the various strategies used in agriculture have been so far dominating. Use of synthetic chemicals has led to the emergence of several problems like environmental pollution, residual effect in grain and killing of non-target organism(s). Development of resistant strains of plant pathogens are serious problem of diseases management, increase due to the application of only pesticide strategies for plant diseases management. To minimize the chemicals related problems, *Trichoderma* is a best biological weapon for crop protection. *Trichoderma* spp. has been an exceptionally good bio-control agent as well as growth promotor because it is ubiquitous easy to isolate and grow rapidly on many substrates, affects wide range of plant pathogens and compatible with some agrochemicals. The combined use of bio-control agents and pesticides has attracted much attention in successful crop production to combat the pest. The success of bio control agents is dependent on its compatibility with other disease management system. However, the compatibility of *Trichoderma* to chemicals needs confirmation before its use in sustainable agriculture.

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