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Compatibility of *Trichoderma* spp. with fungicides and efficiency against *Rhizoctonia solani*

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Abstract
Laboratory experiment was conducted to test the compatibility of *Trichoderma,* a potential bio-control agent with commonly recommended fungicides *viz*; three systemic fungicides carbendazim (50% WP), carboxin (75% WP) and tebuconazole (2% DS) and one contact fungicide copper oxychloride (50% WP) by poison food technique (50,100, 250, 500, 1000 and 1500 ppm). These fungicides were also used to check efficacy against *R. solani* causing agent of okra damping off. All four potential bioagents showed compatibility with copper oxychloride but *Trichoderma* sp.-2 also showed highest compatibility as 55.33 per cent inhibition was observed at 1500 ppm followed by *Trichoderma* sp.-9 in which 64.57 per cent was observed. Carbendazim was found best fungicide as it gave complete inhibition of fungal pathogen even at 250 ppm concentration. Among four fungicides tested *in vitro* carbendazim was most effective against *R. solani* but incompatible with bioagents while copper oxychloride was found compatible with bioagents.

Keywords: Okra, damping off, *Rhizoctonia solani*, fungicides, compatibility, fungal inhibition

Introduction
Chemical control is necessary with many diseases at present but are undesirable and even inadequate as a long-term solution to crop and human health. Now a days, Plant disease control through use of biological control agents is continuous process. During the past several years, some notable successes of disease control were achieved through introduction of microorganisms in the laboratory, glass house and fields. It is now widely recognized that biological control of plant pathogen is a distinct possibility for future and can be successfully exploited in the modern agriculture especially within the frame work of integrated pest management system. Some soil-borne root infecting fungi are difficult to eradicate because they produce resting structure like sclerotia, chlamydospores or oospores for their survival for a longer period of time under adverse environmental conditions. Use of fungicides for the control of soil borne diseases are costly and also produce environment and health hazards to men and also adversely affect the beneficial microorganisms in soil. This has devastated the effect of plant pathologist towards alternate methods in developing biological control. Integral use of microbial antagonists with reduced dose of chemical fungicides has shown promising results. Integration of biocontrol agents and commonly used fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists individually.

Material and Methods
In vitro screening of *Trichoderma* spp. and *Rhizoctonia solani* for compatibility with fungicides
Three systemic fungicides viz., carbendazim (Bavistin 50% WP), carboxin (Vitavax 75% WP), tebuconazole (Raxil 2% DS) and one non-systemic fungicides viz., copper oxychloride (Blitox 50% WP) were tested at different concentrations against four potential *Trichoderma* isolates which exhibited significantly high inhibition of pathogen and okra damping off causing pathogen, *R. solani* by poisoned food technique. Double strength potato dextrose agar medium was prepared in distilled water and sterilized in an autoclave at 1.05 kg/ cm^2 pressure (121.6 °C) for 20 min. A series of different concentrations viz; 50, 100, 250, 500, 1000 and 1500 ppm

of each fungicide, as per commercial formulation, were prepared separately and added to the medium aseptically before pouring. In check, sterilized water added instead of fungicide solution to double strength medium. Plates with medium were then inoculated with mycelial disc (5 mm) from culture of *Trichoderma* spp. and *R. solani*. The observations on radial growth of *Trichoderma* spp. was recorded after 5 days of incubation. The per cent inhibition in each treatment was calculated as per the formula given by Vincent (1947) [5].

$$I(\%) = \frac{C - T}{C} x\, 100$$

Where;
I (%) = Per cent mycelial inhibition
C = Mycelial growth in control
T = Mycelial growth in treatment

Results and Discussion

Efficacy of different fungicides against *Rhizoctonia solani*

Three systemic fungicides *viz.*, carbendazim (50% WP), carboxin (75% WP) and tebuconazole (2% DS) and contact fungicide copper oxychloride (50% WP) were tested *in vitro* (each @ 50,100, 250, 500, 1000 and 1500) by poison food technique and the data for their effects on the radial growth of *R. solani* shown in table 1 and plate 1.

Table 1: Evaluation of different fungicides against *Rhizoctonia solani*

Fungicide	Per cent mycelial inhibition (concentration in ppm formulation basis)					
	50	100	250	500	1000	1500
Carbendazim (50% WP)	0.00 (1.00) *	73.78 (8.65)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Carboxin (75% WP)	43.11 (6.64)	54.22 (7.43)	74.67 (8.70)	90.67 (9.57)	100.00 (10.05)	100.00 (10.05)
Copper oxychloride (50% WP)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	45.56 (6.82)	68.00 (8.31)	90.57 (9.57)
Tebuconazole (2% DS)	24.67 (5.08)	44.00 (6.71)	55.11 (7.49)	55.78 (7.54)	85.33 (9.29)	100.00 (10.05)
Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
C.D.(p=0.05)	0.212	0.092	0.132	0.111	0.060	0.052

* The figures in parentheses are square root transformed values

The data indicated that all the fungicides inhibited mycelial growth of *R. solani* to varying degree. Carbendazim was the best fungicide as it gave complete inhibition of fungal growth at 250 ppm concentration. However, carboxin and tebuconazole gave cent per cent inhibition at 1000 and 1500 ppm, respectively. Copper oxychloride was found to be less effective at lower concentration at 50 to 250 ppm and gave no inhibition, but deleterious effect increases as concentration increase and gave 45.56, 68.00 and 90.57 per cent inhibition at 500, 1000 and 1500 ppm, respectively. Meena and Chattopadhyay (2002) [3] also reported that carbendazim inhibited *R. solani* even at 50 ppm concentration and proved most effective. Recently, Kadri and Kanzaria (2017) [1] also evaluated *in vitro* efficacy of systemic and non-systemic fungicide against *R. solani* Kühn. They reported that among systemic fungicides, carbendazim (50% WP) gave 82.21, 83.24, 84.49 and 85.41 per cent inhibition followed by tebuconazole (25.9% EC) with 73.29, 81.42, 83.24 and 88.88 per cent inhibition of mycelia at 50, 100, 250 and 500 ppm concentrations, respectively. Copper oxychloride (50% WP) was found second best effective fungicide with 72.47 and 78.98 per cent inhibition at 1500 and 2000 ppm, respectively.

Compatibility of potential bioagents with different fungicides

Three systemic fungicides *viz.*, carbendazim (50% WP), carboxin (75% WP) and tebuconazole (2% DS) and contact fungicide copper oxychloride (50% WP) found effective against pathogen were tested *in vitro* (each @ 50,100, 250, 500, 1000 and 1500) by poison food technique for their effects on potential *Trichoderma* bioagents and data has between presented in table 2, 3, 4 and 5.

Table 2: Mycelial growth inhibition (%) of *Trichoderma* sp.-2 by different fungicides

Fungicide	Per cent mycelial inhibition (concentration in ppm formulation basis)					
	50	100	250	500	1000	1500
Carbendazim (50% WP)	100.00 (10.05) *	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Carboxin (75% WP)	6.89 (2.81)	22.89 (4.89)	41.11 (6.49)	55.33 (7.51)	81.56 (9.09)	100.00 (10.05)
Copper oxychloride (50% WP)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	51.33 (7.23)	55.33 (7.51)
Tebuconazole (2% DS)	69.56 (8.40)	87.78 (9.42)	90.67 (9.57)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD(p=0.05)	0.109	0.081	0.047	0.037	0.056	0.037

* The figures in parentheses are square root transformed values

Results in the table 2 showed that the growth of *Trichoderma* sp.-2 was completely inhibited at all concentrations at 50 to 1500 ppm when media amended with carbendazim. The antagonist isolate showed active growth on the medium containing tebuconazole at 50, 100, 250 ppm, while no growth was observed at 500 ppm concentration and above. With carboxin gradual mycelial inhibition ranging from 6.89 to 81.56 per cent was observed between 50 to 1000 ppm however, complete inhibition was observed at 1500 ppm. Copper oxychloride showed 0.00 to 555.33 per cent inhibition between 50 to 1500 ppm.

Table 3: Mycelial growth inhibition (%) of *Trichoderma* sp.-6 by different fungicides

Fungicide	Per cent mycelial inhibition (concentration in ppm formulation basis)					
	50	100	250	500	1000	1500
Carbendazim (50% WP)	100.00 (10.05) *	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Carboxin (75% WP)	9.33 (3.21)	24.40 (5.00)	42.00 (6.56)	56.89 (7.61)	77.56 (8.86)	100.00 (10.05)
Copper oxychloride (50% WP)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Tebuconazole (2% DS)	3.78 (2.17)	34.22 (5.93)	61.11 (7.90)	94.67 (9.78)	100.00 (10.05)	100.00 (10.05)
Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD(p=0.05)	0.208	0.105	0.072	0.063	0.031	0.000

* The figures in parentheses are square root transformed values

The data on effect of four fungicides on radial growth of *Trichoderma* sp.-6 revealed that Carbendazim was found totally incompatible with antagonist even at low concentration of 50 ppm (Table 3). The mycelial inhibition was observed with tebuconazole upto 500 ppm in range from 3.78 to 94.67 per cent but growth was completely inhibited at 1000 ppm. *Trichoderma* grow easily at 50 to 1000 ppm concentration of carboxin and inhibition range from 9.33 to 77.56 per cent while no growth was recorded at 1500 ppm. The radial growth was observed cent per cent at 50,100 and 250 ppm with copper oxychloride However, its growth was completely inhibited at 500 ppm.

Table 4: Mycelial growth inhibition (%) of *Trichoderma* sp.-9 by different fungicides'

Fungicide	Per cent mycelial inhibition (concentration in ppm formulation basis)					
	50	100	250	500	1000	1500
Carbendazim (50% WP)	100.00 (10.05) *	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Carboxin (75% WP)	31.33 (5.69)	50.89 (7.20)	68.00 (8.31)	88.00 (9.43)	100.00 (10.05)	100.00 (10.05)
Copper oxychloride (50% WP)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	64.67 (8.10)
Tebuconazole (2% DS)	48.67 (7.05)	69.11 (8.37)	88.89 (9.48)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD(p=0.05)	0.078	0.051	0.041	0.029	0.000	0.053

* The figures in parentheses are square root transformed values

Results (Table 4) illustrated that carbendazim had inhibitory effect on growth of *Trichoderma* sp.-9 from 50 to 1500 ppm. Tebuconazole was found sensitive to antagonist as complete growth inhibition was recorded at 500 ppm. Carboxin at 50 to 500 ppm gave up to 88.00 per cent inhibition while complete inhibition was observed at 1000 ppm. The lower concentration of copper oxychloride did not affect the growth of *Trichoderma* as concentration increased growth was reduced and at higher concentration at 1500 ppm growth was inhibited upto 64.67 per cent.

Table 5: Mycelial growth inhibition (%) of *Trichoderma* sp.-11 by different fungicides

Fungicide	Percent mycelial inhibition (concentration in ppm formulation basis)					
	50	100	250	500	1000	1500
Carbendazim (50% WP)	100.00 (10.05) *	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Carboxin (75% WP)	0.00 (1.00)	8.00 (3.00)	36.89 (6.16)	73.56 (8.63)	100.00 (10.05)	100.00 (10.05)
Copper oxychloride (50% WP)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	12.44 (3.67)	68.22 (8.32)	78.89 (8.94)
Tebuconazole (2% DS)	53.78 (7.40)	66.22 (8.20)	88.67 (9.47)	100.00 (10.05)	100.00 (10.05)	100.00 (10.05)
Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD(p=0.05)	0.051	0.104	0.075	0.082	0.045	0.026

* The figures in parentheses are square root transformed values

Data in table 5 revealed that carbendazim showed complete inhibition of mycelial growth of *Trichoderma* sp.-11 even at 50 ppm. Tebuconazole inhibited mycelial growth in range from 53.78 to 88.67 per cent at 50 to 250 ppm but growth was completely inhibited at 500 ppm. Carboxin inhibited growth upto 73.56 per cent at 500 ppm and complete inhibition was found at 1000 ppm. Though increased concentration of the copper oxychloride reduced the radial growth, but 78.89 per cent *Trichoderma* was inhibited at 1500 ppm.

In general, all four potential bioagents showed compatibility with copper oxychloride being maximum with *Trichoderma* sp.-2 which showed highest compatibility i.e. 55.33 per cent inhibition was observed at 1500 ppm followed by *Trichoderma* sp.-9 in which 64.57 per cent was observed. The results of present investigations are almost in accordance with Madhusudhan *et al*. (2010) [2] who had evaluated *in vitro* compatibility between two *T. viride* isolates (T2 and T4) with six fungicides *viz*., carbendazim (50% WP), propiconazole (25% EC), hexaconazole (5% EC), tridemorph (80% EC), chlorothalonil (75% WP) and mancozeb (75% WP) and reported that carbendazim showed cent per cent inhibition even at 50 ppm.

In the study of Ranganathswamy *et al*. (2012) [4], *T. virens* and *T. harzianum* were also less incompatible to copper oxychloride, than the results of present study. However, they had tested at higher concentration (0.3%).

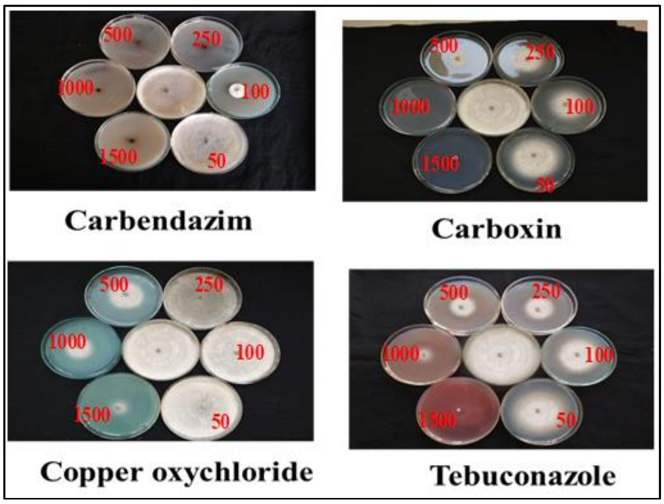

Plate 1: Efficacy of different fungicides against *Rhizoctonia solani*

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