International Journal of Plant Pathology and Microbiology

E-ISSN: 2789-3073
P-ISSN: 2789-3065
www.plantpathologyjournal.com
IJPPM 2024; 4(2): 127-130
Received: 17-09-2024
Accepted: 22-10-2024

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In vitro evaluation of compatibility of *Trichoderma asperellum* with systemic and non-systemic fungicides

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Abstract
Indiscriminate use of pesticides causes detrimental effect on the all forms of life and also disturbed ecological balance of environment. *Trichoderma* emerged as powerful biocontrol agents against soil borne pathogens. *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producer of spores and antibiotics. Therefore, a study was conducted to evaluate compatibility of *Trichoderma asperellum* with commonly used seven systemic fungicides (@ 500, 1000 and 1500 ppm) and seven non systemic fungicides (@ 1500, 2000 and 2500 ppm). The systemic fungicides, Propiconazole, Tebuconazole, Difenoconazole and Carbendazim caused cent per cent (100%) inhibition of *Trichoderma asperellum* and were found incompatible at 500, 1000 and 1500 ppm concentrations. The non systemic fungicides *viz.*, Sulphur, Zineb and Chlorothalonil were found compatible with *T. asperellum* at 1500, 2000 and 2500 ppm concentrations. Propineb was found compatible with *T. asperellum* at 1500 and 2000 ppm concentrations and incompatible at 2500 ppm concentrations. *T. asperellum* was found incompatible with Mancozeb, Copper oxy chloride and Copper hydroxide at all three concentrations.

Keywords: *Trichoderma asperellum,* fungicides, compatibility, inhibition, concentrations

Introduction
Agriculture is facing the destructive activities of numerous plant pathogens from an early time, which leads to the reduction in yield of the crops as well as the aesthetic value (Bastakoti *et al*., 2017) [1]. The plant diseases are one of the major concern not only in the cultivation of field crops but also in loss of farm produce. There is a pressing need to manage diseases to make sure a steady and constant supply of marketable produce for the increasing world population (Sharma *et al*., 2014) [7]. The use of biological control agents for the management of soil borne diseases has been increased widely. The full expression of potential biocontrol is considered in terms of rhizosphere competence, suppression of pathogens, tolerance to pesticides, competitive saprophytic ability and adaptability to environment etc. (Vasundara *et al*., 2015) [9]. A fungi of the genus *Trichoderma* have emerged as most powerful biological protectants for management of soil borne plant diseases (Maurya *et al.*, 2020) [5]. The success and adaptability of *Trichoderma* strains as BCA is due to their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms (Benitez *et al*., 2004) [2]. In an effective Integrated Disease Management, combining antagonists with synthetic or non-synthetic chemicals eliminates the chance of development of resistance within serious pests, reduce the chemical applications and maintain the balance of natural ecosystem (Singh *et al.*, 2021; Kumar *et al.*, 2018) [8, 3]. Therefore, it is required to find the compatible antagonist with commonly used agrochemicals in the management of plant diseases having no deleterious effect on the native bio-control agents (Maheshwary *et al.*, 2020) [4]. Considering the above issue the present study was undertaken.

Materials and Methods
Standard Poisoned Food Technique was followed to assay the compatibility of *Trichoderma asperellum* with the systemic fungicides (@ 500, 1000 & 1500 ppm) and non-systemic fungicides (@ 1500, 2000 & 2500 ppm) (Nene and Thapliyal, 1993) [6]. Details of fungicides are mentioned in Table 1. Based on active ingredient, requisite quantity of the test fungicides was calculated, amended separately and mixed with autoclaved and cooled PDA medium in

conical flask to obtain desired concentrations. This PDA amended with the test fungicides was poured (20 ml / plate) aseptically in Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicides and its test concentrations, three plates / treatment were maintained. After solidification of the PDA medium, all the plates were inoculated separately and aseptically by placing 5 mm mycelial disc in the center obtained from actively growing 7 days old pure culture of *T. asperellum* with the help of flame sterilized cork borer and incubated at temperature of 28±1 ^{0}C. Petri plates filled with plain PDA (Without any fungicides) and inoculated separately with *T. asperellum* was maintained as untreated control.

Treatment Details
Design: CRD
Treatments: Eight
Replications: Three

Table 1: List of systemic and non-systemic fungicides.

Tr. No.	Systemic fungicides	Trade Name	Non systemic fungicides	Trade Name
T_1	Propiconazole 25% EC	Tilt	Copper oxychloride 50% WP	Blitox
T_2	Tebuconazole 25.9% EC	Folicur	Copper hydroxide 53.8% w/w DF	Dupont Kocide 2000
T_3	Difenoconazole 25% EC	Score	Sulphur 80% WDG	Sulphaboost
T_4	Metalaxyl 35% WS	Rampart	Chlorothalonil 75% WP	Kavach
T_5	Thiophanate methyl 70% WP	Roko	Propineb 70% WP	Antracol
T_6	Pyraclostrobin 20% WG	Headline	Zineb 75% WP	Indofil Z-78
T_7	Carbendazim 50% WP	Bavistin	Mancozeb 75% WP	Indofil M-45
T_0	Untreated control	-------	------	------

Observations on radial mycelial growth / colony diameter of the *Trichoderma asperellum* was recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of *Trichoderma asperellum* with the test fungicides over the untreated control was calculated by using the formula given by Vincent, 1927 [10].

$$\text{Per cent inhibition} = \frac{C - T}{C} 100$$

Where,
C = Growth of the test fungus in untreated control plates.
T = Growth of the test fungus in treated plates.

Results and Discussion

Compatibility with systemic fungicides

Radial mycelial growth

The results (Table 2, Plate I) describes that, at 500 ppm concentration, the treatment T_4 (Metalaxyl), T_6 (Pyraclostrobin) and T_5 (Thiophanate methyl) allowed significant mycelial growth of *T. asperellum* in decreasing pattern. The treatment T_4 (Metalaxyl) showed 34.50 mm mycelial growth followed by T_6 (Pyraclostrobin) (17.00 mm) and T_5 (Thiophanate methyl) (11.50 mm). At 1000 and 1500 ppm concentrations, the treatment T_6 (Pyraclostrobin) favoured more mycelial growth of *T. asperellum* than T_4 (Metalaxyl) and T_5 (Thiophanate methyl). The treatment T_6 (Pyraclostrobin) allowed 15.83 and 12.50 mm mycelial growth of *T. asperellum* followed by T_4 (Metalaxyl) (12.50 and 06.00 mm) and T_5 (Thiophanate methyl) (10.00 and 07.66 mm), respectively. The treatment T_1 (Propiconazole), T_2 (Tebuconazole), T_3 (Difenoconazole) and T_7 (Carbendazim) showed no mycelial growth (00.00 mm) of *T. asperellum* and were statistically at par with each other and statistically significant over rest of the treatments and untreated control at all the three concentrations.

Per cent inhibition

The results (Table 2) revealed that, at 500, 1000 and 1500 ppm concentrations, the treatment T_4 (Metalaxyl) inhibited 61.66, 86.10 and 93.36% mycelial growth of *T. asperellum*, respectively. This was followed by treatment T_6 (Pyraclostrobin) which recorded 81.29, 84.18 and 86.11% inhibition, respectively. The treatment T_5 (Thiophanate methyl) showed 87.21, 88.88 and 91.47% inhibition of *T. asperellum*, which was highest as compared to treatments T_4 and T_6, indicating it's incompatibility with *T. asperellum*. The treatments T_4 (Metalaxyl), T_6 (Pyraclostrobin) and T_5 (Thiophanate methyl) tested against *T. asperellum* showed more than 50% mycelial growth inhibition at all three concentrations and found incompatible with *T. asperellum*. The treatment T_3 (Difenoconazole) completely restricted the mycelial growth and showed cent per cent inhibition (100%) of *T. asperellum* and were found completely incompatible.

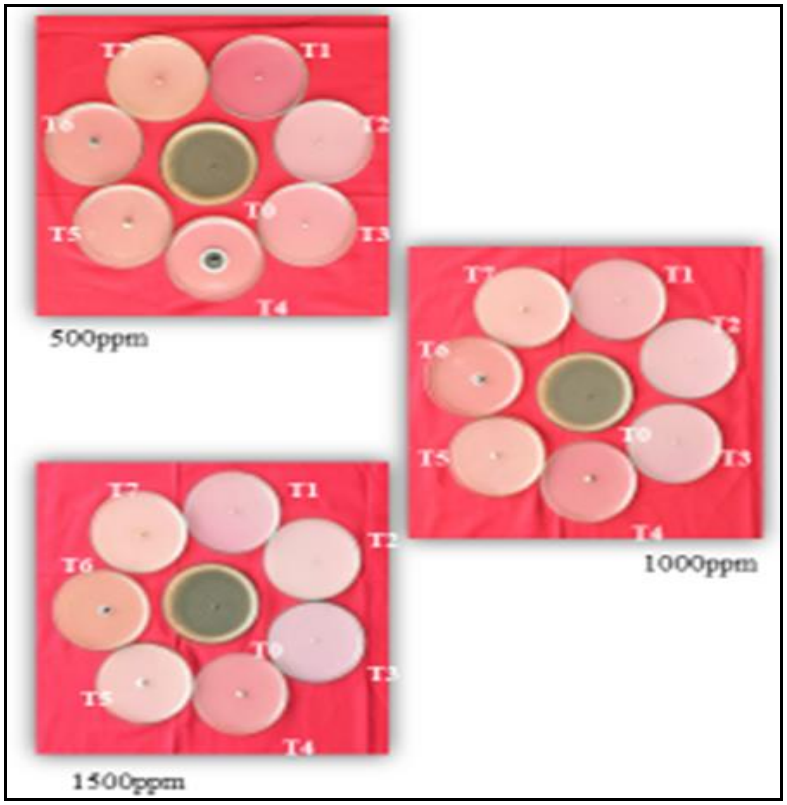
Compatibility with non-systemic fungicides

Radial mycelial growth

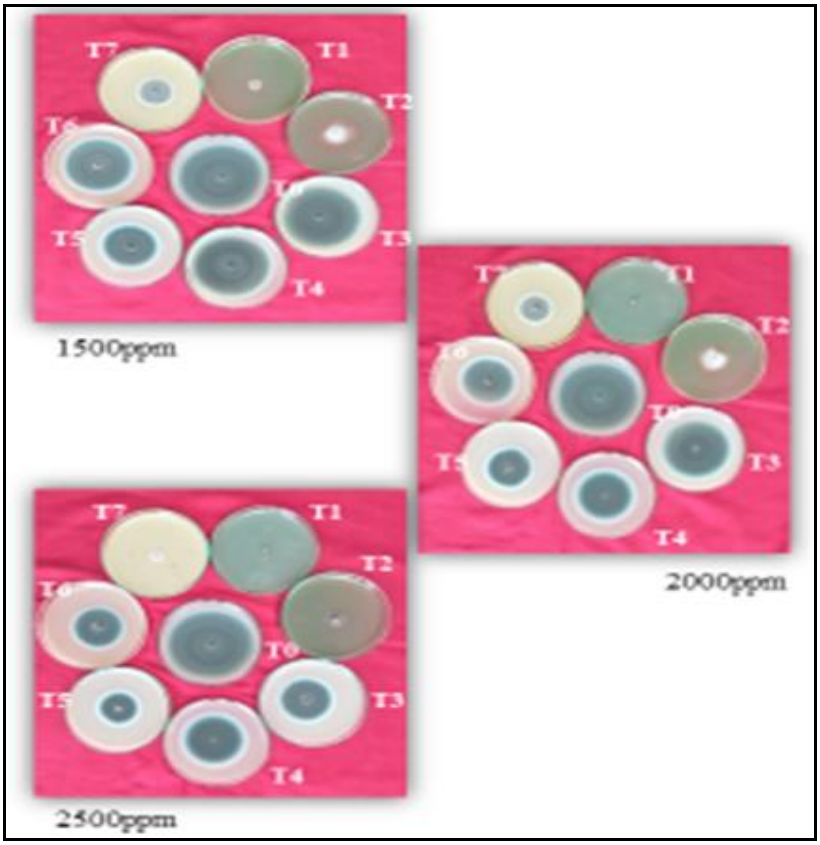
The results (Table 3, Plate I) revealed that, at 1500, 2000 and 2500 ppm concentrations, the treatment T_3 (Sulphur) favoured highest mycelial growth of *T. asperellum* and found safer. Treatment T_3 (Sulphur) showed 80.50, 77.16 and 56.66 mm mycelial growth of *T. asperellum*. It showed least growth at higher concentration as compared to lower concentration. This was followed by treatment T_4 (Chlorothalonil) which allowed 77.83, 70.33 and 66.50 mm mycelial growth of *T. asperellum* with decreasing pattern with increasing concentration. The treatment T_6 (Zineb) and T_5 (Propineb) showed similar trend as that of treatment T_3 (Sulphur) and T_4 (Chlorothalonil) and allowed 71.50 62.50 and 54.50 mm and 60.50, 51.66 and 43.50 mm mycelial growth of *T. asperellum* at all three concentrations tested, respectively. Whereas, the treatment T_2 (Copper hydroxide) and T_7 (Mancozeb) showed moderate mycelial growth of *T. asperellum*. T_2 (Copper hydroxide) allowed 38.66, 33.33 and 24.50 mm mycelial growth followed by T_7 (Mancozeb) which showed 36.50, 35.83 and 13.16 mm mycelial growth of *T. asperellum* at 1500, 2000 and 2500 ppm concentrations, respectively. Treatment T_1 (Copper oxy chloride), at 1500 ppm concentration, showed significant inhibitory effect on the mycelial growth of *T. asperellum* (12.50 mm) and completely restricted the mycelial growth (00.00 mm) at 2000 and 2500 ppm concentrations.

Per cent inhibition: The results (Table 3) revealed that, at 1500, 2000 and 2500 ppm, the treatment T_3 (Sulphur)

(10.55, 14.25 and 37.03%), T_4 (Chlorothalonil) (13.51, 21.84 and 26.10%), T_6 (Zineb) (20.55, 30.92 and 39.44%) and T_5 (Propineb) (32.77, 42.59 and 51.66%, respectively) were found compatible with *T. asperellum* and showed least mycelial inhibition. The treatment T_2 (Copper hydroxide) (57.03, 62.58 and 72.77%), T_7 (Mancozeb) (59.44, 60.55 and 85.36%) and T_1 (Copper oxy chloride) (86.11, 100.00 and100.00%) recorded highest mycelial growth inhibition as compared to other treatments and found incompatible with *T. asperellum*.



Effect of systemic fungicides on growth of *T. asperellum*



Effect of non systemic fungicides on growth of *T. asperellum*

Plate 1: Radial mycelial growth of *Trichoderma asperellum* under systemic and non systemic fungicides, showing inhibition patterns at different concentrations compared to control.

Table 2: Effect of systemic fungicides on *Trichoderma asperellum.*

Tr. No.	Treatment / Conc. (ppm)	Radial mycelial growth (mm)*			Per cent inhibition (%)		
		500	1000	1500	500	1000	1500
T_1	Propiconazole	00.00	00.00	00.00	100.00 (90.00)#	100.00 (90.00)	100.00 (90.00)
T_2	Tebuconazole	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	Difenoconazole	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_4	Metalaxyl	34.50	12.50	06.00	61.66 (51.72)	86.10 (68.10)	93.36 (75.08)
T_5	Thiophanate methyl	11.50	10.00	07.66	87.21 (69.02)	88.88 (70.49)	91.47 (72.99)
T_6	Pyraclostrobin	17.00	15.83	12.50	81.29 (64.35)	84.18 (66.63)	86.11 (68.10)
T_7	Carbendazim	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_0	Untreated control	90.00	90.00	90.00	00.00 (0.00)	00.00 (0.00)	00.00 (0.00)
	S. E. (m) ±	0.33	0.35	0.29	0.39	0.79	0.33
	C. D at 1%	1.01	1.07	0.88	1.19	2.39	1.00

*Mean of three replications
#Figures in parenthesis are angular transformed values

Table 3: Effect of non systemic fungicides on *Trichoderma asperellum.*

Tr. No.	Treatment/Conc. (ppm)	Radial mycelial growth (mm)*			Per cent inhibition (%)		
		1500	2000	2500	1500	2000	2500
T_1	Copper oxychloride	12.50	00.00	00.00	86.11 (59.49)#	100.00 (90.00)	100.00 (90.00)
T_2	Copper hydroxide	38.66	33.33	24.50	57.03 (49.02)	62.58 (52.26)	72.77 (58.52)
T_3	Sulphur	80.50	77.17	56.66	10.55 (18.87)	14.25 (22.16)	37.03 (37.46)
T_4	Chlorothalonil	77.83	70.33	66.50	13.51 (21.53)	21.84 (27.85)	26.10 (30.71)
T_5	Propineb	60.50	51.66	43.50	32.77 (34.93)	42.59 (40.72)	51.66 (45.93)
T_6	Zineb	71.50	62.50	54.50	20.55 (26.94)	30.92 (33.77)	39.44 (38.87)
T_7	Mancozeb	36.50	35.83	13.16	59.44 (50.42)	60.55 (51.07)	85.36 (67.48)
T_0	Untreated control	90.00	90.00	90.00	00.00 (0.00)	00.00 (0.00)	00.00 (0.00)
	S. E. (m) ±	0.65	0.34	0.77	0.72	0.41	0.86
	C. D at 1%	1.95	1.04	2.32	2.17	1.25	2.58

*Mean of three replications
#Figures in parenthesis are angular transformed values

Conclusion

All the systemic fungicides tested against *Trichoderma asperellum* were found incompatible at 500, 1000 and 1500 ppm concentrations. Among the non systemic fungicides, Sulphur, Zineb and Chlorothalonil were found compatible with *T. asperellum* at 1500, 2000 and 2500 ppm concentrations. Propineb was found compatible with *T. asperellum* at 1500 and 2000 ppm concentrations and incompatible at 2500 ppm concentrations. *T. asperellum* was found incompatible with Mancozeb, Copper oxy chloride and Copper hydroxide at all three concentrations.

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