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Compatibility study of fungal and bacterial bio control agents with agro chemicals

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Abstract
Fungicides/combination of fungicides, insecticides and herbicides were tested for their compatibility with *Trichoderma harzianum* and *Pseudomonas fluorescens* under *in vitro*. Carbendazim @ 50, 100, 250 and 500 ppm, and copper oxychloride @ 1000, 1500, 2000 and 2500 ppm concentrations, completely inhibited growth of *T. harzianum*. Methyl-o-demeton at all the four concentrations i.e. @ 250, 500, 1000 and 1500 ppm were found incompatible with 94.44% growth inhibition. However, chlorpyrifos and cartap hydrochloride @ 250 and 500 ppm were moderately compatible. Quizalofop-ethyl at 500, 100, 1500 and 2000 ppm produced incompatible reaction with 94.44% growth inhibition of fungal bioagent *T. harzianum*. At initial two lowest concentrations, pendimethalin @ 500 and 1000 ppm and fenoxyprop-p-ethyl @ 50 and 100 ppm found compatible with *T. harzianum*. All the fungicides/combination of fungicides, insecticides and herbicides tested at all their concentrations were completely compatible with *Pseudomonas fluorescens* isolate-1.

Keywords: Trichoderma, pseudomonas, carbendazim, copper oxychloride, mancozeb + carbendazim, methyl -o – demeton, cartap hydrochloride, chloropyrifos, quizalofop, pendimethalin, fenoxyprop – p – ethyl, compatibility

Introduction
Notable success of disease control with the use of antagonistic microorganism in the laboratory, glass house and field have been achieved during past several years and based on the information, there is a possibility of developing biological control of plant disease under field condition. The commercial formulations of different bio-control agents is already available in the market. However, inadequate information on the performance of the antagonists under varying conditions is a major constraint in the large scale adoption of this technology. There has been growing interest in combining biocontrol fungi with fungicides and information on this kind of integrated control is accumulating more rapidly than on other combination of control components (Lewis and Papavizas, 1991) [6]. A novel blending technique has been reported in which the biocontrol agents were used simultaneously with seed dressing fungicides without toxic effect on antagonist (Papavizas and Lumsden, 1980). Integrated seed treatment with chemicals and compatible antagonists not only protects the seed and seedlings from soil - borne infection but also provides protection from seed borne inoculum. Compatible fungicides are therefore essential for integrated management (Dubey and Patel, 2001) [3]. Considering this, present study was ndertaken to find out the compatibility of agrochemicals with *T. harzianum* and *P. fluorescens* (isolate-1) with a view to formulate an integrated approach.

Materials and Methods
The effect of nine agro chemicals as per table 1, on the growth of *T. harzianum* was studied using poisoned food technique (Mayer, 1962) [7]. Potato dextrose agar medium without any of the agrochemicals was served as control. Colony diameter of the test organism in each treatment along with control was measured (mm) and recorded after every 24 hours, till the test organism occupied the full Petri plate in the control. To determine the compatibility of *P. fluorescens* with same agrochemicals, 25 to 50,000 µg/ml concentrations were prepared in double distilled water. Double strength nutrient agar was used as medium for both the bacteria. 20 ml of nutrient agar containing desired concentration was poured in Petri plates and left over night to observe contamination, if any. Thereafter, sterilized paper discs dipped in overnight cultures was utilized to inoculate the plates. The per cent inhibition of growth over control was calculated by Vincent's formula (1947) [17].

$$I = \frac{C - T}{C} X\ 100$$

Where,
I = Per cent inhibition of mycelium growth
C = Radial growth of bio agents in control
T = Radial growth of bio agents in treatment

Table 1: Compatibility of agrochemicals with bio agent (*in vitro*)

Sr. No.	Technical/active ingredient	Concentration in (ppm)			
		1	2	3	4
1.	Mancozeb 50% + Carbendazim 25% (75% WP)	50	100	250	500
2.	Copper oxychloride 50% WP	1000	1500	2000	2500
3.	Carbendazim 50% WP	50	100	250	500
4.	Methyl -o – demeton 25% EC	250	500	1000	1500
5.	Cartap hydrochloride 50% SP	250	500	1000	1500
6.	Chloropyrifos 50% EC	250	500	1000	1500
7.	Quizalofop – ethyl 5% EC	500	1000	1500	2000
8.	Pendimethalin 30% EC	500	1000	1500	2000
9.	Fenoxyprop – p – ethyl 9.3% EC	50	100	250	500
10.	Control				

Results and Discussion

The data in Table 2 revealed that out of three fungicides evaluated for compatibility, carbendazim @ 50, 100, 250 and 500 ppm and copper oxychloride @ 1000, 1500, 2000 and 2500 ppm concentrations, more or less inhibited growth of *T. harzianum*. Combination of mancozeb 50% + carbendazim 25% @ 50 and 100 ppm concentration was found to be compatible with *T. harzianum*, however this combination at 250 and 500 ppm concentration was not compatible with promising bioagent *T. harzianum*.

Table 2: Compatibility of promising agrochemicals with promising fungal bioagent *Trichoderma harzianum* (*in vitro*)

Agrochemicals and Concentrations (ppm)	Per cent growth inhibition				
	1	2	3	4	Mean
Copper oxychloride @1000, 1500, 2000 and 2500	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)
Carbendazim @50, 100, 250 and 500	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)
Mancozeb 50% + Carbendazim 25% @50, 100, 250 and 500	0.00 (0.00)	0.00 (0.00)	76.37 (94.44)	76.37 (94.44)	38.18 (38.21)
Methyl-o-demeton @ 250, 500, 1000 and 1500	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)
Chlorpyrifos @ 250, 500, 1000 and 1500	50.81 (60.06)	55.25 (67.50)	58.91 (73.34)	65.35 (82.61)	57.58 (71.26)
Cartap hydrochloride @ 250, 500, 1000 and 1500	57.88 (71.73)	61.72 (77.55)	64.44 (81.39)	71.50 (89.93)	63.88 (80.62)
Quizalofop-ethyl @ 500, 1000, 1500 and 2000	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)	76.37 (94.44)
Pendimethalin @ 500, 1000, 1500 and 2000	55.76 (68.34)	62.98 (79.37)	63.05 (79.46)	75.92 (94.08)	64.43 (81.37)
Fenoxyprop-p-ethyl @ 50, 100, 250 and 500	48.94 (56.86)	66.64 (84.28)	71.72 (90.16)	71.76 (90.20)	64.76 (81.82)
Mean	51.88 (61.90)	55.48 (67.88)	63.73 (80.41	66.63 (84.27)	
	Agrochemicals (A)		**Conc. (C)**		**A x C**
S.Em. ±	0.53		0.18		1.06
C. D. at 5%	1.50		0.51		2.99
C.V.%	3.10				

Values outside parenthesis are arcsine transformed and inside the parentheses, are re-transformed values

Among the three insecticides evaluated for compatibility, methyl-o-demeton at all the four concentrations i.e. @ 250, 500, 1000 and 1500 ppm were found incompatible with promising fungal biocontrol agent *T. harzianum* with 94.44% growth inhibition. However, chlorpyrifos and cartap hydrochloride at their lower concentrations i.e. @ 250 and 500 ppm were moderately compatible with *T. harzianum*, but with increased concentrations i.e. @ 1000 and 1500 ppm, they also found incompatible with fungal bio control agent *T. harzianum* showing inhibition up to 89.93%. Under same progression, out of three herbicides found promising under previous study, quizalofop-ethyl at all four concentrations i.e. 500, 1000, 1500 and 2000 ppm produced incompatible reaction with *T. harzianum* showing 94.44% growth inhibition of fungal bioagent *T. harzianum*. Pendimethalin and fenoxyprop-p-ethyl at their initial two lowest concentrations found moderately compatible with *T. harzianum*, but showed incompatible response with increase in concentrations with 94.08% growth inhibition of *T. harzianum*.

Ramarethinam *et al.* (2001) [14] reported that mancozeb 75% WP and copper oxychloride w/w each @ 100 ppm and 500 ppm did not inhibit *T. viride*. However, copper oxychloride @ 1000 ppm completely inhibited its growth. Fungicides carbendazim 50% WP, hexaconazole 5% EC, propiconazole 25% EC and a weedicide metalachlor 50% EC completely inhibited growth of *T. viride* even @ 100 ppm. Desai and Kulkarni (2004) [2] reported cent per cent growth inhibition of *T. harzianum* with alachlor, carbendazim, chlorpyrifos, glyphosate and thiram. Lal and Maharshi (2007) [5] reported that carbendazim and thiophanate methyl each @ 500 ppm, completely inhibited mycelial growth of both of the test bioagents, *T. harzianum* and *T. viride*, but thiram and streptocycline showed less toxicity and better compatibility. Imidacloprid, endosulfan and chlorpyrifos were less compatible with the test bioagents. Pendimethalin, fluchloralin and oxyfluorfen reduced the growth of both bioagents, but pendimethalin was most toxic. Rakholiya (2010) [13] also found that carbendazim, propiconazole, chlorothalonil, zineb and carbendazim + mancozeb inhibited cent per cent mycelial growth of *T. harzianum*. These finding are in corroboration with Saravanan *et al*. (2014) [15], Mohammadi (2015) [8] and Pranab Dutta *et al*. (2017) [12].

The data in Table 3 clearly indicates that out of three fungicides evaluated for compatibility, all fungicides/combination of fungicides at all their concentrations found completely compatible with *Pseudomonas fluorescens* isolate-1. The highest radial growth inhibition 8.89% of *Pseudomonas fluorescens* isolate-1 at all the concentrations was observed in the treatment of copper oxychloride. Among the three insecticides evaluated for compatibility, methyl-o-demeton at all the four concentrations i.e. @ 250, 500, 1000 and 1500 ppm were found compatible with promising bacterial bio control agent *Pseudomonas fluorescens* isolate-1 with maximum growth inhibition of 7.78% by cartap hydrochloride. Same progression was also observed in case of herbicides, all the three molecules at their four concentrations tested, produced compatible reaction with *Pseudomonas fluorescens* isolate-1 showing 7.13% maximum growth inhibition by fenoxyprop-p-ethyl.

P. fluorescens was found to be more tolerant to fungicides than fungi and this might be due to the reason that some bacteria might use pesticides as nutrient source and hence can tolerate higher concentrations of chemicals (Aislabie and Jones, 1995) [1]. According to Shahida *et al.* (2010) [16], *P. fluorescens* was compatible with potassium phosphonate, but incompatible with BM. Keshgond and Naik (2013) [4] have noticed that carbendazim had minimum inhibition when mancozeb and captan showed complete inhibition of *P. fluorescens*. Mohiddin and Khan (2013) [9] found that *P. fluorescens* was compatible with fungicides like thiram, mancozeb, captan and carbendazim. A study by Pandian *et al*. (2013) [10] had proved that *P. fluorescens* was compatible with lower concentration of copper hydroxide.

Table 3: Compatibility of promising agrochemicals with promising bacterial bio agent *Pseudomonas fluorescens* isolate-1 (*in vitro*)

Agrochemicals and their concentrations in ppm	Per cent growth inhibition				
	1	2	3	4	Mean
Copper oxychloride @1000, 1500, 2000 and 2500	17.5 (8.89)	17.5 (8.89)	17.5 (8.89)	17.5 (8.89)	17.5 (8.89)
Carbendazim @50, 100, 250 and 500	15.95 (7.55)	15.95 (7.55)	15.95 (7.55)	15.95 (7.55)	15.95 (7.55)
Mancozeb 50% + Carbendazim 25% @50, 100, 250 and 500	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)
Methyl-o-demeton @ 250, 500, 1000 and 1500	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)
Chlorpyrifos @ 250, 500, 1000 and 1500	14.30 (6.11)	14.30 (6.11)	14.30 (6.11)	14.30 (6.11)	14.30 (6.11)
Cartap hydrochloride @ 250, 500, 1000 and 1500	16.20 (7.78)	16.20 (7.78)	16.20 (7.78)	16.20 (7.78)	16.20 (7.78)
Quizalofop-ethyl @ 500, 1000, 1500 and 2000	14.31 (6.11)	14.31 (6.11)	14.31 (6.11)	14.31 (6.11)	14.31 (6.11)
Pendimethalin @ 500, 1000, 1500 and 2000	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)	13.64 (5.56)
Fenoxyprop-p-ethyl @ 50, 100, 250 and 500	15.49 (7.13)	15.49 (7.13)	15.49 (7.13)	15.49 (7.13)	15.49 (7.13)
Mean	13.45 (5.41)	13.45 (5.41)	13.45 (5.41)	13.45 (5.41)	13.45 (5.41)
	Agrochemicals (A)	Conc. (C)	A x C		
S. Em. ±	0.17	0.06	0.35		
C. D. at 5%	0.49	0.17	NS		
C.V.%	4.52				

Values outside parenthesis are arcsine transformed and inside the parentheses, are re-transformed values

Conclusion

On the basis of this investigation we can conclude that fungal bio control agent under the study are cannot be applied to crops along with fungicides or insecticides or herbicides. While bacterial bio control agent was found compatible with agrochemicals.

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