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COMPATIBILITY OF TRICHODERMA ATROVIRIDE WITH FUNGICIDES AGAINST BLACK ROT DISEASE OF TEA: AN IN VITRO STUDY

ROMILA THOUDAM*
B.K.DUTTA**

*Microbial & Agricultural Ecology & Biodiversity Conservation laboratory, Dept. of Ecology and Environmental Science, Assam University, Silchar, India
**Microbial & Agricultural Ecology & Biodiversity Conservation laboratory, Dept. of Ecology and Environmental Science, Assam University, Silchar, India

ABSTRACT

The potential of seven fungicides against Corticium theae, causal pathogen of Black rot disease of tea was evaluated under in vitro condition. The potential of the biocontrol agent isolated from the phyllosphere of tea and tea environment against the pathogen was evaluated under in vitro condition. To be integrated into a management system, the biocontrol agent Trichoderma atroviride must be compatible with other management practices. For managing black rot disease of tea through integrated approach, studies were conducted on the compatibility of the biocontrol agent, T. atroviride with the fungicides. In vitro evaluation of the seven fungicides against C.theae revealed that Tetraconazole was the most effective in suppressing the radial growth of the pathogen followed by Tebuconazole. The effectiveness of the fungicides increased with the increase in their concentration and it was found to be statistically significant. On the other hand, T.atroviride was found to be highly compatible with all the seven fungicides evaluated, except Carbendazim. Percentage inhibition of mycelial growth among the concentrations and incubation period differed significantly. Present investigation suggests that compatible fungicides can be used with Trichoderma in an IDM package simultaneously to control plant pathogens of tea (i.e. Corticium theae).

KEYWORDS: Biocontrol Agent, Compatibility, IDM, in Vitro, Phyllosphere

INTRODUCTION

New generation fungicides are most frequently used in crops as seed treatment, soil drenching and foliar sprays. To develop an effective disease management programme, compatibility of potential bioagents with fungicides is essential. The combination of biocontrol agents with fungicides would provide similar disease suppression as achieved with fungicide use (Monte, 2001). It would eliminate the chance of resistance development and would reduce the fungicide application. Fungicide resistance problems, concerns regarding pesticide residues and revocation of registration of certain widely used fungicides have led to increased activity in the development of bioagents against the plant pathogens. It is therefore,

proposed to identify the compatibility of the potential bioagents with commonly used fungicides for the eco-friendly management of the tea diseases. Since fungicides should have inhibitory effect on the pathogen but should not have deleterious effect on the antagonists, an understanding of the effect of fungicides on the pathogen and the antagonists would provide information for the selection of fungicides and fungicide resistant antagonists, through compatibility studies in vitro. In addition, this strategy may display even better control of resistant strains of fungal pathogens and may help the commercial growers to reduce the amount of fungicide use, thus lowering the amount of chemical residue in the marketed products. Combined applications of BCAs followed by small quantities of fungicides may help the antagonists and the relative cost of the formulations (Lima et al., 2008).

Keeping the above in view, the present work was undertaken to observe the compatibility of different fungicides with the bio control Agent (BCA) i.e. Trichoderma atroviride in vitro.

Materials and methods

In vitro chemical screening: The effect of various fungicides on the growth of the pathogen in vitro was studied by poisoned food technique (Adam and Wong, 1991). Different concentration of fungicides was prepared by dissolving the requisite quantity of each fungicide in warm potato dextrose agar medium before autoclaving. After autoclaving the medium was then dispensed uniformly into 90 mm diameter petriplates and inoculated at the centre with the 2 mm mycelial discs of the pathogen taken from 7 day old culture. Pathogen inoculated in unamended medium served as control. Each treatment was maintained in triplicate. The inoculated plates were incubated at 28 ± 2ºC for 3 days and the diameter of the fungal colony was measured by measuring the two opposite circumference of the colony growth at 3 days interval for 15 days.

Antagonism studies

To ascertain whether antagonism existed between the test fungi and the pathogen, the standard dual culture method was employed. A 4 mm disc of the antagonistic fungi from 7 days old culture plate was placed in the petridishes containing sterile Potato dextrose agar medium at 2 cm apart from the pathogen. Three replicates were prepared for each fungus. Respective controls were also made without the test fungi. All the plates were separately incubated at 25 ± 1 ^{0} C for 7 days and the antagonistic colony interaction were examined thereafter.

Compatibility of fungicides with biocontrol agents

The native antagonistic fungal isolates from tea agroecosystem phyllosphere /atmosphere was identified as Trichoderma atroviride (8927.12) by the Indian type culture collection, New Delhi. They were tested for their compatibility with the fungicides through poisoned food technique (Adam and Wong, 1991). Different concentrations of fungicides were prepared by dissolving the requisite quantity of each fungicide in warm potato dextrose agar medium, before autoclaving. After autoclaving the medium was then dispensed uniformly into 90 mm diameter petriplates and inoculated at the centre with the 2 mm mycelial discs of the pathogen taken from 7 days old culture. Antagonists inoculated in unamended medium served as control. The experiment was maintained in triplicate. The plates were incubated at 28±2ºC for 3 days and diameter of the fungal colony was determined by measuring the two opposite circumference of the colony growth at 3 days interval for 15 days.

Results and Discussion:

TABLE 1: The inhibitory effect of the fungicides on the radial growth of Corticium theae in vitro.

TREATMENT	NO OF DAYS				
Tebuconazole	3rd day	6th day	9th day	12th day	15th day
Control	21.33±0.01	47.66±1.22	65.33±0.78	87.33±0.19	89.33±0.51
1 ppm	17±0.89	19±0	25.8±2.17	31.5±1.52	35.09±1.47
10 ppm	14.1±0.75	15±0.49	19.3±0.96	24.6±1.5	31.66±2.28
50 ppm	11.33±0.98	12.3±0.85	18.3±2.42	24.6±1.25	28.5±2.29
100 ppm	9.8±1.96	11.4±1.02	12.6±1.05	18.5±2.29	20±1.75
F-test	13.65	11.22	25.1	3.5	177.18
Carbendazim					
1 ppm	29±1.44	34±1.38	41.6±2.33	42.5±1.81	45.02±2.33
10 ppm	18.3±1.96	20.3±0.9	34±1.38	36.3±2.33	38.24±1.07
50 ppm	17±1.15	20.3±0.51	21±0.68	32±2.17	35.72±1.15
100 ppm	14±0.76	17.6±0.51	20.33±0.60	24.3±0.9	29.66±1
F-test	17.44	8.78	11.39	40.64	62.14
Mancozeb					
1 ppm	28±0.28	33.33±1.73	37.66±0.38	41±0.86	45±0.76
10 ppm	19±1.8	21.66±1.26	25.33±0.76	28.33±1.85	32.66±0.1
50 ppm	17±0.76	19±1.04	23.66±0.76	28.33±1.85	32.66±0.1
100 ppm	15±0.76	18.25±0.38	22.32±0.96	25.33±0.33	28.66±0.57
F-test	20.99	7.44	20.59	541.79	669.24
Captan					
1 ppm	19±0.86	22.66±0.38	29.76±2.3	31±0.86	35.66±1.54
10 ppm	17.33±0.96	18.63±0.57	25.66±2.11	28±1.6	31.33±1.54
50 ppm	11.66±1.07	18.6±2.36	24.33±2.87	27.66±1.54	30.67±1.73
100 ppm	9.33±1.64	15.66±1.73	21.66±2.11	28.33±0.96	25±1.44
F-test	18.15	7.94	16.44	515.4	348.01
Carbendazim+Mancozeb					
1 ppm	24±1.15	30.66±1.15	37±2	37.12±2	40.66±1.52
10 ppm	20±2.3	28±1.52	29.33±0.57	39.66±0.57	42.33±0.57
50 ppm	14.33±1.54	20.33±1.83	23.66±0.57	25.33±0.38	38.66±4.5
100 ppm	10.33±0.5	11±0.28	22.3±2	28±2.3	32.66±1.15
F-test	14.28	8.62	18.11	323.12	107.47
Sulphur+copper					

1 ppm	23.66±2.08	28.32±2.22	35.33±2.45	37±2.3	39±1.73
10 ppm	20±2.5	26.33±1.95	29.66±1.15	35±1.73	40.22±2.24
50 ppm	19.22±1.28	22.23±1.28	28.33±1.73	32±0.57	36±1.15
100 ppm	13.66±1.15	14.33±1.15	15±0.86	26.23±1.02	29.66±1.15
F-test	4.54	6.82	19.7	316.25	264.45
Tetraconazole					
1 ppm	20.66(±0.66)	29(±3.04)	36(±2.56)	39.3(±1.85)	45.33(±1.67)
10 ppm	23.83(±2.3)	23.83(±2.3)	28.83(±1.73)	33.16(±1.24)	50.33(±3.07)
50 ppm	14.83(±1.52)	18.16(±1.15)	21.33(±0.38)	24.83(±1.15)	29.5(±1.15)
100 ppm	10.33(±0.38)	14.83(±1.15)	15.83(±1.15)	16(±1.15)	17.55(±1.15)
F-test	15.48	7.09	21.26	502.95	244.78

Data are the mean of three replicates for each concentration. Significant at P> 0.05 level of significance

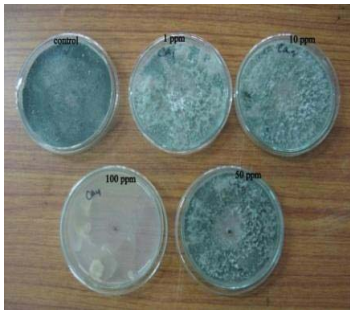
Table 2: In vitro antagonism of fungal spp. against Corticium theae

Sl.no	Test mycoflora	Control (mm)	Interaction(mm)	% growth inhibition of Corticium theae
1.	Trichoderma atroviride Karsten	81.6(±0.49)	22.8(±0.18)	72.05

TABLE 3: Observations on the compatibility of T.atroviride with fungicides in vitro.

TREATMENT	NO OF DAYS				
Tebuconazole	3rd day	6th day	9th day	12th day	15th day
Control	90	90	90	90	90
1 ppm	64±0.9	74±0.81	90	90	90
10 ppm	61±2.65	67±0.73	90	90	90
50 ppm	57.3±0.78	61.6±0.68	71.6±0.68	90	90
100 ppm	53.3±0.62	59±0.56	65±0.45	90	90
F-test	4.3	3.82	10.9	-	-
Carbendazim					
1 ppm	46.6±0.08	56.6±0.08	68.6±0.08	88.3±0.16	90
10 ppm	0	0	0	0	0
50 ppm	0	0	0	0	0
100 ppm	0	0	0	0	0
F-test	10514.28	11264.28	12503.71	4294	-
Mancozeb					
1 ppm	90	90	90	90	90
10 ppm	81.1±0.44	83.5±0.24	88±0.15	90	90
50 ppm	78.3±0.16	80.6±0.24	86±0.15	90	90
100 ppm	71±1.87	73.6±1.91	76.3±2.05	90	90
F-test	12.345	13.66	19.94	-	-
Captan					
1 ppm	90	90	90	90	90
10 ppm	90	90	90	90	90
50 ppm	90	90	90	90	90
100 ppm	90	90	90	90	90
F-test	-	-	-	-	-
Carbendazim + Mancozeb					

1 ppm	79±0.2	85±0.17	90	90	90
10 ppm	0	0	0	0	0
50 ppm	0	0	0	0	0
100 ppm	0	0	0	0	0
F-test	2696.5	3833.33	-	-	-
Sulphur + Copper					
1 ppm	74±0.5	90	90	90	90
10 ppm	68±0.25	69.6±0.24	75.3±0.31	90	90
50 ppm	65.3±0.26	68.3±0.31	71±0.17	90	90
100 ppm	53.6±0.23	57.6±0.12	64±0.2	90	90
F-test	20.023	48.41	38.7	-	-
Tetraconazole					
1 ppm	74±0.23	80.6±0.16	87.6±0.14	90	90
10 ppm	69±0.26	73±0.28	78.6±0.23	90	90
50 ppm	64.3±0.29	70.3±0.18	74.3±0.2	90	90
100 ppm	61.3±0.13	62.3±0.23	6.95±0.05	90	90
F-test	27.8	27.68	32.67	-	-



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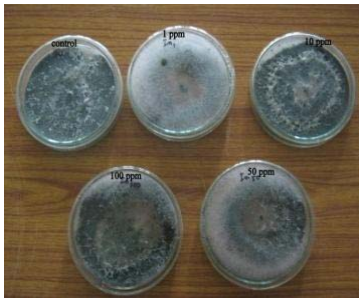

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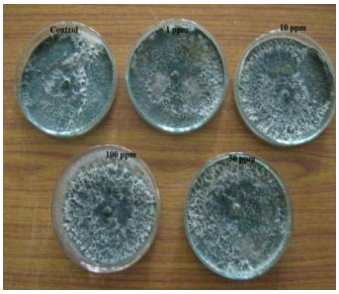

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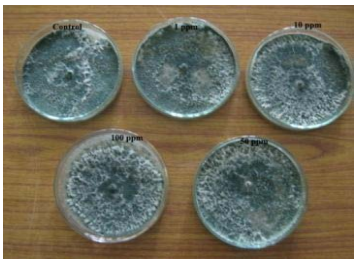

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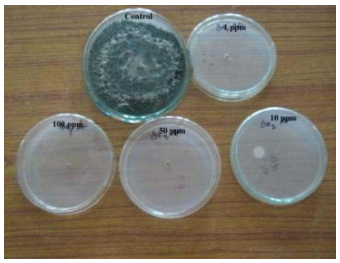

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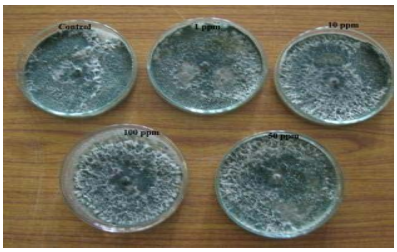
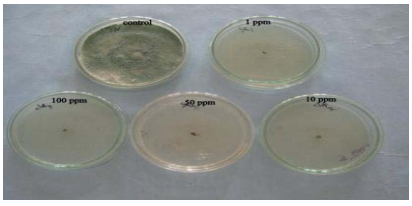


Sulphur + Copper



Carbendazim + Mancozeb

Plate 1: Compatibility of the tested fungicides with the antagonist Trichoderma atroviride

In vitro studies indicated the suppressive effect of various fungicides on the growth of the mycelium of Corticium theae (Table 1). Suppressive effect of various fungicides was expressed as percent inhibition of the radial growth of the fungus. It can be seen that the mycelial growth of Corticium theae was inhibited on the PDA containing petriplates amended with the different concentration of fungicides as compared to control. Three days after inoculation free growth of the pathogen was observed in the control plates (21.33 mm). The plates containing PDA amended with seven fungicides separately, in respective treatments caused significant reduction in the radial growth of the pathogen. The inhibitory effect varied with the different fungicides tested. Among the tested fungicides, Tetraconazole was found to be the most effective in suppressing the radial growth of the pathogen followed by Tebuconazole. The effectiveness of the fungicides increased with the increase in their concentration and this was found to be statistically significant. The potential of the chemicals increased in the ascending order Tetraconazole > Tebuconazole > Captan > Mancozeb > Carbendazim > Sulphur and Copper > Carbendazim and Mancozeb.

The result in table 2 shows the inhibition of the radial growth of the pathogen C. theae with T.atroviride when grown in dual culture. T.atroviride inhibited the growth of the pathogen, C. theae upto 77.33 %.

The compatibility test of different fungicides with the antagonists isolated from the tea phyllosphere and air of the tea plantation area was done in vitro. The experiment was set up to find out whether the antagonistic fungi are resistant /compatible with the test fungicides or not. The radial mycelial growth of Trichoderma atroviride was 100 % inhibited in all the concentrations used (i.e. 1, 10, 50 and 100 ppm), when grown in PDA amended with Carbendazim (Bavistin) and Carbendazim + Mancozeb (Saaf). However, there was luxuriant growth of the antagonist occupying the entire area of the petriplate (i.e. 90 mm) at 1 ppm concentration of PDA amended with Captan (Captaf). It can also be seen that with the increasing number of days and with the increase in the concentration of the fungicides, there was gradual increase in the mycelia growth. Captan (Captaf) was found to be highly compatible with T.atroviride indicating that this chemical can be sprayed integrating with the biocontrol agent to control the leaf disease (i.e. black rot). Domarck (Tetraconazole), Folicur (Tebuconazole), Sulphur + Copper (Suco) and Mancozeb (Indofil M-45) were also found to be compatible with T.atroviride. Carbendazim (Bavistin) was highly compatible with T.

atroviride at 1 ppm concentration, while at 10, 50 and 100 ppm concentrations; there was complete inhibition of the mycelial growth of the antagonist (table 3).

Since, fungicides should have deleterious effect on the pathogen but not to the antagonists, an understanding of the effect of fungicides on the pathogen and the antagonist would provide information on the selection of fungicides and fungicide resistant antagonists for compatibility studies (Malathi et al., 2002). The idea of combining biocontrol agents (BCA) with fungicides is for the establishment of desired microbes in the rhizosphere (Papavizas and Lewis, 1981) and on the phyllosphere. Further, the antagonism of BCA was influenced by the addition of fungicides (Kay and Stewart, 1994; Naar and Kecskes, 1999). Earlier reports suggest that biocontrol agents that can tolerate a certain level of fungicides were mixed with agrochemicals, resulting in eradication of the diseases (De Cal et al., 1994).

In the present investigation it was observed that A. flavus and T.atroviride was highly compatible with all the test fungicides (i.e. Tebuconazole, Tetraconazole, Sulphur + Copper, Carbendazim + Mancozeb, Mancozeb, Captan , Copper Oxychloride and Hexaconazole) except Carbendazim (Table 10.1 and 10.3). In this case Carbendazim at 1 ppm concentration, on the 15th day of inoculation the antagonist occupied the whole area of the petriplate but there was complete inhibition of its growth at 10, 50 and 100 ppm concentration. Results on the susceptibility of Trichoderma to carbendazim supports the earlier findings on the benzimidazole compounds (Ortiz et al., 1996; Viji et al., 1997; Naar and Kecskes, 1999; Silva et al., 1999). Similarly Gowdar et al., (2006) reported maximum inhibition (100%) of Trichoderma sp with carbendazim @ 0.1 and 0.2 per cent concentration at 24 hrs incubation followed by 96.88 and 88.44 per cent inhibition at 24 hrs with thiophanate methyl @ 0.1 and 0.2 per cent concentration, respectively. The minimum inhibition (00.00%) of the test fungus was observed with captan @ 0.1 and 0.2 percent concentration at 48 and 72 hrs. and at 72 hrs. in thiram @ 0.1 per cent concentration. Bagwan (2010) reported that thiram, copper oxychloride and Mancozeb at 0.2 % are compatible with T. harzianum and T. viride.Tapwal et al., (2012) also reported compatibility of Trichoderma sp with Dithane, Bavistin and Ridomil at any level of selected concentration i.e. 50 ppm, 100 ppm, 200 ppm, 300 ppm and highly insensitive to blue copper and captaf. However, Vyas (1994) stated that under pot culture studies Carbendazim gave an additive effect with Trichoderma viride and T. harzianum when applied as soil drench against Rhizoctonia bataticola causing dry root rot of soybean. Compatibility studies on the thiophanatemethyl with Trichoderma revealed that the fungicide at lower concentration improved the antagonistic potential of Trichoderma spp.,

which might be due to weakening of the pathogen by the fungicide. Viji et al., (1997) reported that application of fungicide may metabolically weaken the pathogen and make it vulnerable to potent antagonists. The antagonistic potential of biocontrol agents is expressed in terms of enhanced mode of action as increased hyperparasitism activity. Papavizas (1985) observed that application of biocides in sub-lethal doses, Trichoderma spp are known to proliferate and produce antibiotics in soil. Kay and Stewart (1994) and Naar and Kecskes (1998) also reported that the tolerant biotypes exhibited greater antagonism with the addition of fungicides. Deepthi (2013) reported that the Trichoderma isolate GRHF4 was more compatible with Mancozeb followed by copper oxychloride. Similar results were also obtained by Vijayaraghavan and Abraham (2004). They observed that mancozeb was compatible with Trichoderma sp. Similar results were also observed by Sarkar et al., 2010, who reported Copper oxychloride and copper hydroxide to be highly compatibile with T. harzianum.

Conclusion

Since biocontrol agents cannot manage the disease completely when large scale infection is already established in the field, farmers favoured fungicides for managing the crop diseases. But fungicides are deleterious to the environment and also harmful for the soil, productivity and human and animal health. Due to the disadvantages of fungicides, integrated disease management programs (1 ppm) are recommended, in which judicious use of fungicides and their integration with biocontrol agents is favoured. Since fungicides may have deleterious effect on the pathogen as well as on the antagonists, an understanding of the effect of fungicides on the pathogen and antagonists, would provide information on the selection of selective fungicides and fungicides resistant antagonists for compatibility studies as has been suggested in the present paper.

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