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Studies on potential of biocontrol agents in improving the soil health through absorption of pesticide residue from soil

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The prills with biocontrol agent have a great credibility to act as pesticide filter when combined with different pesticides like Bordeaux mixture (BM), Carbendazim and Nicotine. 0.2% Bordeaux mixture, different concentration of Carbendazim and Nicotine were added in column separately containing pelletized formulation of *Trichoderma harzianum* and control prills (without any biocontrol agent) for two days. Ten ml samples were collected from all these column on 3rd and 7th day in case of BM and every week in case of carbendazim and nicotine and concentration of pesticide were analysed by UV absorption spectroscopy. The elluents collected were studied against the spore germination of *P. parasitica* (P21, P22) and *C. capsici* (DK1, DK2) and observed that the spore germination of the pathogen is reduced in active prills of *Trichoderma harzianum* in comparison to the dummy prills (without *Trichoderma harzianum*). The results thus indicated that application of biocontrol agent reduces toxicity of pesticides by absorbing pesticides from soil and in turn improves the soil health.

Key words : Soil health, fungicides, *Trichoderma harzianum*, prills, *Phytophthora parasitica, Colletotrichum capsici*

INTRODUCTION

The genus *Trichoderma* is known to include several potentially promising hyperparasites/antibiotic producers that have promise against a large number of soil borne plant pathogens. In integrated disease management *Trichoderma* is also used in combination with other fungicides. The fungicide residue in soil and with repeated application may increase the residual toxicity of soil i.e., application of Bordeaux mixture as soil drenching increase the copper toxicity of soil. Though combined application of bioagents and Bordeaux mixture to manage the foot rot of betelvine may reduce the residual toxicity by absorbing copper from soil. The present investigation was carried out to study the potentiality of biological control to increase soil health or the effectiveness of biocontrol agents as pesticide filter.

MATERIALS AND METHODS

To study the potential of biocontrol agent *Trichoderma harzianum* on Bordeaux mixture (BM), carbendazim and nicotine were used to manage the foot rot of betelvine as well as to act as pesticide filter to reduce the residual toxicity of soil.

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Bordeaux mixture (BM) carbendazim and nicotine

Forty gram of pelletized formulation of *Trichoderma harzianum* in alginate clay mixture was taken in glass column 50 cm in length and 8 cm in diameter. In another glass column 40 gram of control prills (without any biocontrol agent) was taken. Hundred ml of 0.2% BM was added to these columns and kept for 2 days. On 3rd and 7th day 10 ml of samples were collected from both of these columns and copper was analysed by atomic absorption spectroscopy. Similarly, 50 gm of carbendazim and 50 ml of nicotine were added to other columns. Each concentration was replicated thrice. Ten ml samples were collected from both these column every week and concentration of pesticide was analysed by UV absorption spectroscopy.Fifty ml elluent of the pesticide at different concentration was taken in conical flasks. Twenty gram of prills containing biocontrol agents were added to these flasks. The experiment was set in triplicate with parallel control sets. In control sets prills, without biocontol agent were used. After 4 weeks 20 ml of aliquot were taken from each flask and filtered. The filtrate was tested against the spore germination of two isolates of*Phytophthora parasitica* (P21 asnd P22) and *Colletotrichum capsici* (DK1 and DK2). The fungicide and plant products used were carbendazim, BM and nicotine. Three different dosages were taken for each fungicide. For carbendazim (Bavistin), 2.5 ppm, 25 ppm, 250 ppm were used and were designated as B 2.5, B 25 and B250. The treatments with filtrates of Bavistin of these concentration were passed through control prills (without biocontrol agents) were donated as B2.5c, B25 c and B250c. In case of Bordeaux mixture, 100ppm,200ppm and 400 ppm concentration were used and the treatment were designated as BM 100, BM200, BM400. The filtrate of BM of the said concentration were passed through biocontrol agents. The control set was designated as BM 100c, BM200c, BM400c. in case of nicotine the treatment were N2000, N 4000, N6000, i.e.the filtrate of nicotine of N2000ppm, N 4000ppm, N6000ppm were passed through biocontrol agents. The control set was denoted as N 2000c, N 4000c N4000c.

RESULTS AND DISCUSSION

Biocontrol agents as pesticide filter

The result showed that (Table 1) with increasing the time the absorption of copper residue by the biocontrol agents is increased to the extent of 30.5% and 50% of the initial level by 3rd and 7th day of impregnation. In the control hardly any copper was absorbed during the same period of time. Therefore prima-facie evidence showed that the biocontrol prills may act as Pesticide Filter. In case of carbendazim it was observed that absorption of fungicide was increased with decrease in concentration (ppm) and with increase in time. Minimum concentration 25 ppm caused maximum absorption (44%) by activated prills in comparison to 250ppm (40%) after 4th week of impregnation (Table 2).

It was also observed that the biocontrol agent enmeshed in polymer prills absorbed 20-32% carbendazim and 20-25% nicotine within one week. Thereafter, the pesticide were found to dissipate gradually as a function of time. Within 4 weeks, 40-44% carbendazim and 45-50% of nicotine absorbed were dissipated. In control prills the concentration of nicotine was reduced to 15-20% and that of carbendazim was reduced to 16-28% within one week. This reduction in pesticide concentration was described mainly due to absorption by the control prills. Thereafter the change in concentration of pesticides was very low (Table 2).

From the results, it would appear that the biocontrol agents absorbed /dissipated copper residue to the extent of 30.5% and 50% of the initial level by 3rd and 7th day and no copper on 3rd day in Bordeaux mixture. Therefore prima facie evidence showed that the biocontrol prills may act as pesticide filter. *Trichoderma harzianum* can inhibit the pathogen *Phytophthora parasitica* in *in vitro* condition (De'Souza *et al,* 2001). Similarly the growth of *Colletotrichum capsici* were also inhibited by *Trichoderma harzianum* both *in vitro* and *in vivo* condition. (De'Souza *et al,* 2001; Roy *et al,* 2002).

In case of nicotine and carbendazim it appears that the biocontrol agent absorbed / dissipated 20-25% of nicotine and 20-32% carbendazim within one week. Thereafter, the pesticide were found to dissipate gradually as a function of time. Within 4 weeks, 45-50% of nicotine and 40-44% carbendazim absorbed were dissipated. In control prills the concentration of nicotine was reduced 15-20% and that of carbendazim was reduced to 16-28% within one week. This reduction in pesticide

Table 1 : Concentration of Copper in prills treated with a combination of biocontrol agent (*T. harzianum*) and Bordeaux mixture

Concentration of the added to the column (ppm)	Concentration of copper in elluent from placebo prills (ppm)		Concentration of copper in elluent from biocontrol prills (ppm)		Concentration of copper absorbed/dissipated
	3rd day	7th day	3rd day	7th day	
2000	2000	1980	1390	1000	Control: 20ppm ai, 3rd day: 30.5%, 7th day: 50%

Table 2 : Concentration of carbendazim and nicotine in biocontrol and inactive prills as a function of time

Fungicides	Concentration in ppm	Type of prills used	Concentration in ppm			
			1st week	2nd week	3rd week	4th week
Carbendazim	250	Control prills[1]	180(28)	176(30)	176(30)	176(30)
		Activated prills[2]	170(32)	162(35)	159(36)	150(40)
	25	Control prills	20.5(18)	20.5(18)	20(20)	20(19)
		Activated prills	20(20)	18(28)	16.5(34)	15(41)
	25	Control prills	21(16)	2.0(20)	2.0(20)	20(18)
		Activated prills	20(20)	1.7(32)	1.5(40)	14(44)
Nicotine	6000	Control prills[3]	5100(15)	4990(17)	4985(17)	4980(17)
		Activated prills[4]	4600(23)	4100(31)	3600(40)	3150(48)
	4000	Control prills	3400(18)	2200(20)	3180(21)	3100(23)
		Activated prills	3202(20)	3000(25)	2504(37)	2200(45)
	2000	Control prills	1600(20)	1550(22)	1545(23)	1542(23)
		Activated prills	1500(25)	1300(35)	1180(41)	1000(50)

Figures in the patenthesis indicate percent absorption of pesticide in the prills
[1] Untreated prills; [2] prills treated with carbendazim, [3] Untreated prills; [4] prills treated with nicotine

concentration was ascribed mainly due to absorption by the control prills. Thereafter the change in concentration of pesticides was very low. The fungicides Carbendazim and Bordeaux mixture also inhibited the growth of *Phytophthora* sp. and *Colletotrichum capsici* (Roy *et al,* 2002).

Elluents containing prills on spore germination of the two test fungi

The elluents obtained after passed through a column containing activated prills were tested for their effects on germination of spore vis-a-vis control prills that contained no biocontrol agent. The experiment was conducted against two *Phytophthora parasitica* (P21 asnd P22) and *Colletotrichum capsici* (DK1 and DK2) isolates.

Phytophthora parasitica

Two isolates of *Phytophthora parasitica* were tested against elluents of active and control prills of *T.harzianum*, and carbendazim, Bordeaux mixture and nicotine at different concentrations showed that both carbendazim and Bordeaux mixture as well as the plant product nicotine when passed through prills containing biocontrol agents, a significant quantity has been held in the prills leading to higher percentage of germination in each concentration when elluents from active prills were used to study germination of spores of two *Phytophthora* isolates than when it was obtained from the dummy prills, though in each case percentage of germination of spores were minimum in higher concentration of fungicide and nicotine. Maximum inhibition of spore germination was recorded at 250 ppm of carbendazim (53.76 and 58.95%), 400 ppm of Bordeaux mixture (70.96% and 58.95%), 6000 ppm of nicotine (82.43 and 84.44%) for active prills against P21 and P22 isolates respectively (Table 3), though maximum inhibition was occurred on control prills, where biocontrol agent was absent in each concentration for both the isolates.

Table 3 : Effect of fungicide and plant product elluent passed through biocontrol agent containing alginate prills on spore germination of *Phytophthora* spp

Fungicide / plant product	Isolate	Concentration (ppm)	Percent of germination over control		Percent of inhibition over control	
			Active	Control	Active	Control
Bavistin (Carbendazim)	P 21	2.5	94.22	91.34	5.78	8.6
		25	87.17	83.15	12.83	16.85
		250	46.24	38.25	53.76	61.75
	P22	2.5	92.5	89.89	7.50	10.11
		25	84.35	79.47	15.65	20.53
		250	41.05	34.65	58.95	65.35
Bordeaux mixture	P21	100	55.27	52.2	44.73	47.8
		200	47.51	37.5	52.49	62.5
		400	29.04	22.05	70.96	77.95
	P22	100	54.93	52.5	45.07	47.5
		200	40.88	40.76	59.12	59.2
		400	41.05	30.33	58.95	69.67
Nicotine	P 21	2000	62.11	51.87	37.89	48.13
		4000	34.75	23.5	65.25	76.5
		6000	17.57	14.38	82.43	85.62
	P22	2000	59.98	54.38	40.02	45.62
		4000	27.49	23.76	72.51	76.24
		6000	15.56	13.85	84.44	86.15

Colletotrichum capsici

Another experiment was designed on the same lines as for *Phytophthora* and described in the previous section, was designed for 2 isolates of *Colletotrichum capsici*, DK1 and DK2. The results showed that (Table 4) both bavistin (carbendazim), Bordeaux mixture and nicotine when passed through prills containing biocontrol agent, the potentiality of both of these fungicide and plant product has been increased against the pathogenic isolate DK1 and DK2 of *C. capsici*. The inhibition of germination percentage over control was maximum with increase in dose (250 ppm, 400 ppm, and 6000 ppm for carbendazim, Bordeaux mixture and nicotine respectively). It was also observed that inhibition of germination was maximum in elluents of control prills in comparison to the elluents of active prills as the control prills elluents contained only on fungicide (71.64%, 75.20% on carbendazim of 250 ppm, 70.10%, 69.80% on Bordeaux mixture of 400 ppm and 82.47% and 83 60% on nicotine of 6000 ppm for both DK1 and DK2 isolates respectively) and active prills elluents contain both fungicide and biocontrol agents (58.85%, 53.50% on Carbendazim of 250 ppm, on Bordeaux mixture of 400 ppm and 78.12%, 80.92% on Nicotine of 6000 ppm for both DK1 and DK2 respectively).The effect of elluents of fungicide and plant product passed through biocontrol agent on spore germination of the 2 test fungus was studied and the ED50 values were tabulated.The results (Table 5) concluded that both Carbendazim, Bordeaux mixture and nicotine when passed through prills containing biocontrol agent, the potentiality of both of these fungicide and plant product had been reduced against the pathogenic isolate P12, P22 of *Phytophthora* sp and DK1, DK2 of *C. capsici.* This may be due to the deposition of fungicide on elluents and spore germination of the isolate P21, P22, of *Phytophthora* sp and DK1, DK2 of *C. capsici.* On that elluents the ED50 value increased compairing with the ED50 value of the pathogens isolates when tested against the same fungicide. The results also indicated that theED50 values of carbendazim, Bordeaux mixture and nico-

Table 4 : Effect of elluents fungicides and plant product passed through biocontrol agent containing alginate prills on spore germination of test isolates *C.capsici*

Fungicide	Isolates	Treatments (ppm)	Percent of germination over control		Percent inhibition over control	
			Active	Control	Active	Control
Bavistin (carbendazim)	DK1	2.5	89.22	86.38	10.78	13.62
		25	84.66	80.45	15.34	19.55
		250	41.15	28.36	58.85	71.64
	DK2	2.5	90.15	89.48	9.85	10.52
		25	86.06	84.35	13.94	15.65
		250	46.5	24.8	53.5	75.2
Bordeaux mixture	DK1	100	61.76	57.05	38.24	42.95
		200	55.38	48.68	44.62	51.32
		400	34.2	29.9	65.8	70.1
	DK2	100	64.34	58.94	35.66	41.06
		200	58.44	47.23	41.56	52.77
		400	37.55	30.2	62.45	69.8
Nicotine	DK1	2000	68.56	56.09	31.44	43.91
		4000	40.28	29.62	59.72	70.38
		6000	21.88	17.59	78.12	82.41
	DK2	2000	64.35	53.56	35.65	46.34
		4000	38.15	24.09	61.85	75.91
		6000	19.08	16.40	80.92	83.60

Dissipation of pesticide residue in /o Betelvine leaves and soils

Table 5 : The ED_{50} value of pesticide elluent passed through biocontrol agent containing alginate prills on spore germination of test isolates *P. parasitica* and *C.capsici*

Fungicide / Plant product	ED50 value(ppm)			
	Isolates of *P. parasitica*		Isolates of *C. capsici*	
	P21	P22	DK1	DK2
Carbendazim	240.8 (122.0)	176.8 (121.90)	202.9 (5.59)	202.9 (5.59)
Bordeaux mixture	199.52 (87.12)	177.82 (79.50)	223.87 (36.60)	223.87 (36.60)
Nicotine	3125.6 (2038.0)	2977.8 (1832.0)	3107.2 (2028.35)	3107.2 (2028.35)

Figures in the parentheses indicate the ED50 value of the fungicide and plant products

tine against *Phytophthora* isolates were 122.0, 121.90, 87.12, 79.50 and 2038.0, 1832.0 ppm respectively whereas combination of bioagents and fungicides as active prills the ED50 values increased for both the isolates (240.8, 176.8 ppm for Carbendazim, 199.52, 177.82 for Bordeaux mixture and 3125.6, 2977.8 ppm for Nicotine). Similar results were also noticed against *Colletotrichum* isolates also. These findings may be indicated that the active prills were acting as Pesticide Filter and this was not just because of the polymer matrix but because of biocontrol agents in the matrix. The alternative potential of this finding is that if prills were treated with fungicide to which the biocontrol agent was tolerant but the pathogen is sensitive, such prills provide a tool for more efficient management of the pathogen through delivery of two components, viz. the biocontrol agent and the pesticide through a single operation. The possibilities are that the biocontrol agent absorbs excess pes-

ticides from soil and reduces the residual toxicity of soil which will increase the beneficial microorganisms in soil and it may increase the available nutrient in soil and ultimately improves the soil health.

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