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Compatibility of Biocontrol Agent *Trichoderma harzianum* with Fungicides, Insecticides and Herbicides (*invitro*)

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A B S T R A C T

An *invitro* compatibility studies were conducted with *Trichoderma harzianum* and some commonly used chemical pesticides. The experiment was laid out in Completely Randomized Design (CRD) with four replications and seven treatments of fungicides, seven treatments of herbicides and seven treatments of insecticides on biocontrol agents *T.harzianum.* The fungicide ametoctradin + diamethomorph was found moderately toxic, while carbendazim + mancozeb, copper oxychloride, azoxystrobin + tebuconazole, captan and thiophenate methyl, proved toxic. Among the herbicides, carfentrazone ethyl was found compatible and glyphosate was proved slightly toxic. oxyflourofen and metribuzin are moderately toxic. The herbicides, sulfentrazone + clomazone and fomesafen + flauzifop-p-butyl, were incompatible. Insecticides, imidachloprid, acetamiprid, spinosad, imamectin benzoate and chlorantraniliprole, were found compatible whereas thimethoxam + lambda cyhalothrin was found slightly toxic.Some chemical pesticides did not affected the growth and development of *T harzianum*. Hence combination of such chemical pesticides with biocontrol agents can provide an additive or synergistic effect in the control of diseases and pests. These chemical pesticides and biocontrol agents can be used in an integrated pest management programme.

Introduction

In agriculture use of biocontrol agents safe and environmentally acceptable alternative to pesticides (Gampala and Pinnamaneni, 2010; Bhai and Thomas, 2010). Combining a fungicide tolerant biocontrol agent with respective fungicides has improved the extent of disease control and reduced the quantity of fungicides required for effective management (Buck, 2004; Ambethgar, *et al*., 2009). Hence, an *in vitro* study will be conducted to assess compatibility of some commonly used, commercially available fungicides, insecticides and herbicides on growth of *T.harzianum.*

Materials and Methods

The present study was conducted at "Plant Pathology and Agricultural Microbiology" section of Rajarshee Chhatrapati Shahu Maharaj, College of Agriculture, Kolhapur, during the year 2020-21. For this study poisoned food technique is used.

After sterilization, allowed medium to cool at near the 40 ºC and precisely measured doses of chemical pesticides are added into the medium into each conical flask respectively. About 20 ml of PDA medium amended with various chemical pesticides is poured into each 9 cm sterilized Petri plate.

Without pesticide amended medium served as control. All the Petri plates allowed to solidifying. Each treatment was performed in four replications.

Chemical pesticides and their concentrations

The *in vitro* bio-efficacy of pesticides was determined by the poisoned food technique (Nene and Thapliyal, 1993). Six different fungicides, six herbicides and six insecticides were selected for this study. All about eighteen chemical pesticides were used according to the recommended application rate for field crops.

After the medium has solidified, biocontrol agents were injected aseptically by transferring a circular 5 mm diameter disc produced from sterilized cork-borer from an actively developing 7 day old culture of the fungal biocontrol agent to the middle of the Petri dish. Observations of the mycelial growth of biocontrol agents were recorded by measuring the diameter (mm) of radial growth by using the measuring scale. Observations of four replications were recorded every 24 hours till the end of 7^{th} days.

The growth inhibition of biocontrol agents was estimated by using the following formula given by Vincent (1947) and per cent inhibition of mycelial growth was obtained.

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

Where,

I = Percent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

Statistical analysis

All laboratory work was carried out in Completely Randomized Design, with four replications and seven treatments each of fungicides, herbicides and insecticides.

Data obtained which in per cent format were transformed in arcsine format. Transformed data was subjected to analysis of variance.

Results and Discussion

Effect of fungicides on *T.harzianum*

Among the fungicides (Table 4), ametoctradin + diamethomorph was inhibited growth by 37.87 per cent and was found to be moderately toxic. *T. harzianum* was found completely incompatible with the fungicides carbendazim + mancozeb, copper oxychloride and azoxystrobin + tebuconazole. Captan and thiophenate methyl also proved toxic to the growth of *T. harzianum*. The fungicide ametoctradin + diamethomorph was found to be moderately toxic and the remaining all fungicides were incompatible with *T. harzianum*.

Bhosale and Borade (2015) also suggested that copper oxychloride was highly incompatible with *T. harzianum*. Saxena *et al.,* (2014) also reported that thiophenate methyl was found incompatible with *T.harzianum*. Bhale and Rajkonda (2015) showed that concentrations of mancozeb above 5000 µg/ml and captan above 500 µg/ml inhibited the mycelial growth of *Trichoderma* spp.

Effect of herbicides on *T.harzianum*

In the case of herbicides (Table 5), only carfentrazone ethyl was found compatible. Glyphosate inhibited mycelial growth by 28.46 per cent and proved slightly toxic. Oxyfluorofen was found moderately toxic followed by metribuzin.

High mycelial growth inhibition was recorded 91.49 per cent, within the treatment sulfentrazone + clomazone followed by fomesafen + flauzifop-p-butyl. Herbicide, sulfentrazone + clomazone found highly incompatible with *T. harzianum*.

Only one herbicide, carfentrazone ethyl, was found compatible with *T.harzianum*, while glyphosate was found slightly toxic. Oxyfluorofen and metribuzin proved moderately toxic. Mohamed and Radwan (2017) also reported that oxyfluorofen and glyphosate are moderately toxic.

Effect of Insecticides on *T.harzianum*

Out of six insecticides (Table 6), imidachloprid was found highly compatible with *Trichoderma harzianum* followed by acetamiprid, spinosad, imamectin benzoate and chlorantraniliprole which showed inhibition of 7.02, 10.63, 18.91 and 21.09 per cent respectively. Thimethoxam + lambda cyhalothrin was slightly toxic and inhibited mycelial growth by 30.44 per cent. Except for the insecticide thimethoxam + lambda cyhalothrin, all insecticides were compatible with *Trichoderma harzianum*. The results of the present investigation are similar with earlier studies (Thiruchchelvan *et al.*, 2013) which showed that chlorantraniliprole, acetamiprid and imidacloprid were compatible with *T.harzianum.*

Ametoctradin + Dimethomorph was found moderately toxic, hence it can be used under critical circumstances in the orchards (Grape) where *T. harzianum* was already applied for management of fungal diseases.

The herbicides, Carfentrazone ethyl was found compatible and glyphosate was slightly toxic, so both can be applied safely in IPM system.

The herbicides, Sulfentrazone + Clomazone and Fomesafen + Flauzifop-p-butyl, were incompatible, so application of both must be avoided in *T. harzianum* applied soils. The insecticides *viz.*, Imidacloprid, Acetamiprid, Spinosad, Emamectin benzoate and Chlorantraniliprole were compatible; hence combined use of all these insecticides will be safer to *T. harzianum*.

Table.1 Concentration of fungicides

S. No.	Treatment	Chemical name	Trade name	Application rate/100 ml
1	T_1	Captan 50% WP	Caftaf	0.3 g
2	T_2	Carbendazim 12% + Mancozeb 63% WP	Starlet	0.3 g
3	T_3	Thiophenate methyl 70% WP	Roko	0.05 g
4	T_4	Copper oxychloride 50% WP	Blitox	0.3 g
5	T_5	Azoxystrobin 11% + Tebuconazole 18.3% W/W SC	Custodia	0.1 ml
6	T_6	Ametoctradin 22% + Dimethomorph 20% EC	Zampro	0.2 ml
7	T_7	Control	-	-

Table.2 Concentration of herbicides

S. No.	Treatment	Chemical name	Trade name	Application rate/100 ml
1	T_1	Fomesafen11.1% W/W + flauzifop-p-butyl 11.1% W/W SL	Fusiflex	0.3 ml
2	T_2	Metribuzin 70% WP	Adrino	0.05 g
3	T_3	Sulfentrazone 28% + Clomazone 30% WP	Authority	0.4 g
4	T_4	Glyphosate 41% SL	Touchdown	0.8 ml
5	T_5	Oxyfluorfen 23.5% EC	Goal	0.2 ml
6	T_6	Carfentrazone ethyl 40% DF	Affinity	0.01 g
7	T_7	Control	-	-

Table.3 Concentration of insecticides

S. No.	Treatment	Chemical name	Trade name	Application rate/100 ml
1	T_1	Chlorantraniliprole 18.5% SC	Coragen	0.03 ml
2	T_2	Imidacloprid 48% SL	Gaucho	0.03ml
3	T_3	Emamectin benzoate 5% WP	Rilon	0.04 g
4	T_4	Spinosad	Tracer	0.03 ml
5	T_5	Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC	Alika	0.1 ml
6	T_6	Acetamiprid 20% SP	Manik	0.05 g
7	T_7	Control	-	-

Fig.1, 2, 3

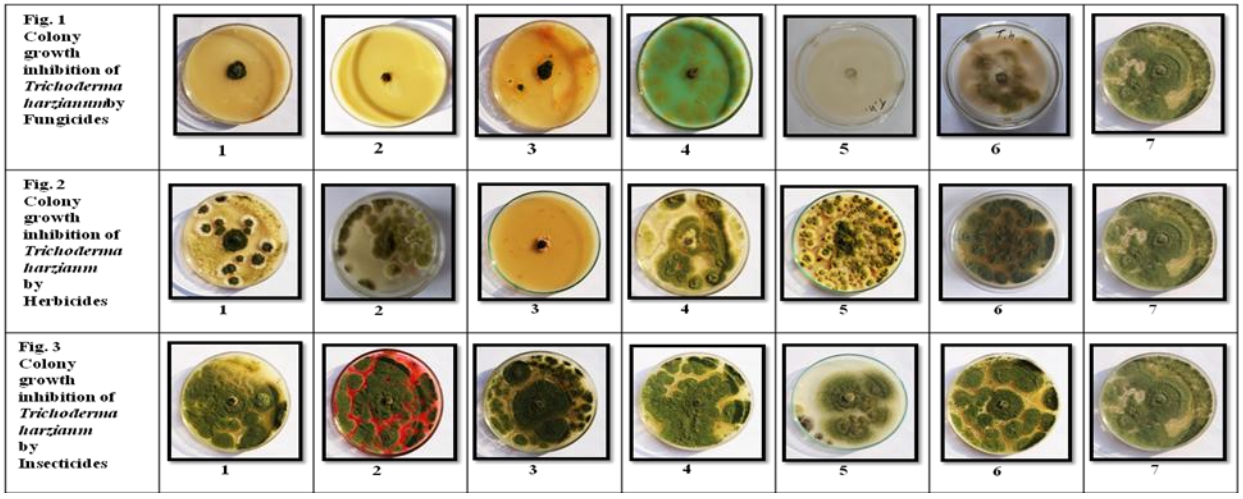

Table.4 Effect of fungicides on the colony growth of *T.harzianum*

Treatment	Average colony diameter of *T. harzianum* (mm) **												Toxicity Level
	24* hr	% Inhib.	48* hr	% Inhib.	72* hr	% Inhib.	96* hr	% Inhib.	120* hr	% Inhib.	144* hr	% Inhib.	
FT_1Captan	1.20	93.89 (75.68)	3.52	86.64 (68.56)	6.90	86.88 (68.75)	7.40	88.01 (69.75)	9.30	88.24 (69.96)	11.55	86.20 (68.19)	Toxic
FT_2Carbendazim + Mancozeb	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	Toxic
FT_3Thiophanate methyl	2.87	85.35 (67.49)	6.30	76.11 (60.73)	9.37	82.17 (65.01)	8.37	86.43 (68.40)	9.12	88.46 (70.19)	12.80	84.71 (67.02)	Toxic
FT_4Copper oxychloride	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	Toxic
FT_5Azoxystrobin+ Tebuconazole	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.0 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	Toxic
FT_6Ametoctradin+ Dimethomorph	2.62	86.62 (68.56)	6.50	75.36 (60.25)	24.50	53.40 (46.92)	33.75	45.32 (42.29)	45.25	42.76 (40.82)	52.00	37.87 (37.98)	Moderately Toxic
FT_7Control	19.62	(0.00)	26.37	0.00 (0.00)	52.57	0.00 (0.00)	61.72	0.00 (0.00)	79.05	0.00 (0.00)	83.70	0.00 (0.00)	-
SEm±	0.11	0.16	0.18	0.29	0.33	0.39	0.46	0.46	0.54	0.54	0.70	0.47	-
CD at 1%	0.32	0.47	0.54	0.87	0.98	1.15	1.37	1.37	1.60	1.59	2.07	1.40	-

Table.5 Effect of herbicides on the colony growth of *T.harzianum*

Treatments	Average colony diameter of *T. harzianum*(mm)**												Toxicity Level
	24* hr	% Inhib.	48* hr	% Inhib.	72* hr	% Inhib.	96* hr	% Inhib.	120* hr	% Inhib.	144* hr	% Inhib.	
HT_1Fomesafen+ Flauzifop-p-butyl	3.90*	80.13 (63.53)**	8.22	68.82 (56.00)	17.77	66.19 (54.42)	20.05	67.52 (55.29)	27.95	64.64 (53.52)	30.27	63.83 (53.03)	Toxic
HT_2Metribuzin	7.45	62.04 (51.93)	17.05	35.36 (36.28)	30.05	42.84 (40.81)	31.77	48.52 (44.12)	33.35	57.81 (49.49)	51.02	39.04 (38.66)	Moderately Toxic
HT_3Sulfentrazone+ Clomazone	1.12	94.27 (76.16)	3.80	85.59 (67.65)	4.90	90.68 (72.24)	5.22	91.54 (73.08)	6.87	91.30 (72.94)	7.12	91.49 (73.06)	Toxic
HT_4Glyphosate	12.80	34.78 (36.00)	20.77	21.23 (27.11)	31.80	39.51 (38.85)	40.22	34.83 (36.11)	53.87	31.85 (34.30)	59.87	28.46 (32.23)	Slightly Toxic
HT_5Oxyfluorofen	5.70	70.96 (57.37)	9.55	63.79 (52.85)	18.72	64.38 (53.32)	23.05	62.66 (52.33)	31.37	60.31 (50.97)	46.62	44.30 (41.67)	Moderately Toxic
HT_6Carfentrazone Ethyl	12.55	36.05 (36.81)	19.12	27.49 (31.38)	35.80	31.91 (34.38)	47.20	23.53 (28.93)	55.12	30.27 (33.36)	75.82	9.41 (17.71)	Compatible
HT_7Control	19.62	0.00 (0.00)	26.37	0.00 (0.00)	52.57	0.00 (0.00)	61.72	0.00 (0.00)	79.05	0.00 (0.00)	83.70	0.00 (0.00)	-
SEm±	0.24	0.92	0.45	1.28	0.63	0.87	1.00	1.06	1.21	1.01	1.55	1.17	-
CD at 1%	0.72	2.72	1.35	3.78	1.86	2.56	2.96	3.12	3.58	2.97	4.57	3.45	-

Table.6 Effect of insecticides on the colony growth of *T.harzianum*

Treatments	Average colony diameter of *T. harzianum* (mm)**												Toxicity Level
	24* hr	% Inhib.	48* hr	% Inhib.	72* hr	% Inhib.	96* hr	% Inhib.	120* hr	% Inhib.	144* hr	% Inhib.	
IT_1Chlorantra-niliprole	10.97	44.08 (41.57)	17.87	32.23 (34.42)	35.65	32.19 (34.47)	51.40	16.73 (23.34)	62.37	21.09 (26.98)	66.05	21.09 (27.14)	Compatible
IT_2Imidacloprid	16.22	17.32 (24.52)	23.45	11.09 (17.33)	38.87	26.06 (30.61)	49.12	20.41 (26.83)	59.12	25.21 (30.03)	78.40	6.33 (14.37)	Compatible
IT_3Emamectin benzoate	14.45	26.37 (30.79)	23.95	9.19 (15.18)	43.30	17.64 (24.26)	49.47	19.85 (26.35)	55.72	29.51 (32.85)	67.87	18.91 (24.75)	Compatible
IT_4Spinosad	13.87	29.30 (32.72)	22.30	15.45 (22.35)	49.87	5.14 (12.65)	54.30	12.03 (20.15)	66.37	16.03 (23.35)	74.80	10.63 (18.61)	Compatible
IT_5Thiamethoxam+ Lambda cyhalothrin	8.10	58.73 (50.12)	13.87	47.39 (43.36)	29.87	43.18 (41.01)	35.90	41.84 (40.28)	47.65	39.72 (39.07)	58.22	30.44 (33.45)	Slightly Toxic
IT_6Acetamiprid	14.62	25.48 (30.26)	20.87	20.85 (26.99)	37.47	28.72 (32.32)	48.30	21.75 (27.65)	56.25	28.84 (32.46)	77.82	7.02 (15.15)	Compatible
IT_7Control	19.62	0.00 (0.00)	26.37	0.00 (0.00)	52.57	0.00 (0.00)	62.72	0.00 (0.00)	79.05	0.00 (0.00)	83.70	0.00 (0.00)	-
SEm±	0.40	1.11	0.58	2.97	1.17	1.80	1.24	1.80	1.77	1.65	2.04	2.25	-
CD at 1%	1.20	3.26	1.71	8.74	3.45	5.31	3.66	5.31	5.21	4.85	6.00	6.63	-

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