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EVALUATION OF COMPATIBILITY OF *TRICHODERMA HARZIANUM* WITH SELECTED PESTICIDES USED IN CARDAMOM, PEPPER AND GINGER

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ABSTRACT

An *in vitro* study was conducted to evaluate the effect of six pesticides on the growth of *Trichoderma harzianum* at two concentrations by poisoned food technique on PDA medium. Observations on radial growth of *T. harzianum* after, 24, 48, 72, 96 and 120 hour intervals were recorded. The progressive inhibition (%) of *T. harzianum* was observed with the insecticide, Imidacloprid 17.8% SL, at the optimal dosage of 1ml/ lit- and higher dosage of 1.5 ml/lit (4.33 & 10.73%) after 96 hours of incubation. Thiamethoxam 25% WG showed moderate inhibition at the optimal dosage of 1gm/ lit and higher dosage of 2gm/lit (14.81 & 32.58%). Chlorpyrifos 50% + Cypermethrin 5 % EC, showed the highest inhibition at the optimal dosage of 2 ml/ lit and higher dosage of 4 ml/lit (78.81 & 82.02%) after 72 hours of incubation. Among the three insecticides Imidacloprid and Thiamethoxam were found to be compatible whereas Chlorpyrifos + Cypermethrin showed only partial compatibility with *T. harzianum*. There was progressive inhibition of *T. harzianum* with the fungicides, Fluopicolide 4.44% + Fosetyl-Al 66.67% at the optimal dosage of 2.5gm/lit (55.95%) and at higher dosage of 4gm/lit (70.73%) after 96 hours of incubation. Oxathiapiprolin 3% + Metalaxyl-M 18% at the optimal dosage of 1.5ml/ lit and higher dosage of 3 ml/lit was found to be highly inhibitory (70.36 & 77.03%) after 96 hours of incubation. Hexaconazole 5% SC showed high inhibition at the optimal dosage of 1.5ml/lit and higher dosage of 3 ml/lit (97.22%) after 96 hours of inhibition. Among the three fungicides, Fluopicolide + Fosetyl-Al at the optimal dosage was moderately compatible with *T. harzianum*. Whereas, Oxathiapiprolin + Metalaxyl-M was found to be partially compatible with *T. harzianum* and Hexaconazole 5% SC fully incompatible. The results would be helpful to delineate the possibility of timeline scheduling for usage of *T. harzianum* as biocontrol agent in GAP.

Keywords: Inhibition, Pesticide, Insecticide, Fungicide, *T. harzianum*.

Introduction

Crop losses due to plant pest and pathogens are a serious concern that affects incomes and food security worldwide (Cerdeira *et al.*, 2017). To reduce the impact of biotic stress due to pest and diseases, applications of pesticides either prophylactic or later incidence are the available crop management options Timothy *et al.* (2019). The view of reducing environmental risk and increasing agricultural and environmental sustainability and sensitive agro-ecosystems, the judicious and conscious integration of pesticides and

biocontrol agents have been the subject of research in the recent years (Dhanya *et al.*, 2016). Recommended doses of insecticides / fungicides along with bio control agents show promising effect on the management of various plant pests than the chemicals alone (Vinit *et al.*, 2012). Therefore, the combined use of bio-control agents and chemical pesticides has enticed much attention as a way to obtain synergistic or additive effects in the control of soil-borne pathogens (Locke *et al.*, 1985). *Trichoderma harzianum* has been identified as a very promising bio-control agent (Deepika *et al.*, 2014). *Trichoderma harzianum* has

been identified as the potential bio-control agent for soil borne disease management in black pepper, cardamom and ginger (Siddhartha *et al.*, 2017). Integration of compatible bio-agent with pesticides may enhance the effectiveness of disease control and provide better management of soil borne diseases (Vinit *et al.*, 2012; Deepika *et al.*, 2014). In recent past many new molecules have been introduced for the pest and disease management which are effective at very low doses and their compatibility with fungal antagonists is yet to be worked out. Keeping above facts in view the present investigations was being undertaken.

Materials and Methods

The present study was conducted at Plant Pathology division of Indian Cardamom Research Institute., Regional Research Station. Sakleshpur, Hassan, Karnataka during the year 2024-25. *Trichoderma harzianum* culture maintained in the Plant Pathology laboratory of ICRI Regional Station, Donigal was used in the current study. Mother culture of *T. harzianum* was prepared by growing on PDA (20 ml). After inoculation with *T. harzianum*, the bottles were incubated for 8-10 days at 24±1°C. Potato Dextrose Agar (PDA) medium was used for growing the fungus and also testing the compatibility of *T. harzianum* with different insecticide and fungicides.

Insecticides and fungicides used for the compatibility study with *T. harzianum* are widely used in managing pest and diseases of prominent spice crops like cardamom, black pepper and ginger. Two levels of concentrations of insecticides and fungicides were prepared on the basis of recommended dose and higher dose for drenching were evaluated by (Poisoned Food Technique) PFT (Table 1 & 2). Entire set of experiment was replicated three times. The requisite concentration of insecticides and fungicides were made by dissolving required volume of sterilized distilled water. The requisite quantity of insecticide and fungicide was incorporated into molten PDA separately, mixed thoroughly by vigorous shaking of flaks just before pouring into petri plates. Twenty millilitre (20 ml) PDA was poured in each Petri plates. The medium was allowed to solidify and then each replicated Petri plates was centrally inoculated with mycelia disc of 5 mm diameter from 7day old culture of *T. harzianum*. Petri plates were incubated at 27 ± 1°C for 4 days. The PDA petri plates without any insecticides and fungicides, inoculated with 5 mm disc of *T. harzianum* served as control. The observation on radial growth of mycelium was taken by measuring the colony diameter (mm) vertically and horizontally at right angle and mean colony diameter was calculated

and the radial growth of the colony was measured after 24 hr, 48 hr, 72 hr, 96 hr and 120 hr of inoculation. The percent mycelial growth inhibition of *T. harzianum* over control was calculated by using following formula:

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

Where, C = Colony diameter (mm) of fungus in Control.

T = Colony diameter (mm) of fungus in Treatment.

I = Percent Inhibition of mycelial growth.

Table 1 : Treatment details of insecticides tested for compatibility with *Trichoderma harzianum* in *in-vitro*:

Treatments	Dosage/L
T ₁ Imidacloprid 17.8% SL	1.0 ml
T ₂ Imidacloprid 17.8% SL	1.5 ml
T ₃ Thiamethoxam 25% WG	1.0 gm
T ₄ Thiamethoxam 25% WG	2.0 gm
T ₅ Chlorpyrifos 50% + Cypermethrin 5% EC	2.0 ml
T ₆ Chlorpyrifos 50% + Cypermethrin 5% EC	4.0 ml
T ₇ Control	-

Table 2 : Treatment details of fungicides tested for compatibility with *Trichoderma harzianum* in *in-vitro*:

Treatments	Dosage/L
T ₁ Fluopicolide 4.44% + Fosetyl Al 66.67%	2.5 gm
T ₂ Fluopicolide 4.44% + Fosetyl Al 66.67%	4.0 gm
T ₃ Oxathiapiprolin 3% + Metalaxyl-M 18%	1.5 ml
T ₄ Oxathiapiprolin 3% + Metalaxyl-M 18%	3.0 ml
T ₅ Hexaconazole 5% SC	1.5 ml
T ₆ Hexaconazole 5% SC	3.0 ml
T ₇ Control	-

The insecticides and fungicides which showed >50 percent reduction of mycelial growth was considered incompatible whereas those showing < 50 percent reduction of mycelial growth were considered compatible with *T. harzianum* (Singh *et al.*, 2012). The radial growth was analysed by one way ANOVA statistical analysis was done by OPSTAT software developed by Chaudhary Charan Singh (CCS) Haryana Agriculture University, Hisar, India. The inhibition (%) was transformed to corresponding angular values and analysed by one way ANOVA.

Results and Discussion

Effect of *in-vitro* study on the compatibility of *Trichoderma harzianum* and insecticide combinations

The effect of three insecticides viz., Imidacloprid 17.8% SL, Thiamethoxam 25% WG and Chlorpyrifos 50% + Cypermethrin 5% EC, at two

level of concentration were studied on *T. harzianum* containing poisoned food medium with the above insecticides in *in-vitro* condition. Inhibition of radial growth of *T. harzianum* was observed during the initial period of 24 hours among all the insecticides. Subsequently inhibition started to reduce in Imidacloprid 17.8% SL and Thiamethoxam 25% WG. *T. harzianum* recorded radial growth of 8.60 cm & 8.03 cm in the treatments T₁ & T₂ with inhibition of 4.43 & 10.73 % respectively. At both the concentrations effect of Imidacloprid 17.8 % SL on growth of *T. harzianum* mycelia was observed to be synergistic even after 96 hours after incubation. Similar trend of observations were also recorded in case of Thiamethoxam 25% WG also. Mycelial radial growth of 7.66 cm & 6.03 cm with percent inhibition of 14.81 & 32.58% was observed after 96 hours of incubation where Thiamethoxam 25% WG was used @ 1 and 2 g respectively. On the contrary at all the time interval of observation inhibition percent was high in treatments where Chlorpyrifos 50% + Cypermethrin 5% EC (T₅ & T₆) was used. At 96 hours after incubation lower radial growth of 2.33 & 2.34 cm with higher inhibition of *T. harzianum* growth 77.73 and 76.65 % was observed in T₅ & T₆ respectively (Table.3).

Among three insecticides Imidacloprid, Thiamethoxam were found to be compatible with *T. harzianum* whereas Chlorpyrifos + Cypermethrin at both concentrations was found to be incompatible with *T. harzianum* (Table 2). The results of present study are in accordance with the earlier of findings that, *T. harzianum* possess tolerance to Thiamethoxam (Prasanna et al., 2002 & Dhanya et al. (2016)) and Imidachlorprid (Singh et al., 2012). The insecticide Chlorpyrifos is already known to be compatible with *T. harzianum* (Stephen et al., 2000; Singh et al., 2014) but (Siddhartha et al., 2017) reported that, when the combination of Chlorpyrifos 50% + Cypermethrin 5% was used, it was found to inhibit the growth of *T. harzianum*. Therefore, it implies that the Cypermethrin is inhibitory to *T. harzianum*.

Effect of *in-vitro* study on the compatibility of *Trichoderma harzianum* and fungicide combinations.

The effect of three fungicides viz., Fluopicolide 4.44% + Fosetyl Al 66.67%, Oxathiapiprolin 3% +

Metalaxyl – M 18% and Hexaconazole 5% SC at two concentrations : recommended dose and higher than the recommended dose are studied on *T. harzianum* using poisoned food medium in *in-vitro* condition. Among the chosen fungicides all the fungicides exhibited incompatible behaviour at both the doses with *T. harzianum*.

T. harzianum was found to be incompatible with fungicide Fluopicolide 4.44% + Fosetyl Al 66.67% (T₁ & T₂). At both the doses, *T. harzianum* growth inhibited > 50% after 96 hours of incubation (55.93 & 70.73 %). Whereas similar response of *T. harzianum* was also observed with Oxathiapiprolin 3% + Metalaxyl – M 18%, and Hexaconazole 5% SC at both the dosages of the treatments. Oxathiapiprolin 3% + Metalaxyl – M 18%, showed 70.36 & 77.03 % inhibition and Hexaconazole 5% SC showed 97.22 percent inhibition after 96 hours incubation (Table 4). Results of the current study are in line with the earlier reports of Soumik et al. (2010) that, incompatibility of hexaconazole with *T. harzianum*. In present study an effort has been made to check the compatibility of *T. harzianum* with insecticides and fungicides at optimal and higher doses. Results show *Trichoderma harzianum* promise as part of a replacement strategy for toxic insecticides and fungicides because of the toxicity and environmental impacts as results of applying huge amount of insecticides and fungicides. It is important to study the compatibility of *Trichoderma* with low doses of pesticide, which may be able to lead to a synergistic effect resulting from suppression of competitive soil micro flora. Among the three insecticides Imidacloprid 17.8% SL of recommended and higher dosage were found to be highly compatible with *T. harzianum*. Chlorpyrifos 50% + Cypermethrin 5% EC at both the tested dosages was found to be partial compatibility with *T. harzianum*.

Among the tested fungicides Flupicolide 4.44% + Fosetyl Al 66.67%, Oxathiapiprolin 3% + Metalaxyl-M 18% were found to be moderately compatible whereas Hexaconazole 5% SC was found to be highly incompatible with *T. harzianum*. This information can therefore be used for selecting appropriate chemicals with their dosages for application along with bioagents in farmer's field. However further investigations are recommended for field application.

Table 3: *In-vitro* study on the compatibility of *Trichoderma harzianum* and insecticide combinations

Treatments	Dosage / L	Radial growth of colony (cm)					Inhibition (%)			
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	24 hrs	48 hrs	72 hrs	96 hrs
T ₁ Imidacloprid 17.8% SL	1.0 ml	1.28	4.63	6.63	8.60	8.69	25.80 (30.48)	11.05 (18.73)	6.02 (13.67)	4.43 (11.87)
T ₂ Imidacloprid 17.8% SL	1.5 ml	1.10	3.43	6.20	8.03	8.24	34.25 (35.70)	33.43 (34.71)	12.09 (19.96)	10.73 (18.13)
T ₃ Thiamethoxam 25% WG	1.0 gm	1.50	4.26	5.70	7.66	7.89	13.40 (21.39)	17.84 (24.81)	19.44 (25.67)	14.81 (22.41)
T ₄ Thiamethoxam 25% WG	2.0 gm	1.10	4.00	5.33	6.03	6.39	36.54 (37.16)	23.02 (28.63)	24.60 (29.70)	32.58 (34.77)
T ₅ Chlorpyrifos 50% + Cypermethrin 5% EC	2.0 ml	0.77	1.08	1.50	2.00	2.33	54.46 (47.57)	79.11 (62.78)	78.81 (62.57)	77.73 (61.83)
T ₆ Chlorpyrifos 50% + Cypermethrin 5% EC	4.0 ml	0.25	0.96	1.26	2.10	2.24	85.52 (67.61)	81.30 (64.39)	82.02 (64.96)	76.65 (61.10)
T ₇ Control		1.73	5.20	7.06	9.00	-	-	-	-	-
SE (m) ±		0.06	0.24	0.22	0.23	0.25	4.51 (2.70)	5.31 (3.53)	3.04 (2.53)	2.71 (2.40)
CV		10.52	12.59	0.25	6.38	7.26	18.77 (11.70)	22.47 (15.70)	14.17 (12.17)	12.99 (11.90)

Figures in parenthesis indicate arc sin transformed values

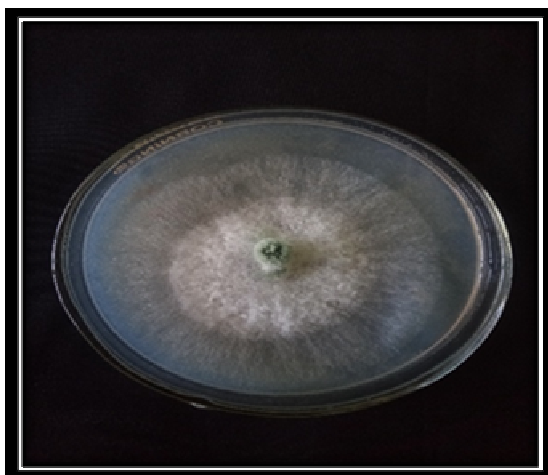
Table 4: *In-vitro* study on the compatibility of *Trichoderma harzianum* and fungicide combinations

Treatments	Dosage / L	Radial growth of colony (cm)					Inhibition %			
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	24 hrs	48 hrs	72 hrs	96 hrs
T ₁ Fluopicolide 4.44% + Fosetyl Al 66.67%	2.5 gm	0.82	1.53	3.56	3.96	5.43	51.82 (46.03)	68.73 (56.01)	49.48 (44.61)	55.92 (48.44)
T ₂ Fluopicolide 4.44% + Fosetyl Al 66.67%	4.0 gm	0.56	1.10	2.00	2.53	4.03	66.58 (54.68)	77.42 (61.63)	71.60 (57.81)	70.73 (57.32)
T ₃ Oxathiapiprolin 3% + Metalaxyl-M 18%	1.5 ml	0.70	1.03	1.56	2.66	5.00	58.83 (50.08)	79.21 (62.88)	76.78 (61.34)	70.36 (57.04)
T ₄ Oxathiapiprolin 3% + Metalaxyl-M 18%	3.0 ml	0.63	0.83	1.36	2.06	4.33	62.89 (52.48)	82.98 (65.67)	80.58 (63.88)	77.03 (61.35)
T ₅ Hexaconazole 5% SC	1.5 ml	0.25	0.25	0.25	0.25	0.25	85.25 (67.39)	94.86 (76.89)	96.45 (79.11)	97.22 (80.37)
T ₆ Hexaconazole 5% SC	3.0 ml	0.25	0.25	0.25	0.25	0.25	85.25 (67.39)	94.86 (76.89)	96.45 (79.11)	97.22 (80.37)
T ₇ Control	-	1.70	4.93	7.06	9.00	-	-	-	-	-
SE (m) ±	-	0.05	0.15	0.27	0.29	0.17	2.47 (1.46)	1.36 (0.99)	4.28 (2.59)	3.64 (2.17)
CV	-	12.48	19.26	20.45	0.88	9.24	6.26 (4.50)	2.85 (2.59)	9.44 (6.99)	8.07 (5.87)

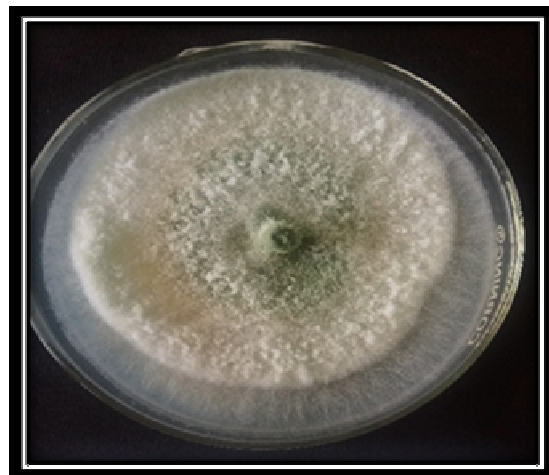
Figures in parenthesis indicate arc sin transformed values

PLATE 1

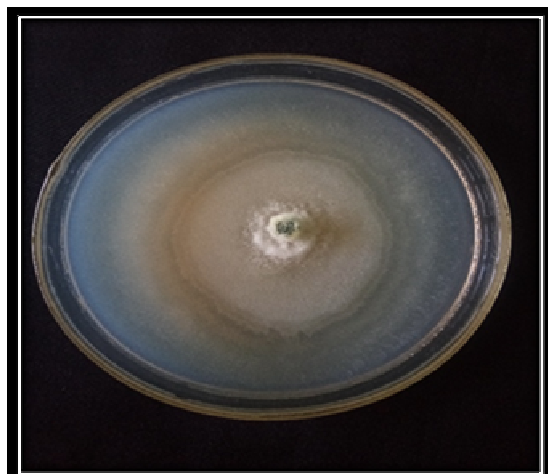
In-vitro study on the compatibility of *Trichoderma harzianum* and insecticide combinations



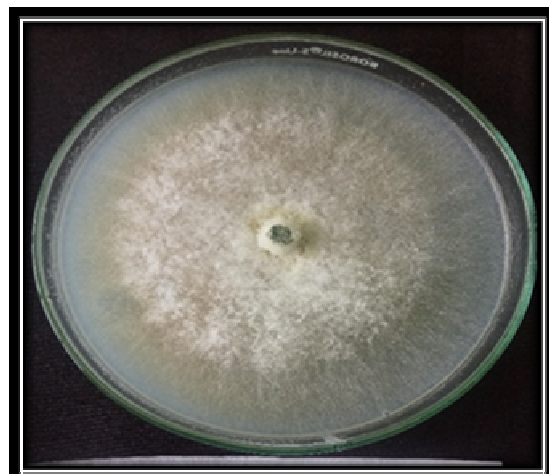
T₁ Imidachloropid - 96 hrs



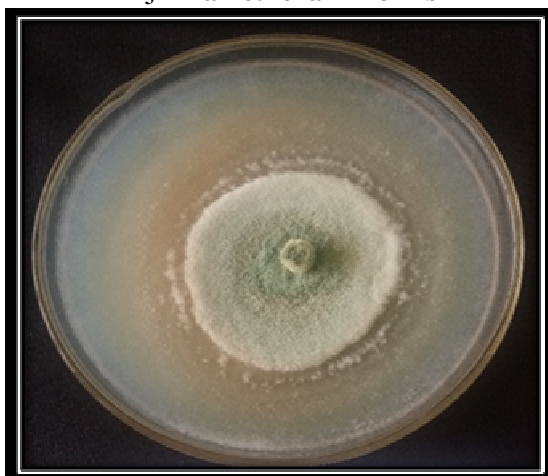
T₁ Imidachloropid- 120 hrs



T₃ Thiamethoxan - 48 hrs



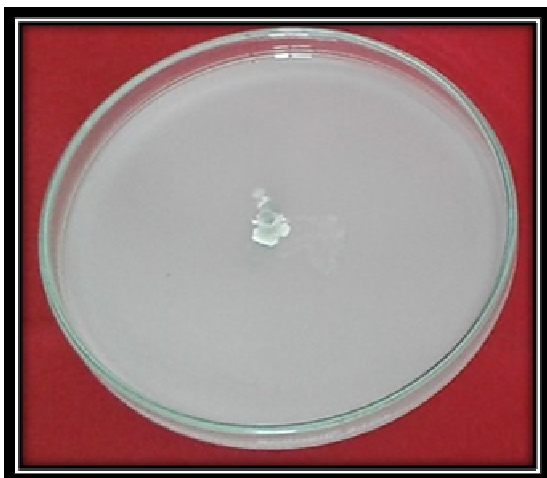
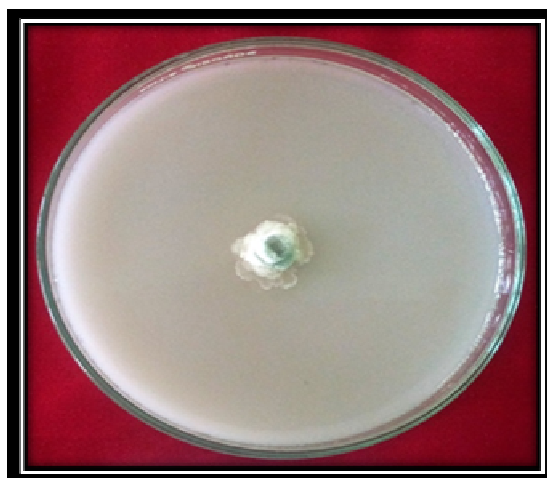
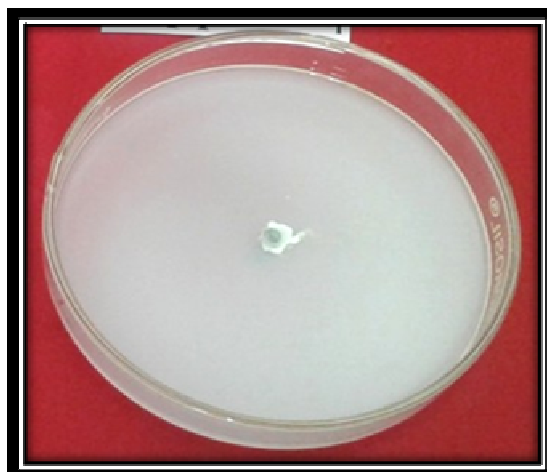
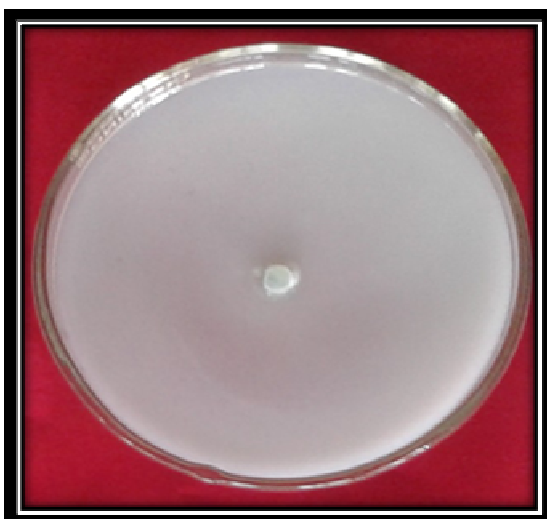
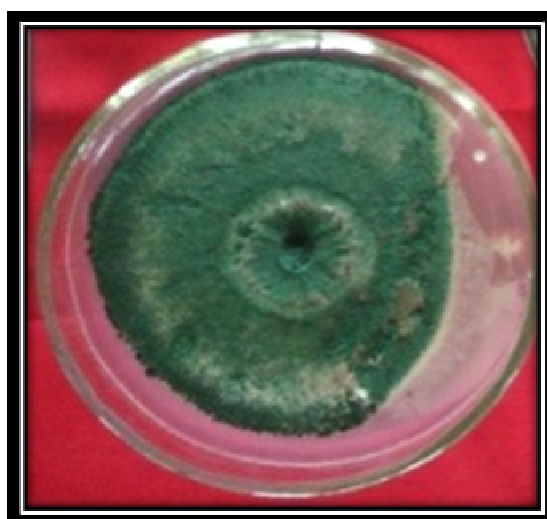
T₃ Thiamethoxan - 96 hrs



T₅ Hexaconazole - 120 hrs



Control

PLATE 2*In-vitro* study on the compatibility of *Trichoderma harzianum* and fungicide combinations**T₁ Fluopicolide + Fosetyl AI - 48 hrs****T₁ Fluopicolide + Fosetyl AI - 120 hrs****T₃ Oxathiapiprolin + Metalaxyl-M - 24 hrs****T₃ Oxathiapiprolin + Metalaxyl-M - 48 hrs****T₅ Chlorpyrifos + Cypermethrin - 96 hrs****Control**

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