

Original Research Article

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## Evaluation of Substrates for Mass Production of *Trichoderma harzianum* and its Compatibility with Chlorpyrifos + Cypermethrin

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### ABSTRACT

Coir pith compost, rice husk and sugarcane bagasse with and without 5% Jaggery solution as nutritional supplement was evaluated for the mass production of *Trichoderma harzianum* in comparison with coffee husk (parchment). On incubation up to 60 days, maximum number of propagules were recorded in coir pith compost ( $43 \times 10^{10}$ ), coir pith compost + 5% jaggery ( $32.33 \times 10^{10}$ ) and sugarcane bagasse ( $21 \times 10^{10}$ ) followed by rice husk ( $38 \times 10^{10}$ ) and rice husk + 5% jaggery ( $23.33 \times 10^{10}$ ). Coir pith compost and sugarcane bagasse recorded more population than other substrates and hence could be used in mass production. In another experiment, the compatibility of *T. harzianum* and insecticide combination, Chlorpyrifos 50% + Cypermethrin 5% was studied by poisoned food technique on PDA medium at concentrations of 55, 83, 110, 138, 165, 193 and 220 ppm. In the first set, the insecticide was added to the autoclaved medium (hence not heated) and in the second set the insecticide was autoclaved along with the medium. In both the cases, the colony growth was very slow initially for 5 days, however continued to grow as evident from the measurements on 10th and 15th day. Thus the insecticide was partially compatible with *T. harzianum* in *in-vitro* studies at all the concentration levels. Later, *T. harzianum* and Chlorpyrifos + Cypermethrin were incorporated into soil taken in containers so as to get the above insecticide concentrations and studied the compatibility by monitoring the population by serial dilution plate technique. After 30 days, it was found that the propagules in all the insecticide concentrations were on par with each other. Hence it is implied that the insecticide did not affect the population of *T. harzianum* in *in-vivo* conditions and found compatible for field application.

#### Keywords

Chlorpyrifos, compatibility, Cypermethrin, Mass production, Substrates and *Trichoderma*.

#### Article Info

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### Introduction

*Trichoderma harzianum* has been identified as the potential biocontrol agent for soil borne disease management in black pepper, cardamom and ginger. Several studies were conducted to evaluate various substrates for its mass production (Rini *et al.*, 2007; Prakash *et al.*, 1999; Saju *et al.*, 2002). Coffee husk

(Parchment) is considered as a very good substrate for the mass production and field application especially in the Western Ghats area of Karnataka. However, during off-seasons there is scarcity of coffee husk and an alternative is sought for uninterrupted production of the bioagent formulation.

Therefore, this study was conducted to evaluate other organic substrates suitable for mass multiplication of *T. harzianum*. Attempts were also made to enhance the population on these substrates using nutritional supplements.

For effective management of diseases, the compatibility of potential bioagents with insecticides is essential (Ashwini *et al.*, 2012). Integration of compatible bioagent with pesticides may enhance the effectiveness of disease control and provide better management of soil borne diseases (Vinit *et al.*, 2012; Thiruchelvan *et al.*, 2013; Saxena *et al.*, 2014). In black pepper, the insecticide combination of Chlorpyrifos 50% and Cypermethrin 5% is being used as drench to control mealy bugs and termites. It is established that the Chlorpyrifos is compatible with *T. harzianum* (Stephen *et al.*, 2000). The compatibility of Cypermethrin with *T. harzianum* is not studied so far. In view of the above, a study was undertaken to evaluate the compatibility of *T. harzianum* and combination of insecticide Chlorpyrifos 50% + Cypermethrin 5% *in-vitro* and *in-vivo*.

## Materials and Methods

### Preparation of mother culture of *T. harzianum*

*T. harzianum* maintained in the Plant Pathology laboratory of ICRI Regional Station, Sakaleshpur was used. This isolate is being mass produced and used for disease management in spices. Mother culture of *T. harzianum* was prepared by growing on PDA (20 ml) taken in low cost bottles. After inoculation with *T. harzianum*, the bottles were incubated for 8-10 days at 24+1°C. Then distilled water was added to each bottle and harvested the spores into a beaker. Six bottles were used to prepare 1 liter of spore suspension.

### Growth and sporulation of *T. harzianum* on organic substrates

Coir pith compost, rice husk and sugarcane bagasse are the various organic substrates used in this study in comparison with coffee husk (parchment). The substrates were sprinkled with water or 5% jaggery solution so as to obtain 50% moisture, filled (250 g) in poly propylene bags, sealed and sterilised. After reaching room temperature, each bag was inoculated with 10 ml of spore suspension of *T. harzianum* taken in a sterile syringe and mixed thoroughly for uniform distribution of spores. Then the bags were incubated at room temperature. The population of *T. harzianum* was analyzed by serial dilution plate technique (SDPT) 15 days after inoculation (DAI), 30 DAI and 60 DAI. Aliquots (1 ml) from the suitable dilution was mixed with Rose Bengal Agar (RBA) in petri plates and incubated for colony formation and recorded after 7-8 days. The number of colony forming units (cfu) was then converted to log values and analyzed by ANOVA using MSTATC software. Duncan's Multiple Range Test (DMRT) did the mean comparison.

### Treatments

- T1 Coir pith compost
- T2 Coir pith compost + 5% Jaggery
- T3 Sugarcane bagasse
- T4 Sugarcane bagasse + 5% Jaggery
- T5 Rice husk
- T6 Rice husk + 5% Jaggery
- T7 Coffee husk parchment (control)

Replications: 3

Number of bags per replication: 12

### *In-vitro* study on the compatibility of *T. harzianum* and insecticide combination Chlorpyrifos 50% + Cypermethrin 5%

The compatibility of *T. harzianum* and insecticide combination, Chlorpyrifos 50% + Cypermethrin 5% was studied by poisoned

food technique on PDA medium. Requisite quantity of the insecticide was added to the PDA in order to get final concentrations of 55, 83, 110, 138, 165, 193 and 220ppm. In the first set of experiment, the insecticide was added to the autoclaved medium and then dispensed into petri plates (hence the insecticide was not heated). In the second set of experiment, the insecticide was added to the medium and then autoclaved and dispensed into the petri plates. The petri plates were then inoculated with 5 mm diameter mycelial disc of 3 days old culture of *T. harzianum* and incubated at room temperature. There were 8 treatments with 3 replications for each concentration of the insecticide. The radial growth of the colony was measured after 1 day, 2 days, 3 days, 4 days, 5 days, 10 days and 15 days of inoculation. The inhibition (%) of the growth of *T. harzianum* due to the presence of insecticide was calculated using the formula, (Radial growth of TH in control plate – Radial growth in treated plate) / Radial growth in control plate) x 100

The radial growth was analyzed by ANOVA and Duncan's Multiple Range Test (DMRT) did the mean comparison using the MSTATC software. The inhibition (%) was transformed to corresponding angular values and analyzed by ANOVA and DMRT.

#### ***In-vivo* study on the compatibility of *T. harzianum* and combination of insecticide Chlorpyrifos 50% + Cypermethrin 5% in soil**

100 g of shade dried soil was taken in plastic cups of 11.5 cm height x 11.5 cm diameter. Requisite quantity of the insecticide was added to the soil in order to get final concentration 55, 83, 110, 138, 165, 193 and 220 ppm. 1 g of *T. harzianum* grown on coffee husk (parchment) was added to each cup and maintained at  $24 \pm 2^\circ\text{C}$  in a shade

house for 30 days. Distilled water (5-7 ml) was added weekly twice to retain the moisture in each cup. The population of *T. harzianum* was analyzed by serial dilution plate technique (SDPT) at 0 hour, 15 days after inoculation (DAI) and 30 DAI. The number of colony forming unit (cfu) was converted to corresponding log values and analyzed by ANOVA using MSTATC software. Duncan's Multiple Range Test (DMRT) did the mean comparison.

### **Results and Discussion**

#### **Growth and sporulation of *T. harzianum* on organic substrates**

The growth and sporulation of *T. harzianum* on organic substrates like coir pith compost, rice husk, sugarcane bagasse etc was studied. The inoculated substrates were incubated and analyzed the cfu at periodical intervals. After 15 days of incubation colony forming units of *T. harzianum* in each treatment was found to be on par with each other and recorded colonies up to  $10^{10}$  dilution. After 30 days, coffee husk (T<sub>7</sub>) showed maximum number of cfu per gram of the substrate ( $48 \times 10^{10}$ ) followed by coir pith compost (T<sub>1</sub> /  $24.33 \times 10^{10}$ ), sugarcane bagasse + 5% jaggery (T<sub>4</sub> /  $26 \times 10^{10}$ ) and rice husk (T<sub>5</sub> /  $43.33 \times 10^{10}$ ) which were on par with each other. On further incubation up to 60 days, coir pith compost (T<sub>1</sub> /  $43 \times 10^{10}$ ), coir pith compost + 5% jaggery (T<sub>2</sub> /  $32.33 \times 10^{10}$ ) and sugarcane bagasse (T<sub>3</sub> /  $21 \times 10^{10}$ ) recorded maximum number of cfu followed by rice husk (T<sub>5</sub> /  $38 \times 10^{10}$ ) and rice husk + 5% jaggery (T<sub>6</sub> /  $23.33 \times 10^{10}$ ). In all the substrates, colonies were found in  $10^{10}$  dilution and survived even up to 60 days of incubation (Table 1). Coir pith compost (T<sub>1</sub>) and sugarcane bagasse (T<sub>3</sub>) recorded more population than other substrates and hence could be used as alternate media in mass production. Earlier studies reported excellent multiplication of *T.*

*harzianum* on coffee husk (Prakash *et al.*, 1999; Saju *et al.*, 2002).

***In-vitro* study on the compatibility of *T. harzianum* and insecticide combination Chlorpyrifos 50% + Cypermethrin 5%**

When the insecticide was added to the autoclaved PDA and inoculated with *T. harzianum*, the radial growth of the colony was least in T<sub>7</sub> (0.64 cm) followed by T<sub>6</sub> (0.75 cm) and T<sub>5</sub> (0.86 cm) which were on par with each other after 5 days of incubation as compared to control (T<sub>8</sub> / 9.87 cm). Among T<sub>1</sub> - T<sub>4</sub> growth of *T. harzianum* could be observed after 2 days, but in T<sub>5</sub> and T<sub>6</sub> growth could be seen only after 3 days and in T<sub>7</sub> after 4 days. The inhibition (%) of colony growth after 4 days of incubation was highest in T<sub>7</sub> (91.66), T<sub>6</sub> (91.53) and T<sub>5</sub> (91.04) (Table 2). Similarly, after 5 days of incubation,

inhibition (%) was highest in T<sub>7</sub> (97.78) followed by T<sub>6</sub> (90.39). This was followed by T<sub>5</sub> (88.99) and T<sub>4</sub> (87.98) which were on par with each other. Even though the colony growth was very slow initially for 5 days, it continued to grow as evident from the measurements on 10th and 15th day.

When the insecticide was added to PDA, autoclaved and then inoculated with *T. harzianum*, the radial growth of the colony was least in T<sub>7</sub> (0.70 cm) and T<sub>6</sub> (0.68 cm) and T<sub>5</sub> (0.69 cm) which were on par with each other after 5 days of incubation. The inhibition (%) of colony growth after 5 days of incubation was on par with each other in all the treatments (Table 3). Similar to the previous set of experiment, even though the *T. harzianum* was inhibited initially, it continued to grow as evident from the measurements on 10th and 15th day.

**Table.1** Population (cfu) of *Trichoderma harzianum* on different substrates at periodical intervals

Treatments	No. of cfu x 10 <sup>10</sup>		
	15 days	30 days	60 days
T <sub>1</sub> Coir pith compost	52.00 (11.72) <sup>a</sup>	24.33 (11.51) <sup>ab</sup>	43.00 (11.63) <sup>a</sup>
T <sub>2</sub> Coir pith compost + 5% Jaggery	43.66 (11.31) <sup>a</sup>	26.00 (11.40) <sup>abc</sup>	32.33 (11.65) <sup>a</sup>
T <sub>3</sub> Sugarcane bagasse	56.00 (11.74) <sup>a</sup>	23.66 (11.20) <sup>c</sup>	21.00 (11.61) <sup>a</sup>
T <sub>4</sub> Sugarcane bagasse + 5% Jaggery	44.66 (11.63) <sup>a</sup>	26.00 (11.49) <sup>ab</sup>	24.00 (11.43) <sup>b</sup>
T <sub>5</sub> Rice husk	23.33 (11.36) <sup>a</sup>	43.33 (11.45) <sup>ab</sup>	38.00 (11.54) <sup>ab</sup>
T <sub>6</sub> Rice husk + 5% Jaggery	27.33 (11.42) <sup>a</sup>	35.66 (11.28) <sup>bc</sup>	23.33 (11.54) <sup>ab</sup>
T <sub>7</sub> Coffee husk (Parchment)	32.33 (11.51) <sup>a</sup>	48.00 (11.53) <sup>a</sup>	37.33 (11.26) <sup>c</sup>
CD (p=0.05)	0.4605	0.2320	0.1258

cfu: colony forming units

Figures in parenthesis indicate logarithmic values

Means followed by the same alphabet(s) are not significantly different as per Duncan's Multiple Range Test

**Table.2** *In-vitro* study on the compatibility of *Trichoderma harzianum* and insecticide combination Chlorpyrifos 50% + Cypermethrin 5% added after autoclaving

Treatment (Chlorpyrifos + Cypermethrin)	Radial growth of colony (cm)							Inhibition (%)				
	1 day	2 day	3 day	4 day	5 day	10 day	15 day	1 day	2 day	3 day	4 day	5 day
T <sub>1</sub> 55 ppm	0.25 <sup>b</sup>	0.60 <sup>b</sup>	0.85 <sup>b</sup>	1.13 <sup>b</sup>	1.50 <sup>b</sup>	2.48 <sup>a</sup>	4.43 <sup>a</sup>	91.37	85.71 (67.78) <sup>b</sup>	86.64 (68.55) <sup>d</sup>	85.12 (67.30) <sup>d</sup>	80.96 (64.06) <sup>e</sup>
T <sub>2</sub> 83 ppm	0.25 <sup>b</sup>	0.72 <sup>b</sup>	0.77 <sup>b</sup>	0.87 <sup>bc</sup>	1.22 <sup>bc</sup>	2.02 <sup>ab</sup>	4.50 <sup>a</sup>	91.37	82.85 (65.60) <sup>b</sup>	87.94 (69.74) <sup>cd</sup>	88.45 (70.16) <sup>bc</sup>	84.47 (66.73) <sup>d</sup>
T <sub>3</sub> 110 ppm	0.25 <sup>b</sup>	0.60 <sup>b</sup>	0.76 <sup>b</sup>	0.90 <sup>bc</sup>	1.20 <sup>bc</sup>	1.66 <sup>bc</sup>	3.50 <sup>b</sup>	91.37	85.71 (67.78) <sup>b</sup>	87.99 (69.71) <sup>cd</sup>	88.15 (69.83) <sup>c</sup>	84.77 (67.01) <sup>d</sup>
T <sub>4</sub> 138 ppm	0.25 <sup>b</sup>	0.60 <sup>b</sup>	0.61 <sup>b</sup> <sup>c</sup>	0.72 <sup>c</sup>	0.94 <sup>bc</sup>	1.41 <sup>cd</sup>	2.23 <sup>c</sup>	91.37	85.71 (67.78) <sup>b</sup>	90.45 (71.98) <sup>bc</sup>	90.47 (71.95) <sup>ab</sup>	87.98 (69.69) <sup>c</sup>
T <sub>5</sub> 165 ppm	0.25 <sup>b</sup>	0.25 <sup>c</sup>	0.6b <sup>c</sup>	0.68 <sup>c</sup>	0.86 <sup>c</sup>	1.44 <sup>cd</sup>	2.22 <sup>c</sup>	91.37	94.04 (75.82) <sup>a</sup>	90.61 (72.15) <sup>bc</sup>	91.04 (72.50) <sup>a</sup>	88.99 (70.57) <sup>c</sup>
T <sub>6</sub> 193 ppm	0.25 <sup>b</sup>	0.25 <sup>c</sup>	0.53 <sup>bc</sup>	0.64 <sup>c</sup>	0.75 <sup>c</sup>	1.08 <sup>de</sup>	1.61 <sup>cd</sup>	91.37	94.04 (75.82) <sup>a</sup>	91.65 (73.25) <sup>b</sup>	91.53 (73.06) <sup>a</sup>	90.39 (71.92) <sup>b</sup>
T <sub>7</sub> 220 ppm	0.25 <sup>b</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup>	0.63 <sup>c</sup>	0.64 <sup>c</sup>	0.77 <sup>e</sup>	0.97 <sup>d</sup>	91.37	94.04 (75.82) <sup>a</sup>	96.08 (78.46) <sup>a</sup>	91.66 (73.19) <sup>a</sup>	91.78 (73.26) <sup>a</sup>
T <sub>8</sub> Control	2.90 <sup>a</sup>	4.20 <sup>a</sup>	6.39 <sup>a</sup>	7.60 <sup>a</sup>	7.87 <sup>a</sup>	-	-	-	-	-	-	-
CD (p=0.05)	0.110	0.207	0.375	0.363	0.626	0.556	0.767	-	2.211	2.704	2.038	1.290

Figures in parenthesis indicate arc sin transformations

Means followed by the same alphabet(s) are not significantly different as per Duncan's Multiple Range Test

**Table.3** *In-vitro* study on the compatibility of *Trichoderma harzianum* and insecticide combination Chlorpyrifos 50% + Cypermethrin 5% added before autoclaving

Treatment (Chlorpyrifos + Cypermethrin)	Radial growth of colony (cm)							Inhibition (%)				
	1 day	2 day	3 day	4 day	5 day	10 day	15 day	1 day	2 day	3 day	4 day	5 day
T <sub>1</sub> 55 ppm	0.25 <sup>b</sup>	0.60 <sup>b</sup>	0.82 <sup>b</sup>	1.23 <sup>b</sup>	1.45 <sup>b</sup>	2.25 <sup>a</sup>	3.23 <sup>a</sup>	91.37	85.71	87.16 (68.99) <sup>b</sup>	83.76 (66.36) <sup>a</sup>	81.55 (64.84) <sup>a</sup>
T <sub>2</sub> 83 ppm	0.25 <sup>b</sup>	0.60 <sup>b</sup>	0.85 <sup>b</sup>	1.06 <sup>bc</sup>	1.23 <sup>bc</sup>	1.96 <sup>a</sup>	2.22 <sup>b</sup>	91.37	85.71	86.59 (68.47) <sup>b</sup>	85.96 (68.01) <sup>a</sup>	84.34 (66.67) <sup>a</sup>
T <sub>3</sub> 110 ppm	0.25 <sup>b</sup>	0.60 <sup>b</sup>	0.71 <sup>b</sup>	0.82 <sup>bcd</sup>	0.85 <sup>bc</sup>	0.96 <sup>b</sup>	1.03 <sup>c</sup>	91.37	85.71	88.83 (70.45) <sup>b</sup>	89.20 (70.81) <sup>a</sup>	89.12 (70.81) <sup>a</sup>
T <sub>4</sub> 138 ppm	0.25 <sup>b</sup>	0.60 <sup>b</sup>	0.69 <sup>b</sup>	0.80 <sup>bcd</sup>	0.82 <sup>bc</sup>	0.86 <sup>b</sup>	0.91 <sup>c</sup>	91.37	85.71	89.19 (70.78) <sup>b</sup>	89.46 (71.08) <sup>a</sup>	89.54 (71.20) <sup>a</sup>
T <sub>5</sub> 165 ppm	0.25 <sup>b</sup>	0.25 <sup>c</sup>	0.62 <sup>b</sup>	0.68 <sup>cd</sup>	0.69 <sup>c</sup>	0.69 <sup>b</sup>	0.74 <sup>c</sup>	91.37	94.04	90.29 (71.82) <sup>b</sup>	91.04 (72.56) <sup>a</sup>	91.23 (72.73) <sup>a</sup>
T <sub>6</sub> 193 ppm	0.25 <sup>b</sup>	0.25 <sup>c</sup>	0.60 <sup>b</sup>	0.61 <sup>d</sup>	0.68 <sup>c</sup>	0.75 <sup>b</sup>	0.82 <sup>c</sup>	91.37	94.04	90.61 (72.15) <sup>b</sup>	91.97 (73.54) <sup>a</sup>	91.36 (72.83) <sup>a</sup>
T <sub>7</sub> 220 ppm	0.25 <sup>b</sup>	0.25 <sup>c</sup>	0.25 <sup>c</sup>	0.63 <sup>cd</sup>	0.70 <sup>c</sup>	0.71 <sup>b</sup>	0.75 <sup>c</sup>	91.37	94.04	96.08 (78.46) <sup>a</sup>	91.66 (73.19) <sup>a</sup>	91.11 (72.63) <sup>a</sup>
T <sub>8</sub> Control	2.90 <sup>a</sup>	4.20 <sup>a</sup>	6.39 <sup>a</sup>	7.60 <sup>a</sup>	7.87 <sup>a</sup>	-	-	-	-	-	-	-
CD (p=0.05)	0.110	0.156	0.327	0.449	0.749	0.337	0.377	-	-	3.816	13.16	16.91

Figures in parenthesis indicate arc sin transformations

Means followed by the same alphabet (s) are not significantly different as per Duncan's Multiple Range Test

**Table.4** *In-vivo* study on the compatibility of *Trichoderma harzianum* and combination of insecticide Chlorpyrifos 50% + Cypermethrin 5% in soil

Treatment (Chlorpyrifos + Cypermethrin)	No. of cfu x 10 <sup>10</sup>		
	0 Hour	15 DAI	30 DAI
T <sub>1</sub> 55 ppm	10 (10.97) <sup>a</sup>	14 (11.12) <sup>a</sup>	10 (11.00) <sup>a</sup>
T <sub>2</sub> 83 ppm	2 (10.35) <sup>b</sup>	12 (11.07) <sup>a</sup>	11 (11.02) <sup>a</sup>
T <sub>3</sub> 110 ppm	4 (10.61) <sup>ab</sup>	14 (11.12) <sup>a</sup>	9 (10.93) <sup>a</sup>
T <sub>4</sub> 138 ppm	6 (10.75) <sup>ab</sup>	3 (10.51) <sup>a</sup>	5 (10.63) <sup>a</sup>
T <sub>5</sub> 165 ppm	9 (10.95) <sup>ab</sup>	6 (10.64) <sup>a</sup>	3 (10.51) <sup>a</sup>
T <sub>6</sub> 193 ppm	7 (10.85) <sup>ab</sup>	5 (10.68) <sup>a</sup>	4 (10.58) <sup>a</sup>
T <sub>7</sub> 220 ppm	6 (10.74) <sup>ab</sup>	5 (10.68) <sup>a</sup>	4 (10.54) <sup>a</sup>
T <sub>8</sub> Control	13 (11.11) <sup>a</sup>	17 (11.23) <sup>a</sup>	15 (11.15) <sup>a</sup>
CD (p=0.05)	0.606	1.006	0.743

cfu: colony forming units; Figures in parenthesis indicate logarithmic values  
Means followed by the same alphabet(s) are not significantly different as per Duncan's Multiple Range Test

The concentration of insecticide for soil drenching in black pepper is 200 ml per 100 liters that would give 83 ppm solution. Hence, concentration below and above the recommended dose were studied here. The insecticide Chlorpyrifos was already known to be compatible with *T. harzianum* (Stephen *et al.*, 2000). However, in this study when the combination of Chlorpyrifos 50% + Cypermethrin 5% was used it was found to slow down the radial growth of *T. harzianum* initially for 5 days. Therefore it implies that the Cypermethrin is effecting the growth of *T. harzianum* initially even at 55ppm. It is possible since the *T. harzianum* comes in direct contact with the insecticide molecules during *in-vitro* studies. However, the fungus continued to grow after initial suppression as evident from the measurements on 10th and 15th day. This shows the partial compatibility of the insecticide combination and *T. harzianum* in *in-vitro* condition.

***In-vivo* study on the compatibility of *T. harzianum* and combination of insecticide Chlorpyrifos 50% + Cypermethrin 5% in soil**

The number of cfu recorded at 0 hour slightly varied in different treatments. This might be

due to handling error and such variations are not expected since uniform quantities were added in each treatment. The cfu in all the treatments were on par with each other at 15 DAI which showed that the addition of insecticide into soil did not affect the population of *T. harzianum*. Colonies were noted even at 10<sup>10</sup> dilution (Table 4). Similarly at 30 DAI, the cfu present in all the treatments were also on par with each other. Colonies were present even at 10<sup>10</sup> dilution. Hence it is implied that the insecticide did not affect the population of *T. harzianum* in *in-vivo* conditions. Stephen *et al.* (2000) noted similar observations with *T. harzianum* and Chlorpyrifos.

The combination of insecticide has entered in to the market very recently. The *in-vitro* study conducted here gives an indication that care should be taken to avoid direct contact of the *T. harzianum* and the insecticide so that benefits of both are made available for the protection of black pepper. One strategy that is employed with other incompatible chemicals is that there would be a time gap given in between the application of *T. harzianum* and the chemical so that the direct contact is avoided thereby damage to its

propagules minimized. It may be further noted that even though the insecticide showed partial incompatibility with *T. harzianum* in *in-vitro* test, the concentration is further diluted when we drench the same to the root zone which consists of ~500 kg of soil and receives ~5 liter of drenching solution. That is why in *in-vivo* studies the insecticide doses could not affect the *T. harzianum* propagules and showed compatible for field application.

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