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Compatibility study of insecticides recommended for the management of tea mosquito bug *Helopeltis* spp. with bio-fungicide, *Trichoderma harzianum*

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Abstract
The present investigation was conducted to study the lethal effect of insecticides recommended for management of tea mosquito bug, *Helopeltis* spp. on bio-control agent, *Trichoderma harzianum*. Effect of six insecticides (Bifenthrin 10% EC, Clothianidin 50% WDG, Flonicamid 50% WG, Imidacloprid 17.8% SL, Thiamethoxam 25% WG and Quinalphos 25% EC) with three doses (lower, recommended and higher) on colony growth, antagonism potential and sporulation of *Trichoderma harzianum* were studied. Quinalphos 25% EC showed high inhibitory effect in all three doses. Recommended dose of Quinalphos caused 72.77, 58.18 and 97.16 per cent reduction in mean colony growth, antagonism potential and sporulation of *Trichoderma harzianum* respectively. Slight deleterious effect in highest dose (150 ppm) of Bifenthrin 10% EC was recorded with 13.33, 5 and 9.83 percent inhibition in mean colony growth, antagonism potential and sporulation respectively. All other insecticides under investigation were found non toxic at all three doses and were at par with control which indicated the compatibility of these insecticides with *Trichoderma harzianum*. All the compatible insecticides can be utilized in Integrated Pest Management programme with recommended doses along with *Trichoderma harzianum* without causing any deleterious effect.

Keywords: compatibility, *Trichoderma harzianum*, spore count, insecticides, dual plate

1. Introduction
In cashew, it causes 30 to 50 per cent annual crop loss due to inflorescence blight and immature nut fall [4-7]. Around 80 per cent of the tea plantations are being affected by this pest which in turn reducing the productivity to the tune of 10-50 per cent [8]. In cocoa, it causes up to 85 per cent yield loss if infestation synchronized with pod development stage [9]. Use of synthetic insecticides is the only successful and widely accepted practice for the management of TMB. Insecticides belonging to organophosphate, neonicotinoid and synthetic pyrethroid groups are commonly recommended for TMB management. Organophosphate insecticides including carbaryl, monocrotophos, phosalone, phosphamidon, quinalphos, dimethoate and dichlorvos are recommended for the control of *H. antonii* in cashew [4, 10, 11, 12]. Synthetic pyrethroids *viz*., decamethrin (0.002%), permethrin (0.01%), cypermethrin (0.0075%) are recommended against TMB, *H. antonii* [13].
Despite the use of fungicides, *Trichoderma* spp. is extensively engaged as a bio-control agent for the management of many phyto-pathogens [14]. Different strains of *Trichoderma* spp. are employed for the sustainable management of *Phytophthora* diseases in cocoa, cashew nut and tea. In Cameroon, various strains of *T. asperellum* were found to be mycoparasitic and considerably lowering the percentage of diseased pods caused by *P. capsici*, *P. citrophthora*, and *P. palmivora* during field screening in cocoa [15]. Krauss and Soberanis [16] found that *P. palmivora* was most susceptible to mycoparasitism exhibited by mycoparasitic mixtures including *Trichoderma* spp. *Trichoderma virens* reduced the black pod incidence and increased yield up to 15 per cent by using the strain mixtures [17]. *T. harzianum* exhibits mycoparasitic and enzymatic activity towards the target besides induced systemic resistance in host plants (Harman *et al.*, 2004) [14].
Chemical insecticides recommended for management of TMB in cocoa as well as tea ecosystem may affect the population of beneficial *Trichoderma* present in the ecosystem.

Integrated pest management (IPM) is evolved as an effective strategy for management of diseases and pests in sustainable manner. IPM includes utilization of all the available management techniques in a compatible way for the management of target pest.

In this context, there is an urgent need to check the compatibility of all the recommended chemical insecticides against TMB with *T. harzianum* to avoid detrimental effect on soil micro-flora and to conserve the beneficial microorganisms for sustainable pest management. Hence, the present investigation was formulated to explore the possibilities of using insecticides and *T. harzianum* in combination by following principle of integrated pest management in a compatible manner.

2. Materials and Methods

2.1 Culture

Fungal biocontrol agent, *T. harzianum* (CPTD28) and oomycete pathogen, *Phytophthora palmivora* were obtained from Plant pathology section, ICAR-CPCRI regional station, Vittal, Karnataka to carry out present investigation. All cultures were maintained in potato dextrose agar (PDA) plates at 28 ± 2 ºC.

2.2 Insecticides

Central insecticide board (CIB) recommended insecticides for the management of TMB were included in the present investigation. A six test insecticides with three different concentrations (lower, recommended and higher) were used in this compatibility study. Details of insecticides along with concentrations are given in the Table 1.

Table 1: Details of insecticides with different concentrations used

S. No.	Test insecticide	Concentrations use (in ppm)
1	Bifenthrin 10% EC	50, 100 and 150
2	Flonicamid 50% WG	100, 150 and 200
3	Clothianidin 50% WDG	100, 120 and 140
4	Imidacloprid 17.8% SL	90, 110 and 130
5	Thiamethoxam 25% WG	42.5, 62.5 and 82.5
6	Quinalphos 25% EC	200, 250 and 300

2.3 *In-vitro* Compatibility Test for Insecticides and *T. harzianum*

Poison food technique was used to assess the lethal effects of different test insecticides against the bio-control agent, *T. harzianum* [18]. Desired concentrations of insecticides were amended in PDA media and 5mm sized mycelial plugs of 5-days old *T. harzianum* culture was inoculated in insecticides amended PDA plates. All the inoculated plates were incubated at 28 ± 2 ºC. PDA plates minus insecticides served as control. A total of 18 treatments with six insecticides (three doses of each) were maintained. Colony morphology and mycelial growth were observed routinely and mean colony inhibition percentage was calculated using the following formula [19].

Mean colony inhibition (%) = C-T/C*100

Where 'C' and 'T' are the radial growth of *T. harzianum* in cm in control plate and test plate respectively. The experiment was replicated thrice to get the accuracy.

2.4 Dual Plate Assay

Effect of the test insecticides on the antagonistic activity of *T. harzianum* against *P. palmivora* was studied using dual plate assay [20]. 5mm sized mycelial plugs from 5 days old culture plates of *T. harzianum* and *P. palmivora* were placed in opposite direction (5mm away from the periphery) on insecticide (three different doses) amended carrot agar (CA) plates. Dual and *P. palmivora* inoculated plates without insecticides served as control. All the inoculated plates were incubated at 20±2 °C for 10 days. The antagonism level of *T. harzianum* was assessed by using standard method given by Bell and co-workers [21].

Radial growth of *T. harzianum* and *P. palmivora* in dual plates were recorded once *P. palmivora* reached full growth in control plates. Antagonistic potential of *T. harzianum* was assessed by calculating mean colony inhibition of *P. palmivora* using the following formula.

Mean colony inhibition in% (P) = C-T/C*100

'P' is considered as 100 per cent antagonistic potential of *T. harzianum* and further used to calculate the effect of insecticidal doses on antagonistic potential of *T. harzianum.* Where 'C' is the radial growth of *P. palmivora* in CA plates containing only pathogen and 'T' is the radial growth of *P. palmivora* in dual control plates in cm.

Colony diameter of *P. palmivora* was recorded from control (dual plate minus insecticides) and treatment (dual plate with respective insecticidal dose) after nine days of inoculation. Mean colony inhibition of *P. palmivora* was calculated using following formula:

Mean colony inhibition in% (Q) = C-T/C*100

Where 'C' is the radial growth of *P. palmivora* in control dual plates and 'T' is the radial growth of *P. palmivora* in insecticide amended dual plates in cm.

'Q' is considered as mean colony inhibition of *P. palmivora* due to *T. harzianum* in presence of insecticides and further compared with 'P' (100 per cent antagonism potential of *T. harzianum*) to find out the per cent reduction in antagonism potential (R) due to different insecticidal doses as follows:

R = 100-(Q*100/P)

2.5 Spore count assay

Spore counting of *T. harzianum* in control and test insecticides treated plates were done using Haemocytometer [22]. A drop of filtered conidial suspension was placed on the engraved grid and allowed for 1-2 minutes to settle the spores at the bottom. Cover glass was put over the grid to avoid formation of air bubbles. Since, *T. harzianum* producing small conidia, spores observed in the middle square of haemocytometer is considered for calculation of spores/ ml using the following formula:

Spores/ml = Number of spores counted on the middle square of the grid * 10000

Effect of test insecticides on sporulation *T. harzianum* was further studied by calculating per cent reduction of spores in various treatments by following formula:

Spore reduction (%) = X-Y / X*100

Where 'X' is the number of spores/ml in control plate and 'Y' is the number of spores/ml in treated plate.

2.6 Statistical analysis

To confirm the results, all the experiments were repeated

thrice at different time intervals. Data was analyzed by one-way analysis of variance (ANOVA) using SPSS software. Differences between treatment mean values were determined following DMRT test at P<0.05. *In-vitro* compatibility (bio-fungicide and insecticides) experiment was conducted in complete randomized design (CRD).

3. Results and Discussion

3.1 *In-vitro* Compatibility Test for Insecticides and *T. harzianum*

Colony growth of *T. harzianum* was recorded after 96 h of incubation under *In-vitro* compatibility test. The results resulted that Quinalphos 25% EC showed high inhibitory effect against *T. harzianum* in all three doses *viz.*, 200, 250 and 300 ppm with per cent inhibition of mean colony growth as 70.55, 72.77 and 76.66 respectively (Fig 1). In case of Bifenthrin 10% EC, highest dose i.e., 150 ppm showed a slightly high inhibitory effect with 13.33 per cent inhibition in mean colony growth. Rest of the insecticides was at par with control for all three doses showed the compatibility of these insecticides with *T. harzianum* (Table 2).

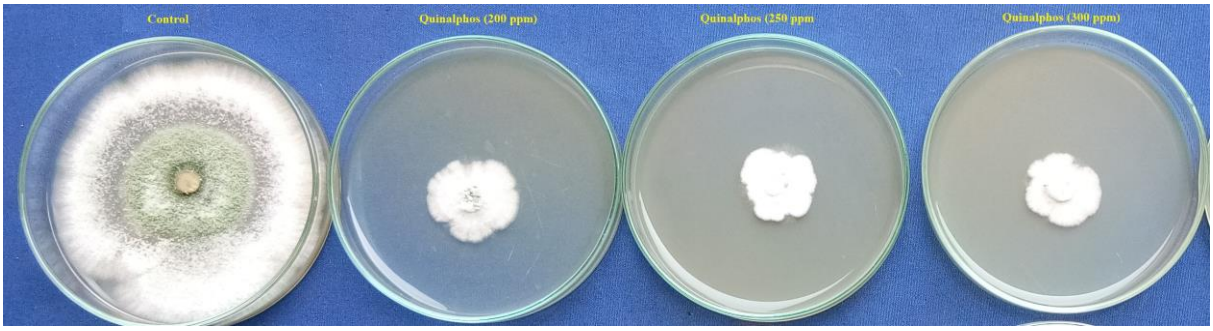

Fig 1: Colony growth of *T. harzianum* in Quinalphos treated doses

Table 2: Effect of insecticidal doses on *T. harzianum* on PDA medium 96 h after incubation.

Treatments	Insecticides (dose)	Mean colony growth (cm)	Inhibition (%)
T1	Control	9.00[a]	0
T2	Quinalphos (200ppm)	2.65[c]	70.55
T3	Quinalphos (250ppm)	2.45[cd]	72.77
T4	Quinalphos (300ppm)	2.10[d]	76.66
T5	Bifenthrin (50ppm)	8.80[a]	2.22
T6	Bifenthrin (100ppm)	8.95[a]	0.55
T7	Bifenthrin (150ppm)	7.80[b]	13.33
T8	Imidacloprid (90ppm)	8.76[a]	2.66
T9	Imidacloprid (110ppm)	8.85[a]	1.66
T10	Imidacloprid (130ppm)	8.67[a]	3.61
T11	Clothianidin (100ppm)	8.95[a]	0.55
T12	Clothianidin (120ppm)	8.75[a]	2.77
T13	Clothianidin (140ppm)	8.82[a]	2
T14	Flonicamid (100ppm)	8.90[a]	1.11
T15	Flonicamid (150ppm)	8.95[a]	0.55
T16	Flonicamid (200ppm)	8.75[a]	2.77
T17	Thiamethoxam (42.5ppm)	8.85[a]	1.66
T18	Thiamethoxam (62.5ppm)	8.80[a]	2.22
T19	Thiamethoxam (82.5ppm)	8.70[a]	3.33

Figures having same letter as superscripts in a column indicate the values are not significantly different according the DMRT at 0.05∞

3.2 Dual Plate Assay

As per Bell and co-authors [21] antagonistic classification, studies on the dual culture activity indicated that *T. harzianum* CPTD28 used in this study exhibits class *I* level (*Trichoderma* grew, overlapped the *P. palmivora* colony and covered the whole media surface), of antagonism.

Studies on the effect of insecticidal doses over *T. harzianum* antagonistic activity confirmed that Quinalphos 25% EC significantly reduced the antagonistic potential of *T. harzianum* in all three doses (200, 250 and 300 ppm) with 55.81, 58.18 and 61.81 per cent respectively. Bifenthrin 10% EC with 150 ppm exhibited 5 per cent reduction in antagonistic potential. Whereas, in Flonicamid *T. harzianum* grown more profusely and overgrew *P. palmivora* (Fig 2). Rests of the insecticides were at par with control which indicated no reduction in antagonistic potential of *T. harzianum* (Table 3).

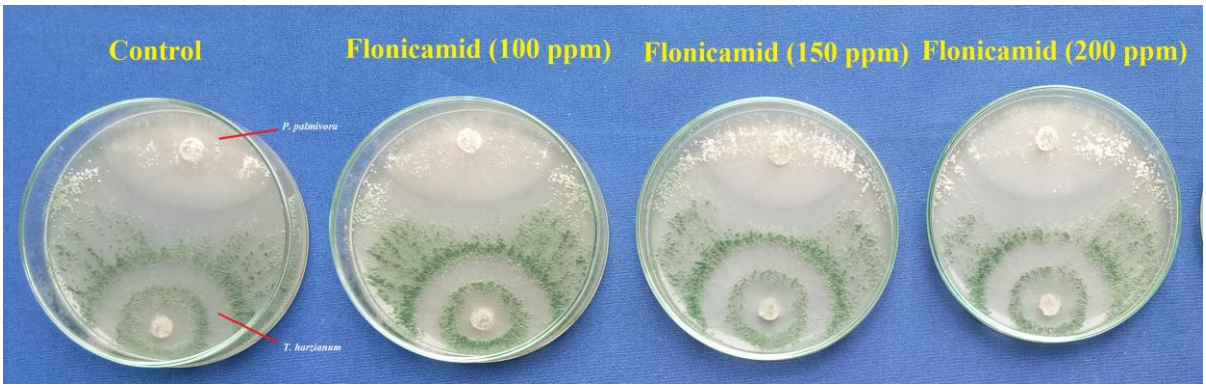

Fig 2: Colony growth of *T. harzianum* over *P.palmivora* in Flonicamid treated dual plates

Table 3: Effect of insecticidal doses on antagonistic potential of *T. harzianum*

Treatments	Insecticides (dose)	Mean colony growth (cm)	Reduction in antagonistic potential (%)
T1	Control	3.50de	0
T2	Quinalphos (200ppm)	6.50b	55.81
T3	Quinalphos (250ppm)	6.70ab	58.18
T4	Quinalphos (300ppm)	6.90a	61.81
T5	Bifenthrin (50ppm)	3.50de	-0.00
T6	Bifenthrin (100ppm)	3.47def	-0.54
T7	Bifenthrin (150ppm)	3.82c	5.90
T8	Imidacloprid (90ppm)	3.55d	0.90
T9	Imidacloprid (110ppm)	3.42def	-1.45
T10	Imidacloprid (130ppm)	3.52de	0.45
T11	Clothianidin (100ppm)	3.50de	0.01
T12	Clothianidin (120ppm)	3.22f	-5.09
T13	Clothianidin (140ppm)	3.50de	-0.00
T14	Flonicamid (100ppm)	3.45def	-0.91
T15	Flonicamid (150ppm)	3.45def	-0.91
T16	Flonicamid (200ppm)	3.53de	0.54
T17	Thiamethoxam (42.5ppm)	3.41def	-1.54
T18	Thiamethoxam (62.5ppm)	3.31def	-3.45
T19	Thiamethoxam (82.5ppm)	3.27ef	-4.18

Figures having same letter as superscripts in a column indicate the values are not significantly different according the DMRT at 0.05∞.

3.3 Spore count assay

Spore count assay indicated that significant variation in sporulation of *T. harzianum* among different treatments. Significant reduction of sporulation was recorded in Quinalphos 25% EC treated plates with 436.66, 424.33 and 409.66 mean number of spores ml^{-1} for 200, 250 and 300 ppm respectively as compared to control (14942 spores ml^{-1}) (Fig 3). Bifenthrin 10% EC with 150 ppm dose resulted in slight reduction of sporulation (9.83 per cent) as compared to control. No reduction in sporulation was observed for other treatments (Table 4).

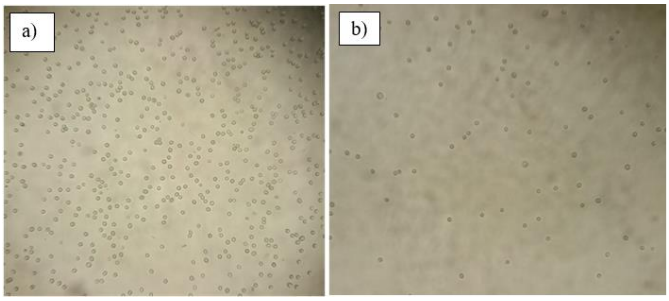

Fig 3: Spores of *T. harzianum*; a. Control, B. Quinalphos (300 ppm)

Table 4: Effect of different insecticidal doses on sporulation of *T. harzianum*

Treatments	Insecticides (dose)	Spore Count (mean numbers ml^{-1})	Spore reduction (%)
T1	Control	14942.00^{a}	0
T2	Quinalphos (200ppm)	436.66c	97.07
T3	Quinalphos (250ppm)	424.33^{c}	97.16
T4	Quinalphos (300ppm)	409.66^{c}	97.25
T5	Bifenthrin (50ppm)	14884.00^{a}	0.388
T6	Bifenthrin (100ppm)	14811.00^{a}	0.876
T7	Bifenthrin (150ppm)	13472.00^{b}	9.83
T8	Imidacloprid (90ppm)	14905.66^{a}	0.24
T9	Imidacloprid (110ppm)	14841.33^{a}	0.67
T10	Imidacloprid (130ppm)	14502.00^{a}	2.94
T11	Clothianidin (100ppm)	14907.00^{a}	0.23
T12	Clothianidin (120ppm)	14896.33^{a}	0.30
T13	Clothianidin (140ppm)	14820.00^{a}	0.81

T14	Flonicamid (100ppm)	14894.66[a]	0.31
T15	Flonicamid (150ppm)	14834.33[a]	0.72
T16	Flonicamid (200ppm)	14563.00[a]	2.53
T17	Thiamethoxam (42.5ppm)	14939.00[a]	0.020
T18	Thiamethoxam (62.5ppm)	14867.33[a]	0.49
T19	Thiamethoxam (82.5ppm)	14811.33[a]	0.87

Figures having same letter as superscripts in a column indicate the values are not significantly different according the DMRT at 0.05∞.

Results of Quinalphos toxicity with respect to per cent inhibition in mean colony growth of *T. harzianum* supports the earlier findings of Sarkar and co-authors, 2010, who reported that 300 ppm dose of Quinalphos, resulted in 75.5 per cent reduction in mycelial growth of *T. harzianum*. Our study confirmed that Imidacloprid is highly compatible with *T. harzianum* which is in harmony with the reports of Bindu Madhavi [23] and Thube [24]. Also Thiamethoxam is highly compatible with *T. harzianum* which is supported by the findings of Patel and co-authors [25]. In parallel to the results of this study, recommended dose of Bifenthrin showed no toxic effect; but higher doses resulted into negative effect on *T. harzianum* [26]. Two newer molecules *viz*., Flonicamid and Clothianidin included in our study showed no negative effect on *T. harzianum* even at higher doses; hence, these chemicals are considered as safer and highly compatible with *T. harzianum*.

Conclusion

Quinalphos 25% EC is highly toxic even at lower doses and responsible for significant reduction in mean colony growth, antagonistic potential and sporulation of *T. harzianum*. Bifenthrin 10% EC at higher dose is slightly toxic and to some extent reduced all the bio-control properties of *T. harzianum*. Quinalphos 25% EC and Bifenthrin 10% EC are not compatible with *T. harzianum* and may also affect the native beneficial fauna; hence, they cannot be included in any integrated pest management (IPM) and integrated disease management (IDM) programme. All other insecticides (Clothianidin, Imidacloprid, Flonicamid and Thiamethoxam) are highly compatible with *T. harzianum* even at higher doses and can be safely use in any IPM/IDM programme.

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