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Research Article

Compatibility of *Cordyceps javanica* (OM321438) strain with botanicals and insecticides against tea mosquito bug, *Helopeltis theivora* Waterhouse

SUDESHNA RAY[1], PURNIMA DAS[1*], S. RAHMAN[1], MANURANJAN GOGOI[2], H. K. DEVA NATH[3], MUDASIR GANI[4], PRIYANKA SAIKIA[1], PARTHA P. G. DAS[1] and BANNA USHASRI[1]

[1]Department of Entomology, Assam Agricultural University, Jorhat, Jorhat MB and OG Part – 785013, Assam, India
[2]Department of Tea Husbandry and Technology, Assam Agricultural University, Jorhat, Jorhat MB and OG Part – 785013, Assam, India
[3]AAU- Zonal Research Station, Shillongoni, Assam Agricultural University, Jorhat, Jorhat MB and OG Part – 785013, Assam, India
[4]Division of Entomology, Faculty of Horticulture, SKUAST-Kashmir, Shalimar, Srinagar – 190025, Jammu and Kashmir, India
**Corresponding author E-mail:* purnima.dashazarika@gmail.com

ABSTRACT: In present study, the compatibility of a native entomopathogenic fungus (EPF), *Cordyceps javanica* strain (OM321438) with three different botanicals viz., neem oil, pongamia oil and jatropha oil and insecticides viz., clothianidin 50WDG, thiamethoxam 25WG and profenofos 50EC at three different concentrations *viz.* recommended dose, half of the recommended dose and more than the recommended dose and efficacy of the best compatible combinations were evaluated under laboratory conditions against tea mosquito bug, *Helopeltis theivora* which is one of the major mirid pests of tea. Among the tested botanicals and insecticides, neem and pongamia oil at half of the recommended dose, clothianidin 50WDG at all doses and thiamethoxam 25WG at half of the recommended (0.01%) and recommended dose (0.02%) were found to be compatible with *C. javanica* ($1x10^7$ conidia/ml). The highest mortality (100%) of *H. theivora* was recorded on combined treatment of *C. javanica* with thiamethoxam 25WG at half of the recommended dose after 72 HAT whereas 70% mortality was observed in the combination of *C. javanica* with neem oil at half the recommended dose after 120 HAT. Moreover, *C. javanica* alone caused 85% mortality after 120 HAT.

KEYWORDS: Botanicals, compatibility, *Cordyceps javanica, Helopeltis theivora,* tea crop

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ABBREVIATIONS: %: Percentage; @: At the rate; °C: Degree centigrade; BOD: Biological Oxygen Demand; conc.: Concentration; EPF: Entomopathogenic fungus; HAT: Hours after Treatment; IPM: Integrated Pest Management; *i.e.*: That is; cm: Centimetre; ml: Millilitre; mins.: Minutes; PDA: Potato Dextrose Agar; pH: Negative logarithm of H^+ ion concentration; RD: Recommended dose; RH: Relative humidity; TMB: Tea mosquito bug; *viz.*: Vidilicet (namely)

INTRODUCTION

Tea, *Camellia sinensis* L. (O.) Kuntze (Family-Theaceae) is one of the most popular evergreen perennial nonalcoholic beverages in the world known as the "queen of beverages" (Prasad *et al.*, 2013). Tea originated in South-West China and was commercialized in India by the British during the 17th century. The Assam variety of tea is named *Camellia sinensis var. assamica.* The economic part of tea is the tender shoots of the plant, which typically consist of two to three leaves and a bud.

In India, tea is grown mostly in Assam, West Bengal, Tamil Nadu, and Kerala. However, a small portion of tea is grown in other states such as Karnataka, Tripura, Himachal Pradesh, Uttaranchal, Arunachal Pradesh, Manipur, Sikkim, Nagaland, Meghalaya, Mizoram, and Bihar (Borbora & Singh, 1994). Assam tea is one of the World's most famous high-quality teas, accounting for approximately 51.90 per cent of the country's total tea production. In India, both large plantations and small gardens are used to produce tea. In 2022, Assam alone accounted for 337690.35 ha of land and 672.14 million kg of production, whereas India covered

636557.10 ha and total production was 1344 million kg (Tea Statistics, Tea Board India, 2022).

Being a long-duration monoculture crop, it is susceptible to a number of biotic (diseases and insect pests) and abiotic (nutrient deficiency, drought and salinity) stresses that ultimately affect the annual crop loss. Amongst these constraints, biotic stresses are considered the major significant constraint. For example, arthropod pests associated with the tea crop are the most detrimental ones; there are 1031 species worldwide, but only 3% of them are widely distributed (Hazarika *et al*., 2009). In North-East India, 176 different kinds of insect and mite pests have been documented so far. Among the insect pests, mirids are the main insect pests in Asian nations, where they cause agricultural losses of between 11.00 and 100.00% (Hazarika *et al*., 2009; Hazarika *et al*., 2020). Among the mirids, the Tea Mosquito Bug (TMB), *Helopeltis theivora* Waterhouse (Hemiptera: Miridae), has been predicted to be the greatest enemy to tea growers (Watt & Mann, 1903). TMB was first recorded on tea in Java in 1847 (Rao, 1970), whereas it was first noted in Cachar, India, in 1865 (Watt & Mann, 1903) and spread to various districts of Assam and Darjeeling during 1869 (Borbora & Singh, 1994), causing trouble for the tea industry.

The TMB causes severe harm to both the quantity and quality of the tea. Nymphs and adults of TMB suck cell sap from sensitive stems, leaves, and buds to create reddish brown, circular feeding holes or lesions that range in size from 0.29 to 2.51 mm. The saliva of nymphs and adults is rich in enzymes like pectinase, lipase, proteinase and carbohydrate, in reaction to salivary enzymes, which, in the presence of salivary amino acids, detoxify the protective chemicals of the host, the inside of the ring changes from transparent light brownish in hue to dark brown, sunken, and eventually desiccates, after 24 hours (Das *et al.*,2019). The most severely impacted tea plants are stunted and have a darker shade of green (Hazarika, 2009). TMB has destroyed up to 55% of the crop in Africa (Rattan, 1992) and 11 to 100% of the crop in Asia (Muraleedharan 1992a, 1992b).

To control these insect pests, a variety of insecticides were used in an injudicious manner, particularly in Northeast India's tea estates. The indiscriminate use of insecticides, has become harmful to the environment along with other negative consequences such as the resurgence of primary pests, secondary pest outbreaks and development of pesticide resistance and residual toxicity. Therefore, finding alternatives to chemical insecticides for pest control is necessary, especially in light of biological control techniques.

In recent years, researchers have concentrated on developing bio-pesticides based on entomopathogenic fungi. They are host-selective with no or little effect on non-target organisms and large-scale production of entomopathogenic fungus is possible. To effectively use entomopathogenic fungi as a biocontrol agent, it is necessary to have a full knowledge of their biology and mode of action on the target pest.

Beauveria bassiana (Bals.) Vuill*, Aspergillus flavus* and *Lecanicillium lecanii* are the most significant and widely used entomopathogenic fungi that have been successfully used to manage Tea mosquito bug. Numerous studies revealed that *H. theivora* as well as *Drosophila melanogestar* experienced effective encapsulation in the presence of large haemocyte loads (Baruah and Hazarika, 2006). In addition to these, there are a few entomopathogenic strains which are effective against different groups of insects, but their bio-efficacy, and compatibility with botanicals and insecticides against TMB and other tea pests is yet to be evaluated. However, Kaur 2022 extensively studied *C. javanica,* a native strain against TMB. Hence, the present investigation aimed to explore the efficacy of *C. javanica* (OM321438) in combination with commonly used botanicals and insecticides in the tea ecosystem against TMB, with the logic to formulate an effective pest management strategy in chemocentric agriculture.

MATERIALS AND METHODS

The laboratory experiments were carried out to study the effect of entomopathogenic fungus, *C. javanica* alone and in combination with botanicals and insecticides against *H. theivora* in the Physiology Laboratory, Department of Entomology, Assam Agricultural University, Jorhat (26.75° N- Latitude and 94.22° E-Longitude) during 2022-2023. The commercial formulations of three common insecticides used against TMB in Assam *viz.,* chlothianidin 50 WDG (dantatsu), thiamethoxam 25 WG (actara), and profenofos 50 EC (celcron)and three botanicals *viz.,* neem oil (neem seed oil), pongamia or karanj seed oil (pure karanja oil) and jatropha seed oil were used in this study.

Isolation and pure culture of *C. javanica*

To increase the virulence, initially, *C. javanica* was passed through the adults *H. theivora,* which were collected from the Experimental Garden for Plantation Crops (Organic tea plot), Department of THT, Assam Agricultural University, Jorhat-13. The pathogen was re-isolated from the infected cadavers on Potato Dextrose Agar medium and maintained a pure culture for further study.

Compatibility study of EPF with botanicals and insecticides

Incorporation of botanicals and insecticides in PDA medium

The *in vitro* compatibility of botanicals and insecticides with EPF was determined by using the poison food technique (Nene and Thapliyal, 1978) at three different doses of botanicals and insecticides *viz.,* half of the recommended dose, Recommended Dose (RD) and more than the recommended dose. In this technique, the PDA medium was poisoned with botanicals and insecticides, where 100 ml PDA medium was poured in 250ml conical flasks for each botanical and insecticide and then autoclaved at 121°C and 15 psi for 30 min. Then it was allowed to cool down to about 45°C. The stock solutions of botanical and insecticide were prepared with sterilised distilled water and diluted in required concentrations and incorporated in the PDA medium at required concentrations. The tested concentrations for neem oil (0.5, 1.0 and 1.5%), pongamia oil and jatropha oils (0.5%, 1.5% and 2.5%) and for thimethoxam 25 WG and chlothianidin50 WDG (0.01%, 0.02% and 0.04%) and for profenofos 50 EC (0.06%, 0.125% and 0.25%).

Each flask was well shaken to ensure appropriate mixing before it was poured into 90 mm sterilised petri plates and allowed to solidify for about one hour. Botanicals and insecticide-free medium were served as a control. Each petri plate was inoculated with 0.8 cm (8 mm) inoculums disc cut out with the help of sterilized cork borer from 10 days culture plates of *C. javanica* and transferred to the centre of each petri plate having the desired media. Then the inoculated plates were sealed with cellophane tape and placed in a BOD incubator at 26±1°C temperature for 15 days for complete growth and observations were recorded on biological parameters of the EPF such as mycelial growth, conidial density and sporulation.

Mycelial growth

The mycelial growth of the fungus was studied by placing a bit of size 0.8cm diameter of the fungus at the centre of the respective treated medium of botanicals and insecticides by using a cork borer of size 0.8cm diameter. Similarly, a set of Petri plates of untreated medium containing similar fungal bits of the same size were kept as control and placed in a BOD incubator at 26±1°C temperature. Five plates (replications) per treatment were maintained and measured by a caliper scale for mycelial growth. Five orthogonal diameters of the culture plates were measured three Days After the Inoculation (DAI) at intervals of 24 h to 15 days (Kaur, 2022; Das *et al.*, 2011).

Conidial density and spore germination

To determine the conidial density of the fungus in different treatments, the fungus was grown on both treated and untreated media for seven days in BOD maintained at 26°C temperature. The culture tube of each treatment was suspended in 10 ml distilled water with Tween-80 (0.023%) in a 20 ml test tube. The conidial density of $1x10^7$conidia/ ml spore dilution was calculated by following the method described by Das *et al.* (2011) using a Neubauer haemocytometer under a light microscope at a magnification of 400 X. The germination test of viable conidia was carried out by using a previously described procedure (Francisco *et al.*, 2006; Das *et al.*, 2011). A 400X phase contrast microscope was used for direct observation to calculate the germination rate.

Percent inhibition over control

The per cent inhibition of the EPF over the untreated control 15 DAI, was calculated for the respective botanicals and insecticides by following the formula given by Vincent's equation (1927):

$$I = [(C-T)/C]x100$$

where, I = Per cent inhibition, C= Radial growth of fungus in control (mm), T= Radial growth of fungus in treatment (mm).

Compatibility classification based onthe T toxicity model

The toxicities of botanicals and insecticides against *C. javanica* were calculated by using the formula proposed by Alves *et al.* (1998); T = [20(VG) + 80 (SP)]/100,where, VG=Vegetative growth and SP= Sporulation value. In this formula, T values between 0 and 30 signify products as very toxic; from 31 to 45 as toxic; from 46 to 60 moderately toxic and above 60 considered as compatible with fungus.

Insect rearing

Wooden frame insect rearing cages were used for the maintenance of the *H. theivora* population under laboratory conditions. Gravid female adults were collected from the Experimental Garden for Plantation Crops (Organic tea plot), Assam Agricultural University, Jorhat and released on the tea twigs for egg laying. Newly emerged adults were collected from the cage and utilized for bio-efficacy tests.

***In vitro* bio-efficacy test**

The most effective compatible combinations of EPF with botanicals and insecticides at lower concentrations (half of the recommended) were selected for their efficacy against TMB**.**Moreover, selected oils and insecticides were used at their recommended doses as an individual treatment against *H. theivora* to compare their efficacy with the compatible ones along with an untreated control under laboratory conditions. The treatments were as follows: T_1: *C. javanica*(1x 10^7 conidia ml^{-1}) +clothianidin 50WDG (0.01%); T_2: *C. javanica*(1x 10^7 conidia ml^{-1}) + thiamethoxam 25WG (0.01%); T_3:*C. javanica*(1x 10^7 conidia ml^{-1}) + Neem oil

(0.5%); T_4: *C. javanica*(1x 10^7 conidia ml^{-1}) + Pongamia oil (0.5%); T_5: *C. javanica*(1x 10^7 conidia ml^{-1});T_6: clothianidin 50WDG at 0.02%; T_7:thiamethoxam 25WG at 0.02%;T_8: Neem oil at 1.0%; T_9: Karanj oil at 1.5% and T_{10}:Control.

Tea mosquito bug adults were collected from the Experimental Garden for Plantation Crops (Organic tea plot), under the Tea Husbandry Department, Assam Agricultural University, Jorhat-13 and reared inthe laboratory at room temperature (30±1°C, RH 85-90%). The bioefficacy of the best compatible combination dose of insecticides and botanicals was tested against adult TMB by following the methodology as suggested by Akmal *et al.,* 2013. To find out the efficacy of each treatment, a set-up was made by using conical flasks containing a glass chimney (4.5 cm in diameter at the top and 7 cm bottom respectively with a height of 23 cm) with muslin cloth at the mouth portion to prevent the exit of the adult tea mosquito bugs and filter paper at the base and put tea twigs containing two leave one bud on the conical flasks. Then, different concentrations of the selected treatments were sprayed on the twigs having the test insects using a hand glass atomizer. A control set was also kept where water containing Tween 80 was sprayed. Each treatment was replicated five times containing a total 250 numbers of test insects. Mortality data in each treatment were recorded at 24, 48, 72, 96 and 120 hours of exposure. The per cent mortality in each treatment was recorded and the observed mortality in the control was corrected by using Abbott's formula (1925) as follows.

$$\text{Corrected mortality} = \frac{\%\text{ mortality in treatment} - \%\text{ mortality in control}}{100 - \%\text{ mortality in control}} \text{x} 100$$

Moreover, observations were made on feeding punctures after 18 hours of treatments. Insect cadavers were collected and transferred to sterilized petri plates with moist filter paper for fungal growth.

STATISTICAL ANALYSIS

The data on radial growth, conidial density and spore germination of entomopathogenic fungus were statistically analyzed with CRD and subjected to Analysis of Variance (ANOVA). The mortality data obtained from the treatments after 24, 48, 72, 96 and 120 h were subjected to angular transformation and data on feeding spots after 18 h of treatments were analyzed through Analysis of Variance (ANOVA) using Completely Randomized Design (CRD) and averages were compared using the Duncan Multiple Range Test (DMRT) (0.05%).

RESULTS

In vitro compatibility study

Among the botanicals tested, the highest mycelial growth of the EPF, 15 days after inoculation (DAI) was recorded in neem oil (60.00 mm) and the lowest (42.50 mm) was recorded in jatropha oil at a concentration of 0.5%. In the case of insecticides, clothianidin 50 WDG at half of the RD (0.01%) recorded the highest (76.00 mm) mycelial growth, whereas profenofos 50 EC showed the lowest mycelial growth (24.60 and 8.00 mm) both at halfof the RD and RD (0.06% and 0.125%). The conidial density and spore germination were recorded at 1x10^7conidia/ml spore dilution of *C. javanica* for all the combinations along with control. Among all tested botanicals, the highest conidial density and spore germination of the EPF was observed at neem oil at 0.5%, *i.e.,* 5.52 x 10^7 conidia/ml and 65.94%, whereas, the lowest conidial density was recorded at jatropha oil at 2.5% (2.16 x 10^7 conidia/ml and 36.54%). In the case of insecticides higher conidial density and sporulation of the EPF was observed at clothianidin 50 WDG (7.52 x 10^7 conidia/ml and 79.22%), whereas the highest reduction in conidial density (1.9 x 10^7conidia/ml and 22.06%) showed by profenofos 50 EC at more than RD (0.25%). Moreover, according to Vincent's equation (1927), among botanicals, the minimum per cent inhibition of the fungus was recorded when treated with neem oil at 0.5% (31.19) and maximum inhibition per cent was recorded at jatropha oil at 2.5% (71.56). However, in the case of insecticides the minimum per cent inhibition was recorded in clothianidin 50WDG at 0.01% (12.84) and the highest inhibition per cent was recorded in the medium treated with profenofos 50 EC (90.37 and 90.83) both at RD and more than RD. The inhibition percentage was found to be gradually increasing with the increase in the concentrations of botanicals and insecticides **(**Table 1, Figures 5-7).

Compatibility classification

The compatibility classification was calculated based on the "T" toxicity index given by Alves *et al.* (1998) using values of 20% vegetative growth and 80% sporulation, which showed the variable responses of the EPF with botanicals and chemical insecticides. Data presented in Table 2 revealed that among the botanicals, neem and pongamia oil were found to be compatible at half of the RD and moderately toxic at both recommended and more than-recommended doses. Jatropha oil was found to be moderately toxic to toxic. Among the insecticides, clothianidin was found to be compatible in all three doses, thiamethoxam was found to be compatible both in half of the RD and RD. However, profenofos was found to be toxic to very toxic with *C. javanica*. Overall, among the insecticides and oils tested in the present study, chlothianidin, thiamethoxam, neem oil and pongamia oil were found to be the most compatible with the EPF at half of the recommended doses. Thus, the compatible treatments, *i.e.,* the EPF strain with these selected insecticides and

Table 1. Radial growth, conidial density and sporulation of *C. javanica* at 1x 10^7 conidia ml^{-1} spore dilution on different treatments of botanical and insecticide formulations

Treatments		Radial growth (mm)	Inhibition %	Conidial density (1x 10^7conidia ml^{-1})	Sporulation (%)
T_1	*C. javanica*+ Neem oil (0.5%)	60±1.76	31.19	5.52±0.11	65.94±0.71 (54.29)
T_2	*C. javanica*+ Neem oil (1.0%)	45±0.95	48.39	4.3±0.14	61.74±1.03 (51.79)
T_3	*C. javanica*+ Neem oil (1.5%)	33±0.84	62.16	3.9±0.07	54.98±0.74 (47.86)
T_4	*C. javanica*+ Pongamia oil (0.5%)	49.2±2.31	43.58	4.98±0.3	63±0.37 (52.54)
T_5	*C. javanica*+ Pongamia oil (1.5%)	43.8±0.97	49.77	3.46±0.12	58±1.85 (49.60)
T_6	*C. javanica*+ Pongamia oil (2.5%)	30±0.32	65.60	2.98±0.12	54.96±1.06 (47.85)
T_7	*C. javanica*+ Jatropha oil (0.5%)	42.5±0.45	51.26	4.29±0.07	48.12±1.03 (43.92)
T_8	*C. javanica*+ Jatropha oil (1.5%)	30.6±0.68	64.91	2.68±0.13	40.36±1.36 (39.44)
T_9	*C. javanica*+ Jatropha oil (2.5%)	24.8±0.2	71.56	2.16±0.08	36.54±1.35 (37.19)
T_{10}	*C. javanica*+ Clothianidin 50WDG (0.01%)	76±2.28	12.84	7.52±0.09	79.22±0.68 (62.88)
T_{11}	*C. javanica*+ Clothianidin 50WDG (0.02%)	49.2±0.37	43.58	5.96±0.15	74.42±0.53 (59.62)
T_{12}	*C. javanica*+ Clothianidin 50WDG (0.04%)	35.6±0.75	59.17	4.98±0.07	70.58±0.78 (57.15)
T_{13}	*C. javanica*+ Thiamethoxam 25WG (0.01%)	60.6±2.52	30.50	7.26±0.09	73.2±1.16 (58.82)
T_{14}	*C. javanica*+ Thiamethoxam 25WG (0.02%)	40.8±1.86	53.21	5.46±0.09	70.04±1.21 (56.81)
T_{15}	*C. javanica*+ Thiamethoxam 25WG (0.02%)	31.4±0.51	63.99	4.6±0.11	66.1±1.24 (54.39)
T_{16}	*C. javanica*+ Profenofos 50EC (0.06%)	24.6±0.51	71.79	3.4±0.07	36.68±0.96 (37.27)
T_{17}	*C. javanica*+ Profenofos 50EC (0.125 %)	8.4±0	90.37	2.6±0.16	29.2±1.22 (32.71)
T_{18}	*C. javanica*+ Profenofos 50EC (0.25%)	8±0	90.83	1.9±0.12	22.06±0.73 (28.01)
T_{19}	Control	88.8±0.58	-	8.06±0.05	83.5±1.40 (66.03)
S.Ed(±)		**1.780**	**-**	**0.175**	**0.952**
C.D. (P=0.05)		**3.552**	**-**	**0.35**	**1.899**

* Means ± SD are based on 5 replications and are separated by DMRT ($p<0.05$)

botanicals could simultaneously be used for the management of insect pests of tea.

Bio-efficacy

In vitro efficacy study of the best compatible combinations *viz., C. javanica* (1x10^7conidia/ml) +clothianidin 50WDG (0.01%), *C. javanica* (1x10^7conidia/ml) +thiamethoxam 25WG (0.01%), *C. javanica* (1 x 10^7conidia/ml) + neem oil (0.5%) and *C. javanica* (1x10^7conidia/ml) + karanj oil (0.5%) were tested against tea mosquito bug along with individual treatments at their RDs by following topical spraying method. The per cent mortality of *H. theivora* was recorded at 24, 48, 72, 96 and 120 Hours After Treatment (HAT) (Table 3). The mortality of *H. theivora* was found to be significantly

Table 2. T-values and compatibility classification of botanicals and insecticides with *C. javanica*

Treatment	T value	Classification
Neem oil		
0.50%	64.75	Compatible
1%	58.39	Moderately toxic
1.50%	50.58	Moderately toxic
Pongamia oil		
0.50%	60.24	Compatible
1.5%	55.16	Moderately toxic
2.50%	49.97	Moderately toxic
Jatropha oil		
0.50%	46.99	Moderately toxic
1.5%	38.41	Toxic
2.5%	34.19	Toxic
Clothianidin 50WDG		
0.01%	78.58	Compatible
0.02%	69.38	Compatible
0.04%	63.58	Compatible
Thiamethoxam 25WG		
0.01%	70.68	Compatible
0.02%	64.19	Compatible
0.04%	59.16	Moderately toxic
Profenofos 50EC		
0.06%	34.26	Toxic
0.125%	24.96	Very toxic
0.25%	19.28	Very toxic

different among the treatments on different days. Among all the treatments, the highest mortality, *i.e.,*100% was recorded in the combined application of *C. javanica* at 1 x 10^7 conidia/ml + thiamethoxam 25WG at 0.01 per cent at 72 HAT which was followed by the combined treatment of *C. javanica* at 1 x 10^7 conidia/ml +clothianidin 50WDG at 0.01 per cent at 96 HAT. Moreover, the EPF alone also showed promising results as causing 85 per cent mortality at 120 HAT whereas; the lowest mortality (35.00%) was recorded in individual treatment of pongamia oil which was followed by combined treatment of *C. javanica* at 1 x 10^7 conidia/ml + pongamia oil at 0.5% (40.00%).

The feeding punctures of TMB were recorded after 18 hours of treatments (Table 4). *C. javanica* at 1 x 10^7conidia/ml + thiamethoxam 25WG at 0.01per cent treated twigs had a smaller number of feeding punctures (20.60 nos.) followed by *C. javanica* at 1x10^7conidia/ml +clothianidin 50WDG at 0.01 per cent (25.8 nos.), whereas *C. javanica* at 1 x 10^7conidia/ml alone showed 34.40 nos. of feeding puncture. However, all the recommended botanicals and insecticides alone recorded-thiamethoxam 25WG (27.00 nos.), clothianidin 50WDG (32.00 nos.), pongamia oil (49.00 nos.), neem oil (43.00 nos.) and the highest number of feeding punctures were recorded in the untreated control (73.80 nos.).

DISCUSSION

The tea mosquito bug, *H. theivora* is one of the major sucking pests in the tea ecosystem causing severe damage to the annual tea production across the country. The output of the present study would be a great boon for tea growers as mirids cause up to 100% yield reduction (Hazarika *et al.,* 2009). The result of the experiment revealed that among the botanicals tested, neem oil and pongamia oil were showed better compatibility with the EPF only at half of the RDs, whereas jatropha oil did not show any compatibility and classified

Table 3. Effect of different treatments against *H. theivora*

Treatments		Dose (%) 24HAT	% Mortality of *H. theivora* at different exposure period				
			48HAT	72HAT	96HAT	120HAT	
T_1	*C. javanica* (1x 10^7 conidia ml^{-1}) + Clothianidin 50 WDG	0.01%	20±8.94 (26.57)	56±4.00 (48.45)	88±4.89 (69.73)	100 (90.00)	100 (90.00)
T_2	*C. javanica* (1x 10^7 conidia ml^{-1}) + Thiamethoxam 25 WG	0.01%	40±6.33 (39.23)	84±7.48 (66.42)	100 (90.00)	100 (90.00)	100 (90.00)
T_3	*C. javanica* (1x 10^7 conidia ml^{-1})+ Neem oil	0.5%	4±4.00 (11.53)	16±7.483 (23.58)	40±6.33 (39.23)	58±6.63 (49.60)	70±9.35 (56.79)
T_4	*C. javanica* (1x 10^7 conidia ml^{-1})+ Pongamia oil	0.5%	0±0.00 (0.33)	4±4.00 (11.54)	16±7.48 (23.58)	28±8.00 (31.95)	40±6.12 (39.23)
T_5	*C. javanica* (1x 10^7 conidia ml^{-1})	-	4±4.00 (11.54)	24±7.48 (29.33)	48±4.89 (43.83)	66±6.00 (54.33)	85±6.12 (67.21)
T_6	Clothianidin 50 WDG	0.02%	8±4.89 (16.43)	28±4.89 (31.95)	56±4.00 (48.45)	67±4.36 (54.94)	85±6.12 (67.21)
T_7	Thiamethoxam 25 WG	0.02%	20±8.94 (26.57)	36±9.79 (36.87)	60±8.94 (50.77)	87±5.385 (68.87)	100 (90.00)
T_8	Neem oil	1.0%	0±0.00 (0.33)	4±4.00 (11.54)	16±7.48 (23.58)	37±7.00 (37.47)	50±7.91 (45)
T_9	Pongamia oil	1.5%	0±0.00 (0.33)	0±0.00 (0.33)	8±4.89 (16.45)	24.80±3.878 (29.87)	35±6.12 (36.27)
T_{10}	Control (Water)	-	0±0.00 (0.33)	0±0.00 (0.33)	0±0.00 (0.33)	4±4.00 (11.54)	16±7.48 (23.57)
S.Ed (±)			**6.834**	**7.514**	**7.3**	**6.231**	**7.579**
C.D. (P=0.05)			**13.864**	**15.242**	**14.808**	**12.640**	**15.375**

*HAT- Hours After Treatment*Mean are based on 5 replications and are separated by DMRT ($p<0.05$)
*Sample size: 25 insects/treatments*Data within the parentheses are angular transformed value

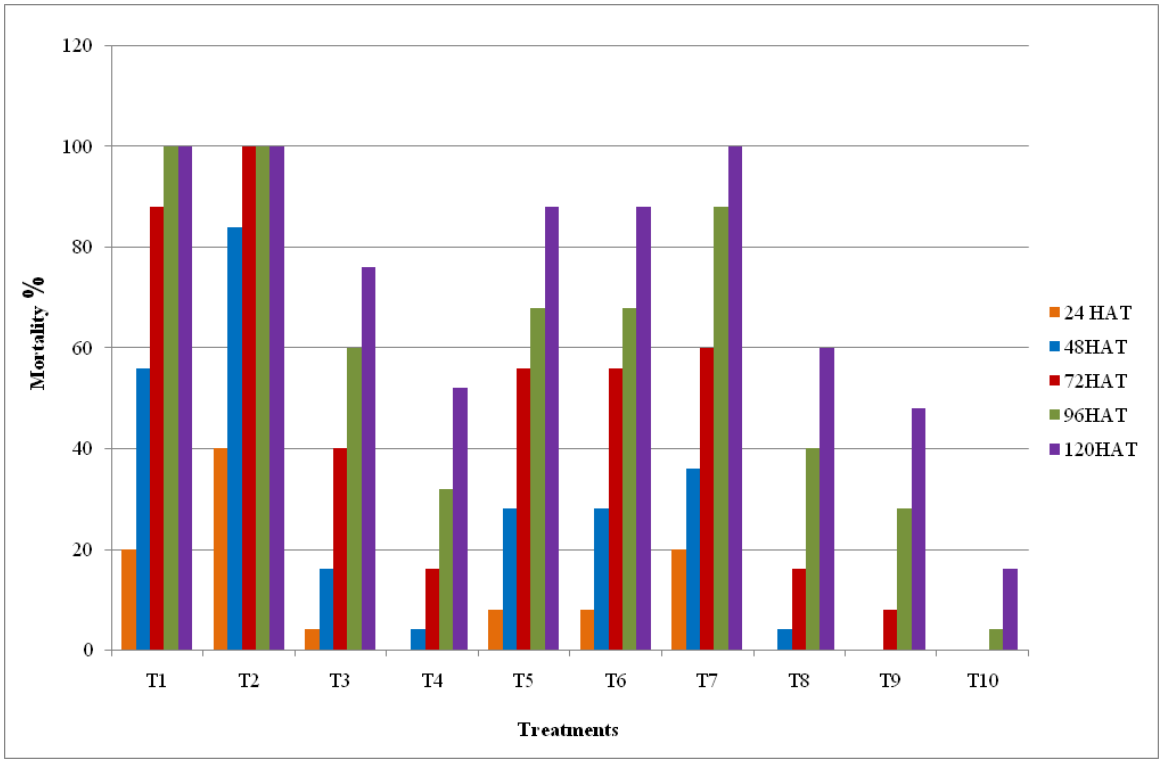


Figure 1. Efficacy of different treatments against *Helopeltis theivora*.

Table 4. Feeding punctures/twig of different treatments against *H. theivora*

Treatments		Dose (%)	No. of Feeding punctures/ twigs 18 HAT
T_1	*C. javanica* (1x 10^7 conidia ml^{-1}) + Clothianidin 50 WDG	0.01%	25.8±1.69
T_2	*C. javanica* (1x 10^7 conidia ml^{-1}) + Thiamethoxam 25 WG	0.01%	20.6±1.72
T_3	*C. javanica* (1x 10^7 conidia ml^{-1})+ Neem oil	0.5%	35.8±0.92
T_4	*C. javanica* (1x 10^7 conidia ml^{-1})+ Pongamia oil	0.5%	48.8 ±2.31
T_5	*C. javanica* (1x 10^7 conidia ml^{-1})	-	34.4±0.68
T_6	Clothianidin 50 WDG	0.02%	32±1.14
T_7	Thiamethoxam 25 WG	0.02%	27±1.00
T_8	Neem oil	1.5%	43±1.09
T_9	Pongamia oil	1.5%	49±3.59
T_{10}	Control (Water)	-	73.8±1.32
S.Ed (±)			**2.473**
C.D. (P=0.05)			**5.017**

*HAT- Hour After Treatment
*Mean are based on 5 replication
*Sample size: 1 insects/replication, 5 insects/ treatments

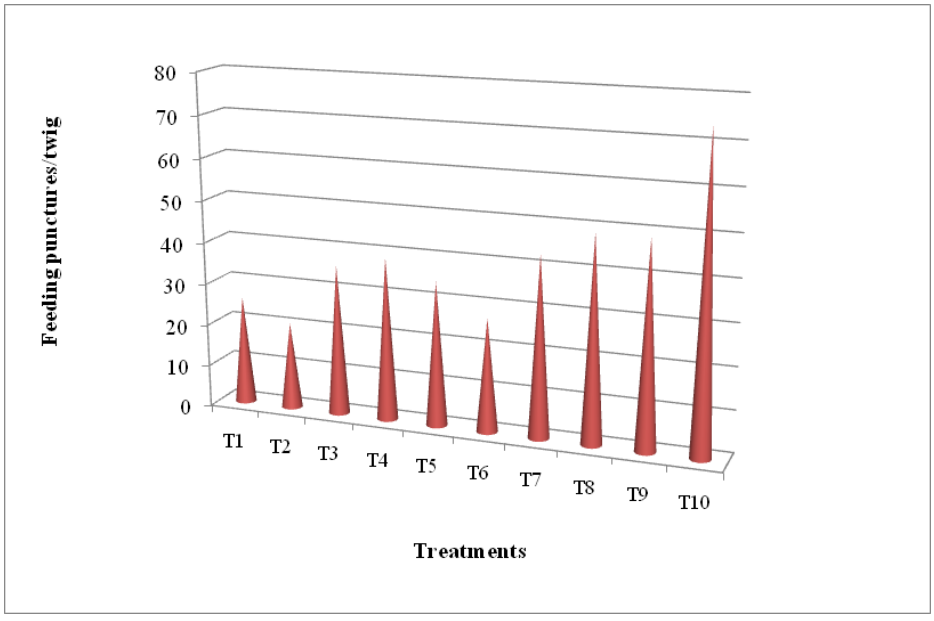


Figure 2. Number of feeding punctures/twig of treatments against *Helopeltis theivora*.

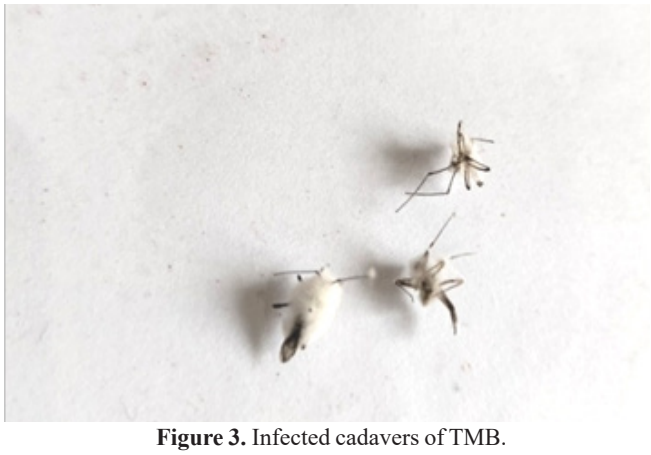


Figure 3. Infected cadavers of TMB.



Figure 4. Pure culture of EPF.

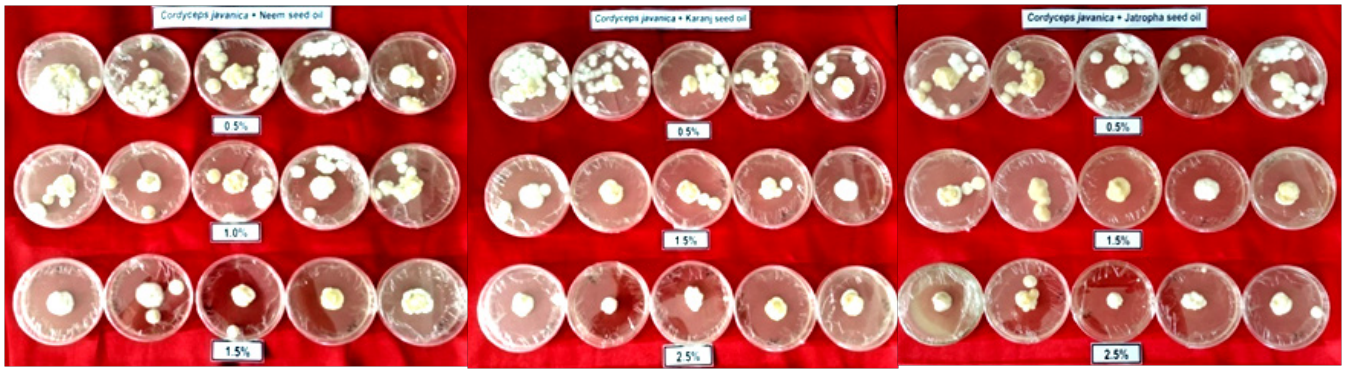


Figure 5. Compatibility of *Cordyceps javanica* with botanicals at different concentrations (15 DAI).

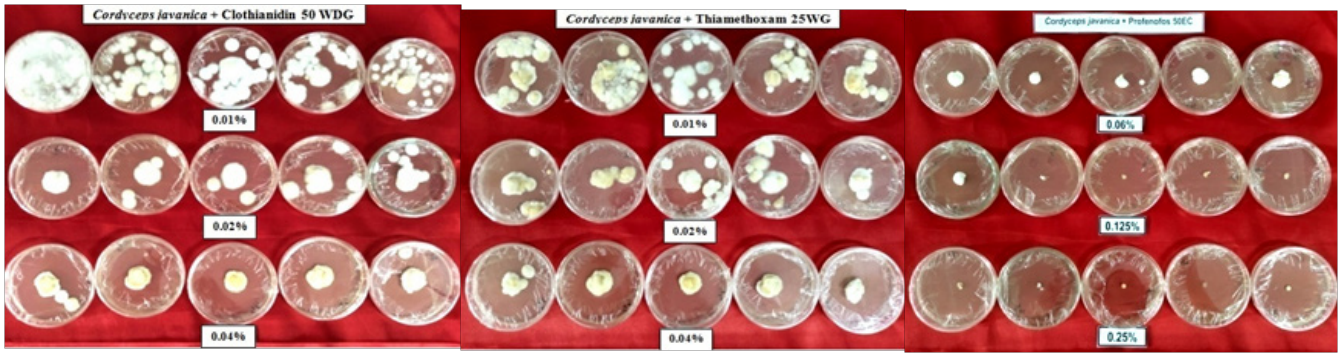


Figure 6. Compatibility of *Cordyceps javanica* with insecticides at different concentrations (15 DAI).



Figure 7. Growth of *Cordyceps javanica* on PDA media (15 DAI).

as moderately toxic to toxic (Tables 1, 2). The findings of the present study were similar to the findings of Depieiri *et al.* (2005), where reported that all tested concentrations of neem oil reduced vegetative growth and conidiogenesis of the fungus, *B. bassiana*. The present findings also corroborate the findings of Sain e*t al.* (2019) who reported that neem oil at the half and full recommended doses was compatible with different strains of *Codyceps javanica, B. bassiana* and *Metarhizium anisopliae* at different levels of compatibility classification as mentioned as moderately toxic and toxic, respectively. Band *et al.* (2021) and Sain e*t al.* (2021) reported that pongamia oil reduces the growth of *M. anisopliae, C. javanica* and *B. bassiana*. The incompatibility of pongamia oil with the tested entomopathogenic fungus might be due to the active ingredients in the oil, such as karanjin, quercitin, pinnatin, saponin, and pongamol, which have fungal-toxic properties and exert antagonistic effect and results in significant antifungal activity preventing the mycelial growth of many pathogenic fungi (Visalakshy *et al.*, 2005; Moore, 2013).

The incompatibility of jatropha oil was greater as compared to neem and pongamia oil. It might be due to the seeds of jatropha which contain 47% fat and various anti-nutritional factors such as saponin, phytate, trypsin inhibitor, and cyanogenic glycosides (Makkar *et al.*, 1997; Kumar & Sharma 2008; Rakshit *et al.*, 2008). However, it consists of various terpenoids present as secondary metabolites of

Jatropha. To date, 65 types of terpenes have been explored in jatropha oil (Devappa *et al.*, 2011). Phorbol Esters (PEs) are a group of tigilane diterpenes which are a major toxic constituent of jatropha (Adolf *et al*., 1984; Makkar *et al*., 1997). Moreover, the incompatibility of *C. javanica* and jatropha oil might be due to the intolerance of the fungus to the excessive oil. Therefore, it was also clear from the experiment that inhibition induced by jatropha oil to the EPF was more than neem oil and pongamia oil.

Among the insecticides, clothianidin 50 WDG and thiamethoxam 25 WG showed greater compatibility with the EPF, whereas profenofos 50 EC was found to be toxic to very toxic. Similar results have been reported by Yadav *et al.* (2019), in the case of *B. bassiana* and *Metarhizium brunneum* where they reported that a lower dose of clothianidin 50WDG did not significantly inhibit radial growth, conidial density and sporulation of the fungi *B. bassiana* and *M. brunneum*, respectively. However, higher concentrations showed greater reductions in radial growth, conidial density and sporulation of both fungi. The present findings also confirm the earlier result of Oliveira *et al.* (2003) and Neves *et al.* (2001) in respect of *B. bassiana* and *M. anisopliae* where they reported that thiamethoxam 25WG was found to be compatible with the fungi at lower doses in respect of the above-mentioned biological parameters. According to their report, it has been clear that wettable powder formulations of insecticide do not inhibit the growth of the fungi. Profenofos 50EC at the lethal dose showed greater inhibition to the growth of the fungus *Metarhizium anisopliae* and appeared as highly toxic, as reported by Amutha *et al.* (2012). Similar results were also obtained for inhibition of profenofos 50EC at half and full recommended doses with different strains of EPF, *i.e.*, *C. javanica, B. bassiana, M. anisopliae* and most of the strains were found to be very toxic to toxic at these doses. (Sain *et al.,* 2022*; Joshi et al.,* 2018*; Akbar et al.,* 2012*; Amutha et al.,* 2010). Anderson and Roberts (1983) reported that wettable or flowable formulations did not cause any inhibition of fungal growth but it increased colony numbers, whereas, emulsifiable concentrate formulations inhibited germination of *B. bassiana*. Similarly, Moino Jr. and Alves (1998) pointed out a high compatibility of *B. bassiana* and *M. anisopliae* with imidacloprid and fipronil as both formulations contained water-dispersible granules.

According to the present *in vitro* bioefficacy test, the best treatment was the compatible combinations of EPF and insecticides, *C. javanica* + thiamethoxam 25WG and *C. javanica* + clothianidin 50 WDG showing the highest percentage of mortality. The present investigation focuses on the fact that the combination of any two inputs always gives better results as compared to a single one. The reason might be due to the synergistic effect of two inputs (insecticides and EPF) against the pest. Nawaz *et al.* (2022) supported the present findings where they studied the compatibility and synergistic interactions of fungi, *Metarhizium anisopliae* and insecticides against sucking pest, *Aphis gossypii.* They found that the combination mixtures of *M. anisopliae* and insecticides were significantly more toxic than the individual treatments due to the synergistic effect of the combined mixtures of both compounds as the fungus can utilize the substances of insecticides as secondary nutrients (Moino Jr. & Alves, 1998). The findings of Mohsin *et al*. (2020), Halder *et al.* (2021) and Puzari *et al.* (2015) also corroborate with the present study, where they reported that compatible combined treatment of EPF and insecticides were found to be more effective than their treatment.

Moreover, the compatible combinations of EPF and botanicals, *C. javanica* with Neem oil showed better results than the individual treatment of neem oil and pongamia oil. In a similar line of work, Halder *et al.* (2021) reported that the lowest population of jassid and whitefly was recorded in the combined treatment of *L. lecanii* and neem oil at half of their recommended concentrations.

Moreover, the aqueous leaf extract of *Mimosa pudica* with *B. bassiana* showed effective results against *Polyphagotarsonemus latus* (Puspitarini *et al.*, 2022). Similar kinds of work carried out by Rehman *et al*. (2020), using neem extract, eucalyptus extract and tobacco extract with *B. bassiana* against *Tribolium castaneum*, where neem extract with *B. bassiana* at their maximum concentration recorded the highest mortality of the larvae.

The present study also shows that *C. javanica* ($1x10^7$ conidia ml^{-1}) alone recorded 85.00 per cent mortality at 120 HAT and when combined with thiamethoxam 25WG and clothianidin 50 WDG at half of the RD, it causes 100 per cent mortality of *H. theivora* at 72 and 96 HAT, respectively. However, the combination of *C. javanica* with neem and pongamia oil recorded lower per cent (70.00 and 40.00 per cent) as compared to the individual treatment of *C. javanica* 120 HAT, respectively. From this investigation, it could be concluded that, though the EPF was found to be compatible with neem and pongamia oil, their combined treatments did not show promising results as compared to the combined treatment with insecticides.

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