



Research Article

Compatibility of commercially available biopesticidal formulations with Temperature Tolerant strain of *Trichogramma chilonis* (TTT) in pre- and post-release situations in laboratory

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ABSTRACT: Laboratory experiments were conducted to evaluate the effect of some commercially available biopesticidal formulations, such as *Bacillus thuringiensis* var. *kurstaki* (2g/l), *Bacillus sphaericus* (2g/l), *Beauveria bassiana* (5g/l), *Metarhizium anisopliae* (5g/l), *Lecanicillium lecanii* (5g/l) on the Temperature Tolerant strain of *Trichogramma chilonis* (TTT). Two sets of experiments were conducted, in the first experiment, all the test biopesticides were sprayed on the egg cards prepared from *Corcyra cephalonica* eggs (pre-release situation) and percent parasitisation and adult emergence were recorded; in the second one, test bio pesticides were sprayed on the parasitized cards (post release situation) and adult emergence was recorded. The results revealed that percent parasitisation and adult emergence from treated cards in both the experiments are on par with the untreated control, hence they are considered safe and can be used along with TTT.

KEYWORDS: Biopesticides, compatibility, Temperature Tolerant *Trichogramma* (TTT)

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INTRODUCTION

Biopesticides are alternatives to chemical pesticides and offer potent tools to create a new generation of sustainable agriculture products. Biopesticides are employed in agriculture and used for the purpose of insect control, disease control, weed control, nematode control and plant physiology and productivity. They are often important components of Integrated Pest Management (IPM) programmes and have received much practical attention as substitutes to synthetic chemical plant protection products, as these biopesticides are ecofriendly and facilitate the control of pests in agricultural crops (Shashi Prabha *et al.*, 2016).

Among various components of biological control, egg parasitoids belonging to the *Trichogrammatidae* family are known to be effective biocontrol agents against many pests across crops (Takada *et al.*, 2001). *Trichogramma* is a minute wasp and is used for pest control on numerous crops; these include cotton, sugarcane, vegetables, sugar beets and also in orchard and forest trees (Hassan, 1993). Some of the pests against which the *Trichogramma* is considered as a viable

biocontrol option include Cotton bollworm (*Helicoverpa armigera*), Brinjal shoot borer (*Leucinodes orbonalis*), Diamond back moth (*Plutella xylostella*), Maize stem borer (*Chilo partellus*), Stem borer of Rice (*Scirpophaga incertulas*), Leaf folder of Rice (*Cnaphalocrocis medinalis*) etc.

Apart from this parasitoids and predators, several microbial pesticides are also being used against many pests in IPM programmes as a part of biological control. Microbial biopesticides include several microorganisms like bacteria, fungi, baculoviruses, and nematode-associated bacteria that act against invertebrate pests in agro-ecosystems. The biopesticide sector is expanding rapidly, and many discoveries are being transformed into new biopesticide products, fueling a growing global market offer (Ruiu, 2018).

Despite their proven safety individually, very little information is available on the compatibility among these biological inputs. It is, therefore, necessary to assess the compatibility of these bio agents with the commercially

available bio pesticial formulations. In view of this, a study was planned to evaluate the compatibility between *Trichogramma* and commonly used biopesticides.

MATERIALS AND METHODS

For evaluating the compatibility of commonly used biopesticidal formulations with TTT, the following bio pesticial formulations with their recommended doses were used for the study.

Inoculum cultures of TTT were procured from the ICAR Base Culture Maintenance Unit (ICAR-BCMU) located at AICRP of Biological Control, PJTSAU, Rajendranagar, Hyderabad. Similarly, the inoculum cultures of Rice moth *Corcyra cephalonica* (Stainton) were also procured from the same unit for development, maintenance and scaling up of the cultures of both test parasitoid and its laboratory host insect.

All the Standard Operating Procedures (SOPs) were followed to maintain healthy cultures of *C. cephalonica* to ensure timely supply of required number of host insect cultures for the proposed experiments.

Maize grains were used for the rearing of *C. cephalonica*. Grains were coarsely milled such that each grain is broken into three to four pieces and they were heat sterilized in the oven at 100°C for 30 minutes. Then the grains were sprayed with 0.1 percent formalin to prevent the growth of moulds and were filled into plastic tubs @ 2.5Kg/tub and were infested with 1cc of *C. cephalonica* eggs. The tubs were sealed with muslin cloth and kept in racks. Moths started emerging from the 40th day and continued till 70th day. Moths were collected daily from the tubs and were transferred to an oviposition cage for mating and egg-laying. Eggs were then collected and cleaned to remove the dust particles by using mesh sieves and were used for the maintenance of *Trichogramma* cultures.

In the first experiment, where the evaluation of compatibility of TTT with the treatment biopesticides in the pre-release situations in the laboratory, egg cards

were prepared out of counted number of *Corcyra* eggs by collecting the cleaned eggs from the *Corcyra* production unit. These eggs were glued to the paper card as per the standard procedure prescribed for producing egg cards. UV incubation of the freshly prepared egg cards was done before treatment applications. Then each test biopesticide was weighed on a single pan electronic balance with a sensitivity of 0.001g as per required field concentrations, then mixed with a litre of water into the glass jars and poured into the atomizer and after repeated agitations, the test biopesticides were sprayed on respective egg cards of each test bio pesticides. Care was taken not to drench the egg card while spraying.

Each treatment was replicated five times to facilitate statistical analysis through a Completely Randomised Design (CRD). Proper labelling was done indicating the treatment number, replication number and date of parasitization on each card. After proper drying, the treated egg cards with different treatmental formulations were exposed to egg parasitoids for 24 hours as per the standard procedures of parasitization. After ensuring effective parasitization for 24 hours, the adults were removed from the tube containing the *Tricho* card and the *Tricho* cards were observed for uniform blackening after three to four days of exposure to parasitoids. From the sixth day after parasitization each unit consisting of *Tricho* cards treated with test formulations was observed for the emergence of adults of *Trichogramma*. The no. of adults being emerged everyday were counted from the sixth to the 15th day of parasitization. The cumulative no. of the emerged parasitoid adults in each unit was taken as a base for working out percent parasitization in different treatments. The counting was done by keeping a white paper sheet and placing the adults which were immobilized by putting them in a deep freezer condition for a short period in order to make them amenable to for counting using a thin brush.

In the second experiment, where the compatibility of test biopesticides is evaluated in post-release situation firstly eggcards were prepared by the same procedure as discussed above, but in this experiment, eggcards were exposed to parasitoids before the spraying of the test

Table 1. Test biopesticides used for compatibility studies with Temperature Tolerant strain of *Trichogramma chilonis* (TTT)

S. No.	Name of Biopesticide	Recommended dosage
1	<i>Bacillus thuringiensis var. kurstaki</i>	2g/l
2	<i>Bacillus sphaericus</i>	2g/l
3	<i>Beauveria bassiana</i>	5g/l
4	<i>Metarhizium anisopliae</i>	5g/l
5	<i>Lecanicillium lecanii</i>	5g/l
6	Insecticidal check (Quinalphos 25% EC)	2ml/l
7	Untreated control	-

formulations. After three to four days of parasitization, duly ensuring the uniform development of black colour in *Tricho* cards they were sprayed with field concentration on the test bio pesticidal formulations. During the spraying of treatments, care was taken not to drench the *Tricho* cards with treatmental formulation and also ensuring proper drying of the card before being into the tube for adult emergence. As in the earlier procedure, each unit was observed for adult emergence from the sixth to the 15th day of parasitization. Percent parasitization was worked out by comparing the cumulative no. of adults that emerged from each unit, with the total no. of the eggs on *Trichocards* of different treatments.

The above experiments (pre- and post-release situations) were conducted in a Completely Randomized block Design (CRD) with five replications for each treatment. The results in percentage proportions were subjected to angular transformation for analysis and thus obtained mean values were subjected to Duncan's test.

RESULTS AND DISCUSSION

Pre-release situation

The results obtained with regard to percent parasitisation in pre-release situations suggested that *Metarhizium anisopliae* is found to be least effective with 93.8 percent parasitisation followed by both *Lecanicillium lecanii* and

Bacillus sphaericus with 92 percent parasitisation. Least parasitisation among the biopesticides was found to be in *Bacillus thuringiensis var. kurstaki* with 91.8 percent parasitisation. However, all the bio pesticidal formulations are statistically on par with each other and resulted in an equal amount of parasitisation i.e. evidenced in the untreated control (91.2%). The insecticidal check however was found to be significantly detrimental in reducing the percent parasitisation to least levels (22.8%) and differed significantly with all the eco-friendly biopesticides in terms of percent parasitisation.

Almost similar trends were observed with regard to per cent adult emergence where in highest adult emergence of 79.2 percent was observed in *M. anisopliae* followed by *B. sphaericus* with 78.4 percent adult emergence while the least adult emergence of 74.8 percent was observed in *L.lecanii* treatment, despite the fact that they were all differing significantly different from least adult emergence noticed in case of an insecticidal check, where only 8.8 percent adult emergence was witnessed, eventhough all the test biopesticides resulted into percent adult emergence which is statistically on par with percent adult emergence (77.8%) achieved in the untreated control (Table 2 and Figure 1).

Table 2. Compatibility of commercially used biopesticides on Temperature Tolerant *Trichogramma chilonis* (TTT) in terms of percent parasitisation and percent adult emergence under pre-release conditions in laboratory

S. No.	Treatment	Recommended dosage	Parasitization (%)	Adult emergence (%)
1.	<i>Bacillus thuringiensis var kurstaki</i>	2g/l	90.80 ^a (72.37)	77.00 ^a (61.37)
2.	<i>Bacillus sphaericus</i>	2g/l	92.00 ^a (73.65)	78.40 ^a (62.33)
3.	<i>Beauveria bassiana</i>	5g/l	90.60 ^a (72.25)	76.20 ^a (60.88)
4.	<i>Metarhizium anisopliae</i>	5g/l	93.80 ^a (76.10)	79.20 ^a (62.91)
5.	<i>Lecanicillium lecanii</i>	5g/l	92.00 ^a (73.99)	74.80 ^a (59.87)
6.	Insecticidal check (Quinalphos 25% EC)	2ml/l	22.80 ^b (28.42)	8.80 ^b (17.15)
7.	Untreated Control	-	91.20 ^a (72.79)	77.80 ^a (61.94)
	CD	-	4.17	3.43
	SE(m)	-	1.43	1.17
	CV	-	4.77	4.77

*Values followed by common alphabet did not differ significantly among themselves.

*Values mentioned in parentheses are angular transformed values.

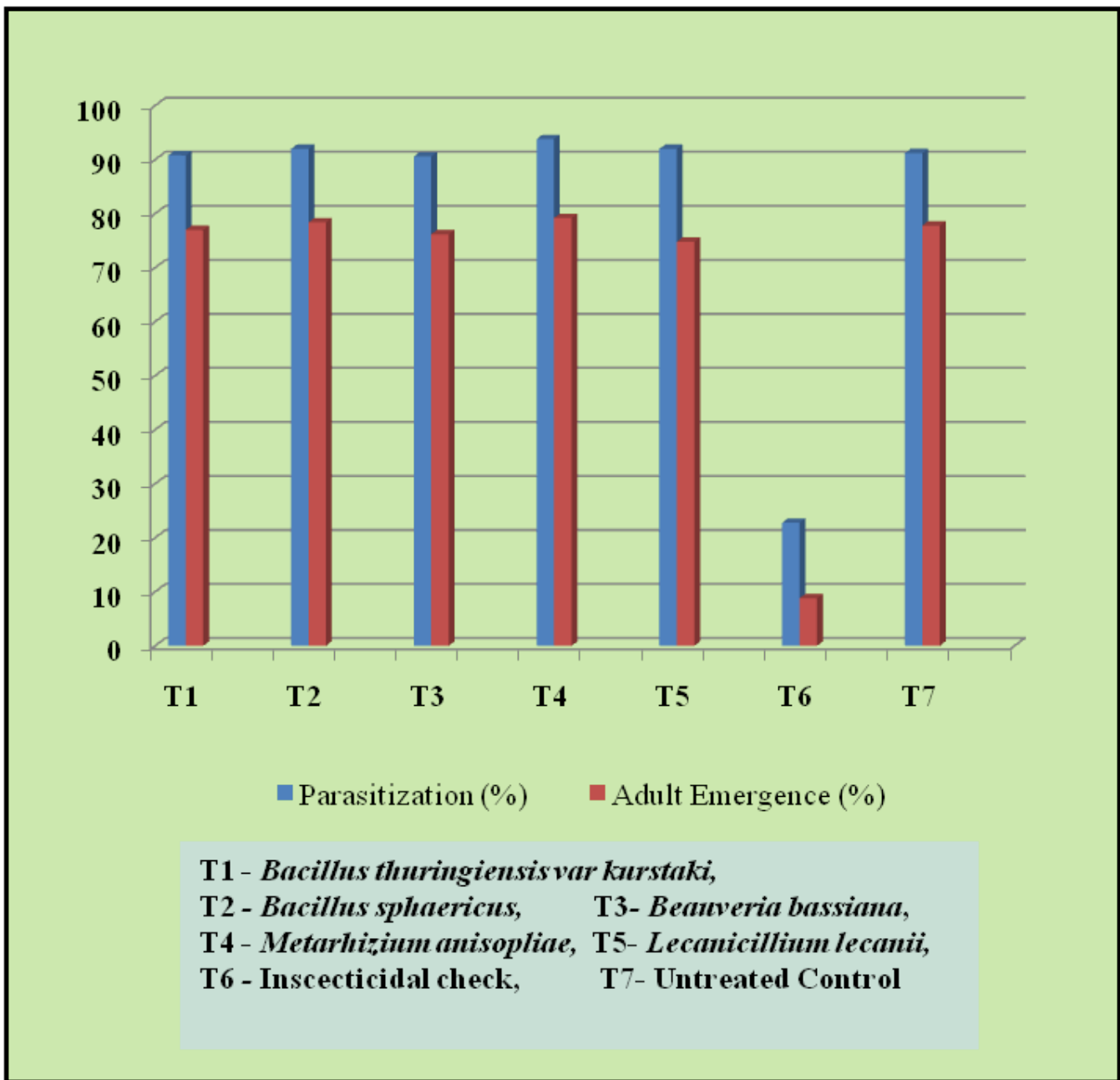


Figure 1. Impact of test biopesticides on parasitisation and adult emergence of Temperature Tolerant *T. chilonis* (TTT) in pre release situation under laboratory conditions.

Post-release situation

The results of post release situation clearly demonstrated that among the test biopesticides which were sprayed on *Tricho* cards, maximum percent adult emergence was observed from the cards sprayed with *Beauveria bassiana* (78.4%) followed by *L. lecanii* (77.8%) and *B. thuringiensis* var *kurstaki*, *B. sphaericus* with the adult emergence of 77.0 percent. Cards sprayed with *M. anisopliae* have shown the least adult emergence among the biopesticides (73.8%). Detrimental effect was observed in the cards treated with insecticide,

with the least adult emergence of 10 percent. However adult emergence in all the test biopesticides is statistically on par with the untreated control, where 75.2 percent adult emergence was observed (Table 3 and Figure 2).

The overall results of the two studies clearly demonstrated that all the test biopesticides were exceptionally compatible with Temperature Tolerant *T. chilonis* (TTT) in comparison with insecticidal check where, both percent parasitisation and adult emergence were detrimentally affected.

Table 3. Compatibility of commercially used biopesticides on Temperature Tolerant *Trichogramma chilonis*(TTT) in terms of percent adult emergence under post-release conditions in laboratory

S. No.	Treatment	Recommended dosage	Adult emergence (%)
1.	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	2g/l	77.00 ^a (61.37)
2.	<i>Bacillus sphaericus</i>	2g/l	77.00 ^a (61.40)
3.	<i>Beauveria bassiana</i>	5g/l	78.40 ^a (62.35)
4.	<i>Metarhizium anisopliae</i>	5g/l	73.80 ^a (59.20)
5.	<i>Lecanicillium lecanii</i>	5g/l	77.80 ^a (61.99)
6.	Insecticidal check (Quinalphos 25%EC)	2ml/l	10.00 ^b (18.35)
7.	Untreated Control	-	75.20 ^a (60.16)
	C.D.	-	3.48
	SE(m)	-	1.198
	C.V.	-	4.87

*Values followed by common alphabet did not differ significantly among themselves.

*Values mentioned in parentheses are angular transformed values.

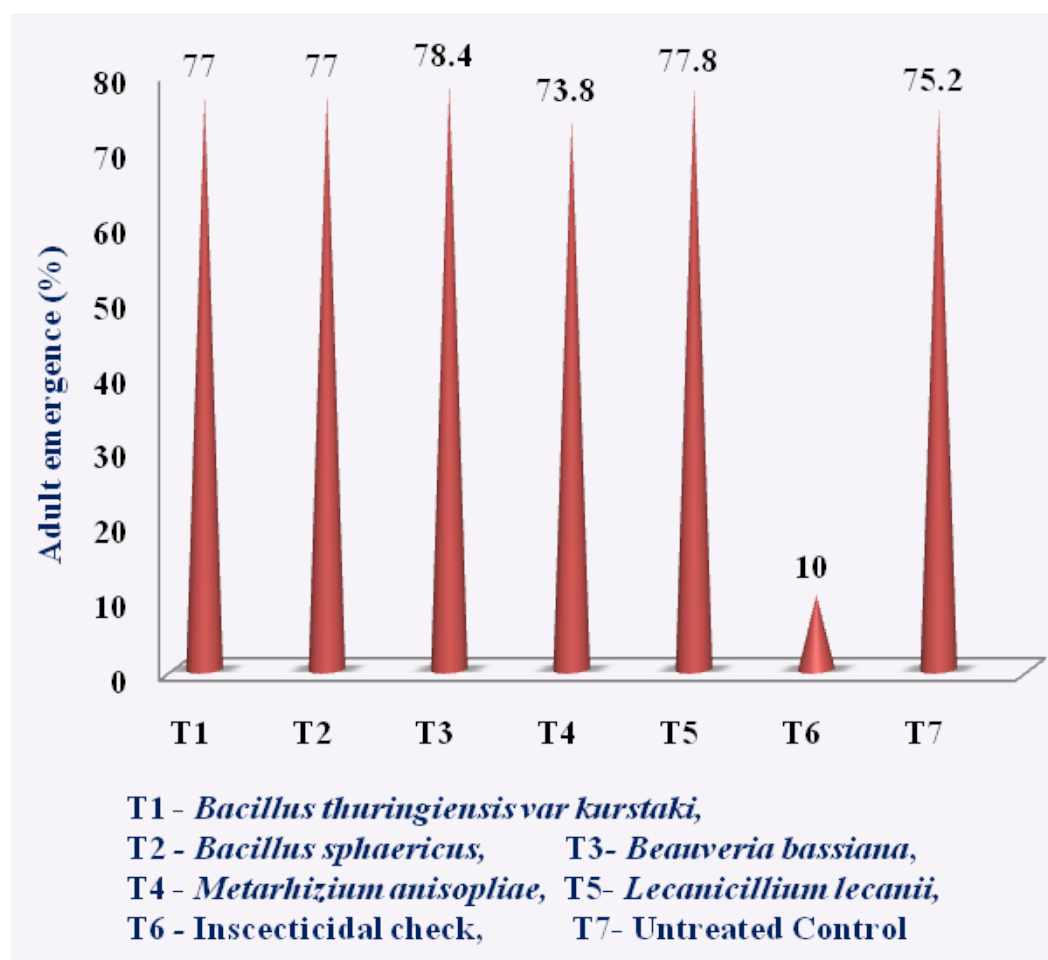


Figure 2. Influence of test biopesticides on adult emergence of Temperature Tolerant *T. chilonis* (TTT) in post release situation under laboratory conditions.

Srinivasan and Babu (2000) concluded from their studies that different commercial formulations of *Bt* are safe for three species of *Trichogramma* viz., *T. chilonis*, *T. japonicum* and *T. brasiliensis*. Derakhshan *et al.* (2007) reported that there is no significant effect on different biological parameters such as percent parasitism, adult emergence and adult duration of *T. chilonis* when treated with *L. lecanii* in pre- and post-release conditions. Polanczyk *et al.* (2010) reported that biopesticides (*B. bassiana* and *M. anisopliae*) did not affect the evaluated parameters of *Trichogramma atopovirilia* (Oatman and Platner).

Adult emergence of *T. chilonis* was found to be between 81.79-93.66 percent when treated with biopesticides, viz., *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *M. anisopliae* hence it is concluded that they are safe to the parasitoid (Patel and Pramanik, 2012). The results from the studies of Khan *et al.* (2014) show that the percent reduction in both emergence and parasitism relative to the controls was less than 10 percent for all doses and life stages treated, indicating that *HaNPV* is harmless to the emergence as well as parasitism efficiency of *T. chilonis*. The results of the present studies are in line with the above findings.

It is concluded from the above studies that all the test biopesticides (*Bacillus thuringiensis* var. *kurstaki*, *Bacillus sphaericus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*) were not harmful against Temperature Tolerant *T. chilonis* (TTT) in both pre- and post-release situations, hence the mentioned biopesticides and Temperature Tolerant *T. chilonis* (TTT) can be used together for pest management in Integrated pest management programmes in different crops.

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