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Evaluation of Toxicity of Botanical and Microbial Insecticides to Egg Parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae)

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Insecticides hamper the effectiveness of *Trichogramma chilonis* Ishii not only through the direct adult mortality but also reduces the parasitisation capability and per cent emergence from the parasitised host eggs which have been exposed to insecticides in field. In the present investigation, the treatments comprised biopesticides, viz. *Bacillus thuringiensis* var *kurstaki 5% WP* @ 0.5% and 0.1%, *M. anisopliae* (2×10^9 spores/g) @ 0.5%, *Beauveria bassiana* (2×10^9 spores/g) @0.5%, *Ha*NPV (1 X 10^9PIB/ml) @ 0.2% synthetic insecticide cartap hydrochloride 50% SP @ 0.1% and aqueous leaf extract of neem and Parthenium (3% and 5%) were investigated. Results revealed that the very less parasitisation (0.00 to 8.67%) and adult emergence (0.0% to 28.33%) were recorded from cartap hydrochloride 50% SP @ 0.1% + *Ha*NPV (0.2%), followed by the combination of aqueous neem leaf extract (5%) + Btk (0.1%) causes lowest parasitisation (10.00% to 27.33%), whereas lowest adult emergence (25.33% to 48%) were recorded from Parthenium leaf extract (3%) + Btk (0.1%) among botanicals and microbial insecticides. On the contrary maximum parasitisation (52% to 71.67%) and emergence (34.33% to 83%) recorded from *Ha*NPV (1 X 10^9PIB/ml) @ 0.2%, followed by combination of *Ha*NPV (1 X 10^9PIB/ml) @ 0.2% + Btk (0.1%) resulting 50% to 60% parasitisation and 35% to 75.67% emergence which show very less toxicity to T. chilonis Ishii.

Keywords: *Trichogramma chilonis* Ishii, *Bacillus thuringiensis* var *kurstaki, Beauveria bassiana, M. anisopliae,* microbial insecticides, Botanical insecticides.

In integrated pest management (IPM), it is important to determine which insecticides are compatible with key biological control agents and to identify the possible disruptive effects on beneficial insects. Many studies concerning the effect of pesticides on oviposition behavior were conducted on parasitoids because of the direct linkage between oviposition and parasitism rate and consequently pest regulation. The indirect perturbation in oviposition behavior may be induced by the repellent effect of pesticides, which can reduce the chances that a natural enemy will find a suitable host or oviposition site and also by occurrence of uncoordinated movements after pesticides exposure (Umoru *et al*., 1996, Desneux *et al*. 2004). Hence, we need to tackle the crop production problems due to insect pest in a cost effective and environment friendly way through the use of botanical and microbial insecticides. The present investigations were carried out to know the safer and effective combinations of botanicals

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and microbial insecticides to *Trichogramma chilonis* in laboratory conditions.

Trichogramma spp. includes extremely tiny parasitic wasps, which belong to the family Trichogrammatidae. *Trichogramma* parasitizes eggs of Lepidopterans by laying one or more eggs inside the eggs of Lepidopteron insects. The eggs of *Trichogramma spp.* hatch into small larvae, which feed on the embryo of the moth eggs, thereby killing it. There are three larval instars, all sacciform. At the beginning of the third larval instar, the host egg turns black due to the deposition of black granules at the inner surface of the chorion, an invaluable diagnostic character for parasitized eggs (Raghuram and Singh,1999). After 8-10 days of feeding and growth, an adult wasp chews out through the shell of the moth egg. It then copulates with the opposite sex and starts searching for fresh Lepidopteron eggs for further egg-laying, *Trichogramma* also reproduces parthenogenetically it is induced by bacteria (*wobachia*), in the absence of these bacteria the wasp reproduce sexually (Stouthamer and Kamer,1994). Visual, olfactory and tactile cues are used by the parasitoid to find out their hosts. Volatile compound emanating from the scales of *Corcyra cephalonica* and *Helicoverpa armigera* moth such as Hexatriacontane, docosane and monacosane increased the activity of *Trichogramma chilonis* (Ananthakrishnan, 1993).

Many *Trichogramma* specieses are generalized egg parasitoid with a broad host range including Lepidoptera, Diptera, Coleoptera, Hymenoptera, Neuroptera and Megaloptera (Mansfield and Mill, 2004). The adult wasps can destroy up to 98% of the host eggs. A large number of species, sub-species and strain of *Trichogramma spp.* are distributed throughout the world in diverse habitats and parasitizing eggs of over 200 insect species belonging to 70 families and 8 orders (Flanders and Quednau, 1960). In India, about 26 trichogrammatids are recorded of which *Trichogramma chilonis* Ishii is of significant importance (Tyagi and Khan, 1993).

The inundative or inoculative releases of *Trichogramma spp.* in the field to reduce the pest population which ultimately reduces the reliance on chemicals for the control of lepidopteron insect pest of field crops. The insecticide resistance has limited the effectiveness of many chemical insecticides; an intensive effort has been made to find out alternate methods of control (Nathan *et al.*, 2004). A public concern about pesticide residues, in agricultural commodities, has led to a progressive increase of interest in alternative approaches for the control of insect pests and disease causing pathogens. The application of microbial and botanical insecticides is increasing mostly because of greater environment awareness and food security concerns.

Botanical insecticides have long been advertised as attractive substitutes to synthetic chemical-insecticides, for controlling many insect pests because botanicals reputedly pose little threat to the environment or to the human health. The botanical mixtures are the best alternative to conventional pesticides to deal with problems of resistance, resurgence and residues (Raguraman and Singh, 1997). Pyrethrum and neem are well established commercially (Isman, 2006). Botanical and microbial insecticides are highly effective, safe, and ecologically acceptable. Plants produce a diversity of biologically active substances that affect the growth and development of other organisms and can, also provide protection against the herbivores. These plant products discourage or prevent an attack from the non-adapted organisms and play an important role in the ecology and physiology of the phytophagous insects (Sukumar, 1993).

Chemical preparations from the leaves and seeds of the Indian neem tree, *Azadirachta indica* A. Juss. (Meliaceae), have been shown to have harmful effects on the insects (Schumutterer, 1990). Neem seed kernel extracts (NSKEs) have suppressed the feeding, growth, and reproduction aspects of the pest insects and have, thus, been used in many integrated pest management (IPM) programme (Ascher *et al.*, 1996). Thus, keeping in view the above stated facts, the present investigation carried out with the objective to find out the effects of plant extracts and microbial insecticide combinations on parasitization and emergence of *Trichogramma chilonis* Ishii.

MATERIALS AND METHODS

Mass production of *Corcyra cephalonica*

The factitious host (*Corcyra cephalonica*) of *Trichogramma chilonis* Ishii was

taken from bio-control laboratory of CSAUA&T Kanpur and the nucleus culture of *T. chilonis* Ishii obtained from IISR, Lucknow (Utter Pradesh). Rearing was done on the milled maize grains. The grains were sterilized for 1 hour at 100^0 C in a hot air oven and than sprayed with 0.1% formalin to prevent the growth of moulds as well as to increase the grain moisture up to 15-16%. Baked groundnut 100gms, 5gms Yeast and 0.05gms streptomycin sulphate were mixed with 2.5 kg of grain. Each *Corcyra* rearing cage (2.5 kg grain/ cage) was charged with 0.5 cc eggs of *Corcyra cephalonica* (1cc = 20,000 eggs). The charged cages were kept for development of larvae at 30 ± 2^0 c and 60 ± 5 % R.H. Adult moths started emerging after 40 days of charging and could be collected up to 90 days continuously. Adult moths were fed daily with honey 50% (in distilled water) + vitamin E solution and kept in oviposition cages for mating and oviposition. 0-24 hour's old *Corcyra cephalonica* eggs were exposed to UV rays of 15 watt for 1 hr at a distance of 12-15 cm to prevent egg hatching. The sterilized eggs were pasted equidistantly with Arabic gum on paper cards of 21cm x 30cm size having thirty 7x2 cm rectangles. These egg cards were placed in a polythene cover along with the nucleus culture of *T. chilonis* parasitised eggs of *Corcyra cephalonica* (6:1).

Preparation of plant extracts

The plant species were collected from C.S.A. University campus and were washed with water until all dust particles were removed and were dried in shade. After that grounded with mortar and pestle into fine powder. Than 10 g powder of each plant species were taken in a beaker and 100 ml of distilled water was added. After 24 hrs, the mixture was passed through a coarse muslin cloth. The desired quantity of distilled water was then added into filtered plant material in order to make the volume to 100 ml. Thus, 10 %(w/v) stock solution of each plant extract was prepared. The required concentration of each of the plant extracts were made by adding pre-determined quantity of distilled water at the time of testing. The other microbial and synthetic insecticides viz. BIO-DART(*Bacillus thuringiensis* var *kurstaki 5% WP*), Metarrhizium®(*M. anisopliae* 2×10^9 spores/ g), Boverin (*Beauveria bassiana*, 2×10^9 spores/g), Heli-Cide (HaNPV 1 X 10^9PIB/ml) and cartap hydrochloride 50% SP were brought from local market of Kanpur, which were being conventionally used for management of insect pest of crops.

The toxic effect of plant extracts and microbial insecticides on parasitization of Corcyra eggs by *T. chilonis* Ishii Investigations to evaluate the toxic effect of plant extracts and microbial insecticides were carried out under laboratory conditions (Table 1). The egg cards 2.0 x 2.5 cm with 50 eggs per card, were sprayed with spray fluid using micro pipettes at the rate of 1 ml per card in different combinations of insecticides, cards were sprayed first with plant extracts (or mixtures of plant extracts) and air dried followed by spray of microbial insecticides solutions and air dried again. After spraying, the cards were introduced into glass vials of 15 x 2.5 cm. After giving the treatment the egg cards were exposed to 5 pairs of *Trichogramma chilonis* Ishii adults per cards of 50 eggs for 24 hrs at different time intervals 0 day after treatment (DAT), 1^{st}, 2^{nd}, 3^{rd} and 4^{th} days after treatment. Streak of diluted honey 50% (in distilled water) was provided on the inner wall of the glass vials as food for the adults at mouth of the vials and were plugged with cotton. Each treatment was replicated three times and the experiment was conducted at 26 ± 2^0 c and 65 ± 5 % R.H. in B.O.D. incubator. In untreated control, egg cards were sprayed with distilled water only. On the basis of total number of eggs provided and number of eggs parasitized, the per cent parasitization was calculated. The parasitized eggs were distinguished by blackening of the eggs (Raghuram and Singh,1999).

The toxic effect on adult emergence of *T. chilonis* Ishii from parasitised Corcyra eggs

The egg cards of 2cm x 2 cm size having 50 parasitised eggs of *Corcyra* were treated with spray fluid using micro pipettes at the rate of 1 ml per card same as treated for testing the toxic effects of plant extracts and microbial insecticides on parasitization of Corcyra eggs by *T. chilonis* Ishii. The treated egg cards were air dried under shade for 10 minutes and kept inside the tube for adult emergence in control only water was sprayed. The experiment was conducted at 26 ± 2^0 c and 65 ± 5 % relative humidity in lab conditions and the experiment was replicated three times. The number of adults emerged from eggs treated with different insecticides were recorded after 8^{th}, 9^{th}, 10^{th}, 11^{th} and 12^{th} day after treatment.

RESULTS AND DISCUSSION

Toxic effects on the parasitization of insecticide treated eggs by *Trichogramma chilonis* Ishii

The number of parasitised eggs by *Trichogramma chilonis* Ishii after different periods of time treated with different insecticides is given in Table 1. The minimum parasitisation was observed on 0th day after treatment (DAT) and no parasitisation on the same day was recorded from T_{11} (Cartap hydrochloride @0.1% + *Ha*NPV @ 0.2%). Whereas minimum parasitisation was recorded from T_4, T_8, T_{10}, T_5 and T_9, and these were statistically at par, T_2, T_3, T_1 and T_6 which were also statistically nonsignificant followed by T_7, T_{12}, T_{13} and T_{14}. On the 4th DAT minimum parasitisation (8.67%) was recorded from T_{11} (cartap hydrochloride 50% SP+ *Ha*NPV @ 0.2%) followed by $T_5 < T_2 < T_{10} < T_6 < T_4 < T_9 < T_3 < T_8 < T_7 < T_1 < T_{12} < T_{13}$ and T_{14}. An increased parasitisation was recorded at 1st, 2nd, 3rd and 4th day after treatment (DAT) from each treatment. On

Table 1. The effect of insecticides on parasitisation of *C. cephalonica* eggs by *T. chilonis*

S. No.	Treatment	Per cent Parasitization at different days after treatment (DAT)*				
		0th DAT	1st DAT	2ndDAT	3rd DAT	4th DAT
1.	NLE (3%) + *B. bassiana* (0.5%)	16.67ef (24.08)	20.67hi (27.02)	36.00d (36.86)	37.33d (37.65)	40.67d (39.60)
2.	NLE (5%) + *B. bassiana* (0.5%)	16.00ef (23.46)	22.67fg (28.42)	27.33k (31.51)	28.00j (31.93)	28.67l (32.36)
3.	NLE (3%) + *M. anisopliae* (0.5%)	16.67ef (24.08)	27.33de (31.51)	32.67ef (34.84)	34.00e (35.65)	35.33g (36.46)
4.	NLE (5%) + *M. anisopliae* (0.5%)	08.00 g (16.34)	21.33gh (27.49)	31.33g (34.02)	32.00fg (34.43)	32.00i (34.44)
5.	NLE (5%) + *Btk* (0.5%)	10.00 g (18.37)	22.67fg (28.40)	26.00l (30.63)	26.67j (31.08)	27.33m (31.51)
6.	NLE + PLE (1:1) 2.5% + *Btk* (0.1%)	18.00e (25.07)	23.33f (28.87)	28.67ij (32.36)	30.67gh (33.61)	31.33ij (34.02)
7.	NLE + PLE (1:1) 2.5% + *B. bassiana* (0.5%)	22.00d (27.96)	28.67d (32.36)	33.33e (35.25)	36.67d (37.25)	38.67e (38.43)
8.	PLE (3%) + *B. bassiana* (0.5%)	08.00g (16.34)	17.33jk (24.59)	30.00h (33.20)	32.67ef (34.84)	36.67f (37.25)
9.	PLE (3%)+ *M. anisopliae* (0.5%)	10.00 g (18.37)	18.67j (25.56)	32.00fg (34.43)	32.67ef (34.84)	34.00h (35.65)
10.	PLE (3%)+ *Btk* (0.1%)	08.00g (16.42)	16.00kl (23.57)	29.33hi (32.78)	30.00hi (33.20)	30.67jk (33.61)
11.	Cartap hydrachloride (0.1%) + *Ha*NPV (0.2%)	00.00h (00.00)	04.67m (12.41)	08.00m (16.42)	08.00k (16.42)	08.67n (17.09)
12.	*Ha*NPV (0.2%) + Btk (0.1%)	50.00bc (44.98)	52.67c (46.51)	57.33c (49.20)	58.67c (49.97)	60.00c (50.75)
13.	*Ha*NPV (0.2%)	52.00b (46.13)	56.00b (48.43)	60.67b (51.14)	68.67b (55.94)	71.33b (57.61)
14.	Control	60.00a (50.76)	62.67a (52.32)	72.00a (58.04)	75.33a (60.20)	76.67a (61.10)
	CD at 5%	3.201	1.549	1.312	1.381	1.170
	SE(m)	1.459	0.749	0.460	0.474	0.402
	CV	7.32	3.20	2.203	2.194	1.809

Btk = Bacillus thuringiensis var. kurstaki, *HaNPV= Helicoverpa armigera* Nuclear polyhydrosis virus, PLE:-*Parthenium* leaf extract

*Average of three replications

Figures within parentheses are angular transformed values in a column, means followed by same alphabet are not significantly different

the contrary findings also suggest that the toxicity effect of insecticides reduced significantly up to 4 DAT. The effect of all the tested insecticides declined as the days after treatment increased. Patel and Pramanik (2012) corroborates our findings who reported maximum (80 % to 85 %) parasitization of Corcyra eggs which were treated with *B. bassiana* (0.5%) and *M. anisopliae* respectively and lowest parasitisation (8.89 per cent) were observed in eggs treated with cartap hydrochloride (0.1%) + *M. anisopliae* (0.5%). In the present study parasitisation was significantly reduced in egg cards treated with aqueous parthenium leaf extract (3%) and microbial insecticides followed by aqueous neem leaf extract (5%) and microbial insecticides. In an another study Khan and Tiwari (2001) reported that *Parthenium hysterophorous* caused 48.00% parasitization and 65.33% emergence of *Trichogramma chilonis* and Singh (2007) studied the safety aspect of biopesticides to the bioagent *Trichogramma chilonis* and found *annona* 0.25% and neem 0.05% safer to egg parasitoid.

On the other hand, it was observed the

Table 2. The effect of insecticides on adult Emergence of T. chilonis Ishii from Parasitized eggs of *Corcyra cephalonica*

S. No.	Treatment	Per cent Parasitization at different days after treatment (DAT)*				
		0th DAT	1st DAT	2ndDAT	3rd DAT	4th DAT
1.	NLE (3%) + *B. bassiana* (0.5%)	38.33 a (38.18)	48d (43.83)	51.33c (45.75)	55.33 cd (48.05)	68c (55.55)
2.	NLE (5%) + *B. bassiana* (0.5%)	40.33 ab (39.39)	47.67 d (43.64)	50.67 c (45.36)	54.67 d (47.66)	60.33de (50.95)
3.	NLE (3%) + *M. anisopliae* (0.5%)	36 cd (36.83)	44.67 e (41.92)	45.33 d (42.30)	57.33cd (49.20)	62.67d (52.32)
4.	NLE (5%) + *M. anisopliae* (0.5%)	40.67a (39.58)	47.33 d (43.45)	52.33 c (46.32)	54.67 d (47.66)	57.67e (49.40)
5.	NLE (5%) + *Btk* (0.5%)	40.33 ab (39.39)	51c (45.55)	52.33 c (46.32)	54 e (47.28)	55f (47.85)
6.	NLE + PLE (1:1) 2.5% + *Btk* (0.1%)	36.33bcd (37.05)	41f (39.78)	50.67 c (45.36)	55.67cd (48.24)	55.67 f (48.23)
7.	NLE + PLE (1:1) 2.5% + *B. bassiana* (0.5%)	39.67abc (40.18)	43.33 e (35.25)	47.33d (43.45)	59c (50.17)	56.33 f (48.62)
8.	PLE (3%) + *B. bassiana* (0.5%)	25.33e (30.15)	33.33h (35.25)	35.67f (36.65)	50.67f (45.36)	52.33g (46.32)
9.	PLE (3%)+ *M. anisopliae* (0.5%)	24.33 e (29.50)	37.33g (37.65)	42.67e (40.76)	48.33fg (44.03)	55.33 f (48.05)
10.	PLE (3%)+ *Btk* (0.1%)	25.33 e (30.19)	35.33h (36.45)	41.67 e (40.18)	45.67gh (42.49)	48h (43.83)
11.	Cartap hydrachloride (0.1%) + *Ha*NPV (0.2%)	0f (0.00)	0i (0.00)	0g (0.00)	12i (20.25)	28.33i (32.08)
12.	*Ha*NPV (0.2%) + Btk (0.1%)	35 d (36.25)	51.67c (45.94)	65.67b (54.16)	66.33 b (54.52)	75.67b (60.49)
13.	*HaNPV* (0.2%)	34.33 d (35.83)	62.67b (52.32)	65.67 b (54.12)	68.33b (55.74)	83 a (65.72)
14.	Control	40.67a (39.59)	64.67a (53.51)	68.67a (55.96)	75.33a (60.22)	85.33a (67.58)
	C.D.	4.31	1.996	2.421	2.705	3.818
	SE(m)	1.478	0.686	0.831	0.929	1.311
	C.V.	7.594	2.998	3.379	3.408	4.434

Btk = Bacillus thuringiensis var. kurstaki, *HaNPV= Helicoverpa armigera* Nuclear polyhydrosis virus, PLE:-*Parthenium* leaf extract

*Average of three replications

Figures within parentheses are angular transformed values

In a column, means followed by same alphabet are not significantly different

parasitization was significantly higher in *Ha*NPV (0.2%) and *Ha*NPV (0.2%) + *Btk* (0.1%) followed by combinations of aqueous leaf extracts and *B. bassiana* (0.5%), *M. anisopliae* (0.5%), and *Btk* (0.1%) and it can be suggested that plant extracts have repellent or deterrent effects on *T. chilonis* Ishii which causes reduced parasitisation of corcyra eggs. Babasaheb *et al.,* 2009 also reported that *Ha*NPV @ 1× 10^9 POBs/ml and *Bacillus thuringiensis* @ 2 ml/ lit. were safer to the parasitoid whereas neem oil (1.0%) and lamdacyhalothrin (0.0015%) reduced the parasitization. Similarly Kaur, Amandeep et al. (2012) found *Bacillus thuringiensis* (0.2%) as a safer microbial insecticide to *Trichogramma chilonis*.

Toxic effects of botanical and microbial insecticides on emergence of *Trichogramma chilonis* Ishii

Results on per cent emergence of *T. chilonis* Ishii adult as influenced by microbial and botanical mixture are presented in Table 2. The *T. chilonis* Ishii wasp started to emerged out at significant rate from 8th DAT and up to 12th DAT. On the 8th DAT minimum emergence were recorded from T_{11} (Cartap hydrochloride @0.1% + *Ha*NPV @ 0.2%) followed by $T_9 < T_{10} < T_8 < T_{13} < T_{12} < T_3 < T_6 < T_1 < T_7 < T_5 < T_2 < T_4$ and T_{14}. The combination of PLE (3%) with microbial agents (*B. bassiana* (0.5%), *M. anisopliae* (0.5%) and *Btk*,0.1%) significantly reduced the adult emergence from parasitised *Corcyra* eggs. Adult emergence increased as days after treatment increased indicates the slower development of *T. chilonis* Ishii inside the host eggs. The adult emergence from parthenium leaf extract treated eggs was significantly lower at 11th and 12th days after treatment. The findings of Sankarganesh and Khan (2006) concur our results, they evaluated effects of 12 weed species extracts *T.chilonis* Ishii reared on *Corcyra cephalonica* eggs. They reported lowest per cent parasitisation and per cent emergence from *P.hysterophorous* 3% by *Trichogramma chilonis* Ishii. In an another study Sattar *et al*.(2011) also endorse the present investigation they reported neem oil as harmless to the egg of *T.chilonis* Ishii but exerted slightly harmful effect on larval development and female fecundity. The maximum adult emergence was reported from *Ha*NPV @ 0.2% (83%) which was statistically at par over the control (untreated) and the combination of *Ha*NPV (0.2%) + Btk (0.1%) emerged as most promising combination of microbial insecticides having less toxic effect on adult emergence of *T.chilonis* Ishii while combination of neem leaf extract and microbial insecticides stood at 2nd place showing high adult emergence of adult parasitoid *T.chilonis* Ishii.

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