Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 1263-1267

Received: 01-03-2019
Accepted: 03-04-2019

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Safety studies on certain pesticides and biopesticides against green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Petersen)

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Abstract
An experiment was conducted on safety studies on certain pesticides and biopesticides against Green lacewing, *Chrysoperla zastrowi sillemi* (Esben- Peterson) under laboratory conditions, which were carried out at Agricultural Entomology Laboratory at Vanavarayar Institute of Agriculture, Pollachi during December 2018 – March 2019. The data on per cent mortality of third instar grubs due to effect of pesticides and biopesticides shows that the treatment *Bacillus thuringiensis* 0.025% recorded 0 per cent grub mortality of third instar grubs and emerged as the best treatment. Next superior treatments in order of safeness were *Pseudomonas fluorescens* 0.5%, *Nomuraea rileyi* 0.5%, Abamectin 1.9 EC, *Metarrhizium anisopliae* 0.5%, *Lecanicillium lecanii* 0.5%, NSKE 5%, Thumbai leaf extract 5%, Neem-Azal 1%, Pungam oil 3%, *Beauveria bassiana* 0.5%, Novaluron 10 EC, Tobacco leaf extract 5%, Imidacloprid 17.8 SL, 3G mixture 5%, Thiamethoxam 25 WG, Lambda cyhalothrin 5 EC which recorded 8.9, 13.7, 16.6, 17.0, 18.4, 22.1, 23.4, 25.0, 31.3, 40.8, 44.2, 49.8, 78.9, 88.7 per cent mortality, respectively. Acephate 75% SP was the most toxic to the third instar grubs, which recorded 100 per cent grub mortality.

Keywords: *Chrysoperla*, bio-safety, synthetic insecticides, botanicals, microbial pesticides

1. Introduction
The golden eyes or aphid lion, *Chrysoperla zastrowi sillemi* (Neuroptera: Chrysopidae), commonly called as green lacewing. The grubs of green lacewings are effective predators of insect eggs and soft bodied insects such as aphids, whiteflies, thrips and spider mites found in many agricultural systems [11, 19]. The adults were free living feeds on nectar, pollens and honey. Existence of such naturally occurred biological control agents is one reason that many plant feeding insects do not ordinarily become economic pests. Because of its benefits in pest management, it is considered as an important bio-control agent in Integrated Pest Management (IPM) programs. Hence, it is recommended as the first line of defence in an IPM program [10]. Though it has plenty of benefits in IPM, the presence of Green lacewing in the field is rare due to indiscriminate use of non selective agrochemicals [17]. Early insecticides are considered as a backbone of pest management program and hence used as a sub-ordinate with biological control methods. The use of such insecticides is unavoidable in insect pest management programs especially when the pest exceeds the economic threshold level (ETL). Though it has benefits, frequent and over use of such agrochemicals in pest control leads to adverse toxic effect to natural enemies such as predators and parasitoids. In some rare cases, these natural enemies developed resistance to such harmful insecticides [22, 26]. Hence, use of targeted insecticides in pest control is important and they should have no adverse effects on beneficial organisms such as predators and parasitoids [8, 18].
Consequently, it is necessary to understand the adverse impact of such pesticides utilized in pest control with augmentative and conservative biological control. Thereby, few studies have been conducted to find out the impact of pesticides on green lacewing, which are responsible for maintaining populations in the field. In present era of pesticides, maintenance of biological components is very tough as farmer demand fast management and hence, use of pesticides is inevitable. This could be accomplished by use of comparatively safer pesticides at safer concentration to make a mean-win scenario. However, scanty information is offered on these aspects a number of them are as follows.

In the studies made on aphid control, sub-lethal doses of insecticides found as safe on *C. zastrowi sillemi* immature stages but sub-lethal effects may exist that reduce the effectiveness of green lacewing progeny [4]. The outcome of the experiment conducted to study the toxic effect of spinosad on *C. carnea* showed that spinosad is less toxic effect on adult longevity and fecundity with no impact on eggs and pupae [12]. Also, pyriproxyfen and tebufenozide were found to be less toxic, whereas azadirachtin and diflubenzeuron were highly toxic to *C. carnea* third instar larvae [7, 13, 14]. Biosafety test of Neem seed extract on *C. carnea* was also found a safe to in comparison to nine insecticides where chlorpyrifos, deltamethrin and cypermethrin were found highly toxic to *Chrysoperla* [25]. A research work was done to analyse the toxic effect of neem-based pesticides found that was no mortality of *C. carnea* due to like NSKE 5 per cent, Neemark, Achook, and Nimbecidine each at 0.003 per cent and neem oil at 1 per cent [3, 29].

Taking those above findings in thought it is very important to access the bio-safety of pesticides and biopesticides against natural enemies as pesticides play a significant role in our Indian agriculture. Knowledge on use of those pesticides and biopesticides will be helpful to integrate the natural enemies effectively with pesticides and biopesticides there by helps in maintaining the sustainability of agro ecosystem. Keeping in the view of above facts, Synthetic pesticides, Botanicals and Microbial pesticides were evaluated for their toxicity against third instar grubs of *C. zastrowi sillemi under* laboratory conditions with the aim to find out suitable pesticides and biopesticides which are less hazardous.

2. Materials and Methods

The laboratory investigations were conducted in order to find out the bio-safety of some synthetic insecticides, botanicals and microbial pesticides on the third instar grubs of *C. zastrowi sillemi*. The commercial formulations were procured from the market. The mass multiplication of *C. zastrowi sillemi* was carried out in the Agricultural Entomology laboratory, Vanavarayar Institute of Agriculture, Pollachi. The nucleus culture eggs of *C. zastrowi sillemi* were obtained from Biocontrol laboratory, Tamilnadu Agricultural University, Coimbatore. The mass culturing of *C. zastrowi sillemi* was done by rearing it on eggs of Rice moth, *Corcyra cephalonica*, as a factitious host.

2.1 Production of Rice moth, *Corcyra cephalonica* eggs

To obtain a continuous availability of feed for grubs of *C. zastrowi sillemi* throughout the experimental work period. Rearing of Rice moth was done in Agricultural Entomology laboratory, Vanavarayar Institute of Agriculture, Pollachi.

Heat sterilized broken pearl millet grains (2.5 kg) along with a hundred grams of groundnut powder and 5 g yeast powder are taken as a mixture in plastic troughs (45 × 15 cm). Streptomycin sulphate (0.05%) was sprayed on the mixture at 15 ml per trough to prevent bacterial infection. Wettable Sulphur is added at 5 g per trough as a prophylactic measure against storage mites. *Corcyra* eggs (0.5 cc/ trough) are inoculated into the prepared cumbu grain media and covered with khada cloth supported with rubber bands. The moths started emerging after 45 days of the inoculation of eggs. They were collected by the help of specimen tubes and transferred in the mating chambers, which were provided with 10% honey solutions as diet. The eggs were collected daily and passed through mesh sieve, and then these sieved eggs were UV sterilized to kill the embryo and then used as food for rearing of *Chrysoperla* grubs.

2.2 Rearing of *Chrysoperla zastrowi sillemi* Grubs

The eggs of *Chrysoperla* were counted using a hand lens and transferred in to grub rearing drum at the rate of 250 eggs per drum, provide paper strips for avoiding cannibalism and these drums were covered with khada cloth. After 2 days, the eggs of *Corcyra cephalonica* were inoculated as feeding material for the *Chrysoperla* grub in the laboratory.

2.3 Exposure of *Chrysoperla zastrowi sillemi* grubs to treatment solutions

The glass vials are initially sprayed and coated with treatment solution and air dried. This was followed by inoculation of third instar grubs of *C. zastrowi sillemi*. Mortality of grubs was recorded at 6, 12 and 18 hours after treatment and per cent mortality of grubs have been worked out [21].

2.4 Experimental layout

The experiments were laid out in Completely Randomized Design. The treatments are grouped as Synthetic insecticides, Botanicals and Microbial pesticides; each group consists of 7 treatments including untreated check with each four replications. The list of treatments is given in the Table 1.

Table 1: List of Treatments used

Synthetic Insecticides	Botanicals	Microbial Pesticides
T_1 - Acephate 75% S.P	T_1 - Neem-azal 1%	T_1 - *Beauveria bassiana* 0.5%
T_2 - Imidachloprid 17.8 SL	T_2 - NSKE 5%	T_2 - *Lecanicillium lecanii* 0.5%
T_3 - Abamectin 1.9 EC	T_3 - Tobacco leaf extracts 5%	T_3 - *Metarrhizium anisopliae* 0.5%
T_4 - Novaluron 10 EC	T_4 - 3G Mixture* 5%	T_4 - *Nomuraea rileyi* 0.5%
T_5 - Lambda cyhalothrin 5 EC	T_5 - Thumbai leaf extracts 5%	T_5 - *Bacillus thuringiensis* 0.025%
T_6 - Thiamethoxam 25 WG	T_6 - Pungam oil 3%	T_6 - *Pseudomonas fluorescens* 0.5%
T_7 – Untreated Control	T_7 – Untreated Control	T_7 – Untreated Control

*3G Mixture – Ginger, Garlic and Green Chilli Mixture

2.5 Observation

The experiments were subjected for recording observations. The observations were noted separately during the experimental period based on the mortality of grub after treatment and per cent mortality was worked out.

$$\text{Per cent mortality of grubs} = \frac{\text{No. of dead grubs}}{\text{Total no. of grubs}} \times 100$$

2.6 Data Analysis

The data on mortality per cent were transformed into arcsine values before statistical analysis. Further, the treatment means were differentiated statistically by performing the Least Square Means test (LSD) at $p < 0.05$ levels (Gomez and Gomez, 1984) [6]. Data of the per cent mortality in all treatments in third instar grubs were analysed by one way ANOVA analysis using AGRES Software.

3. Results and Discussion

3.1 Influence of Synthetic pesticides on the third instar grubs of *C. zastrowi sillemi*

The results are presented in Table 2. The results show that Abamectin 1.9 EC recorded the least grub mortality with 8.5, 14.4 and 14.6 per cent at 6, 12 and 18 hours after treatment respectively. It was followed by Novaluron 10 EC which made 28.5, 34.3 and 40.8 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. Imidacloprid 17.8 SL showed 32.3, 48.2 and 48.5 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. Thiamethoxam 25 WG showed 75.1, 78.9 and 79.7 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. Lambda cyhalothrin 5 EC recorded 60.2, 88.7 and 89.0 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. Acephate 75% SP have resulted in 100 per cent grub mortality in all the treatment intervals *viz.,* 6, 12 and 18 hours after treatment respectively. From Table 2, it was found that the descending order of safety of Synthetic insecticides was Abamectin 1.9 EC > Novaluron 10 EC > Imidacloprid 17.8 SL > Thiamethoxam 25 WG > Lambda cyhalothrin 5 EC > Acephate 75% SP. The present findings are in conformity with Nasreen *et al.* [16] who reported that the effect of Abamectin showed 10 per cent grub mortality in *C. zastrowi sillemi.* Preetha *et al.* [24] reported that Imidacloprid caused less than fifty per cent grub mortality on *C. zastrowi sillemi.* Nitharwal *et al.* [20] determined that Thiamethoxam and Acephate were moderately toxic against the *Chrysoperla* grubs but the present finding is not in accordance, showing that Acephate is highly toxic to *Chrysoperla* grubs. This may be due to species difference or variation in environmental conditions. The present investigations are in agreement with Amarasekare *et al.* [1] who determined that none of the larva survived to adult after treatment with Lambda cyhalothrin. According to the recommendation of International Organization for Biological Control [8], the Chemicals Abamectin 1.9 EC, Novaluron 10 EC and Imidacloprid 17.8 SL fall under the Class I which are harmless; Thiamethoxam 25 WG, Lambda cyhalothrin 5 EC fall under the Class III which are moderately harmful and Acephate 75% SP comes under the Class IV indicating that it is a harmful insecticide.

Table 2: Influence of Synthetic pesticides on the third instar grubs of *C. zastrowi sillemi*

Tr. No.	Name of Synthetic insecticide	Dose/ Lit	Grub mortality % after			Toxicity class as per IOBC scale
			6 hrs	12hrs	18hrs	
T_1	Acephate 75% SP	0.78 g	100.0 (89.46)g	100.0 (89.46)g	100.0 (89.46)g	IV
T_2	Imidachloprid 17.8 SL	0.3 ml	32.3 (34.62)d	48.2 (44.00)d	48.5 (44.16)d	I
T_3	Abamectin 1.9 EC	0.047 ml	8.5 (16.93)b	14.4 (22.26)b	14.6 (22.44)b	I
T_4	Novaluron 10 EC	0.27 ml	28.5 (32.25)c	34.3 (35.85)c	40.8 (39.70)c	I
T_5	Lambda cyhalothrin 5 EC	1 ml	60.2 (50.86)e	88.7 (70.62)f	89.0 (70.37)f	III
T_6	Thiamethoxam 25 WG	0.4 g	75.1 (60.05)f	78.9 (62.65)e	79.7 (63.21)e	III
T_7	Control	-	0.0 (0.54)a	0.0 (0.54)a	0.0 (0.54)a	-
	SE d (±) CD @ 5%		0.25 0.53	0.37 0.78	0.66 1.38	

*Figures in the parenthesis are arcsine transformed values, hrs-Hours, Lit-litre.

3.2 Influence of Botanicals on the third instar grubs of *C. zastrowi sillemi*

The results are presented in Table 3. The results show that Neem-azal 1% recorded the least grub mortality with 10 per cent at 6 hours after treatment; but it did not maintain the least grub mortality at 12 and 18 hours after treatment which caused 14.4 and 14.6 per cent grub mortality respectively. NSKE 5% recorded 12 per cent grub mortality at 6 hours after treatment; however it showed the least toxicity and 14.0 and 18.4 per cent grub mortality at 12 and 18 hours after treatment respectively. Thumbai leaf extracts 5% showed 10.3, 18.1 and 22.1 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. Pungam oil 3% showed 10.4, 20.3 and 25.0 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. Tobacco leaf extracts 5% recorded 30.0, 37.3 and 44.2 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. 3G Mixture 5% has resulted in the highest grub mortality among the tested botanicals with 40.6, 49.3 and 49.8 per cent grub mortality in all the treatment intervals *viz.* 6, 12 and 18 hours after treatment respectively. From Table 2, it was found that the descending order of safety of botanicals was NSKE 5% > Thumbai leaf extract 5% > Neem-azal 1% > Pungam oil 3% > Tobacco leaf extracts 5% > 3G Mixture 5%. The present findings are in conformity with Atalla *et al.* [2] who reported that the Neem-azal had no side effects on *Chrysoperla.* Mukesh *et al.* [15] found that NSKE 5% caused 4 per cent grub mortality on *C. zastrowi sillemi*; the present findings are not in agreement with the previous studies this may be due to species difference or variation in environmental conditions. Tesfaye *et al.* [27] detected that NSKE 5% resulted in 7.20 per cent grub mortality on *C. zastrowi sillemi*; the present findings are not in accordance with the previous studies this may be due to species difference or variation in environmental conditions. El Wakeil *et al.* [5] narrated there were no serious side effects of NSKE on parasitism and emergence rates of *Trichogramma* sp. and *Chrysoperla* sp which of natural enemies of *Helicoverpa armigera.* According to the recommendation of International Organization for Biological Control [8], all the treated botanicals fall under Class I i.e. they are harmless.

Table 3: Influence of Botanicals on the third instar grubs of *C. zastrowi sillemi*

Tr. No.	Name of Botanical	Dose/ Lit	Grub mortality % after			Toxicity class as per IOBC scale
			6 hrs	12hrs	18hrs	
T_1	Neem-azal 1%	1 ml	10.0 (18.46)b	20.9 (27.22)d	23.4 (28.95)d	I
T_2	NSKE 5%	50 g	12.7 (20.31)d	14.0 (21.99)b	18.4 (25.42)b	I

T_3	Tobacco leaf extracts 5%	50 g	30.0 (33.23)e	37.3 (37.63)e	44.2 (41.70)f	I
T_4	3G Mixture 5%	50 ml	40.6 (39.55)f	49.3 (44.60)f	49.8 (44.87)g	I
T_5	Thumbai leaf extracts 5%	50 ml	10.3 (18.70)bc	18.1 (25.20)c	22.1 (28.04)c	I
T_6	Pungam oil 3%	30 ml	10.4 (18.84)c	20.3 (26.73)d	25.0 (30.00)e	I
T_7	Untreated Control	-	0.0 (0.54)a	0.0 (0.54)a	0.0 (0.54)a	-
	SE d (±) CD @ 5%		0.16 0.33	0.30 0.63	0.19 0.41	

*Figures in the parenthesis are arcsine transformed values, hrs-Hours, Lit-litre.

3.3 Influence of Microbial pesticides on the third instar grubs of *C. zastrowi sillemi*

The results are presented in Table 4. The results show that *Bacillus thuringiensis* 0.025% recorded the least grub mortality with 0 per cent in all the treatment intervals *viz.*, 6, 12 and 18 hours after treatment respectively. *Lecanicillium lecanii* 0.5% caused the next least grub mortality with 3.0 per cent grub mortality at 6 hours after treatment but it did not continue to be the second best treatment resulting in 13.1 and 17.0 at 12 and 18 hours after treatment respectively. *Nomuraea rileyi* 0.5% showed 3.0, 10.6 and 13.7 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. *Pseudomonas fluorescens* 0.5% resulted in 5.7 per cent grub mortality at 6 hours after treatment; however it managed to prevail as the next best treatment to *Bacillus thuringiensis* 0.025% showing 7.9 and 8.9 per cent grub mortality at 12 and 18 hours after treatment respectively. *Metarrhizium anisopliae* 0.5% recorded 10.3, 13.1 and 16.6 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. *Beauveria bassiana* 0.5% have resulted in 13.3, 29.0 and 31.3 per cent grub mortality at 6, 12 and 18 hours after treatment respectively. *Beauveria bassiana* 0.5% caused the highest mortality among all the tested microbial pesticides. From Table 4, it was found that the descending order of safety of Microbial pesticides was *Bacillus thuringiensis* 0.5% > *Pseudomonas fluorescens* 0.5% > *Nomuraea rileyi* 0.5% > *Metarrhizium anisopliae* 0.5% > *Lecanicillium lecanii* 0.5% > *Beauveria bassiana* 0.5%. The present findings are in close conformity with the previous studies. Nasreen *et al.* [16] reported that *Bacillus thuringiensis* against *Chrysoperla* showed zero per cent grub mortality even after 48 hours of treatment, which is in accordance with the present investigations. Ventura *et al.* [28] depicted that *Metarrhizium* resulted in 17 per cent grub mortality on *Chrysoperla* sp. Ignoffo *et al.* [9] determined that the fungus *Nomuraea rileyi* is relatively safe to predators and parasitoids including *Chrysoperla*, *Apanteles* sp., etc. Person *et al.* [16] detected there was no effect of the fungus *B. bassiana* on egg viability of *Chrysoperla* but third instar grubs of *Chrysoperla* were affected. According to the recommendation of International Organization for Biological Control [8], all the treated Microbial pesticides fall under Class I i.e. they are harmless.

Table 4: Influence of Microbial pesticides on the third instar grubs of *C. zastrowi sillemi*

Tr. No.	Name of Microbial pesticide	Dose/ Lit	Grub mortality % after			Toxicity class as per IOBC scale
			6 hrs	12hrs	18hrs	
T_1	*Beauveria bassiana* 0.5%	5 ml	13.3 (21.43)f	29.0 (32.61)e	31.3 (34.03)e	I
T_2	*Lecanicillium lecanii* 0.5%	5 ml	3.0 (10.02)b	13.1 (21.22)d	17.0 (24.31)d	I
T_3	*Metarrhizium anisopliae* 0.5%	5 ml	10.3 (18.74)e	13.1 (21.24)d	16.6 (24.04)d	I
T_4	*Nomuraea rileyi* 0.5%	5 g	3.0 (10.47)c	10.6 (18.98)c	13.7 (21.72)c	I
T_5	*Bacillus thuringiensis* 0.025%	0.25 g	0.0 (0.54)a	0.0 (0.54)a	0.0 (0.54)a	I
T_6	*Pseudomonas fluorescens* 0.5%	5 ml	5.7 (13.81)d	7.9 (16.32)b	8.9 (17.36)b	I
T_7	Untreated Control	-	0.0 (0.54)a	0.0 (0.54)a	0.0 (0.54)a	-
	SE d (±) CD @ 5%		0.12 0.26	0.15 0.31	0.22 0.46	

*Figures in the parenthesis are arcsine transformed values, hrs-Hours, Lit-litre.

4. Conclusion

Abamectin 1.9EC is the safest chemical pesticide for the third instar grubs of the predator, *C. zastrowi sillemi* among the treated synthetic pesticides. NSKE 5% is the safest botanical for the third instar grubs of *C. zastrowi sillemi* among the treated botanicals. *Bacillus thuringiensis* is the safest microbial pesticide for the third instar grubs of *C. zastrowi sillemi*. Although *Pseudomonas fluorescens* and *Nomuraea rileyi* has shown less toxicity, indicating that they are safer microbial pesticides, however, are toxic when compared to *Bacillus thuringiensis* and untreated check. The above mentioned pesticides and biopesticides can be effectively utilised in IPM strategies with a view of safety to the natural enemy, *C. zastrowi sillemi*. Acephate 75% SP is the most harmful treatment for the third instar grub of *C. zastrowi sillemi* apart from Lambda cyhalothrin 5 EC.

5. Acknowledgement

The authors are very much grateful to the course teachers, friends and institute management for their kind help, valuable suggestions, profound source of inspiration and constant ambitious encouragement for us.

6. References

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