Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(6): 1820-1823

Received: 04-09-2017
Accepted: 05-10-2017

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Safety of some newer insecticides on egg hatchability and fecundity of *Chrysoperla carnea* under laboratory condition

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Abstract
An experiment was carried out to study the effect of insecticides *viz.* diafenthiuron 50 WP, clothianidin 50 WDG, Acetamiprid 20 SP, Imidacloprid 17.8 SL, Thiamethoxam 25 WG, Flubendiamide 20 WG, Indoxacarb 15.8 EC, Spiromesifen 22.9 SC on the egg hatchability and fecundity rate of *Chrysoperla carnea* in the bio-control laboratory, College of Agriculture, Nagpur during 2016-17. Clothianidin was found most harmful to with 13.00, 30.29, 58.18 per cent egg hatchability during 48, 72 and 96 hrs respectively, and with a fecundity rate of 212.67 eggs/female. Whereas, diafenthiuron was found most safe with 28.33, 47.50, 78.37 per cent eggs hatchablity during 48, 72, 96 hrs respectively and with fecundity rate of 355.33 eggs/female.

Keywords: *Chrysoperla carnea*, insecticides, eggs, adult, toxicity

1. Introduction
Biological control is one of the oldest, economical, sustainable and environment friendly means of managing the pests [2]. The importance of natural enemies (parasites, predators and pathogens) for combating pests in agro-ecosystems is coming into closer focus based on modern investigations [2]. Presence of predators and parasitoids in field crops, orchards and vegetables has been a subject for many studies for reducing the insecticide usage and thereby environmental pollution [3, 23]. Sometimes the role played by the predators it reduces the need of pesticide application. Among several predators identified as biocontrol agents the Green lacewing, *Chrysoperla carnea* (Stephens) play a potential role in combating the insect pest damage.
They may be used virtually in any cropping system. They are often already present in the general area of a crop and may be manipulated to increase their numbers, either augmentation or conservation [13]. Lacewings have a high reproductive rate, relatively a long adult life, and along ovipositon period [13].
Green lacewing is a native North American species [7].*Chrysoperla carnea* is predominant species of green lacewing that is not predacious in the adult stage. Larval stage is predatory stage while in some species adults are also predators [11]. These lacewings larvae are considered generalist predators; the larvae are sometimes called aphid lions and have been reported to eat between 100 to 600 aphids each [21]. The purpose of this work was to evaluate the safety of eight insecticides on egg hatchability and fecundity of *Chrysoperla carnea* under laboratory conditions.

2. Materials and Methods
Laboratory studies were conducted to find out the toxicity of newer group of insecticides on *Chrysoperla sp.* (carnea- group). The rearing of the host insect and predator was done under controlled room temperature and relative humidity conditions ranging between 24 ± 2°c and 60 ± 5%, respectively and commercial formulations of insecticides used for the study were procured from the market.
Mass multiplication of Chrysopa was done in the laboratory to obtain healthy culture of the test predator. The initial culture was obtained from the already established culture of Chrysopa in the Biocontrol laboratory at Entomology Section, College of Agriculture, Nagpur and it was further multiplied on the factitious laboratory host i.e. eggs of rice moth, *Corcera cephalonica.*

2.1 Production of *Corcyra cephalonica* eggs

To obtain the eggs of *Corcyra cephalonica* throughout the experimental period, rearing of rice moth was done in the biocontrol laboratory. The culture was maintained on Sorghum based artificial diet with following ingredients for one tray (15x30 cm).

Crushed Sorghum grain – 2.5 kg

Groundnut kernel (powder) – 100 g (To enrich the diet with protein)

Yeast powder – 5 g

Micronized wettable Sulphur 80% – 5 g (As a prophylactic measures against mites)

Streptomycin sulphate – 0.05% (15 ml/tub)

Sorghum grain free from any infestation and infection were ground to make 3-4 pieces of each grain and heat sterilized in hot air oven at 100 ^{0}C for 30 min. The various ingredients listed above were added and mixed well to obtain a homogenous mixture. The mixture was sprayed with 0.05% Streptomycin sulphate. Plastic basins (15x30 cm) were used as rearing trays for these insects. Each tray was filled with 2.5 kg of diet material. These were inoculated with nucleus egg culture of *Corcyra cephalonica* (0.5 cc /tray) and secured with tight lids having fine mesh at the centre. They were also covered with the muslin cloth as precautionary measures for preventing the entry of any foreign insect in the culture.

The moths started emerging after 40 days of inoculation. They were collected daily by opening the trays under nets with the help of specimen tubes and transferred in the mating chambers were provided with 10% honey solutions as diet. The eggs were collected daily and passed through mesh sieve, and then these sieved eggs were sterilized under UV rays 15 watt for 45 min, keeping at 20 cm distance apart between lamp and eggs. These eggs were then used as food for rearing of predator (Chrysopa).

2.2 Treatment of eggs

Eggs (24 hours old) of Chrysopa were obtained on an artificial egg laying substrate (black paper) in their natural form. Paper sheet was cut into strips so that each strip must have 20 eggs and then dipped in the dilutions or water for 3 seconds. The treated eggs strips were placed on paper tissues for 2 hours to absorb extra dilution and air-dry. The eggs were then individually placed in Petri dishes. Irradiated eggs of *Corcyra cephalonica* sprinkled in each Petri plate, as food for hatching larvae of Chrysopa. The number of eggs hatched was noted after 48, 72, and 96 hrs of treatment. Eggs were observed for 2 more days to note any delayed hatching.

To study the per cent hatchability, counted numbers (twenty in each Petridish) of one-day-old eggs were kept in numbered petri dishes. All the Petridishes were kept under constant observation for recording the hatching of the eggs. The per cent hatchability was calculated based on total number of eggs kept for study and number of eggs hatched was noted after 48hrs, 72 hrs& 96 hrs after application of treatments.

2.3 Fecundity of Adults

Observations of effect of insecticides on adults of Chrysopa were recorded by keeping the adults exposed for 4 days in treated vials. Fecundity of adult females were noted after every 24hrs till the end. Female adults that are live were transferred to common chamber for each treatment separately for fecundity observations (The total number of egg laid by the female during the entire oviposition period). The fecundity was determined by working out the average number of eggs laid by a female. Females of *C. Carnea* started oviposition on the black paper sheet and everyday black paper sheet and nylon net with eggs were taken out after transferring the Chrysopa adults to another mating chamber. Then total numbers of eggs laid were counted daily at 24-hour intervals and the process was continued till the death of female adults.

2.4 Statistical analysis

The data generated in respect of per cent leaf infestation due to leaf miner on citrus was transformed into square root value when value in between 0-30% as per Gomez and Gomez, (1984) and then subjected to statistical analysis to test the level of significance of treatment.

3. Results and Discussion

3.1 Effect of various insecticides on per cent eggs hatchability of Chrysopa

In the present study, the data collected on per cent hatchability of eggs of Chrysopa obtained under different treatments at different HAT (hours after treatments) was recorded and presented in Table 1.

From Table-1 it was found that the ascending order of toxicity of insecticide towards egg hatchability was diafenthiuron <imdacloprid <flubendiamide <acetamiprid <thiamethoxam <spiromesifen<indoxacarb<clothianidin during all the three hours under observation.

Earlier workers in agreement with our present study reported that, the egg phase is the most tolerant to the action of pesticide [9]. [16] Recorded 81.63 per cent egg hatchability in diafenthiuron 50 WP@ 200 g a.i./ha. [19] Reported that flubendiamide was safe to the natural enemies [10]. [15] Observed that imidacloprid @ 0.28 ml/l and thiamethoxam @2 g/l caused 84.62%, 82.61% egg hatchability of *C. carnea* respectively, under laboratory conditions. [17]. Recorded an egg mortality of 20.00% (i.e. 80.00% hatchability) after treatment with indoxacarb under laboratory conditions [19] Reported a high toxicity (mortality > 80%) in clothianidin against *C. rufilabris* under laboratory condition. Our results regarding Steward are in concurrence with **[4]**. Whose findings showed that it has slight residual toxicity against the C. carnea larvae? However, we differ from the observation made by [8] regarding Steward which showed moderate toxicity against larval development. Our findings regarding Spinosad are in Conformity with [4] who reported no residual toxicity of these insecticides on C. carnea larvae. However, [5] reported the same results on C. Externa regarding Emamectin benzoate and Spinosad. The findings of the [18]. are in conformity with our findings regarding Emamectin benzoate. Our results regarding the residual effect of Emamectin benzoate are also similar to that of [14].

3.2 Effect of various insecticides on the fecundity of Chrysopa (eggs/female)

The data on average number of eggs laid per female under different treatments are presented in Table 2. All the tested insecticides had affected the fecundity of Chrysopa significantly at 5 per cent level of significance over untreated control.

Table 1: Effect of various insecticides on per cent egg hatchability of Chrysopa.

Tr. No	Treatments	Dose/l.	Eggs hatchability (%)		
			48 hrs	72 hrs	96 hrs
T_1	Clothianidin 50 WDG	0.04g	13.00 (21.13)	30.29 (33.40)	58.18 (49.72)
T_2	Acetamiprid 20 SP	0.2 g	21.20 (27.42)	40.66 (39.64)	70.90 (57.35)
T_3	Imidacloprid 17.8 SL	0.20 ml	25.00 (30.00)	44.30 (41.73)	75.06 (60.07)
T_4	Thiamethoxam 25 WG	0.2 g	20.37 (26.85)	39.43 (38.88)	69.87 (56.73)
T_5	Flubendiamide 20 WG	0.5ml	23.33 (23.81)	43.09 (41.03)	73.00 (58.69)
T_6	Difenthiuron 50 WP	0.20 g	28.33 (32.14)	47.50 (43.57)	78.37 (62.31)
T_7	Indoxacarb 15.8 EC	1.26 ml	16.67 (24.12)	34.80 (36.15)	60.00 (50.77)
T_8	Spiromesifen 22.9 SC	1.31 ml	18.33 (25.35)	36.25 (37.25)	65.20 (53.85)
T_9	Control (Water spray)		33.78 (35.55)	53.21 (46.83)	96.00 (78.46)
	'F' test		Sig.	Sig.	Sig.
	SE (m) ±		1.67	2.39	2.06
	CD at 5%		4.86	6.96	6.00

(Values in the parenthesis are arc sine transformed)

Table 2: Effect of various insecticides on fecundity of Chrysopa.

Tr. No	Treatments	Dose/lit.	Fecundity (eggs/ female)			
			RI	RII	RIII	Mean
T_1	Clothianidin 50 WDG	0.04 g	213.00	212.00	213.00	212.67
T_2	Acetamiprid 20 SP	0.2 g	241.00	246.00	260.00	249.00
T_3	Imidacloprid 17.8 SL	0.20 ml	267.00	267.00	260.00	264.67
T_4	Thiamethoxam 25 WG	0.2 g	252.00	252.00	251.00	245.00
T_5	Flubendiamide 20 WG	0.5 ml	320.00	319.00	320.00	326.33
T_6	Difenthiuron 50 WP	0.20 g	356.00	355.00	354.00	355.33
T_7	Indoxacarb 15.8 EC	1.26 ml	242.00	242.00	247.00	243.33
T_8	Spiromesifen 22.9 SC	1.31 ml	310.00	309.00	309.00	309.33
T_9	Control	Water	420.00	413.00	410.00	414.33
	'F' test					Sig.
	SE (m) ±					2.36
	CD at 5%					6.86

(Values in the parenthesis are arc sine transformed)

Maximum reduction in fecundity was observed due to clothianidin 50 WDG @ 0.04 g/l (212.67 eggs per female) followed by indoxacarb15.8 EC @ 1.26 ml/l >aceatamiprid 20 SP @ 0.2 g/l >thiamethoxam25 WG @ 0.2 g/l>imidacloprid 17.8 SL @ 0.20 ml/l >spiromesifen 22.9 SC@ 1.31 ml/l >flubendiamide 20 WG @ 0.5 ml/l >diafenthiuron 50 WP @ 1.2 g/l and control with on an average of 243.33, 249.00, 245.00, 264.67, 309.33, 326.33, 355.33 and 414.33 eggs per female, respectively. Earlier workers in accordance with our results reported that mean fecundity of adult females varied significantly and affected by the insecticide treatment of Imidacloprid when compared with the untreated check [22, 8]. Reported that the highest and the lowest rates of fecundity were 540 – 49 and 206 – 42 in control and indoxacarb, respectively [6] reported that indoxacarb caused a simultaneous reduction in fertility of adult of lady bird beetle, *Harmonia axyridis* (Pallas) in laboratory [12]. Reported the fecundity *of M. boninensis* was not affected due to various treatments of insecticides like cypermethrin, diamethoate and thiamethoxam, as there were no significant differences in the eggs laid by the females emerged from various insecticidal treatments.

4. Conclusion

Difenthiuron and flubendiamide was found safe towards egg laying of adult female and eggs hatchability of *C. carnea* and can be successfully implemented during IPM programme Where as Clothianidin was found most harmful to both parameters.

6. Acknowledgement

Author is very much grateful to Dr. P. S. Neharkar Associate Professor of Agri. Entomology, Nagpur, Professor of Agri. Entomology, Shri. R. W. Gawande, Dr. H. R. Sawai, Assistant Professor of Agri. Entomology, Shri. V. N. Nandanwar Assistant Professor of Agri. Entomology and Shri. N. V. Lavhe Assistant Professor of Agri. Entomology, College of Agriculture, Nagpur. For their kind help, valuable Suggestions, profound source of inspiration and constant ambitious encouragement.

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