MORTALITY RATES OF FIVE COMMERCIAL INSECTICIDES ON *CHRYSOPERLA CARNEA* (STEPHENS) (*CHRYSOPIDAE: NEUROPTERA*)

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The green lacewing *Chrysoperla carnea* (Stephens) (Chrysopidae; Neuroptera) is a generalist biological agent commonly used to control insect pests. Toxic effects of five commercial insecticides *viz*., carbosulfan, leufenuron, cyfluthrin, methomyl and fenpropathrin were evaluated on green lacewing through laboratory bioassays. At 27 ± 2°C, 65 ±10% RH and 14-h photophase. Insect mortality was determined following insecticide exposure by eggs immersion, larval leaf dip bioassay and by direct adult topical application. Larval mortality was observed for the instar treated and for following instars and pupae. Following insecticide exposure, *C. carnea* mortality was greatest for life stages treated directly and decreased during subsequent life stages. Methomyl, cyfluthrin and fenpropathrin caused about 95% mortality when 1st instar was exposed to chemicals. Methomyl and fenpropathrin remained effective and caused 92% mortality when 2nd instar was exposed to chemicals. All chemicals caused about 60-70% mortality, when applied to 3rd instar. Mortality of adults was highest 57% for fenpropathrin. All materials had greatest effect on longevity and fecundity of adults.
Key words: *Chrysoperla carnea*, insecticides, neuroptera, mortality, egg, larvae, adult, fecundity, longevity.

INTRODUCTION

Biological control agents can cause substantial decrease in pest population numbers (Hassell, 1978). Green lacewing is a generalist and widely distributed predator of many soft bodies insect pests (Geetha and Swamiappan, 1998; New, 1975). Pest management revolution illustrated in the history of biological control in temperate glass houses (Hussey, 1985) can further confirm their importance. Being an entomophagous predator in many crops, with wide use in biological control in glasshouse crops and its ability to be easily reared in large numbers, the common green lacewing, *Chrysoperla carnea* (Stephens) is taken as a representative of Chrysopidae (Greve, 1984; Morrison, 1985) to be used in biocontrol program.

However, pesticides are also used in modern agriculture, as biological control alone most often does not solve all pests and disease problems. Combining biological control with pesticide use was the cornerstone on which the concept of integrated control was founded (Perkins and Garcia 1999), insecticides such as organophosphates, carbamates, and synthetic pyrethroids are generally highly toxic to biological control agents, due to their broad spectrum of activity (Croft, 1990). Other insecticides that do not appear to kill natural enemies, may also have sub-lethal effects, such as altered behavior, reduced reproduction, and reduced longevity, on natural enemies (Jacobs *et al*., 1984; Elzen, 1989; Croft, 1990; Longley and Jepson, 1996). Croft and Brown (1975) reviewed that indiscriminate use of pesticides not only results in the development of insecticide resistance but also eliminates the natural enemies of insect pests. Therefore, it is important to examine the possible disruptive effects of candidate insecticides on beneficial insects, and to determine the insecticides compatible with key biological control agents (Stapel *et al*., 2000).

The most crucial requirement for pesticides is that they must be compatible with biological control. Therefore, only those pesticides should be used that are most selective and which have no adverse effects on beneficial organisms (Hassan, 1989; Cross and Dickler, 1994). In IPM. The compatibility of an insecticide with biological control agents is often examined by tests screening for mortality of natural enemies, but sub-lethal effects on beneficial insects are largely overlooked (Elzen, 1989).

The increase in knowledge is the basis for reducing the undesirable effects of pesticides applications, which among others, is an important principle of integrated production (Cross and Dickler, 1994). Many workers have revealed the importance of *C. carnea* in biological control and the inevitability of chemicals to be applied in synchronization as a prerequisite of IPM. The purpose of work reported here was to evaluate effects of the pesticide on eggs and larvae of *C. carnea* that could help to find compatible predator life stage with selected insecticides and vice versa.

MATERIALS AND METHODS

The experiments were conducted under laboratory conditions at 27 ± 2°C and 60 ± 5% RH and 14-h photoperiod, in Insect Rearing laboratory of University College of Agriculture, Bahauddin Zakariya University Multan. Table 1. outlines the five commercial insecticides tested at this work and their distributors. All solutions were prepared in distilled water by serial dilution for

egg and larval treatment. The insecticide solution for adult application was prepared from technical grade insecticide diluted with acetone. Control population was treated with pure acetone solution.

Table 1. Insecticides selected for the treatment of egg and larvae of *Chrysoperla carnea.*

Insecticide	Active ingredient	Company	Recommended field dose/acre	Concentration (ppm)
Advantage 20EC	Carbosulfan	FMC S. A. International	1000ml	2000
Match 050EC	Leufenuron	Syngenta	800ml	4000
Bulldock 25EC	β Cyfluthrin	Bayer Crop Sciences	330ml	825
Lannate 40SP	Methomyl	Jaaffer Brothers	250gms	1000
Danitol 30EC	Fenpropathrin	Nitchiman	250ml	750

Chrysoperla carnea

C. carnea adults were collected from maize crop and kept in AM-Tech *C. carnea* adult rearing cage (Ashfaq *et al*., 2004). Food, water and oviposition substrate was provided according to the recommendations of Ashfaq *et al*, 2004. *C. carnea* was reared to get F3 generation eggs to get sufficient number of uniform age eggs and larvae for the experiments. Processed eggs of S*itotroga cerealella* (Olivier) were obtained from Insect Rearing laboratory of University College of Agriculture, Bahauddin Zakariya University Multan and used as a food for the larvae.

Treatment of eggs

Eggs (24 hours old) of *C. carnea* were obtained on an artificial egg laying substrate (Morocco paper) in their natural form. Paper sheet was cut into strips so that each strip must have 250 eggs and were 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 (5 cm diameter, 0.5cm deep). Measured quantity (12 mgs) of processed eggs of *S. cerealella* was sprinkled in each Petri plate, for hatching larvae. The number of eggs hatched was noted after 48 hrs of treatment. Eggs were observed for 2 more days to note any delayed hatching.

Treatment of larvae

Leaf dip bioassay was used to treat the larvae, as it more closely approximates the field exposure. Fresh, medium size and unsprayed leaves of cotton (*Gossypium hirsutum*) variety NIAB-Krishma were collected from 60 days old plants. Leaves were cut into circular discs of 1.25cm diameter size with cork borer. Leaf discs were dipped for 5 seconds into insecticide solutions and in water for control. All treated and control discs were allowed to air dry and placed in Petri plates (1.25 cm dia. and 0.75 cm deep). Water moistened filter paper was placed underneath of each leaf disc. The larvae of 1st, 2nd and 3rd instars were placed in Petri plates separately. Measured quantity (24 mgs) of processed eggs of *S. cerealella* was sprinkled in each Petri plate as a food for larvae. Mortality data were recorded at the end of each exposed and subsequent instars and pupae, Number of pupae not changed into adults within seven days was considered dead. We considered larvae dead if they no longer moved or twitched when being touched 2-3 times with a brush.

Treatment of adults

A drop of 0.5µl of insecticide solution was applied on the thorax with an Arnold micro-applicator (Burkard, UK). The treated adults per replication were shifted separately in the plastic jars (11 cm diameter and 21cm deep) and the adults were provided with food (containing yeast, honey and water 1:1:0.5), streaked on paper strips having length 22cm and width 2.5cm. Fecundity and longevity of survived adults were noted after every 24 h.

Data collection and statistical analysis

Mortality data was collected at the end of each life stage treated directly and subsequent life stages. A complete randomized design was used. Mortality of adults was observed 48 hours after treatment. There were six treatments including one control, with five replications, each having 10 specimens. Greater number of eggs and larvae were taken than needed, ensuring an adequate number of specimens for bioassay. The test was not considered valid, if there was more than 12% mortality in control (Hassan, 1989). Percent mortality was corrected through Abbot's formula:

$$\overline{p}_{\text{corr}} = \frac{\overline{p}_{\text{exp}} - \overline{p}_{\text{cont}}}{1 - \overline{p}_{\text{cont}}}$$

where $\overline{p}_{\text{cont}}$ is the mean control response, $\overline{p}_{\text{exp}}$ is the mean experimental treatment response, and $\overline{p}_{\text{corr}}$ is the mean experimental treatment response corrected for control response (Rosenheim and Hoy, 1989). The mean responses were calculated by averaging the observed proportion responding across replicates:

$$\bar{p} = \frac{1}{I} \times \sum_{i=1}^{I} r_i/n_i$$

where r_i is the number of dead flies out of n_i total flies in replicate i = 1, 2,..., I. The mortality values were corrected using a mean control mortality of water. Data for actual values of fecundity and longevity was analyzed by single factor one way ANOVA to detect differences in overall toxicity of the material. Means were distinguished using the least significant difference test (LSD) after a significant F-test at P = 0.05 (SAS Institute 1996).

RESULTS

Egg mortality

Corrected mortality caused by different insecticides to the eggs of *C. carnea* is presented in Table 2. All materials induced low ovicidal effects. Out of five insecticides only two caused more than 20% mortality. Methomyl and carbosulfan remained the most active and presented 27.90 % and 21.90% egg mortality after 48 hours of treatment respectively.

Table 2. Percent corrected egg mortality of *Chrysoperla carnea* caused by different insecticides 48 h after treatment.

Treatment	Egg Mortality
β Cyfluthrin	11.91
Leufenoron	6.91
Methomyl	27.90
Carbosulfan	21.90
Fenpropathrin	12.90
Control	0.00

Larval mortality

When 1st instar larvae were treated with insecticides (Table 3); high mortality was recorded during first instar which was reduced sharply during 2nd and 3rd instar and during pupal stage. Out of five insecticides three remained most effective. Methomyl, cyfluthrin and fenpropethrin caused round about 90 % mortality during first instar. More than 65 % mortality was recorded in leufenoran and carbosulfan treated larvae during first instar. Over all mortality was approximately 95% in methomyl and fenpropathrin and about 75% in all other insecticides.

When 2nd instar larvae were treated with insecticides (Table 4); percent mortality was about 50-60 % in all treatments during 2nd instar, which was reduced up to half during 3rd instar and greatly reduced during pupal stage. Over all mortality was high in all treatments. It was maximum (92.5 %) in fenpropathrin and methomyl and approximately more than 75% in all other treatments.

When 3rd instar larvae treated with insecticides (Table 5)**;** approximately 70 % mortality was noted in cyfluthrin and fenpropathrin, round about 62 % in leufenoran and carbosulfan, 53 % mortality was caused by methomyl during 3rd instar, which was reduced from 2-5 % during pupal stage. Final mortality was approximately 70 % inall treatments except methoyl with 53 %.

Adult mortality

When adults were treated with insecticides (Table 6); fenpropathrin remained most active and caused 57 % adult. Remaining four insecticides caused approximately 40 % mortality 24 hours after treatment.

Adult longevity

Insecticide exposure badly affected the longevity of adults. It was significantly different (F = 552.36, P = 0.05) among treatments. Shortest longevity (22 days) was recorded in cyfluthrin treated adults, compared with control where fecundity was 65 days.

Adult fecundity

Female fecundity was sharply reduced in all treatments. It was significantly different among treatments (F= 15559.16, P = 0.05). Minimum fecundity was recorded in cyfluthrin (419 eggs/female) treated adults. It was reduced up to half for adults treated with chemicals, compared with 1117 eggs/female for adults treated with water.

Table 3. Corrected percent mortality of larvae and pupae of *Chrysoperla carnea* treated as 1st instar larvae with leaf residual exposure of five insecticides.

Treatment	% Mortality				
	1st Instar	2nd Instar	3rd Instar	Pupae	Total
Cyfluthrin	79.48	5.00	5.00	0	89.48
Leufenoron	68.84	5.00	2.5	7.5	68.84
Methomyl	87.05	5.00	0	0	87.05
Carbosulfan	66.53	2.5	2.5	0	66.53
Fenpropathrin	94.74	2.5	0.00	2.5	94.74
Control	0.00	0.00	0.00	0.00	0.00

Table 4. Corrected percent mortality of larvae and pupae of *Chrysoperla carnea* treated as 2nd instar larvae with leaf residual exposure of five insecticides.

Treatment	% Mortality		
	2nd Instar	Pupae	Total
Cyfluthrin	55.0	7.5	80.0
Leufenoron	45.0	7.5	72.5
Methomyl	62.5	2.5	92.5
Carbosulfan	47.5	2.5	82.5
Fenpropathrin	65.0	2.5	92.5
Control	0.00	0.00	0.00

Table 5. Corrected percent mortality of larvae and pupae of *Chrysoperla carnea* treated as 3rd instar larvae with leaf residual exposure of five insecticides.

Treatment	% Mortality		
	3rd Instar	Pupae	Total
Cyfluthrin	71.79	5.12	76.91
Leufenoron	61.53	2.56	64.09
Methomyl	53.84	2.56	56.40
Carbosulfan	64.10	5.12	69.22
Fenpropathrin	69.23	2.56	71.79
Control	0.00	0.00	0.00

(1994) that insect growth regulators, Cascade (flufenoxuron), Dimlin (diflubenzuron), Nomolt (teflubenzuron) and Insegar (fenoxycarb) were moderately/very harmful against *Chrysoperla carnea* under field conditions. Among neurotoxic insecticides, Baythroid (cyfluthrin) was highly toxic, while Evisect (thiocyclame) and Imidan (phosmet) were only slightly affected the larvae. Leufenoron showed harmless effects on egg, but caused high mortality at larval stage. Similar results were recoded by Bueno and Freitas (2000); they stated that leufenoron presented no adverse effects on egg survival. However, it caused high mortality to neonate larvae from treated eggs as well as treated 1st and 2nd instar larvae. Pyrethroid was found less toxic to egg and larvae that described by El-Maghraby *et al*. (1994) was true in our studies for eggs but not for larvae, as (pyrethroids) Cyfluthrin and fenpropethrin were found highly toxic to all larval instar of *C. carnea*. Elzen *et al*. (1998) noted that cyfluthrin and spinosad were significantly less toxic to the larvae than other treatments to *C. carnea,* while cyfluthrin was proved highly toxic to the larvae in the present studies with same bioassay technique.

First and 2nd instar larvae were badly affected as compared to third instar that might be due the formation of pupae that reduced their contact with the

Table 6. Corrected percent mortality of *Chrysoperla carnea* adults after exposure of five insecticides, longevity (days) and fecundity (eggs/female) of treated adults.

Treatment	% Adult Mortality	Longevity (days) Mean ± S D	Fecundity (eggs/female) Mean ± S D
Cyfluthrin	36.01	22.6 ± 2.80 e	419.6 ± 2.23 d
Leufenoron	38.15	37.2 ± 2.74 c	804 ± 3.63 b
Methomyl	43.48	48 ± 1.60 b	793.3 ± 2.90 b
Carbosulfan	48.8	29.4 ± 1.52 d	503.2 ± 2.97 d
Fenpropathrin	57.36	37.7 ± 2.31 c	680.2 ± 2.31 c
Control	0	65.2 ± 2.74 a	1117.5 ± 8.98 a

Means sharing the same letter are not statisticaly different (F= 552.36, P=0.05) for longevity, (F= 15559.16, P=0.05) for fecundity.

DISCUSSION

Our results indicated that application of insecticides not only affects the exposed life stage but induced lethal effects to the subsequent life stages. All selected chemicals presented low to moderate toxicity to eggs and adults while caused high mortality to larvae of each instar. Cyfluthrin and fenpropathrin proved consistently toxic to all larval instars except eggs. Cyfluthrin caused maximum reduction in longevity and fecundity when adults were exposed to insecticides.

Toxicity of leufenoron (IGR) on larvae noted in our studies was in conformation with the findings of Vogt

poisoned surface. Our results that carbamates (methomyl and carbosulfan) proved toxic to the larvae of *C. carnea* were in favour with the findings of Guven *et al*. (2003). They found that Ekalix (quinalphos), Afidrex (dimethoate) Korvin (carbaryl), Deltanet (furathiocarb) and Lannate (methomyl), Flambo (profenophos + cypermethrin), Zipak (bifenthrin + amitraz) showed high toxicity resulting in mortality rate of 100 %. Salama, *et al*. (1990) described that Lannate (methomyl) was proved toxic to *C. carnea* larvae in soyabean field conditions. It means that methomyl remained toxic even in field conditions.

Fenpropathrin caused high adult mortality 65% as compared to other insecticides. Ulahôa *et al.* (2002) stated that fenpropathrin caused 100% mortality to *C. externa* adults (closely related species of *C. carnea*), that was not true in case of *C. carnea* adults in our studies. While Bozsik (1995) found fenpropathrin slightly harmful for common green lacewing adults when applied at field recommended doses that was in favor of our results.

Insect growth regulator leufenoran was noted harmless to the adults. Similar result were stated by Medina *et al*. (2003), they applied three novel insecticides including a growth regulator and found it harmless to green lacewing adults. All materials reduced the fecundity and longevity of adults indicated that chemicals induced sub lethal effects to the adults that effected the normal functioning of adults.

Although data obtained from laboratory toxicity studies have been sufficient to decide upon the use of insecticides in IPM (in cases where mortality was low in laboratory experiments) (Barrett *et al*. 1994), semi-field and field studies are still needed. More research on the effects of insecticides on lacewings, and other predators and parasitoids under different conditions is also needed to elucidate how to use chemicals in IPM programs.

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