

Effects of Bio-Pesticides on Biology of *Chrysoperla carnea* F. (Neuroptera: Chrysopidae)

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Abstract: The experiment was conducted on effects of bio-pesticides on biology of *Chrysoperla carnea* F. under laboratory conditions. The neem and datura leaf extracts were used as bio-pesticides and their effect was compared with confidor at 26±2°C, 65±5% R. H and photoperiod (16L: 8D) in the Department of Entomology, Sindh Agriculture University, Tandojam, Pakistan during 2014. The results shows that the incubation periods of eggs of *C. carnea* feeding on *Aphis gossypii* treated with neem, datura and confidor was 2.2, 2.5 and 3.6 days respectively. The result indicated that the total larval developmental period was 17.03, 13.3 and 15.09, respectively. The pupal period of *C. carnea* was 8.82 on neem, 10.9 on datura and 12.33 days on confidor. The result further revealed that the pre oviposition period of *C. carnea* was 6.35 on neem, 5.5 on datura and 3.6 on confidor. The oviposition period was 34.42 on neem, 30.6 on datura and 26.4 on confidor. The post oviposition period was significantly different was 8.5 days on neem 6.9 on datura and 4.7 on confidor. The maximum fecundity per female of *C. carnea* was 448.38 days on neem, 435.67 on datura and 413.67 on confidor. Similarly, maximum egg hatching percentage of *C. carnea* was recorded on neem followed by datura and confidor. However, the maximum egg mortality (37.65%) was recorded on confidor. However, minimum mortality of 1st, 2nd and 3rd instar larvae was recorded due to neem leaf extracts followed by datura and confidor. The pupal mortality was seen more on neem followed by datura and confidor. The highest adult mortality was obtained on neem followed by datura and confidor insecticide.

Keyword: *Chrysoperla carnea* F., Bio-pesticides, Biology, Synthetic pesticides.

INTRODUCTION

The Green lacewing, *Chrysoperla carnea* (Stephens) belongs to order Neuroptera, family Chrysopidae and genus Chrysoperla. This order consists of a group of insects with rather soft bodies, biting mouthparts and two pairs of very similar membranous wings which are usually held roof-like along the abdomen at rest. It has long been assumed to be a single morphologically identical species with a Holarctic distribution [1]. The adult *C. carnea* are greenish in colour. It is found in most of the environments throughout the world. They are pale green, about 12-20 mm long with long antennae and bright, golden or copper-coloured eyes. They have large, transparent, pale green wings and a delicate body. These adults are active fliers, particularly during the evening and night and have a characteristic, fluttering flight [2, 3]. Adults have a strong flight urge it may fly for 3 to 4 hours. The larvae of *C. carnea* are brownish in colour. Mature third instar larvae spin round, parchment like silken cocoons usually in hidden places in plants and pupate inside cocoons. Larvae grow from <1 mm to 6-8 mm. Emergence of adults occur in 8-10 days. There may be two to several generations per year [4-7].

The eggs of green lacewing are oval in shape and secured under the leaves in field condition and under the surface of cage in laboratory condition by long slender stalks. Each female of green lacewing lays several hundred eggs at the rate of two to five per day, which normally laid in darkness. Oval shaped eggs are protectively laid singly at the end/ tips of long silken stalks, resembling miniature cattails growing from the plant foliage, these are pale green, turning grey in 2-3 days. After 6-7 days eggs hatch out, the larvae which are very active, have three instars, and are grey or brownish, alligator-like with well-developed legs and large pincers with which they suck the body fluids of the prey. Their agricultural importance lies in their carnivorous habits. Adults feed only on pollen, nectar and aphid honeydew. At larvae stage some are terrestrial, feeding on jassids, psyllids, Aphis, coccids, mites etc., and others are aquatic. It is rare in the tropics to find a large colony of Aphis without some neuropterans larvae feeding on them [8, 9]. One larva may devour as many as five hundred Aphides in its life and there is no doubt that they play an important part in the natural control of many small homopterous pests [10, 11]. *Chrysoperla* spp., especially *C. carnea* and *Chrysoperla rufilabris*, are sold commercially by numerous producers and suppliers [12-14] to control insect pests. Green lacewing is an example of one of these species that is not predacious in the adult stage; larval stage is predatory stage while in some species

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adults are also predators [15-17]. Larvae of *C. carnea* are a voracious predator of exposed eggs, small larvae of beetle and lepidopterous pests. It also feed on slow moving, soft-bodied arthropods such as aphid, jassids, thrips, whitefly, scales, mealy bugs and mites [18].

The more recent evidence suggests that it is not a single species but instead a complex of several to many biological species characterized by different male courtship songs [19, 20]. In Japan, the indigenous green lacewing is widely distributed and has been categorized as *C. carnea* [21]. However, *C. carnea* was revised to *Chrysoperla nipponensis* (Okamoto) by Brooks [22] based on external morphological differences such as the color of the gradate cross-veins, which are black in *C. nipponensis* and green in *C. carnea*. Its courtship song also differs from the other *carnea* group species [23, 24]. In 1996, the green lacewing designated as *C. carnea* was imported from Germany on a test basis. It was registered as a biological predator in 2001 and is now on the market in Japan. The two species can now meet in the same habitat. Serious concerns over the non-target impact of introduced exotic natural enemies on native ecosystems have been raised by a number of prominent ecologists and conservation biologists [25-27]. Mochizuki and Mitsunaga [28] showed that there were negligible non target impacts from interspecific predation between the introduced and the indigenous green lacewings. Several cryptic biological species co-occur in Germany, including the true *C. carnea*, all of which are morphologically difficult to identify [29].

Biological control agents such as predators and parasitoids are usually more sensitive to pesticides than the target pests. The adverse impact of insecticides on predators can be decreased /controlled through timing of insecticide application, choice of insecticide and dosage [30]. Selective insecticides can minimize the likelihood of development of resistance in pest [31]. Ferreira et al., [32] reported that emamectin benzoate was classified as harmless, spinosad as slightly harmful, and chlorpyrifos as harmful to first instar larvae of *Chrysoperla externa* (Hagen). (Balasurbramani and Swami appan [33] observed the persistent toxicity of chlorpyrifos and found that it was toxic for eight days *Chrysoperla carnea* in the laboratory. Medina et al., [34] tested three novel insecticides viz. spinosad, tebufenozide and azadiractin against eggs and pupae of *Chrysoperla carnea* and found them safe, only azadiractin caused a slight reduction in the number of pupae and adults. Bueno and Freitas [35] studied side effects of two insecticides,

abamectin and lufenuron on the eggs and larvae of *Chrysoperla externa*. Lufenuron presented no adverse effect on egg survival. However, it induced high mortality in neonate larvae from treated eggs. Lufenuron treated 1st and 2nd instar larvae could not molt. In 3rd instar, high pupal mortality occurred. William et al., [36] concluded that for conservation of predator populations, spinosad represents one of the most judicious insecticides available. According to Medina et al., [37] pyriproxifen and tebufenozide proved to be harmless to adult survival, whereas spinosad reduced the number of adults of *Chrysoperla carnea* after 72 hours of treatment. According to Godoy et al., [38] deltamethrin was toxic to the adult *Chrysoperla externa* while lufenuron reduced the survival rate of egg when sprayed on females. Toxicity of new chemistry insecticides on *Chrysoperla carnea* has received much attention of farmers and researchers as a biological pest control agent due to its polyphagous and voracious nature, vast geographical distribution [39] and tolerance to some pesticides [31]. *C. carnea* reported to give 100 percent lepidopteron pest control in fields, orchards and green houses. In spite of all these benefits, *Chrysoperla carnea* has almost been eliminated from fields due to frequent use of some non-selective insecticides [40].

Keeping in above view about the importance of *C. carnea*, therefore, the study on effects of bio-pesticide on biology of *C. carnea* would be carried out under laboratory conditions and the result will be suggested to new researchers and mass rearing laboratories for farmers.

MATERIALS AND METHODS

The biology of *Chrysoperla carnea* F. was studied under laboratory conditions, in the Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University Tandojam, Pakistan during 2014. The biological parameters of *C. carnea* F. were determined on aphids treated with neem, daturaleaf extracts were used as bio-pesticides and their effect was compared with confidor. The experiment had three replications, each replication consists over 50 larvae of *C. carnea*. These larvae were confined in the glass Petri dish at 26±2°C temperature, 65±5% relative humidity and 16L: 8D photoperiod.

Preparation of Neem and Datura Extracts

Fresh leaves of neem and datura were brought in the laboratory. The leaves were grind in a pestle and mortar. Grinded leaves were squeezed in muslin cloth

to obtain 100% extract. The pure extract was then kept in to a sterilized bottle. Finally the required concentrations were prepared by adding distilled water with the ratio of Neem (30ml/70 ml water) and Datura (30 ml/ 70 ml).

Biology of *C. carnea*

For this purpose, ten fresh leaves of cotton were collected from field and brought into the laboratory than cleaned with fresh water than soaked under 100 watt bulb. The leaves were sprayed with prepared concentration of Neem and Datura (30 ml/70 ml water), whereas, Confidor 70 SL (0.25/100ml water) for 10 seconds. After treatment, these leaves were dried under shade at laboratory conditions. The leaves were put in glass Petri dishes and 50 aphids were released on them into twenty petridishes separately, and 2 larvae of *C. carnea* were released in each Petridish. The larvae were fed on aphids, till pupation and determine the biological parameters such as; larval period (days), pupal period (days), adult (days), total food consumption, survival and oviposition, respectively. The biological parameters larval period (days), total food consumption and survival were recorded daily.

When the larvae became pupae in each treatment, then they were transferred into separate Petri dishes. When adults emerged they were transferred to rectangular cages made of 6cm thick, transparent plastic sheet. Then cages were maintained for each treatment. Artificial foods containing yeast + sugar + honey + distilled water in ratio of 8:4: 2:1 were provided in food bowls (0.5 cm diameter) engraves in the upper side of 2 mm thick and 22 cm long plastic rods running width wise at the opposite ends inside the cage. A black granulated paper underside the removable top of the cage was provided as an oviposition substrate. The eggs were collected from the sheet with razor at early

morning. The eggs were kept in plastic jars for hatching and further propagations. The period of time from egg laying to hatching was considered incubation period; from hatching till spinning of cocoon was designated the larval period and from cocoon formation and coming out from pupal case as pupal period. The time after emergence of adults and start of oviposition was considered as pre ovipositional period, the period of egg laying was considered oviposition and post-oviposition period of female was recorded as period between the days of female ceased egg laying to the day of death. The period of survival of each male and female was recorded regularly in order to record longevity (days). Biology of *C. carnea* were subjected to statistical analysis, using analysis of variance to assess the significance of the treatments, while LSD was employed to compare the treatment means, following the statistical methods suggested by USA student package software, Statistix-8.1.

RESULTS

Egg Incubation Period

The results (Table 1) showed that the incubation period of eggs of *C. carnea* feeding on aphids was not significantly different from each other ($F= 8.09$; $DF= 2, 2$; $P < 0.0393$). It was 2.2 ± 2.4 on neem, 2.5 ± 1.9 on datura and 3.6 ± 2 days on confidor, respectively.

The result indicated that first instar of larval period of *C. carnea* feeding on aphids was not significantly difference ($F= 1.06$; $DF= 2, 2$; $P < 0.4284$). Duration of first larval instar was 3.11 ± 1.2 on neem, 2.77 ± 0.33 on datura and 2.66 ± 0.57 days on confidor, respectively. The result of second instar of larval period of *C. carnea* feeding on aphids was significantly made a small different ($F= 0.80$; $DF= 2, 2$; $P < 0.5119$). Duration of second larval instar was 4.2 ± 0.35 on neem, 3.7 ± 0.14 on datura and 3.33 ± 0.47 on confidor. The result of 3rd

Table 1: Influence of Neem, Datura and Confidor on Biological Parameters of *C. carnea* on Aphids under Laboratory Conditions

Parameters	Neem	Datura	Confidor
Egg Incubation Period	2.2 ± 2.4	2.5 ± 1.9 ab	3.6 ± 2 a
1st instar	3.11 ± 1.2	2.77 ± 0.33 a	2.66 ± 0.57 a
2nd instar	4.2 ± 0.35	3.7 ± 0.14 a	3.33 ± 0.47 a
3rd instar	7.52 ± 0.38	6.83 ± 0.24 a	5.5 ± 0.41 a
Total Larval Period	17.03 ± 3.12	13.3 ± 2.13 a	15.09 ± 2.05 a
Pupal Period	8.82 ± 0.33	10.9 ± 0.36 a	12.33 ± 0.29 a

Mean ± SE followed by same letter not significantly ($P < 0.05$) different from each other by LSD method.

Table 2: Influence of Neem, Datura and Confidor on Reproductive Parameters of *C. carnea* Fed on Aphids under Laboratory Conditions

Parameters	Neem	Datura	Confidor
Pre Oviposition Period	6.35 ± 0.62	5.5±0.88 ab	3.6±0.40 a
Oviposition Period	34.42 ± 0.75	30.6±0.90 a	26.4±0.60 a
Post Oviposition	8.5 ± 0.13	6.9±0.05 ab	4.7±0.20 a
Total	49.27 ± 11.5	43.0±14.10	34.7±13.75
Fecundity	448.38 ± 12.5	435.67±14.19 a	413.67±13.05 a
Fertility %	92.61 ± 2.88	86.9±2.68 a	72.1±3.31 a

Mean ± SE followed by same letters are not significantly ($P < 0.05$) different from each other by LSD method.

instar of larval period of *C. carnea* feeding on aphids was significantly made a small different ($F = 0.65$; $DF = 2, 2$; $P < 0.5686$). Duration of third larval instar was 7.52 ± 0.38 on neem, 6.83 ± 0.24 on datura and 5.5 ± 0.41 days on confidor, respectively. The complete larval developmental period was 17.03 ± 3.12 on neem, 13.3 ± 2.13 on datura and 15.09 ± 2.05 on confidor, respectively.

The pupal period of *C. carnea* was statistically significant and different on various hosts ($F = 2.33$; $DF = 2, 2$; $P < 0.2136$). However, cocoon period of *C. carnea* was 8.82 ± 0.33 on neem, 10.9 ± 0.36 on datura and 12.33 ± 0.29 days on confidor, respectively.

Reproductive Attributes

Feeding of *C. carnea* larvae on aphids significantly affected its pre-oviposition period, oviposition period, post oviposition period, fecundity and fertility of eggs.

Pre Oviposition Period

The results of pre oviposition period of *C. carnea* feeding on different hosts was significantly made a small different ($F = 10.97$; $DF = 2, 2$; $P < 0.0238$). Duration of pre oviposition period was 6.35 ± 0.62 on

neem, 5.5 ± 0.88 on datura and 3.6 ± 0.40 on confidor, respectively.

Oviposition Period

The results of oviposition period of *C. carnea* feeding on different hosts was significantly made a small different ($F = 1.03$; $DF = 2, 2$; $P < 0.4345$). Duration of oviposition period was 34.42 ± 0.75 on neem, 30.6 ± 0.90 on datura and 26.4 ± 0.60 on confidor, respectively.

Post Oviposition Period

The results of post oviposition period of *C. carnea* feeding on different hosts was significantly made a small different ($F = 12.78$; $DF = 2, 2$; $P < 0.0183$). Duration of post oviposition period was 8.5 ± 0.13 on neem 6.9 ± 0.05 on datura and 4.7 ± 0.20 on confidor, respectively.

Fecundity

Feeding on fresh aphids, the larvae of *C. carnea*, significantly not affected its fecundity ($F = 3.07$; $DF = 2, 2$; $P < 0.1559$). The maximum mean fecundity per female of *C. carnea* was 448.38 ± 12.5 on neem, 435.67 ± 14.19 on datura and 413.67 ± 13.05 on confidor.

Table 3: Influence of Neem, Datura and Confidor on Mortality % of *C. carnea* on Different Hosts under Laboratory Conditions

Parameters	Mortality %		
	Neem	Datura	Confidor
Eggs	4.82 ± 4.2	7.69 ± 3.10	37.65 ± 11.79
1st instar	4.52 ± 3.81	5.57 ± 2.32	35.6 ± 14.17
2nd instar	2.1 ± 1.55	3.43 ± 1.65	29.6 ± 8.87
3rd instar	9.72 ± 1.05	15.42 ± 1.17	39.6 ± 10.79
Pupal	8.42 ± 2.1	11.11 ± 1.66	23.08 ± 12.2
Adult	12.35 ± 1.88	17.79 ± 2.21	18.46 ± 4.79

Mean ± SE followed by same letter not significantly ($P < 0.05$) different from each other by LSD method.

Table 4: Influence of Neem, Datura and Confidor on Survival % of *C. carnea* on Different Hosts under Laboratory Conditions

Parameters	Survival %		
	Neem	Datura	Confidor
Eggs	95.18 ± 2.1	92.31±1.32 a	62.35±0.96 a
1st instar	95.48 ±1.87	94.43±2.47 a	64.4±2.01 a
2nd instar	97.8 ±1.66	96.57±1.63 a	70.43±2.01 a
3rd instar	90.28 ± 1.82	84.58±1.09 a	60.42±1.03 a
Pupal Survival %	91.58 ± 1.21	88.89±1.01 a	76.92±1 a
Adult Survival %	87.65 ± 0.25	82.21 ± 0.34 a	81.54±0.13 a

Mean ± SE followed by same letter not significantly ($P < 0.05$) different from each other by LSD method.

Fertility %

Similarly, percentage of fertility of eggs of *C. carnea* significantly ($F = 2.56$; $DF = 2, 2$; $P < 0.1920$), maximum fertility of eggs of *C. carnea* was recorded when fed on aphids 92.61±2.88% on neem followed by 86.9±2.68% on datura and 72.1±3.31% on confidor.

Mortality of *Chrysoperla carnea*

Mortality of Eggs

Analysis of data indicated a significant effect of hosts on the mortality of *C. carnea*. The minimum mortality of eggs recorded on neem (4.82±4.2%) followed by datura (7.69±3.10%). However, the maximum mortality of eggs (37.65±11.79%) was recorded on confidor.

Mortality of Larvae

The mortality of 1st instar larvae recorded on neem was 4.52±3.81%, datura 5.57±2.32% and confidor 35.6±14.17%. Mortality of 2nd instar larvae was recorded on neem 2.1±1.55%, datura 3.43±1.65% and 29.6±8.87% on confidor. Mortality of 3rd instar larvae recorded on neem 9.72±1.05%, datura 15.42±1.17% and 39.6±10.79% on confidor.

Mortality of Pupae and Adult

Mortality of pupae recorded on neem 8.42±2.1%, datura 11.11±1.66% and confidor 23.08±12.2%. Mortality of adult recorded on neem 12.35±1.88%, datura 17.79±2.21% and confidor 18.46±4.79%.

Survival Percentage of *C. carnea*

Analysis of data indicated a significant effect of hosts on the survival of *C. carnea* eggs ($F = 5.32$; $DF = 2, 2$; $P > 0.0747$), 1st instar larvae ($F = 0.36$; $DF = 2, 2$; $P > 0.7195$), 2nd instar larvae ($F = 0.11$; $DF = 2, 2$; $P > 0.8952$), 3rd instar larvae ($F = 1.43$; $DF = 2, 2$; P

> 0.3403), pupae ($F = 0.94$; $DF = 2, 2$; $P > 0.4636$) and adult ($F = 1.74$; $DF = 2, 2$; $P > 0.2863$).

Survival of Eggs

The survival of eggs recorded on neem 95.18±2.1%, datura 92.31±1.32% and 62.35±0.96% on confidor.

Survival of Larvae

The survival of 1st instar larvae recorded on neem 95.48±1.87%, datura 94.43±2.47% and 64.4±2.01% on confidor. Survival of 2nd instar larvae was recorded on neem 97.8±1.66%, datura 96.57±1.63% and 70.43±2.01% on confidor. Survival of 3rd instar larvae recorded on neem 90.28±1.82%, datura 84.58±1.09% and 60.42±1.03% on confidor.

Survival of Pupae and Adult

Survival of pupae recorded on neem 91.58±1.21%, datura 88.89±1.01% and 76.92±1.0% survival on confidor. Survival of adult recorded on neem 87.65±0.25%, datura 82.21±0.34% and 81.54±0.13% on confidor.

DISCUSSION

The experiment was conducted on effects of bio-pesticides on the biology of *Chrysoperla carnea* F. was studied under laboratory conditions at Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam, Pakistan during 2014. It was observed from the experiments, that the life of *C. carnea* is larger on confidor and smaller on neem and datura.

In the present study larvae were fed on aphids, the average duration of the first, second and third, total larval instar period were: 17.03 ± 3.12 days on neem, 13.3±2.13 days on datura and 15.09±2.05 on confidor,

respectively. Venzon & Carvalho [41] observed 13.9± 0, 07 days, for *C. cubana* this value stage was 12.7 days and 15 days. Barbosa *et al.* [42] observed the larvae were fed eggs of *S. cerealella* the average duration of the first, second and third instars were: 5.1 ± 0.03; 4.3 ± 0.05 and 4.5 ± 0.05 days, respectively. Moraes [43], studying *C. cubana* larvae fed *A. kuehniella* eggs plus *Toxoptera* spp. measured an average first instar duration of 4.7 days, while Silva *et al.* [44] verified an average duration of 4.0 days. Results for the second instar are similar to those found by Santa-Cecilia *et al.* [45] for *C. cubana* larvae fed *A. kuehniella*, and to those obtained by Núñez [46] for *C. cincta* larvae fed *S. cerealella*. For the third instar, the average duration in *C. everes* was different from the duration found.

In present study neem and datura was found less toxic than confidor against first, second, and third instar larvae of *C. carnea* as well as pupae and adults are also affected by confidor. The mortality of first instar was 35.06±14.17%, second instar 29.6±8.87%, third instar 39.6±10.79%, pupal mortality was 23.08±12.2% and adult mortality was 18.46±4.79% on Confidor. Neem and datura were safe bio-pesticides with less than 8% larval mortality. It showed that abamectin was non-toxic to adults of *C. carnea* [47]. Spinosad, abamectin and flufenoxuron were safer insecticides causing less than 5% mortality after 48 hrs of treatment. Impidachloprid was moderately toxic causing 21.25% mortality. Mortality of second instar *C. Carnea* larvae followed by chlorpyrifos, thiodiacarb, indoxacarb and prophenophos with mortality of 98.75, 98.70, 92.94 and 72.45%, respectively. Abamectin had shown selectivity for *C. carnea* [48-50]. Giolo *et al.*, [47] tested abamectin, deltemethrin, methoxyfenozide, phosmet and trichlorfon and compared with dimethoate as a standard against predator *C. carnea* under laboratory. The cumulative mortality upto adult emergence was 31.4, 0.0, 2.9, 22.9, 11.4 and 94.3% for abamectin, deltmethrin, methoxyfenozide, phosmet, trichlorfon and dimethoate, respectively.

RECOMMENDATIONS

The neem and datura was found least toxic for the activity of *C. carnea*. Therefore, it is suggested that farmers should spray with these extracts and release the *C. carnea* predator for the suppression of aphids in the field crops.

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