Toxicological impact of new chemistry insecticides on *Chrysoperla carnea* under laboratory conditions

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Abstract

The present study regarding the comparative toxicity of different insecticides against life stages of *Chrysoperla carnea* was conducted in the eco-toxicological laboratory of Entomological Research Institute (ERI), Ayub Agricultural Research Institute (AARI), Faisalabad. Cotton is a major crop in Pakistan; however, it is damaged by various insect pests. *Chrysoperla carnea* is a natural enemy of numerous insect pests, including whitefly, aphid, jassid, and it helps to control many crop-destroying pests. Many insecticides used to control insect pests also reduce beneficial insect populations. Cotton leaves were collected and a 2cm leaf disks was dipped in insecticides solution. Larval mortality was assessed by using leaf dip bioassay while adult mortality was assessed using a glass vial bioassay. The data from all treatments were collected and analyzed using statistical software (statistix 8.1). Flubendiamide, Chlorantraniliprole, and Spinetoram had mortality rates of 28, 36, and 48%, respectively, while Indoxacarb and Lufenuron had 62 and 72%, and Emamectin benzoate had a high mortality rate of 88%. In adult's treatment after three hours (3h) Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole and Flubendiamide showed 20, 16, 20,10,8 and 2 % mortality while after forty-eight hours (48h) showed 78, 70, 64, 40, 30 and 22 % mortality respectively. Flubendiamide and chlorantraniliprole are recommended as safer insecticides than others, although Indoxacarb, Lufenuron, and Spinetoram are considered less to moderately hazardous, and Emamectin benzoate has highly harmful effects.

***Keywords*:** *Chrysoperla carnea*, Leaf dip bioassay, Glass vial bioassay, Insecticides, Mortality.

1. Introduction

Chrysoperla carnea, also known as the green lacewing, is a predator species in the order Neuroptera. (Nadeem et al., 2014; Wu et al., 2022). Their predatory nature makes them important for agriculture. The larvae are crucial predators, particularly for aphid, jassid, psyllid, aphid, coccid, mite, and crape-myrtle bark scale populations (Alghamdi et al., 2018; Rehman et al., 2020; Sultan et al., 2021; Wu et al., 2022). In its lifetime, one larva may consume up to 500 aphids, and there is no doubt that they play a significant role in the natural management of numerous minor homopterous pests (Hussain et al., 2012; Sultan et al., 2021). Adult green lacewings are required for population survival in the field because they feed on nectar, pollen, and sugar excretions of insects (such as aphids) (Borah et al., 2012; Sarwar, 2014). Additionally, it can feed on several soft-bodied insects. It has a lot of commercial potential and might be used in combination with other insect pest management strategies to combat several agricultural pests (Jokar and Zarabi, 2012; Menon et al., 2015; Alghamdi et al., 2018).

The pest management revolution heavily emphasizes the employment of biological control agents to combat pests, such as green lacewing. *C. carnea* is an entomophagous predator with a large population that grows quickly (Wu et al., 2022). In the agriculture sector, numerous chemical pesticides are used to get rid of harmful insect pests (Zia Ullah et al., 2017). However, a lot of pesticides are harmful to these helpful insects because they change their life cycles, lower their ability to reproduce, and decrease their lifespans (Zia Ullah et al., 2017; Choyon et al., 2022; Divekar, 2023). Nowadays chemical controls are extensively applied to get rid of insect pests to improve the agriculture yield (Hussain et al., 2012). On the other hand, due to their physiological similarities, insecticides typically cause the death of both pest and their natural predators (Hussain et al., 2012; Zia Ullah et al., 2017). In this context, insecticides that interact well with biological control agents are valuable tools in an IPM program, hence study of their influence on natural enemies is critical (Ayubi et al., 2013; Zia Ullah et al., 2017).

The most prevalent forms of toxicological testing on natural enemies, however, are acute toxicity and sublethal effects (Shoeb et al., 2017). Acute toxicity is measured after a brief exposure to a chemical molecule (for example, a few hours to a few days), with death as the endpoint. The median lethal dosage (LD_{50}) or lethal concentration (LC_{50}) is determined in acute toxicological research (Stark and Banks, 2003; Moustafa, 2016). Additionally, the sublethal effects of

insecticides are an important factor in IPM programs because they affect an entomophagous capacity to control its host or prey (Moustafa, 2016; Shoeb et al., 2017).

Farmers and researchers have paid close attention to *C. carnea* as a biological pest management agent because of its polyphagous and voracious character, wide geographic spread, and tolerance to some insecticides (Jolivet et al., 2012; Hussain et al., 2012). According to reports, *C. carnea* have a 100% ability to eradicate lepidopteran pests in fields, orchards, and greenhouses (Rincon, 1999). Despite all these advantages, *C. carnea* has almost completely disappeared from fields because of widespread non-selective insecticide use (Hussain et al., 2012; Zia Ullah et al., 2017). The present study was conducted to evaluate the toxic potential of selective synthetic insecticides against *Chrysoperla carnea* under laboratory conditions.

2. Materials and Methods

The present study was conducted on the comparative toxicity of insecticides against *Chrysoperla carnea* at the Eco-toxicological laboratory, Entomological Research Institute (ERI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan during 2021-2022.

Insecticides were tested against *C. carnea* larvae in the laboratory at 27 ± 1 ^{o}C and 65 ± 5% RH. The experiment was laid out in completely randomized design (CRD) with five replications (including control). Using a micropipette, each pesticide was measured and then dissolved in 500 ml of water in a beaker to create the solution. It was then shake to ensure thorough mixing.

2.1. Rearing of *C. carnea*

Newly hatch larvae of *C. carnea* were reared in *Chrysoperla* rearing lab. Larvae were fed on artificial diet until they reach to 2nd instar larval stage. Artificial diet consists of yeast, eggs, honey and sugar in the ratio of 3:4:2:1, respectively. For rearing of larvae to 3rd instar, these were fed on eggs of *Sitotroga cerealella* in gelatin capsules separately to avoid cannibalism.

2.2. Leaf dip bioassay

Fresh cotton leaves were collected from field, and then was washed with tap water. Leaf discs of 2 cm diameter were prepared, and then dipped in insecticides solution for ten seconds and air dried. Untreated control was dipped in tap water only. Ten leaf discs were considered as repeat and each Petri dish with leaf disc having one larva. The observations on mortality percentage of 1st, 2nd and

3rd instar larvae of *C. carnea* was recorded from each treatment at 3, 6, 12, 24 and 48 hours of post application.

Figure 1: A. Leaves dip in insecticides **B.** Petri dishes contain insecticides dip leaves and larvae

2.3. Glass vial bioassay

Glass vials were wash out and air dried then insecticides was spray in vials. Untreated control was spray by simple water. Adult *Chrysoperla carnea* were transferred into glass vials. Data from each treatment and replication was recorded after specific time intervals.

2.4. Insecticides

Table No 1. Insecticides with their common name, trade name, and recommended field dose/acre

Insecticides/Common name	Trade name	Recommended field dose/acre
Spinetoram	Tracer 120SC	80ml/acre
Lufenuron	Match 150EC	200 ml/acre
Flubendiamide	Belt 480SC	50 ml/acre
Emamectin Benzoate	Proclaim 1.9EC	200 ml/acre
Chlorantraniliprole	Coragen 200SC	50 ml/acre
Indoxacarb	Encore 150SC	175 ml/acre

2.5. Data analysis

The data was subjected to analysis of variance (ANOVA) followed by means separation using Fisher's least significant different (LSD) test at 5%. All statistical analysis was carried out using computer software STATISTIX. The percentage mortality regarding immature and mature stages was calculated by using Abbotts formula.

$$\text{Percentage Mortality} = \frac{\text{Number of died insect}}{\text{Total number of insects}} \times 100$$

3. Results

3.1 Effect of insecticides on *Chrysoperla carnea*

Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole and Flubendiamide was significantly different from one another throughout the duration of the post-treatment intervals concerning the mortality of *C. carnea* larvae.

3.2. Percentage mortality of 1st instar of *Chrysoperla carnea*

The application of flubendiamide was significantly less harmful. After 3 hours, the 1st instar mortality rate was 28%, 26%, 22%, 16%, 10%, and 10%, respectively, compared to 6% in the control group. After 6 hours, mortality rates were 44%, 38%, 24%, 18%, 18%, 16% respectively, compared to 16% in the control group. After 12 hours, the mortality rates were 58, 50, 46, 36, 28, and 24%, respectively, compared to 24% in the control group. After 24 hours, the mortality rates were 76, 66, 58, 42, 34, and 26%, respectively, compared to 26% in the control group. After 48 hours, the mortality rates were 92, 84, 66, 50, 34%, and 30% respectively, compared to 24% in the control group.

3.3. Percentage mortality of 2nd instar of *Chrysoperla carnea*

Over 2nd instar after 3 hours percentage mortality was 20, 20, 12, 10, 6 and 4% respectively while in control 2% mortality was observed. After 6 hours percentage mortality was 38, 34,30,22,16 and 14% respectively while in control mortality was 6%. After 12 hours percentage mortality was 50, 54, 40, 32, 22 and 14% respectively while in control 12 % mortality was observed. After 24 hours percentage mortality was 66, 62, 54, 38, 30 and 24% respectively while in control 16% mortality was observed. After 48 hours percentage mortality was 88, 72, 64, 48, 36 and 28% respectively while in control 18% mortality was observed as shown in Table No.1.

3.4. Percentage mortality of 3rd instar of *Chrysoperla carnea*

Over 3rd instar after 3 hours percentage mortality was 28, 18, 12, 12, 6 and 4% respectively while in control 2% mortality was observed. After 6 hours percentage mortality was 44, 38, 32, 26, 10 and 12% respectively while in control 6 % mortality was observed. After 12 hours percentage mortality was 60, 60, 50, 36, 20 and 20% respectively while in control 14 % mortality was observed. After 24 hours percentage mortality was 68, 72, 62, 50, 30 and 28% respectively while in control 20 % mortality was observed. After 48 hours percentage mortality was 88, 84, 72, 52, 32 and 32% respectively while in control 24 % mortality was observed.

3.5. Percentage mortality of adult of *Chrysoperla carnea*

Over Adult after 3 hours percentage mortality was 20, 16, 20, 10, 8 and 2% respectively while in control 2% mortality was observed. After 6 hours percentage mortality was 36, 30, 28, 20, 14 and 8% respectively while in control 4% mortality was observed. After 12 hours percentage mortality was 50, 44, 40, 28, 20 and 12% respectively while in control 8 % mortality was observed. After 24 hours percentage mortality was 64, 46, 54, 34, 22 and 18% respectively while in control 14 % mortality was observed. After 48 hours % mortality was 78, 70, 64, 40, 30 and 22% respectively while in control 16 % mortality was observed.

Treatments	Post treatment mortality of different instars (%)																			
	1st Instar					2nd Instar					3rd instar					Adult				
	3h	6h	12h	24h	48h	3h	6h	12h	24h	48h	3h	6h	12h	24h	48h	3h	6h	12h	24h	48h
Emamectin benzoate	28 ± 2.17^{a}	44 ± 3.21^{b}	58 ± 3.55^{b}	76 ± 5.23^{c}	92 ± 6.65^{abc}	20 ± 2.34^{abc}	38 ± 1.34^{b}	50 ± 1.54^{abc}	66 ± 5.34^{abc}	88 ±7.43^{d}	28 ± 2.12^{a}	44 ± 4.54^{abc}	60 ± 3.55^{e}	68 ± 2.44^{a}	88 ± 6.65^{abcd}	20 ± 2.22^{ab}	36 ± 4.56^{ab}	50 ± 1.34^{abcd}	64 ± 2.44^{ab}	78 ± 4.24^{abc}
Indoxacarb	26 ± 3.54^{a}	38 ± 2.34^{b}	50 ± 3.44^{b}	66 ± 2.34^{c}	84 ± 6.34^{abc}	20 ± 2.34^{a}	34 ± 4.56^{ab}	54 ± 1.54^{bc}	62 ± 4.24^{abc}	72 ±3.23^{d}	18 ± 3.44^{b}	38 ± 4.56^{d}	60 ± 2.55^{a}	72 ± 5.65^{abc}	84 ± 4.55^{abc}	16 ± 1.34^{e}	30 ± 4.24^{abc}	44 ± 1.54^{abc}	46 ± 2.24^{abc}	70 ± 4.34^{abc}
Lufenuron	22± 2.55^{a}	24 ± 2.21^{b}	46 ± 4.56^{ab}	58 ± 2.44^{a}	66 ± 4.34^{abc}	12 ± 1.34^{a}	30± 2.34^{c}	40 ± 1.54^{abc}	54 ± 3.55^{b}	64 ± 2.44^{abcd}	12 ± 1.25^{ab}	32 ± 2.45^{abc}	50 ± 3.34^{abc}	62 ±1.32^{a}	72 ± 1.25^{cd}	20 ± 1.54^{abc}	28 ± 1.25^{cd}	40 ± 1.44^{abcd}	54 ± 1.17^{a}	64 ± 4.56^{ab}
Spinetoram	16± 1.17^{a}	18 ± 2.11^{c}	36 ± 2.45^{ab}	42 ± 2.12^{ab}	50 ± 4.56^{ab}	10 ± 0.34^{ab}	22 ± 2.45^{d}	32 ± 1.34^{abc}	38 ± 2.35^{d}	48 ± 1.25^{cd}	12 ± 1.22^{ab}	26 ± 1.46^{abc}	36 ± 1.34^{cd}	50 ±2.44^{ab}	52 ± 3.55^{bc}	10 ± 0.42^{ab}	20 ± 3.44^{b}	28 ± 3.45^{d}	34 ± 1.44^{abcd}	40 ± 1.25^{abcd}
Chlorantraniliprole	10± 1.25^{b}	18 ± 2.23^{b}	28 ±1.44^{ab}	34 ± 1.34^{c}	34 ±1.44^{ab}	6 ±0.32^{a}	16 ± 1.22^{abc}	22 ± 1.44^{abcd}	30± 3.34^{b}	36 ± 1.25^{abcd}	6 ±0.32^{a}	10 ± 1.34^{a}	20 ± 1.44^{cd}	30 ± 4.56^{abc}	32 ± 2.35^{d}	8 ± 0.34^{b}	14 ±1.32^{a}	20 ± 1.84b^{c}	22 ± 2.23^{b}	30 ± 1.24^{cd}
Flubendiamide	10 ± 2.54^{b}	16 ± 4.21^{a}	24 ± 1.22^{c}	26 ± 1.76^{ab}	30± 2.22^{a}	4 ±0.21^{b}	14 ± 1.54^{abc}	14 ± 1.25^{b}	24 ± 1.24^{c}	28 ± 0.24^{abc}	4±0.21^{b}	12 ± 0.23^{b}	20 ± 1.44^{abcd}	28± 2.35^{e}	32 ± 1.34^{c}	2 ± 0.54^{b}	8 ± 0.35^{abc}	12 ± 0.54^{abc}	18 ± 1.24^{c}	22 ± 2.54^{abc}
Control	6 ± 0.22^{a}	16 ± 3.89^{b}	24 ± 1.34^{c}	26 ± 1.34^{bc}	24 ± 1.24^{c}	2 ± 0.34^{c}	6 ± 0.54^{abc}	12 ± 3.89^{b}	16 ± 1.14ab^{c}	18 ± 2.11^{abc}	2 ± 0.34b^{c}	6 ± 0.22^{c}	14 ± 2.34^{abc}	20 ± 1.22^{d}	24 ± 2.45^{abc}	2 ± 0.25^{b}	4 ±0.32^{a}	8 ± 0.25^{e}	14 ± 0.63^{b}	16 ± 0.63^{e}

Discussion

The experiment was conducted to evaluate the toxicity of different insecticides against different life stages of *Chrysoperla carnea*. In the different larval instar and adult, some of the insecticides show less toxicity but some proved more susceptible. We used six insecticides to check their toxicity on *Chrysoperla carnea* include Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole and Flubendiamide. However, all the new chemistry insecticides have different mode of action, which are mostly used for their target host, and their effect on the host was greater than the natural enemies. Even though other research focused on how different insecticides affected *Chrysoperla carnea*, this study also looks at how *Chrysoperla carnea* is exposed to and affected by insecticides.

The effect of different insecticides on different life stages of *Chrysoperla carnea* showed significant difference. On 1st instar and adult flubendiamide showed minimum effect and safe insecticides as compared to the other. Deltamethrin and Chlorpyrifos show toxic effect to all stages. Among all insecticides, maximum effect shown by Spinosad on all stages (Hussain et al. 2012). *Chrysoperla carnea* has an adult stage that lasts about 34 days. After 5 days, the first larval instar hatches. The green lacewing has three larval instars, and it takes each of them about 12 days to turn into a pupa. The pupae stage lasts about 10 to 15 days, after which the new adults are ready to start a new cycle. Adults don't eat other insects; they only eat nectar, honeydew, and pollen. Larvae, on the other hand, eat soft-bodied insects (Bansod & Sarode 2000).

According to Zia Ullah et al. (2017) Chlorpyrifos cause 100% mortality on 1st instar, 96.67% mortality on 2nd instar, 93.33% mortality on 3rd instar and 86.67% mortality on adults of *Chrysoperla carnea* but spinosad and flubendiamide are relatively safer than the all others. Imidacloprid cause 10 to 40% mortality and safer for adult, and flubendiamide and spinetoram causing 6-36% and 3-36% mortality respectively and safer for 3rd larval instars safer insecticides are environment friendly and used in order to protect green lacewing.

In all treatments of 1st instar Emamectin benzoate showed 28,44,58,76 and 92% mortality. Indoxacarb showed 26, 38, 50, 66 and 84% mortality. Lufenuron showed 22, 24, 46, 58 and 66% mortality. Spinetoram showed 16, 18, 36, 42 and 50% mortality. Chlorantraniliprole showed 10, 18, 28, 34 and 34% mortality. Flubendiamide showed 10, 16, 24, 26 and 30% mortality after 3, 6, 12, 24 and 48 hours respectively.

On 2nd instar Emamectin benzoate showed 20, 38, 50, 66 and 88% mortality. Indoxacarb showed 20, 34, 54, 62 and 72% mortality. Lufenuron showed 12, 30, 40, 54 and 64% mortality while spinetoram showed 10, 22, 32, 38 and 48% mortality. Chlorantraniliprole showed 6, 16, 22, 30 and 36% mortality. Flubendiamide showed 4, 14, 14, 24 and 28% mortality after 3, 6, 12, 24 and 48 hours respectively. In all treatment of 3rd instar Emamectin benzoate showed 28,44,60,68 and 88% mortality. Indoxacarb showed 18, 38, 60, 72 and 84% mortality. Lufenuron showed 12, 32, 50, 62 and 72% mortality. Spinetoram showed 12, 26, 36, 50 and 52% mortality. Chlorantraniliprole showed 6, 10, 20, 30 and 32% mortality. Flubendiamide showed 4, 12, 20, 28 and 32% after 3, 6, 12, 24 and 48 hours respectively.

Flubendiamide, chlorantraniliprole, and spinetoram were the least toxic of all the insecticides for immature insects. Indoxacarb and Lufenuron were moderately toxic, and Emamectin benzoate was very toxic. In adult's treatments after three hours (3h) Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole and Flubendiamide showed 20, 16, 20,10,8 and 2 % mortality while after forty-eight hours (48h) show 78, 70, 64, 40, 30 and 22 % mortality respectively. All insecticides showed less toxicity to adult after 3 hours while after 48 hours adults mortality was high.

Conclusion

The present study regarding comparative toxicity of different insecticides against different life stages of *Chrysoperla carnea* concluded that in immature and mature stages treatment Flubendiamide and Chlorantraniliprole recommended are as safer insecticides as compared to other while Indoxacarb, Lufenuron and Spinetoram considered as less or moderately toxic.

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