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Toxicity of direct and indirect application of chemical and bioinsecticides on *Chrysoperla carnea* under laboratory conditions

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ABSTRACT

Toxic effects of five compounds, *Bacillus thuringiensis kurstaki*, Abamectin, Fipronil, Pyriproxyfen and *Beauveria bassiana* were evaluated on the green lacewing, *Chrysoperla carnea* under laboratory conditions by direct and indirect techniques. Indirect treatment was dependent on applying each of the tested compounds on eggs of *Sitotroga carealella*, then feeding *C. carnea* larvae on the treated eggs. *Chrysoperla carnea*, larval mortalities were 6.76, 26.67, 15.83, 33.33 and 26.66 % 7 days after feeding on eggs treated with *B. thuringiensis*, Abamectin, Fipronil, Pyriproxyfen and *B. bassiana*, respectively, compared to 6.67 % among the control larvae . Larval durations increased from 7.67 days in control to, 11.0, 9.33, 7.93, 9.03, 9.67 days for the treated larvae, respectively. Pupation rates were 40, 46.67, 0.0, 0.0 and 40 % also, adults' emergence rates were 33.34, 28.57, 0.0, 0.0 and 66.67 %, respectively opposed to 93.34 and 92.86 % for the control. Direct treatments were made by direct spraying on *C. carnea* eggs with the recommended concentration of each of the five compounds. The highest hatchability percentage was 100 % in the control while the lowest was 12 % in Pyriproxyfen treatment. Also the longest incubation period was 5.33 days in *B. thuringiensis* treatment, while the lowest was 2.8 days in the control. Larval mortality was highest (82.5 %) in Fipronil, while the shortest 6.67 % occurred with *B. thuringiensis* treated eggs compared with 6.67 % in the control. Pupal mortality was highest (100 %) in Abamectin and Fipronil treatments, while it was the lowest (15 %) in *B. thuringiensis* treatment compared with 11.76 % in the control.

Key words: *Chrysoperla carnea*, insecticides, mortality, eggs, larvae, adults, fecundity, longevity.

Introduction

Intensive application of chemical pesticides for pests' control resulted many injuries such as; air, water and soil pollution, pests' resistance to pesticides and disturbance of the natural balance between living organisms in the ecosystem. For these reasons, the search alternative and environmentally "friendly" methods for pests' control. An alternative is the use of new biological control methods that are effective against target organisms. Biological control methods include releasing predators which feed on a large numbers of insects. Species which belong to Chrysopidae include important predaceous species of which the adults feed on plant nectar and pollen grains, whereas larvae show preference for feeding on certain soft-bodied prey species such as aphids, whiteflies, thrips and eggs and neonate larvae of lepidopterans and acari (Rimoldi *et al.,* 2008). The green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) also known as and aphid lion, is a cosmopolitan polyphagous and efficient predator commonly found in a wide range of agricultural habitat. It has a wide range of prey with enhanced searching capacity and voracious feeding habits. It constitutes a prominent group of predators due to their amenability to be mass produced and high potentiality to be used in varied ecosystems. Its larvae are voracious and polyphagous predators, feeding on leafhoppers, psyllids, aphids, coccids and mites, of which aphids constitute the most preferred prey, as one *C. carnea* larva may devour as many as 500 aphids in its lifetime, it plays an important part in natural control of sucking pest. Effectiveness of *C. carnea* as a biological control agent has been demonstrated in field crops, orchards and green houses (Hagley and Miles, 1987).

The lacewings considered a key generalist biological control agent, which is used primarily through augmentative periodic releases of larvae for the control of various aphid species in greenhouses and outdoor crops (Medina *et al.,* 2002 and Turquet *et al.,* 2009).

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Conservation of natural enemies in integrated pest management (IPM) programmes is enhanced through habitat manipulation or use of selective insecticides (Hopper, 2003). By definition, selective insecticides are primarily harmful to target pests but relatively harmless to natural enemies, Yu SJ (1988) and their use may increase the effectiveness of biological control. Therefore, the use of selective insecticides enhances conservation of natural enemies and may reduce the likelihood of pest resurgence, Johnson and Tabashnik (1999) and the number of insecticidal applications, Hutchison *et al.,* (2004).

Among predators, there are 114 species of arthropods distributed in nine orders and 31 families (Gerling *et al.,* 2001). However, the identification of predators in field conditions is more difficult because many are generalists, and identification methods are sometimes inefficient (Gerling *et al.,* 2001 and Naranjo, 2001). Laboratory studies are useful for predicting the response of natural enemies to insecticidal applications (Naranjo, 2001). The study presented in this article deals with trials for determining the direct and indirect effects of some insecticidal compounds on the eggs of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) that could help to find a safety compounds to be used in the presence of this beneficial natural enemy.

Materials and Methods

1. Compounds used:

1.1. *Bacillus thuringiensis kurstaki*

Common name:	*Bacillus thuringiensis* kurstaki
Trade name:	Protecoto 9.4 % WP
Recommended concentration	300 gram / 100 L water

1.2. Abamectin

Common name:	Abamectin
Trade name:	Abamax 1.8 % EC
Recommended concentration	40 ml/100 L water

1.3. Fipronil

Common name:	Fipronil
Trade name:	Coach 20 % SC
Empirical formula:	$C12H4Cl2F6N4OS$
Recommended concentration	25 cm/100 L water

1.4. Insect growth regulator, Pyriproxyfen

Common name:	Pyriproxyfen
Trade name:	Anti fly 10 % EC
Empirical formula:	$C20H19NO3$
Recommended concentration	75 cm/ 100 L water

1.5. *Beauveria bassiana*

Common name:	*Beauveria bassiana*
Trade name:	Biosect
Recommended dose	200 gram/100 L water

2-Rearing of *Chrysoperla carnea*:

Chrysoperla carnea adults were placed in a rectangular cage, made of 0.6 cm thickness, transparent plastic sheet. Artificial foods containing yeast + honey +distilled water in ratio of 8: 2:1 were provided in 0.5 cm food bowls engraved 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 of the cage was provided as an oviposition substrate. The eggs were collected from the sheath with every morning during the period of study. The freshly eggs were kept in plastic jars until hatching and further propagations. Experiments were conducted on the developmental duration, survival rate and predatory potential of immature stages of *C. carnea* feeding on *Sitotroga carealella* aphid under controlled condition of 27 ±1 °C, 65 ±5 % relative humidity.

3-Indirect treatment of *Chrysoperla carnea*:

Eggs of *Sitotroga carealella* were spread and stuck on paper sheet. The paper sheet was cut into strips so that each strip must have 100 eggs and were dipped for 5 seconds in the recommended concentration each of the five compounds; *Bacillus thuringiensis kurstaki*, Abamectin, Fipronil, Pyriproxyfen and *Beauveria bassiana* compared with water as control. The eggs were then offered to the larvae of *Chrysoperla carnea* for feeding.

4-Direct treatment of *Chrysoperla carnea* egg:

Chrysoperla carnea freshly eggs were sprayed with the recommended concentration each of the mentioned five compounds compared with spraying with the same volume of distilled water. The number of eggs hatched was noted through 2 days after treatment. Eggs were observed for 2 more days to note any delayed hatching. Also, the mortality, larval duration, pupal duration, pupation rates and adults' emergency rates were recorded.

The compounds are classified according to the recommendation of the international organization for biological control, West Palaearctic Regional Section (IOBC/WPRS) working group (Hassan, 1989) as under:

Harmless (toxicity class1) = less than 50 mortality, slightly harmful (toxicity class2) = 50-79% mortality, moderately harmful (toxicity class3) = 80-89% mortality, harmful (toxicity class4) = more than 90% mortality.

Results and Discussion

Toxicity of different five compounds; *Bacillus thuringiensis kurstaki*, Abamectin, Fipronil, Pyriproxyfen and *Beuveria bassiana* to the predator *Chrysoperla carnea* were investigated by using direct and indirect treatments with the recommended concentration after different intervals (2 and 7days) on the mortality, larval and pupal durations, pupation rates and adults' emergency rates.

1. Effect of indirect treatment of *Chrysoperla carnea:*

Indirect treatment was dependent on applying the treated eggs of *Sitotroga carealella* by each of the five compounds then offered for feeding by the larvae of *Chrysoperla carnea*.

Mortalities among *C. carnea* larvae 2 and 7 days after feeding on treated *S. carealella* eggs are presented in tables 1 and 2.

Data in table 1 and fig. 1 showed that the *C. carnea* larval mortality percentages were 0.0, 26.66, 66.67, 26.67 and 26.67 % after feeding of *S. carealella* eggs treated with *B. thuringiensis*, Abamectin, Fipronil, Pyriproxyfen and *B. bassiana*, respectively, compared with 0.0 % in the control treatment. The values were increased after 7 days from treatments by 6.67, 26.67, 15.83, 33.33 and 26.66 % for the same treatments, respectively, compared with 6.67 % in the control. The same table showed that the accumulated mortality percentages were 6.67, 52.23, 82.50, 60.0 and 38.2 % treated by *B. thuringiensis*, Abamectin, Fipronil, Pyriproxyfen and *B. bassiana* treatments, respectively, compared with 6.67 in the control.

So, the three treatments Pyriproxyfen, Abamectin and *Beauveria bassiana* were found harmless to *C. carnea* feeding larvae and caused less than 50 % mortality, while, the insecticide Fipronil was found moderately harmful and caused more than 80 % mortality.

In case of *B. thuringiensis* treated *S. carealella* eggs, it caused 6.67 % mortality showing non significant effect compared with the control.

Also, the obtained data indicated the increased significant effect of these treatments on the duration of larvae, in the control the larvae need to 7.67 days to reached to the pupa, in the same time this period increased to 7.93 days in case of Fipronil treatment but this increase in period was non significant, while, in case of Pyriproxyfen and Abamectin the larvae needed 9.03 and 9.33 days. The longest larval duration until reached the pupal stage occurred among larvae treated with *B. thuringiensis* (11.0 days).

Table 1: Mortality percent, the duration and total mortality of larval instars of *Chrysoperla carnea* after feeding on eggs of *Sitotroga cerealella* exposure to some treatments.

Treatments	Mortality % after		larval duration (days±SE)	Total mortality %	Class toxicity
	2 days	7 days			5 days
Bacillus thuringiensis kurstaki	0.0	6.67±11.55	11.00±1.15	6.67	1
Abamectin	26.66±11.54	26.67±11.54	9.33±2.08	52.33	1
Fipronil	66.67±11.54	15.83±23.09	7.93±1.53	82.50	3
Pyriproxyfen	26.67±11.55	33.33±11.55	9.03±1.09	60	1
Beuveria bassiana	26.67±11.55	26.66±11.54	9.67±2.08	38.2	1
Control	0.0	6.67±11.54	7.67±0.56	6.67	-
L.S.D	16.77	25.16	1.60		

Evaluation categories: 1=harmless (<50%), 2=slightly harmful (50-79%), 3=moderately harmful (80-89%), 4=harmful (>90%)
IOBC-Standard

Pupal duration, pupation rates and adults emergency from *C. carnea* larvae which were fed on the eggs of *S. carealella* treatments with the five compounds are tabulated in Table 2 and Fig 1.
The pupal duration was affected by treatment with either of the five compounds; the longest period (8.66 days) was obtained by treatment of *S. carealella* eggs by Abamectin and also in case of control, while the shortest (7.00 days) occurred by indirect exposure to *B. bassiana* at the recommended rate. On contrary, no pupa resulted from larvae treated by indirect application of Fipronil or Pyriproxyfen.

In two treatments, Fipronil and Pyriproxyfen, *C. carnea* larvae died before reaching the pupal stage, but in other two treatments, *B. thuringiensis* and *B. bassiana* the pupation rates were 40 and 40 %, while, it was 93.34 % among larvae of the untreated control, (Table, 2 and Fig. 1).

With regard to adult emergence rates, those reached 66.67, 33.34 and 28.57 from *B. bassiana, B. thuringiensis* and abamectin, respectively. However, 92.86 % adults' emergence occurred in the untreated control. On contrary, no adults emergeed from pupal stage when *S. cerealella* eggs were treated by Fipronil or Pyriproxyfen. In similar studies, Mukesh *et al.,* (2013) assayed the impact of insecticides on *Chrysoperla carnea*. From the assayed insecticides, the auther found that four, namely monocrotophos, malathion, chlorpyrifos and carbaryl were found very harmful and caused more than 80% mortality among treated larvae. Pyriproxyfen reduced the survival of the larval stage by 19.5 %, and was harmless (Rugno *et al.,* 2016 and Choma *et al.,* 2006). Pyriproxyfen prevents larvae from developing into adulthood and thus rendering them unable to reproduce (Szabo Liz, 2016). The IGR had low toxicity on larval mortality of *C. carne*a (Duffie *et al.,* 1998). Badawy and Arnaouty (1999) also reported that mortalities at first, second and third instar larvae of *C. carnea* against insecticides belonged to classes' organophosphates and carbamates. Rodolfo *et al.,* (2013) mentioned that abamectin caused significant prolongation in the post embryonic period of *C. externa* compared to the control. Abamectin affected the length of first instar larvae, which was of 4 days and differed significantly from the control.

Shahzad *et al.,* (2015) showed that the incubation period of *C. carnea* eggs deposited after feeding on *Aphis gossypii* treated with neem, datura and confidor was 2.2, 2.5 and 3.6 days, respectively. Their results 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. 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.

Table 2: Pupal duration, pupation rates and adults emergence rates of *Chrysoperla carnea* after feeding on egg of *Sitotroga cerealella* exposed to different treatments.

Treatments	Pupal duration (days±SE)	Pupation rates (%)	Adults' emergence rates
Bacillus thuringiensis kurstaki	7.67±0.58	40	33.34
Abamectin	8.66±0.57	46.67	28.57
Fipronil	0.0	0.0	0.0
Pyriproxyfen	0.0	0.0	0.0
Beuveria bassiana	7.00±1.00	40	66.67
Control	8.66±0.56	93.34	92.86
L.S.D	1.03	-	-

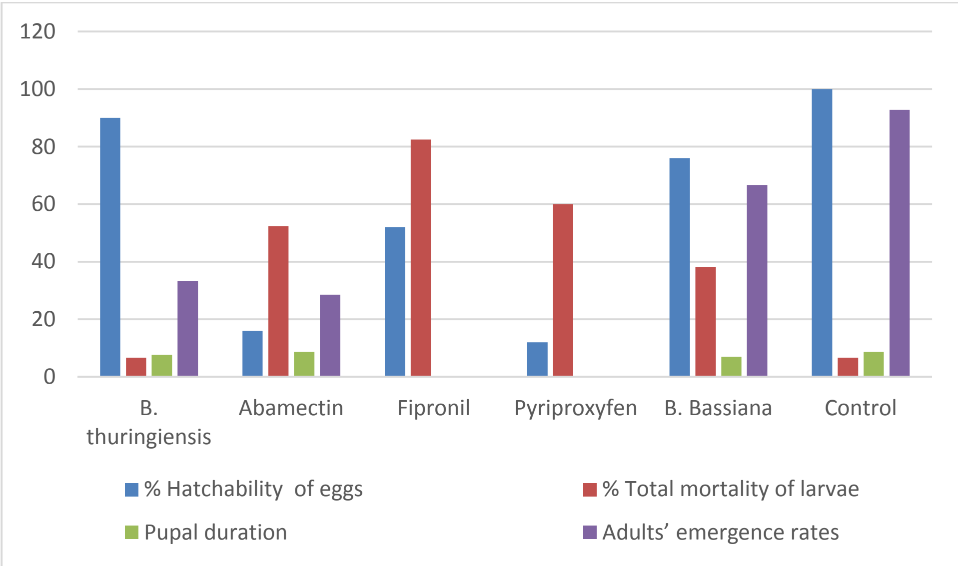

Fig 1: Impact of *Chrysoperla carnea* egg and larvae treatments on biological aspects.

2. Effect of direct treatment of *Chrysoperla carnea*:

Direct treatment depending on spraying the eggs of *C. carnea* by the recommended concentration of five compounds, *B. thuringiensis,* Abamectin, Fipronil, Pyriproxyfen and *B. bassiana*.

2.1. Effect of direct treatment on egg stage of Chrysoperla carnea:

Data in Table 3 and Fig. 1, clearly, demonstrated that all the tested insecticides had affected the hatchability percentage of *C. carnea* eggs being than the untreated control.

The higher effects on hatchability of *C. carnea* eggs were 12 and 16 % found in Pyriproxyfen and Abamectin treatments, while the hatchability percentages recorded 90, 52 and 76 % from the treatments by *B. thuringiensis*, Fipronil and *B. bassiana*, respectively. Incubation period of eggs were affected by the treatments compared with the control (100 % emergence; Table, 3). The effect of tested compounds resulted in prolongation of works on increasing the incubation period of *C. carnea* eggs (3.2- 5.33 days) compared to 2.8 days in the control. Longest incubation period of *C. carnea* eggs was 5.33days in *B. thuringiensis* treatment, but this period was decreased to 4.66, 4.33 and 4.0 days in treatments of Abamectin, *B. bassiana* and Pyriproxyfen, respectively. While the lowest effect on incubation period of *C. carnea* eggs occurred in Fipronil treatment (3.2 days).

Treatments by the tested compounds caused prolongations in the incubation period of *C. carnea* eggs than control. The longest incubation period resulted from *B. thuringiensis* treatment. The duration of this period reached 5.33 ±1.3 (3-7 days). The three treatments Abamectin, *B. bassiana* and Pyriproxyfen were similar in their effects, resulting the incubation periods 4.66 ± 0.88 (3-6), 4.33 ± 0.57 (3-5) and 4 ± 0.5 (3-5) days, respectively. While, the least effect was in Fipronil treatment with 3.2 ± 0.44 (2.5- 4) days compared with 2.8 ± 0.16 (2 – 3) days range period of egg stage in the control.

Also, the tested compounds caused reductions in percentages of hatchability, these reductions reached 88, 84, 48, 24 and 10 % after treatments with Pyriproxyfen, Abamectin, , Fipronil, *B. bassiana* and *B. thuringiensis*, respectively, than control.

Table 3: Effect of direct exposure of *Chrysoperla carnea* eggs chemical and bioinsecticides on some biological aspects.

Treatments (insecticides)	Eggs stage			Larval stage		% Pupal mortality	% Adult emergence
	Hatchability%	incubation (in days)	% Reduction	% Mortality (7 days)	Duration range in days		
Bacillus thuringiensis kurstaki	90	5.33±1.3 (3-7)	10	25	8-10	15	85.0
Abamectin	16	4.66±0.88 (3 - 6)	84	50	7-9	100	0.0
Fipronil	52	3.2±0.44 (2.5-4)	48	86.15	7-9	100	0.0
Pyriproxyfen	12	4.0±0.50 (3 - 5)	88	81.5	8-9	85	15.0
Beuveria bassiana	76	4.33±0.57 (3 -5)	24	21.7	8-9	55	45.0
Control	100	2.8±0.16 (2-3)	0	16.66	7-8	11.76	88.23

2.2. Larval stage of Chrysoperla carnea after treatments off eggs:

All the tested treatments, *B. thuringiensis*, Abamectin, Fipronil, Pyriproxyfen and *B. bassiana* caused higher mortality percentages of larvae of *C. carnea* than the control (Table, 3). As shown in the same Table, larval mortality percentages were 25, 50, 86.15, 81.15 and 21.7 % after treatments with *B. thuringiensis*, Abamectin, Fipronil, Pyriproxyfen and *B. bassiana*, respectively, compared with 16.66 % among the control larvae.

The larval duration was 7-8 days in the control. This period increased to 8-10 days in *B. thuringiensis* treatment, 8-9 days in Pyriproxyfen and *B. bassiana* and 7-9 days in Abamectin and Fipronil treatments.

2.3. Pupal mortality percentage of Chrysoperla carnea developed from treated eggs:

Data in Table 3 and fig. 1 indicated that the highest effect on pupal mortality percentage was in Abamectin and Fipronil treatments with 100 % mortality. This percentage reached 85 % in Pyriproxyfen treatment, 55.0 % in *B. bassiana* and 15 % in case of *C. carnea* eggs treated with *B. thuringiensis.* As for the control, pupal mortality was 11.76 % (Table, 3).

2.4. Adults' emergence percentage of Chrysoperla carnea developed from treated eggs:

Treatments with either of the tested compounds resulted in a decrease in the percentage of emerged *C. carnea* adults than control (Table 3 and Fig. 1). The highest decreased was in Abamectin and Fipronil treatments in which no adult emergence occurred (100 % pupal mortality). This percentage reached 15 % in Pyriproxyfen treatment, 45 % in *B. bassiana* treatment and 85 % in *B. thuringiensis* treatment compared with 88.23 % adults' emergence in the control.

In similar studies carried out by Tillman and Mulrooney (2000) mentioned that spinosad generally did not affect the number of *Geocoris punctipes, Hippodamia convergens,* and

Coleomegilla maculata in the field except for one day after the second application for *G.punctipes.* Jay *et al.,* (2001) showed that imidacloprid and abamectin were highly toxic when applied topically to both parasitoids but were not toxic as one day old residues. The same authors found that insect growth regulators did not cause mortality either as topical applications or residues; however, diflubenzuron caused severe sublethal effects, completely blocking the production of *C. florus* offspring. Biorational pesticides, such as soap, oil, and *B. thuringiensis* products, caused no toxicity to *Colpoclypeus florus* but had a direct impact on *T. platneri* as topical applications through physical immobilization. Also, Fred and Anthony (2003) reported that spinosad was somewhat toxic to *Orius insidiosus* nymphs when applied at field rates and the choice of insecticide material must be dependent on the significant impact on survival of the pests and the predator.

In the same time, Bueno and Freitas (2004); stated that the insect growth regulator, leufenoron presented no adverse effects on egg survival of *C. carnea.* Abida *et al.,* (2007) evaluated the toxic effects of five commercial insecticides, carbosulfan, leufenuron, cyfluthrin, methomyl and fenpropathrin on the eggs of *Chrysoperla carnea* through laboratory bioassays. Insecticide exposure by egg, greatest for life stages treated directly and decreased during subsequent life stages and the insect growth regulators, leufenoron caused 6.91 % mortality on the eggs of *C. carnea*. Gnanadhas *et al.,* (2009) conducted laboratory studies to find out the toxicity of imidacloprid and diafenthiuron to the eggs, larvae and adults of *Chrysoperla carnea*. Imidacloprid at the recommended concentration of 0.28 ml/l caused 15.38% egg mortality, 26.67 and 33.33% larval mortality by ingestion and contact, respectively and 50.00% adult mortality. The egg mortality was about 15.38% and larval mortality of 23.33% and adult mortality of 26.67% occurred by diafenthiuron. Based on the classification given by IOBC/WPRS working group on pesticides and non-target invertebrates, both the insecticides were classified as harmless to *C. carnea*, since the recommended dose caused less than 50% mortality under the laboratory conditions.

Fig. 1, indicated that *Bacillus thuringiensis* kurstaki is the lowest effected on the natural enemy, *C. carnea*. In similar studies, Ana Rodrigo *et al*., (2006) reported that control experiments, Cry1Ac was detected bound to the midgut epithelium of intoxicated *H. armigera* larvae, and cell damage was observed. However, no binding or histopathological effects of the toxin were found in tissue sections of *C. carnea* larvae. These results strongly suggest that the *C. carnea* larval midgut lacks specific receptors for Cry1Ab or Cry1Ac.

Conclusion

Our data suggest that the use of Abamectin, Fipronil, Pyriproxyfen and *Beauveria bassiana* with *Chrysoperla carnea* in integrated pest management (IPM) programs should be carefully evaluated. *Bacillus thuringiensis* kurstaki is the lowest effected on the natural enemy, *C. carnea* larvae, that due to the *C. carnea* larval midgut lacks specific receptors for Cry1Ab or Cry1Ac. So, *B. thuringiensis* prefer to use in integrated pest management unless it effects on the control of the main pest.

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