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Original article

The side effects of ten commercial pesticide formulations on the green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)

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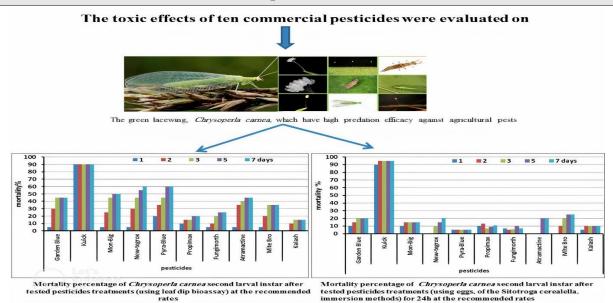
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ABSTRACT

The toxic effects of ten commercial pesticides (Garden Blue, Kuick, Mon-Big, New-Tegrox, Pyra-Blue, Propimax, Fungimorth, Atramactine, Mite Bro; and Kalash) were evaluated on green lacewing, Chrysoperla carnea (C. carnea). The mortality percentage was determined for the tested pesticides by leaf dip bioassay and by eggs, of the Sitotroga cerealella (S. cerealella), immersion (as food). The LC50 values of pesticides varied between pesticides. These values were 520, 120, 6000, 3500, 5600, 5000, 3000, 40, 240; and 650ppm, respectively after 72h post treatments for eggs immersion of S. cerealella. The corresponding LC_{50} in the case of exposure to leaves treated with pesticides were: 200, 80, 210, 230, 260, 1500, 3400, 30, 180, and 400ppm. respectively. The results clearly indicated that direct action by leaf dip technique was more toxic than indirect action by eggs immersion of S. cerealella. All experiments were studied on the 2nd larval instar of C. carnea (1 day old). The rate of application for all the tested pesticides was evaluated at the recommended concentration. The rate of application in the field for 2nd larval instar of the green lacewing was studied in 200L.water fed.⁻¹. The rates were 200cm³, 300gm, lliter, 1liter, 150cm³, 500cm³, 500cm³, 100cm³, 70cm³ and 100gm product as recommended doses. The mortality was recorded 1, 3, 5, and 7 days after exposure to pesticides. Also, these pesticides reduced pupation and adult emergence. The highest effects were recorded with Garden Blue and Kuick (insecticides) with Mon Big, New-Tegrox (herbicides) and with Pyra-Blue (fungicide), and with Atramactine (acaricide).

Graphical abstract



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1. Introduction

Pesticides are considered one of the most effective control methods against target pests but have a negative effect on their natural enemies [1, 2]. Biological control is one of the most significant strategies for pest control; chemical control is still required especially in pest outbreak that causes high economic losses [3].

Plant protection strategies have been recommended, diminishing the use of organic chemical pesticides. Therefore, it is very indispensable to evaluate the side effects of pesticides on insect predators to eliminate the ones that have a high risk of such natural insect enemies [4]. Integrated pest management (IPM) depends on using affordable strategies as well as reducing the risks to agroecosystems [5]. Therefore, natural enemies have been considered as one of the alternatives to pesticides for pest control.

Pesticides have high toxicity to arthropods, as they may kill them or negatively impact several biological aspects [6, 7, 8, 9). Pesticides have negative effects on natural enemies, this motivates the exigency to evaluate their side effects to adjust biological and chemical control programs and also, minimize the negative effects on non-target organisms [10, 11].

Agrochemicals, especially pesticides, can obstruct the efficiency of natural predators causing disruption of the ecosystem [6, 12, 13]. Natural enemies have a high susceptibility to pesticides, and it has been observed that pesticides affect the abundance, species composition and also biology of beneficial insects in agroecosystems (14, 15]. In addition, behavioral impacts of pesticides on beneficial insects have been noticed lately [16, 17, 18, 3]. The behavioral effects of pesticides on natural enemies need still a lot to be studied. Chrysopidae Lacewings family is considered one of the most beneficial insects in agroecosystems, which have high predation efficacy against agricultural pests [19] such as the predator aphid lion *Chrysoperla carnea*.

In this study, we estimated the toxicity and the side effects of certain pesticides (at the recommended dose) on the predator aphid lion *Chrysoperla carnea*.

2. Materials and methods

2.1. Pesticides used

Tested pesticides were conducted as free solutions prepared with distilled water. The field-recommended concentrations for each compound were used. Some information on the used compounds is listed in Table (1).

2.2. Source of aphid lion Chrysoperla carnea

(Stephens)

The green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae) originally was obtained from the Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University. This strain was not previously exposed to any pesticides.

2.3. Rearing technique

Chrysoperla carnea colony was reared at the Department of Plant Protection, Faculty of Agriculture (Cairo), Al-Azhar University laboratory under constant conditions ($25\pm 2C^{\circ}$, $65\pm 5\%$ R.H., and photoperiod 14:10 (L: D)) for one to two successive generations before initiation of experiments [20].

Trade names	Common names	Concentrations and formulations	Rate fed ⁻¹ .in 200 L.water	Source	Туре
Garden Blue	Cyromazine	30% SC	200 cm ³	Tabarak Co.	Insecticides
Kuick	Methomyl	90% SP	300 gm	Sam trade Co.	Insecticides
Mon-Big	Clomazone	48%EC	Liter	Weifangcynda Chemical Co.	Herbicides
New-tegrox	phenmediphamt9,1%+ desmediphamt7,1%+ ethofumesate11,2%	27.4%EC	Liter	Star Chem Co.	Herbicides
Pyra-Blue	pyraclostrobin 10% +tetraconazole 20%	30%EC	150 cm ³	Star Chem Co.	Fungicides
Propimax	Propiconazole	25%EC	500 cm ³	Green-M For Agricultural services	Fungicides
Fungimorth	dimethomorph 9% +mancozeb 60%	69%EC	500 cm ³	Cairo Chem Co.	Fungicides
Atramactine	Abamactin	1.8%EC	100 cm ³	El-Ashkar for trade and agencies	Acaricides
Mite Bro	Bifenazate	43%SC	70 cm ³	El-Helal El-Khasib Co.	Acaricides
Kalash	Hexythiazox	50% WDG	100 gm	El-Moneer Co	Acaricides

•All the pesticide samples were supplied by Agricultural Pesticide Committee (APC), Ministry of Agriculture and Land Reclamation.

Table 1 List of the tested pesticides

2.4. Screening of pesticides

The tested pesticides were evaluated against *C. carnea* 2^{nd} larval instar under laboratory conditions. Stock solutions of formulated pesticides were prepared according to the recommended concentration in distilled water. The control test was conducted by distilled water. The mortality percentage was corrected by Abbott's formula **[21].** The toxic effects of ten pesticides on 2^{nd} larval instar of *C. carnea* were evaluated to calculate the median lethal dose (LC₅₀) values.

The pesticides were classified according to the International Organization for Biological Control, West Palearctic Regional Section (IOBC/WPRS) as the following:

- Toxicity Class I (Harmless) = mortality percent less than 50%.
- Toxicity Class II (Slightly harmful) = mortality percent between 50-79 %.
- Toxicity Class III (Moderately harmful) = mortality percent between 80-89 %.
- Toxicity Class VI (Harmful) = mortality percent between more than 90 % [22].

2.4.1. Direct effect (leaf dip bioassay)

Leaf dip bioassay was used to mimic the field in which the C. carnea adults and larvae were exposed to pesticides. The larvae of C. carnea (1-day old) were kept individually in petri dishes (9cm diam.) for 24h. By leaf dip technique, the healthy leaves of castor bean (Ricinus communis L.) were dipped in different concentration for ten seconds for each pesticide, and then dried at room temperature. Damped filter was put under leaf discs to evade the drying of leaves in the petri dish. Different concentrations were used in each pesticide and each concentration had 50 replicates. Each replicate had one healthy starved second instar larvae. The eggs of Sitotroga cerealella were put into each replicate as food [23]. Mortality data were recorded in all groups every day for 7.0 days, at the end of each larval instar and pupae. Pupae that didn't change into adults within the seven days considered as dead. The survived larvae after pesticides treatments transferred into gelatin capsule along with 0.024gm eggs of S. cerealella until pupation and adult emergence. Pupation and adult emergence rate were evaluated [18].

2.4.2. Indirect effect (Feeding treatment bioassay)

Indirect effect methods were done by dipping the eggs of *S. cerealella* in the ten used pesticides. The 2^{nd} larval instar of *C. carnea* was used (1-day old). The larvae were kept individually in petri dishes (9cm diam.) and exposed to *Sitotroga cerealella* eggs treated with different concentrations of each pesticide for 24hrs. Each concentration had 50 replicate and each replicate had one healthy starved larva. Other 50 replicate were fed on *S. cerealella* eggs treated with distilled water as control. The mortalities percent were

recorded after 1, 2, 3, 5, and 7 days post treatment [24, 25]. In all treatments, mortality percent, pupation and adult emergence were estimated and corrected when needed [26]. The following formula was calculated:-

Pupation % =No. of pupae/total No. of larvae $\times 100$.

Adult emergence % =No. of moths/total No. of larvae $\times 100$.

The toxicity index was calculated according to the following formula:

T. I. =Lc₅₀ of the most effective pesticides/Lc₅₀ of the tested pesticides $\times 100$ [27].

2.5. Statistical analysis

Statistical analysis was evaluated by analysis of variance (ANOVA) and compared by L. S. D. test at 5% level of probability in all experiments according to Duncan's multiple range tests [28].

3. Results

Body The results in table (2) showed the toxicity of the tested pesticides on the 2^{nd} larval instar of *Chrysoperla carnea* by leaf dip technique (direct effect). The data clearly indicated that Atramactine acaricide was a more toxic compound (LC₅₀ values =30 ppm). Also, Kuick was highly effective than other pesticides except Atramactine followed by, Mite Bro, Garden, Mon- Big, New- tegrox, Pyra- Blue, Kalash, then Propimax with LC₅₀ values 80, 180, 200, 210, 230, 260, 400, and 1500ppm, respectively. On the other hand Fungimorth was the least effective one (LC₅₀ =3400 ppm).

The results in table (3) indicated the LC₅₀ values on the 2nd larval instar of *C. carnea* (when eggs of *S. cerealella* were treated with pesticides). From these data, Atramactine was the most effective (LC₅₀= 40 ppm), followed by Kuick, Mite Bro, Garden, Kalash, Fungimorth, New- tegrox, Propimax, then Pyra- Blue with LC₅₀ values 120, 240, 520, 650, 3000, 3500, 5000, 5600pp, respectively. Mon- Big, was the least effective pesticide with LC₅₀ 6000ppm. From tables (2 and 3), the direct effect by leaf dip technique was more toxic than indirect effect by eggs of *S. cerealella* treatment.

The results in table (4) showed mortalities percentage of *C. carnea* on the 2^{nd} larval instar after leaf residual exposure. All pesticides were applied at the recommended rates in the field. The data clearly indicated that Kuick insecticide was significantly more toxic than other pesticides. Kalash acaricide was the least one. No significant differences were found between New-tegrox, Pyra-Blue (fungicides). The toxicity index was harmless in all pesticides except Kuick was harmful.

The results in table (5) showed the effect of ten pesticides on pupation and adult emergence after treatment with dipped leaves. All pesticides significantly reduced pupation. The highest effect on pupation was recorded with Kuick, while Propimax and Kalash were the least effective. The data in this table also indicate the effect of pesticides on adult emergence. The insecticide (Kuick) and fungicide (Propimax) and acaricide (Atramactine) significantly reduced the adult emergence, while Pyra-Blue and Mite Bro had weak effect.

The results in table (6) indicated the effect of pesticides on larval mortality after feeding on treated *S. ceralella* eggs. Kuick insecticide was the most effective followed by Garden Blue. The least effective ones were Pyra-Blue and Mite Bro and Atramactine.

The results in Table (7) showed the effect of ten pesticides on pupation and adult emergence (when 2^{nd} larval instar of *C. carnea* was feed on eggs of *S. cerealella* immersion in all pesticides). The data clearly indicated that all the tested pesticides were significantly reduced the pupation. The Kuick insecticide was more toxic than others followed by Atramactine. The least effective were Propimax, Fungimorth and Kalash. Also, these pesticides significantly reduced the adult emergence except Fungimorth, Propimax and Kalash.

Table 2 Susceptibility of *Chrysoperla carnea* 2nd larval instar to tested pesticides (using leaf dip bioassay) after 72h post-treatment (Direct effect).

Trade names	Concentrations and formulations	LC50 (ppm) (Lower-upper)	Slope ± SE•	Toxicity index••
Garden Blue	30% SC	200 (190-215)	1.60 ± 0.18	15
Kuick	90% SP	80 (75-88)	1.17 ± 0.22	37.5
Mon-Big	48%EC	210 (200-215)	1.60 ± 0.25	14.28
New-tegrox	27.4%EC	230 (225-235)	1.88 ± 0.15	13.04
Pyra-Blue	30%EC	260 (250-270)	1.56 ± 0.26	11.54
Propimax	25%EC	1500 (1490-1520)	0.89 ± 0.21	2
Fungimorth	69%EC	3400 (3380-3410)	1.93 ± 0.17	0.88
Atramactine	1.8%EC	30 (25-35)	1.60 ± 0.19	100
Mite Bro	43%SC	180 (170-195)	2.11 ± 0.25	16.67
Kalash	50% WDG	400 (390-420)	2.20 ± 0.18	7.5

• SE= Standard error

•• Toxicity index = LC_{50} of the most effective pesticides/ LC_{50} of the tested pesticides× 100 at the LC_{50} values [24]

Table 3 Susceptibility of *Chrysoperla carnea* 2nd larval instar to tested pesticides (using eggs, of the *Sitotroga cerealella*, immersion methods) after 72h post treatment (Indirect effect)

Trade names	Concentrations and formulations	LC ₅₀ (ppm) (Lower-upper)	Slope ± SE•	Toxicity index••
Garden Blue	30%SC	520 (480-550)	1.83 ± 0.19	7.69
Kuick	90% SP	120 (113-125)	1.88 ± 0.22	33.34
Mon-Big	48%EC	6000 (5880-5900)	1.73 ± 0.21	0.67
New-tegrox	27.4%EC	3500 (3430-3540)	2.11 ± 0.15	1.15
Pyra-Blue	30%EC	5600 (5580-5650)	2.37 ± 0.28	0.72
Propimax	25%EC	5000 (4930-5010)	1.55 ± 0.21	0.8
Fungimorth	69%EC	3000 (2880-3080)	1.81 ± 0.26	1.34
Atramactine	1.8% EC	40 (35-42)	1.90 ± 0.18	100
Mite Bro	43%SC	240 (235-260)	1.65± 0.19	16.67
Kalash	50% WDG	650 (635-660)	1.93 ± 0.15	6.15

• and •• see footnote of table 1

recomm	nended rates								
Trade names	Concentrations	Rate fed ^{- 1} .in	Corrected mortality percentage at different days				Mean ±SE	Toxicity	
I rade names	and formulation	200L. water	1	2	3	5	7	cla	class*
Garden Blue	30%SC	200cm ³	5	30	45	45	45	34±7.80 ^{bcd}	I (Harmless)
Kuick	90% SP	300gm	90	90	90	90	90	90±0.00 ^a	VI (Harmful)
Mon-Big	48%EC	Liter	5	25	45	50	50	35±8.80 ^{bc}	I (Harmless)
New-tegrox	27.4%EC	Liter	5	30	45	55	60	39±9.92 ^b	I (Harmless)
Pyra-Blue	30%EC	150cm ³	20	35	45	60	60	44±7.60 b	I (Harmless)
Propimax	25%EC	500cm ³	10	15	15	20	20	16±1.90 ^{de}	I (Harmless)
Fungimorth	69%EC	500cm ³	5	10	20	25	25	17±4.06 ^{cde}	I (Harmless)
Atramactine	1.8%EC	100cm ³	5	35	40	45	45	34 ± 7.48^{bcd}	I (Harmless)
Mite Bro	43%SC	70cm ³	5	20	35	35	35	26 ± 6.00^{bcde}	I (Harmless)
Kalash	50% WDG	100gm	zero	10	15	15	15	11±2.91e	I (Harmless)

 Table 4 Mortality percentage of Chrysoperla carnea 2nd larval instar after tested pesticides treatments (using leaf dip bioassay) at the recommended rates

•L. S. D. at 5%=18.43.

Means followed with the same letters are not significantly different at P = 0.05. [27]. *Toxicity class [26]

Table 5 Effect of certain pesticides on pupation and adult emergence of *Chrysoperla carnea* treated at 2nd larval instar (using leaf dip bioassay) at the recommended rates.

Trade names	Concentrations and formulations	Rate fed ⁻¹ .in 200 L.water	Pupation%±SE•	Adult emergency% ±SE•	
Garden Blue	30% SC	200cm ³	45.0±1.30 ^{ef}	40.0±1.30 ^f	
Kuick	90% SP	300gm	10.0±0.89 ^g	10.0±0.70 ⁱ	
Mon-Big	48%EC	Liter	50.0±1.14 ^e	35.0±0.87g	
New-tegrox	27.4%EC	Liter	40.0±1.30 ^f	35.0±1.16 ^g	
Pyra-Blue	30% EC	150cm ³	45.0 ± 1.14^{ef}	$25.0{\pm}1.14^{h}$	
Propimax	25%EC	500cm ³	80.0±1.01 ^b	10.0 ± 0.70^{i}	
Fungimorth	69% EC	500cm ³	70.0±1.70 ^{cd}	50.0±1.10°	
Atramactine	1.8%EC	100cm ³	50.0±0.77 ^e	45.0±0.73 ^d	
Mite Bro	43%SC	70cm ³	65.0±2.21 ^d	55.0±1.66 ^e	
Kalash	50% WDG	100gm	80.0±1.78 ^b	70.0±0.94 ^b	
Control	-	-	95.0±1.55ª	95.0±1.50 ^a	

L. S.D. for pupation at $\overline{5\%}=7.20$; L. S.D. at 5% for adult emergence= 4.19; SE = Standard Error

 Table 6 Mortality percentage of Chrysoperla carnea 2nd larval instar after tested pesticides treatments (using eggs, of the Sitotroga cerealella, immersion methods) for 24h at the recommended rates

Trade names	Concentrations	Rate fed ⁻¹ .in	Co	Corrected mortality percentage at different days				Mean ±SE	Toxicity class*
	and formulations	200 L. water	1	2	3	5	7		
Garden Blue	30% SC	200cm ³	10	15	20	20	20	17±2.0 ^b	1(Harmless)
Kuick	90% SP	300gm	90	95	95	95	95	94±1.0 ^a	4(Harmful)
Mon-Big	48%EC	Liter	10	15	15	15	15	14 ± 1.0^{bcd}	1(Harmless)
New-tegrox	27.4%EC	Liter	Zero	Zero	10	15	20	9±4.0 ^{cde}	1(Harmless)
Pyra-Blue	30%EC	150cm ³	5	5	5	5	5	5±0.0 ^{ef}	1(Harmless)
Propimax	25%EC	500cm ³	10	13	7	9	11	10.0±3.0 ^{cde}	1(Harmless)
Fungimorth	69%EC	500cm ³	7	5	6	10	7	7.0±3.5 ^{de}	1(Harmless)
Atramactine	1.8%EC	100cm ³	Zero	Zero	One	20	20	$5.0{\pm}4.8^{ef}$	1(Harmless)
Mite Bro	43%SC	70cm ³	Zero	10	20	25	25	1.6 ± 4.8^{bc}	1(Harmless)
Kalash	50% WDG	100gm	5	10	10	10	10	9.0± 1.0 ^{cde}	1(Harmless)

. L. S. D. at 5%=7.54.

 $\bullet SE = Standard Error.$

Means followed with the same letters are not significantly different at P = 0.05. [27].

*Toxicity class [26].

Trade names	Concentrations and formulations	Rate fed ⁻¹ .in 200 L. water	Pupation % ± SE•	Adult emergency % ± SE•
Garden Blue	30%SC	200 cm ³	75±2.07 ^d	70±1.40 ^{bc}
Kuick	90% SP	300 gm	10±0.70g	3.0±0.44 ^f
Mon-big	48%EC	Liter	70±1.11 ^e	70.0±1.98°
New-tegrox	27.4%EC	Liter	75±1.51 ^d	70.0±2.26°

150 cm³

500 cm³

500 gm

40 cm³

70 cm3

100 gm

-

Table 7 Effect of certain pesticides on pupation and adult emergence of *Chrysoperla carnea* treated at 2nd larval instar (using eggs, of the *Sitotroga cerealella*, immersion methods) for 24h at the recommended rates

-L.S.D. at 5% for pupation = 3.96

-L.S.D. for adult emergence= 11.80

•SE = Standard error.

Pyra-Blue

Propimax

Fungimorth

Atramactin

Mite bro

Kalash

Control

4. Discussion

30%EC

25%EC

69%EC

1.8%EC

43%SC

50% WDG

The results in this study clarify that, pesticides possibly interfere with the biological control agents such as *Chrysoperla carnea* in the field and caused considerable damage to them. The method of application, type of pesticides, type of active ingredient and mode of action of pesticide are important factors for the toxicity of any pesticide, a possible explanation for this observation is that insecticides and acaricides would cause more mortality of larvae of *C. carnea* than fungicides and herbicides.

Salama *et al.*, (1990) mentioned that *C. carnea* larvae had high toxicity to methomyl in soyabean in fields. Van Emden and Pealall (1996) found that pesticides have undesirable effects on natural predators due to their mode of action, in addition to persistence and non-selectivity. A similar trend was found by Güven and Göven (2003) who reported that *C. carnea* larvae have high susceptibility to methomyl with a percent mortality 100%.

Nasreen et al., (2007) mentioned that C. carnea larval instar exhibited high percent mortality 92% post methomyl and fenpropathrin treatment by using leaf dip bioassay as well as, direct adult topical treatment. Giolo et al., (2009) reported that organophosphorus pho.smet and trichlorfon were considered harmless (Class I) (E<30%) to C. carnea larvae. These variations may be due to the active ingredient concentrations for pesticides, which were lower in the present study, in addition to the metabolic detoxification enzyme in C. carnea, which raise insect resistance. Castilhos et al., (2013) observed that Glyphosate (herbicide), mineral oils, copper oxychloride (fungicides), abamectin captan, and mancozeb were harmless; the tebuconazole fungicides were slightly harmful. The deltamethrin insecticide was moderately harmful (Cass I), while the phosmet, malathion, and dimethoate (insecticides) also, the paraquate dichloride were harmful (Class VI) to C.

externa larvae. Also, they reported that the larvae were sprayed with the above pesticide directly instead of exposure to a treated surface, which supposedly increased the efficiency of the active ingredient. Also, Imam, (2017) found that the direct exposure technique with *B. bassiana* has a higher toxicity than indirect exposure bioassays on the 2^{nd} larval instar of *C. carnea*.

75.0±1.89bc

85±1.48^{a b}

94±1.30^a

70±1.94°

45±0.70^d

90±1.10^a

95±1.16^a

5. Conclusion

According to the results obtained during the present work, we concluded that before using the pesticides for control[strategy must be taken into consideration when releasing the natural enemies, due to the high toxic efficiency of the above-tested pesticides.

Acknowledgments

80±2.00°

90±0.89b

93±1.69^b

 75 ± 1.51^{d}

50±0.89f

90±1.10^b

96±1.04^a

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