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Effects of pesticides on eggs of *Chrysoperla externa* (Neuroptera: Chrysopidae) and consequences on subsequent development

Efectos de los plaguicidas en huevos de *Chrysoperla externa* (Neuroptera: Chrysopidae) y las consecuencias sobre su desarrollo

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Abstract: The effects of six pesticides applied to the coffee crop on eggs and their consequences on the subsequent developmental stages of *Chrysoperla externa* (Neuroptera: Chrysopidae) were evaluated under laboratory conditions. The pesticides and water (control) were sprayed on eggs using a Potter´s tower. After spraying, forty eggs per treatment were individualized in glass tubes and maintained in a climatic chamber, in order to evaluate immature development of this predator. The treatments showed significant differences for egg viability and survival of first-instar larvae. Chlorpyrifos, sulphur and copper oxichlorate reduced the treated egg viability, whereas both sulphur and betacyfluthrin reduced the survival of first-instar larvae. Endosulphan and azociclotin reduced the daily oviposition of this green lacewing species. The harmless products (Class 1, E < 30%), can be recommended for use in integrated pest management programs in coffee crops, in order to preserve this predator.

Key words: *Coffea arabica*. Green lacewing. Pesticides. Selectivity.

Resumen: Los efectos de seis plaguicidas aplicados al cultivo de café sobre los huevos y etapas de desarrollo posteriores de *Chrysoperla externa* (Neuroptera: Chrysopidae), fueron evaluados bajo condiciones de laboratorio. Los plaguicidas y el agua (control) fueron aplicados en los huevos usando una torre de Potter. Después de la aplicación, cuarenta huevos por tratamiento fueron individualizados en tubos de vidrio y mantenidos en cámara bioclimática, con el objetivo de verificar las posibles anormalidades en el desarrollo de este depredador. Los tratamientos mostraron diferencias significativas para la viabilidad de los huevos y la supervivencia del primer instar larval. Chlorpyrifos, azufre y oxicloruro de cobre redujeron la viabilidad de los huevos tratados, mientras que el azufre y el betacyfluthrin redujeron la supervivencia del primer instar larval. Endosulfán y azociclotin redujo la oviposición diaria de esta especie crisopa. Los productos inocuos (Clase 1, E < 30%), pueden recomendarse para su uso en programas integrados de manejo de plagas en los cultivos de café, a fin de preservar este depredador.

Palabras clave: *Coffea arabica*. Crisopas**.** Plaguicidas. Selectividad.

Introduction

The coffee agroecosystem presents optimal conditions for the deployment of integrated control measures, because the perennial nature of the plants favors the increase of predators and parasitoids, which can reach levels capable of reducing arthropod pest populations (Altieri 1994). However, the indiscriminate use of pesticides on this crop has been drastically reducing the populations of beneficial insects (Micheletti 1991).

Insects of the family Chrysopidae (Neuroptera) have been reported as having high predatory capacity and adaptation to different ecosystems (Souza and Carvalho 2002; Costa *et al*. 2010). They have a high biotic potential, feeding on aphids, mealybugs, eggs, larvae and pupae of moths, eggs and adult mites, occurring naturally in coffee (Gravena 1992; Carvalho and Ciociola 1996).

Natural biological control by lacewings is among the most important integrated pest management (IPM) tactics for population regulation of the coffee leaf miner *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae), the coffee ring spot mite *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae) and the coffee red mite *Oligonychus ilicis* (McGregor, 1917) (Acari: Tetranychidae). Preference should be given to the use of selective pesticides, namely, those that control the coffee pests without negatively affecting the natural enemy populations (Gravena 1992). Thus, in order to control *Hypothenemus hampei* (Ferrari, 1867) *L. coffeella* and *Planococcus minor* (Maskell, 1897), this study evaluated the effects of pesticides used in coffee, on eggs of *C. externa* and their consequences on the subsequent stages of development.

Materials and Methods

Tests of physiological selectivity were carried out in accordance with the methodology recommended by the "International Organization for Biological Control of Noxious Animals and Plants" (IOBC), West Palearctic Regional Section (WPRS) (IOBC/WPRS 1992; Hassan 1997; Carvalho *et al*. 2002).

Lacewing adults were collected in a citrus orchard at the Universidade Federal de Lavras (UFLA), taken to the laboratory for identification of species and reared the laboratory. Fourth generation *C. externa* eggs, approximately 12 hours old, were removed from the laboratory rearing cages by cutting the pedicels with the aid of fine-tipped scissors and placed into seven groups of 40, in 15 cm diameter Petri dishes.

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Table 1. Phytosanitary products evaluated for their selectivity to the predator Chrysoperla externa.

Commercial name	Technical name	Dosage (g a.i./L water)	Chemical group
Thiodan 350 CE	Endosulphan	1.750	acid ester. sulf. Diol cyclic
Lorsban 480 CE	Chlorpyriphos	1.200	Organophosphate
Turbo 50 CE	Betacyfluthrin	0.013	Pyrethroid
Kumulus 800 PM	Sulphur	4.000	Sulphur
Peropal 250 PM	Azociclotin	0.310	organo-stannic
Cuprogarb 500 PM	Copper oxychloride	5.000	metallic copper

We evaluated some of the products most used on coffee crops to control pests and diseases, which were applied in the higher concentrations, recommended by the manufacturers, and water application as control. The products, with their respective technical names, trade names and chemical group are presented in table 1.

The products were sprayed directly on the eggs with a Potter's tower at a regulated pressure of 15 lb/pol^2 with an applied chemical spray volume of 1.5 ± 0.5 mL/cm². After application of the compound, the dishes were kept in the laboratory for two hours to reduce egg surface moisture and then were individualized in glass tubes (2.5 cm in diameter and 8.5 cm in height), sealed with PVC film and kept in a climatic chamber at 25 ± 2°C, RH 70 ± 10% and 12 hour of photophase. After hatching, the larvae were fed *ad libitum* every two days with *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) until pupation.

The experimental design utilized was a completely randomized design with seven treatments and ten repetitions with four eggs per plot. The eggs were individualized in tubes because cannibalism by newly hatched larvae is a common problem in green lacewing egg studies (Costa *et al*. 2003; Bezerra *et al.* 2009). For evaluation of larvae, pupae and adult six replications were conducted. We evaluated the egg stage duration and egg viability, duration and larval survival of the first, second and third instars and pupae. The adult sex ratio and fertility were evaluated for a period of four consecutive weeks from the beginning of oviposition.

To evaluate fertility of lacewings reared from sprayed eggs, pupae from each treatment were kept in glass tubes until adult emergence. Adults were allocated to groups comprised of six mating pairs (12 lacewings) per cylindrical PVC cage (10 cm height and 10 cm in diameter). Each cage was lined with filter paper, supported on a tray covered with the same kind of paper, and its top sealed with muslin. The adults were fed a diet of brewer's yeast and honey (1:1 v/v), brushed on a porous material (sponge) set on the end of an 8 ml glass tube containing distilled water, kept on top of each cage according to the methodology of Barbosa *et al.* (2002).

During three consecutive weeks from the beginning of oviposition, the number of eggs on the paper was checked and removed every four days. Additionally, 96 eggs were separated per treatment, which were individualized into micro titer plate compartments used in the ELISA test (Enzyme Linked Immunosorbent Assay). Plates were closed with sheets laminated with PVC and kept under the same environmental conditions described above.

The data from the embryonic, larval, pupae, pre-oviposition, oviposition and pos-reproductive periods, egg viability, larvae and pupae survival were subjected to analysis of variance, the means being grouped by the Scott and Knott test at 5% significance (Scott and Knott 1974). The total effect (E) of each product was assessed in terms of mortality and reproductive aspects during the development of the predator, by the formula E = 100% - (100% - M%) x R1 x R2, proposed by Vogt (1992), where E = total effect (%) M = mortality (%) accumulated from the egg stage until adult emergence in each treatment corrected by the formula of Abbott (1925), where R1 = ratio between the average daily eggs laid per female from the treatment with the product compared to the control and R2 = ratio between the average survival rate of eggs laid per female originated from the treatment with the product

Table 2. Stage duration (days), egg viability, survival (%) (mean ± SE) and sex ratio of larvae and pupae of *Chrysoperla externa*, originating from eggs sprayed with some phytosanitary products.

Treatments	Egg stage		Larvae stage		Sex ratio	Pupae stage	
	Duration	Viability	Duration	Survival		Duration	Survival
Endosulphan	4.6 ± 0.02 a	92.5 ± 3.82 a	8.5 ± 0.14 a	100.0 ± 0.00 a	0.52	10.0 ± 0.07 a	97.5 ± 2.50 a
Chlorpyriphos	4.6 ± 0.02 a	80.0 ± 5.00 b	8.6 ± 0.12 a	97.5 ± 2.50 a	0.51	10.0 ± 0.01 a	100.0 ± 0.00 a
Betacyfluthrin	4.6 ± 0.02 a	95.0 ± 3.33 a	8.5 ± 0.11 a	87.5 ± 5.60 a	0.39	10.3 ± 0.09 a	92.5 ± 5.34 a
Sulphur	4.6 ± 0.03 a	82.5 ± 6.51 b	8.8 ± 0.16 a	90.0 ± 5.53 a	0.43	10.2 ± 0.08 a	94.2 ± 3.94 a
Azociclotin	4.6 ± 0.02 a	92.5 ± 3.82 a	8.8 ± 0.18 a	97.5 ± 2.50 a	0.39	10.1 ± 0.12 a	92.5 ± 3.82 a
Copper oxychloride	4.6 ± 0.02 a	82.5 ± 6.00 b	8.6 ± 0.14 a	96.7 ± 3.33 a	0.52	10.1 ± 0.07 a	96.7 ± 3.33 a
Control	4.6 ± 0.02 a	95.0 ± 3.33 a	8.7 ± 0.10 a	96.7 ± 3.33 a	0.48	10.2 ± 0,05 a	97.5 ± 2.50 a
CV (%)	1.58	15.58	5.03	12.34		2.29	11.30

Means followed by the same letter in column do not differ by Scott and Knott test (P < 0.05). SE = standard error of mean.

Table 3. Duration (days) and survival (%) (mean ± SE) of instars of *Chrysoperla externa*, from eggs sprayed with some phytosanitary products.

Treatments	First instar		Second instar		Third instar	
	Duration	Survival	Duration	Survival	Duration	Survival
Endosulphan	3.0 ± 0.02 a	100.0 ± 0.00 a	3.0 ± 0.05 a	100.0 ± 0.00 a	2.5 ± 0.06 a	100.0 ± 0.00 a
Chlorpyriphos	3.1 ± 0.02 a	100.0 ± 0.00 a	3.0 ± 0.02 a	100.0 ± 0.00 a	2.6 ± 0.11 a	97.5 ± 2.50 a
Betacyfluthrin	3.1 ± 0.02 a	87.5 ± 5.59 b	3.0 ± 0.02 a	100.0 ± 0.00 a	2.4 ± 0.14 a	100.0 ± 0.00 a
Sulphur	3.1 ± 0.02 a	92.5 ± 5.34 b	3.0 ± 0.01 a	100.0 ± 0.00 a	2.7 ± 0.11 a	100.0 ± 0.00 a
Azociclotin	3.1 ± 0.02 a	100.0 ± 0.00 a	3.0 ± 0.02 a	97.5 ± 2.50 a	2.7 ± 0.14 a	100.0 ± 0.00 a
Copper oxychloride	3.0 ± 0.02 a	96.7 ± 3.33 a	3.0 ± 0.04 a	100.0 ± 0.00 a	2.6 ± 0.20 a	100.0 ± 0.00 a
Control	3.0 ± 0.02 a	100.0 ± 0.00 a	3.0 ± 0.01 a	96.7 ± 3.33 a	2.6 ± 0.12 a	100.0 ± 0.00 a
CV (%)	2.50	10.41	1.99	5.02	7.93	3.0

Means followed by the same letter in column do not differ by Scott and Knott test (P < 0.05). SE = standard error of mean.

compared to control. After obtaining the total effect (E), each formulation was framed in toxicity classes proposed by the IOBC (Hassan 1997), as follows: class 1 = harmless (E < 30%), class 2 = slightly harmful (30 ≤ E ≤ 79%), class 3 = moderately harmful (80 ≤ E ≤ 99%) and class 4 = harmful (E > 99%).

Results and Discussion

The duration of the egg stage was not affected by any of the treatments (F = 0.01; df = 6; P = 1.00), with an average of developmental time of 4.6 days for eggs from all treatments (Table 2). This result differ from those obtained by Carvalho *et al*. (2002), who observed an increase in the embryonic period when they used endosulfan at a dose of 1.05 g a.i./liter of water, applied on eggs of *C. externa.* This difference may be due to application methodology, Carvalho *et al.* (2002) used a sprayer adjusted to deliver a volume of 1.7 ± 0.5 ml/cm^2, which was higher than the volume used in our research.

Egg viability was significantly reduced by chlorpyrifos, sulfur and copper oxychloride (F = 1.96; df = 6; P = 0.048), with viability means of 80.0%; 82.5% and 82.5%, respectively, compared with endosulfan, azociclotin, betacyfluthrin and control, which showed averages of the 92.5%; 92.5%; 95.0% and 95.0%, respectively (Table 2). Even with significant differences, egg viability in all treatments was high, similar to studies of Grafton-Cardwell and Hoy (1985), who reported that egg and pupa stages of lacewings are the most tolerant to phytosanitary products.

These results are also similar to that obtained by Godoy *et al.* (2004), who applied the pyrethroid deltamethrin at a dosage of 0.0125 g a.i./liter of water and had mean of 76.7% for egg viability of *C. externa*. They are also in agreement with observations of Carvalho *et al*. (1998) who used growth regulator products, they found viabilities of eggs ranging from 76.6% to 96.6%, and those of Carvalho *et al*. (2002) who applied endosulfan, esfenvalerate, fenpropathrin, trichlorfon and triflumuron in eggs of *C. externa* and found egg viability from 73.3% to 90%.

Viability of eggs treated with the acaricide azociclotin, was 92.5%, similar result to that obtained by Mattioli *et al*. (1992), who used the acaricide fembutatina oxide on eggs of *Ceraeochrysa cubana* (Hagen, 1861) (Neuroptera: Chrysopidae) and observed a viability of 86.8%. For treatment with sulfur, the viability was 82.5%, confirming results obtained by Moraes and Carvalho (1993) who applied sulfur at the same dosage on the eggs of *C. cubana.*

The duration of the larval period and survival of larvae were not affected by the products and the averages ranged from 8.5 to 8.8 days (F = 0.85; df = 6; P = 0.538) and 87.5% to 100.0% (F = 1.50; df = 6; P = 0.192), respectively (Table 2). The products betacyfluthrin and sulfur were responsible for lower survival of first instar larvae, with averages of

Table 4. *Chrysoperla externa* mortality (%), average number of eggs / female, egg viability (%) and total effect on the remaining females (E), followed by toxic class of compounds applied to eggs of green lacewing.

Treatment	Initial number of eggs	M%[1]	Mc%[2]	R'[3]	R"%[4]	E%[5]	Class[6]
Endosulphan	40	12.5	2.8	14.1	93.8	15.22	1
Chlorpyriphos	40	25.0	16.7	13.8	95.0	27.53	1
Betacyfluthrin	40	22.5	13.9	15.7	93.5	16.46	1
Sulphur	40	12.5	2.8	16.2	92.0	23.02	1
Azociclotin	40	20.0	11.1	15.0	94.3	17.27	1
Copper oxychloride	40	20.0	11.1	15.0	90.7	25.61	1
Control	40	12.5	–	15.9	95.2	–	–

[1] Mortality (%) accumulated obtained during of predator development. [2] Mortality (%) corrected by the formula of Abbott (1925). [3] Average number of eggs/female/day. [4] Viability (%) of eggs collected over four consecutive weeks. [5] Total effect of the treatments during of predator development, where E = 100% - (100% - M%) x R1 x R2. [6] Classe toxicity proposed by members of IOBC (Hassan, 1997), as follows: class 1 = harmless (E < 30%).

Table 5. Pre-oviposition period (in days), daily and total oviposition, and viability (%) (± SE) of eggs of *Chrysoperla externa* adults, from eggs sprayed with some pesticides.

	Pré-oviposition period[1]	Oviposition[2]		Viability[1]
		Daily	Total	
Endosulphan	5.1 ± 0.15 a	11.8 ± 0.19 b	366.0 ± 1,32 b	93.4 ± 1.12 a
Sulphur	5.1 ± 0.25 a	14.5 ± 0.16 a	428.1 ± 0,68 a	92.7 ± 0.41 a
Azociclotin	5.3 ± 0.17 a	11.8 ± 0.10 b	357.1 ± 0,89 b	91.1 ± 0.69 a
Copper oxychloride	4.8 ± 0.19 a	15.0 ± 0.12 a	430.9 ± 0,40 a	90.9 ± 0.51 a
Control	4.8 ± 0.21 a	15.5 ± 0.09 a	440.0 ± 0,38 a	95.6 ± 0.48 a
General mean	5.02	13.72	404.2	92.74
F test	0.162	2.519**	2.544**	2.465
CV (%)	11.96	12.68	12.49	4.26

[1] Means followed by the same letter in column do not differ by F test at 5%. [2] Means followed by the same letter in column do not differ by Scott and Knott test ($P < 0.05$).

87.5% and 92.5%, respectively, differing significantly from the other treatments, including the control (100%) survival ($F = 2.40$; df = 6; $P = 0.037$) (Table 3). This may have occurred because this compound has a high residual action, and thus, some larvae when hatched were contaminated by their residues in the chorion (Godoy *et al*. 2004). However, it did not affect the duration of this instar ($F = 7.14$; df = 6; $P = 0.072$). These results resemble those of Godoy *et al.* (2004) who, by applying deltamethrin in eggs of *C. externa*, at a dosage of 0.0125 g a.i. / liter of water, found a significant decrease on first instar larval survival.

The second instar larvae were not affected by the treatments, with an average duration of three days and survival rate of 96.7% for the control; 97.5% for azociclotin and 100.0% for the other treatments ($F = 0.01$; df = 6; $P = 1.00$) (Table 3). The same occurred for the duration of the third instar, where the average period ranged from 2.4 to 2.7 days and mean survival was 97.5% for chlorpyrifos, 100% for the other treatments and for the control 96.7%. Similar results were observed by Godoy *et al.* (2004), where fembutatine oxide was sprayed on *C. externa* eggs at a dose of 0.4 g a.i. / liter of water resulted in 95.0% and 90.0% survival for the second and third larval instars, respectively, and deltamethrin at 0.0125 g a.i. / liter of water, resulting in 100.0% of survival for the two larval instars.

For pupae originating from treated eggs, a toxic effect of products was not found ($F = 2.09$; df = 6; $P = 0.066$), showing duration of 10 to 10.3 days and survival ranging from 92.5% to 100.0% (Table 2).

The sex ratio of adult *C. externa* was not affected by the treatments ($F = 0.68$; df = 6; $P = 0.659$), and ranged from 0.39 to 0.52 (m:f) (Table 2). Although no significant differences occurred, treatments based on betacyfluthrin and azociclotin provided lower values for the sex ratio (> number of males) trends that were also observed by Godoy *et al.* (2004), when treating *C. externa* eggs with deltamethrin.

The products chlorpyrifos and betacyfluthrin presented mortality of 25.0 e 22.0% respectively. 20.0% of mortality was found for adults sprayed with azociclotin and copper oxychloride and only 12.5% of mortality for those treated with endosulfan, sulfur and the control (Table 4).

Taking into consideration the total effect (E) of the compounds on the development stages of *C. externa*, all compounds were classified as class 1 = harmless ($E < 30\%$) (Table 4). Similar results were obtained by Carvalho *et al*. (2002), when spraying eggs of *C. externa* with endosulfan and also with pyrethroids esfenvalerate and fenpropathrin. Godoy *et al.* (2004), when evaluating the total effect (E) of deltamethrin applied on eggs of *C. externa* has framed this product as class 2 = slightly harmful ($30 \leq E \leq 79\%$), disagreeing with the total effect (E) of the pyrethroid betacyfluthrin that this work categorized as class 1. This disagreement, though small, may have been due to tolerance differences of *C. externa* to insecticides of the pyrethroid group, as reported by Grafton-Cardwell and Hoy (1985), which may have been evidenced in our work.

Evaluating the reproductive capacity of survival pairs of *C. externa* , it was found that the products used did not affect the pre-oviposition period, which ranged from 4.8 to 5.3 days ($F = 0.162$; df = 6; $P = 0.096$) (Table 5). These results are similar to those obtained by Figueira *et al*. (2002) who studied the biology of *C. externa* adult; they observed duration of 11.5 days for the pre-oviposition period of females fed with the same diet used in this study.

For the daily mean oviposition in the period evaluated it was observed that females of *C. externa* from eggs sprayed with endosulfan and azocyclotina showed lower daily oviposition, with an average of 11.8 eggs / female / day ($F = 2.51$; df = 6; $P = 0.045$) compared to those sprayed with sulfur, copper oxycloride and water (control), which presented means of 14.5, 15.0 and 15.5 eggs / female / day ($F = 2.51$; df = 6; $P = 0.045$) respectively (Table 5). These results were similar to Figueira *et al*. (2002), who obtained an average of 18.5 eggs/female/day for adults of *C. externa* from no sprayed eggs and adults fed with the same diet.

Endosulfan and azocyclotine did not affect the adult survival, yet somehow influenced reproduction, reducing fecundity ($F = 1.86$; df = 6; $P = 0.032$). These results are similar to those of Ulhôa *et al*. (2002), who found a reduction in oviposition capacity of *C. externa* after application of triflumuron at a dose of 0.038g a.i. L-1 of water. Velloso *et al*. (1999) evaluated growth regulators insecticides on adults of the same green lacewing, they found that buprofezin, cyromazine and pyriproxifen did not affect neither the ability of oviposition nor egg viability, demonstrating that these compounds may act differently in the physiology of adults of this species, compared to the compound triflumuron evaluated by Ulhôa *et al*. (2002).

Egg viability was not affected by any product, with averages ranging from 90.7 to 95.2% (F = 2.46; df = 6; P = 1.00) (Table 4), being similar to the results of Figueira *et al*. (2002) who obtained 87.7% of viability for eggs of *C. externa* that received no spray and was fed with the same diet. Ribeiro *et al*. (1991) also observed similar viability (95.4%) for eggs of the same species, which has not received any spray and was fed with the same diet.

Conclusion

On the toxicity tests in the laboratory, the insects are subject to the maximum exposure to residues of the commercial formulations of pesticides. This is the first step of the sequence of tests recommended by the IOBC / WPRS (Hassan *et al*. 2000). According to classes of toxicity established by the IOBC / WPRS, the insecticide endosulfan (1.75 g/L), chlorpyriphos (1.20 g/L), betacyfluthrin (0.013 g/L), Sulfur (4.00 g/L), copper oxychloride (5.00 g/L) and azociclotin (0.31 g/L) were classified as class 1, selective to eggs of *C. externa* and do not affect the subsequent developmental stages. These products can be recommended for pest management programs in coffee growing in association with this predator seeking its preservation under natural conditions.

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