PHYSIOLOGICAL SELECTIVITY OF INSECTICIDES TO EGGS AND LARVAE OF PREDATOR *Chrysoperla externa* (HAGEN) (NEUROPTERA: CHRYSOPIDAE)

Andrea de Fátima Torres Soares[1], Geraldo Andrade Carvalho[2]

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ABSTRACT: Given the importance of green lacewings as agents of biological pest control, the present study aimed to evaluate the toxicity of insecticides used on coffee crops on the eggs and larvae of *Chrysoperla externa*. The insecticides tested were (g or mL a.i./L) chlorpyrifos (2.25), cartap hydrochloride (1.66), pyriproxyfen (0.33), profenofos/lufenuron (1.33/0.13), fenpropathrin (0.40), triazophos/deltamethrin (0.70/0.02) and zetacypermethrin (0.05). The insecticides, when applied directly on the eggs, caused no adverse effects on the duration of the embryonic period. After the application of triazophos/deltamethrin, pyriproxyfen, profenofos/lufenuron and zetacypermethrin, a reduction in egg viability was induced. The insecticides triazophos/deltamethrin, chlorpyrifos, and profenofos/lufenuron reduced the survival of newly hatched first instar larvae from treated eggs. The first instar larvae that were treated directly were sensitive to the effects of the products used, with the effect of triazophos and chlorpyrifos/deltamethrin being high. The survival of the second instar larvae was reduced by zetacypermethrin, fenpropathrin, profenofos/lufenuron, and cartap hydrochloride. The products chlorpyrifos and triazophos/deltamethrin also did not allow second instar larvae survival. For treated third instar larvae, chlorpyrifos and triazophos/deltamethrin allowed survival of only 20.0 and 57.5%. Eggs and larvae of *C. externa* showed sensitiveness to insecticides chlorpyrifos and triazophos, being needed more studies in semi-field and field conditions for the confirmation or not of the toxicity aiming the conservation of this predator specie on the coffee agroecosystem.

Index terms: *Coffea arabica*, natural enemy, plant protection, side effects.

SELETIVIDADE FISIOLÓGICA DE INSETICIDAS PARA OVOS E LARVAS DO PREDADOR C*hrysoperla externa* (HAGEN) (NEUROPTERA: CHRYSOPIDAE)

RESUMO: Diante da importância dos crisopídeos como agentes de controle biológico de pragas, o presente estudo teve como objetivo avaliar a toxicidade de inseticidas utilizados na cultura cafeeira sobre ovos e larvas de *Chrysoperla externa*. Os inseticidas testados foram (g ou mL i.a./L) clorpirifós (2,25), cloridrato de cartape (1,66), piriproxifem (0,33), profenofós/lufenurom (1,33/0,13), fenpropatrina (0,40), triazofós/deltametrina (0,70/0,02) e zetacipermetrina (0,05). Os produtos fitossanitários aplicados diretamente sobre ovos não causaram efeitos negativos sobre a duração do período embrionário. Após a aplicação de triazofós/deltametrina, piriproxifem, profenofós/lufenurom e zetacipermetrina ocorreu redução na viabilidade dos ovos. Os produtos triazofós/deltametrina, clorpirifós e profenofós/lufenurom provocaram diminuição na sobrevivência de larvas de primeiro instar recém-eclodidas, oriundas de ovos tratados. As larvas de primeiro instar diretamente tratadas foram sensíveis aos efeitos dos produtos utilizados, sendo que clorpirifós e triazofós/deltametrina foram mais tóxicos. A sobrevivência das larvas de segundo instar tratadas foi reduzida por zetacipermetrina, fenpropatrina, profenofós/lufenurom e cloridrato de cartape. Os produtos clorpirifós e triazofós/deltametrina também não permitiram que larvas de segundo instar tratadas sobrevivessem. Para larvas de 3° instar tratadas, clorpirifós e triazofós/deltametrina permitiram a sobrevivência de apenas 20,0 e 57,5%. Ovos e larvas de *C. externa* mostraram-se sensíveis aos inseticidas clorpirifós e triazofós/deltametrina, necessitando de novos estudos em condições de semicampo e campo para confirmação ou não da toxicidade, visando à conservação dessa espécie de predador no agroecossistema cafeeiro.

Termos para indexação: *Coffea arabica,* inimigo natural, proteção de plantas, efeitos colaterais.

1 INTRODUCTION

Although the average productivity of coffee plantations is around 48.64 bags of coffee per hectare in some regions, the average Brazilian productivity is around 28.41 bags per hectare (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2018). Several factors contribute to this drastic difference, among which the most prominent are pests and diseases that attack the crop and cause productivity losses, reduced grain quality, and depletion of plants (POZZA et al., 2010; SILVA et al., 2010).

The coffee agroecosystem hosts a great diversity of arthropod pests such as the coffee leaf miner *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae), coffee berry borer *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytidae), cicada *Quesada gigas* (Olivier, 1790) (Hemiptera: Cicadidae), red coffee mite *Oligonychus ilicis* (McGregor, 1919) (Acari: Tetranychidae), broad mite *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae), false spider mite *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae), among other species (MESQUITA et al., 2016; SILVA et al., 2010; SOUZA; REIS; SILVA, 2007).

[1,2]Universidade Federal de Lavras/UFLA - Departamento de Entomologia/DEN - Cx. P. 3037 - 37.200-000 - Lavras - MG andreaftorres@den.ufla.br, gacarval@den.ufla.br

To control these insect pests, pesticides with high toxicity and broad-spectrum action have been frequently used, which is a major cause of biological imbalances in the agroecosystems. Furthermore, this has caused phenomena such as upwelling and selection of resistant insect-pests populations. In this context, preservation of the natural enemies of these insect pests is one of the most important practices in integrated pest management of coffee crop. The association between chemical and biological methods of control is possible only for pesticides that present selectivity to natural enemies, either physiological or ecological (BUENO et al., 2017; PARRA; REIS, 2013; RIGITANO; CARVALHO, 2001).

Among the various existing species of predator insects, those belonging to the family Chrysopidae play an important role as regulators of the populations of agricultural pests. The green lacewings are predators with high reproductive capacity and great voracity and may feed on the eggs and small caterpillars of moths, aphids, mealybugs, whiteflies, psyllids, among others (AUAD et al., 2007; BARBOSA et al., 2008; GONÇALVES-GERVÁSIO; SANTA-CECÍLIA, 2001; PITWAK; MENEZES JR; VENTURA, 2016). There are reports that *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) is effective for the control of *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae), *L. coffeella* and phytophagous mites on trees, and can be used to regulate the population of these pests (BEZERRA et al., 2006; ECOLE et al., 2002; SILVA et al., 2006).

Given the importance of green lacewings as agents of biological pest control, the present study aimed to evaluate the toxicity of insecticides used in coffee crop on the eggs and larvae of the predator *C. externa*.

2 MATERIAL AND METHODS

The experiments were performed in the laboratory at temperature of 25 ± 2 °C, RH 70±10%, and photophase of 12 h.

The products used are shown in Table 1. The control treatment consisted only of water. The experimental study was a completely randomized design with 8 treatments and 10 replicates, with each plot consisting of 4 eggs or larvae.

Effect of insecticides on eggs of *C. externa*

Eggs of about 24 h old and obtained from the maintenance building were placed in 15 cm diameter Petri dishes for treatment with the products. The compounds were sprayed directly on the eggs using a Potter tower with an application volume of 1.5 ± 0.5 mg/cm^2 and pressure of 15 lb/in^2. Then, the eggs were placed on individual glass tubes of 2.5 cm diameter x 8.5 cm height, and the tubes were sealed using laminated polyvinyl chloride (PVC) film. The surviving larvae that originated from the treated eggs were fed with eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) every day ad libitum.

The parameters evaluated were egg viability (%), duration of embryonic period (days), survival (%) and duration of larval instars (days) and duration of pupae (days).

TABLE 1 - Trade name, active ingredient, chemical group, doses of insecticides and concentrations of the active ingredients of compounds registered for control of *Leucoptera coffeella* in coffee crop (Sistema de agrotóxicos fitossanitários - AGROFIT, 2009), tested in eggs and larvae of *Chrysoperla externa*, under laboratory conditions.

Trade name	Active ingredient	Chemical group	Doses [1]	Concentrations[2]
Astro	Chlorpyrifos	Organophosphate	1.5	2.25
Cartap BR 500	Cartap hydrochloride	Thiocarbamate	1.0	1.66
Cordial 100	Pyriproxyfen	Ether piridiloxipropílico	1.0	0.33
Curyom 550 CE	Profenofos/ Lufenuron	Organophosphate/ Benzoylureas	0.8	1.33/ 0.13
	Fenpropathrin	Pyrethroid	0.4	0.40
Danimen 300 CE	Triazophos/ Deltamethrin	Organophosphate/ Pyrethroid	0.6	0.70/ 0.02
Deltaphos CE	Zetacypermethrin	Pyrethroid	0.04	0.05

[1] Doses (L or kg c.p.ha^{-1}); [2]Concentrations (g or mL a.i.L^{-1} water).

Effect of insecticides on the larvae of *C. externa*

Approximately 24h after hatching or instar change, larvae of the first or second or third instars for each treatment were placed in 15 cm diameter Petri dishes. The products were sprayed directly on the larvae by using the same methodology applied earlier in the egg stage of the predator. Survivors of the treated larvae were fed ad libitum every other day with eggs of *A. kuehniella*.

We evaluated the duration (days) and survival of larval instars (through the presence of exuviae in Petri dishes) and pupae.

Statistical analysis

Data were submitted to Shapiro-Wilk and Bartlett tests in order to verify normality of distribution and homoscedasticity. On confirmation of these assumptions, data were submit ted to analysis of variance (ANOVA), and means were compared using the Scott-Knott test (SCOTT; KNOTT, 1974). In all tests, the level of statistical significance was set at 5%.

3 RESULTS AND DISCUSSION

Side effects of insecticides on eggs of the *C. externa*

The predator eggs treated using pyriproxyfen showed an embryonic period shorter than that for other insecticide treatments (Table 2). Despite the fact that this reduction was statistically significant, the difference while using pyriproxyfen when compared with the control was only 9.6 h, which does not prevent the use of the product in integrated pest management programs, because a short embryonic period can be advantageous. This is because green lacewing eggs are easy prey for other insects. For other compounds, there were no differences in the duration of the embryonic period, which was similar to the results shown by Carvalho et al. (2002) and Vilela et al. (2010a), who treated the eggs of *C. externa* using fenpropathrin at concentrations of 0.09, 0.15, and 0.3 g a.i./L of water, and Ferreira et al. (2005), who assessed the effect of the etofenprox (pyrethroid) and organophosphate chlorpyrifos, both at doses of 150 g c.p./100 L of water.

The viability of eggs was reduced with the usage of triazophos/deltamethrin, pyriproxyfen, profenofos/lufenuron and zetacypermethrin. Treatments using the other insecticides showed a high survival rate (Table 2).

Although the use of some insecticides reduced the egg viability, the morphology of the chorion of green lacewing eggs can hinder the penetration of the chemical molecules, thereby not affect neither the embryonic period nor embryos' survival.

This result is similar to that in the study performed by Grafton-Cardwell and Hoy (1985), which showed that the egg and pupal stages of green lacewings are more tolerant to pesticides. Further, the decrease in viability after the use of juvenoid ether piridiloxypropyl (pyriproxyfen) might be because the embryogenesis was affected, besides suppressing metamorphosis and prolonging the larval period (BUENO et al., 2017; FARIA, 2009).

The reduction in viability of *C. externa* eggs caused by triazophos/deltamethrin, pyriproxyfen, profenofos/lufenuron and zetacypermethrin can be related to the octanol-water partition coefficient (log K_{ow}) of these insecticides (3.34/6.20, 5.5, 4.82/5.12 and 6.6, respectively). High log K_{ow} values implies in higher lipophilicity, facilitating the penetration of a larger amount of the product through the chorion and its translocation to action sites (FERNANDES et al., 2010).

Studies by some authors corroborate the results presented in this work, such as those obtained by Carvalho et al. (2002), which showed a viability of 73.3% when they applied fenpropathrin (0.09 g a.i./L of water) on the eggs of *C. externa*. Vilela et al. (2010a) observed 85.0 and 70.0% viability, respectively, when they tested fenpropathrin at concentrations of 0.15 and 0.30 g a.i./L of water. Godoy et al. (2004) observed a viability of 76.6% for predator eggs treated using deltamethrin (0.0125 g a.i./L of water) and Rimoldi et al. (2008) verified an average of 96.7% viability using cypermethrin (0.025 g a.i./L of water). Preetha et al. (2009) found 81.5% viability of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) eggs treated using the organophosphorus monocrotophos (2 mL c.p./L of water); Gandhi et al. (2005) showed that the use of imidacloprid (350 g c.p./L of water) and endosulfan (350 g c.p./L of water) exhibited viabilities of 43.5 and 55.0 %. The toxic effects of fifteen insecticides, at their highest recommended concentrations for wheat crop, were evaluated by Pasini et al. (2018), who concluded that only etofenprox and imidacloprid + beta-cyflutrin reduced the viability of *C. externa* eggs in 40 and 70%.

TABLE 2 - Duration of the embryonic period (days) and viability (%) (±SE) of treated eggs of *Chrysoperla externa*. Temperature of 25 ± 2°C, RH 70 ± 10% and photophase of 12 hours.

Treatment	Embryonic period (days)	Viability (%)
Chlorpyrifos	5.3 ± 0.14 a	87.5 ± 3.95 a
Cartap hydrochloride	5.4 ± 0.16 a	90.0 ± 3.88 a
Pyriproxyfen	4.8 ± 0.19 b	80.0 ± 5.92 b
Profenofos/Lufenuron	5.3 ± 0.16 a	80.0 ± 5.92 b
Fenpropathrin	5.2 ± 0.12 a	95.0 ± 4.74 a
Triazophos/Deltamethrin	5.1 ± 0.17 a	70.0 ± 6.89 b
Zetacypermethrin	5.3 ± 0.15 a	82.5 ± 5.02 b
Control	5.2 ± 0.14 a	92.5 ± 3.62 a
CV (%)	3.64	9.95

Means followed by the same letter in the column are not statistically different according to the Scott-Knott test ($P < 0.05$).

Although the use of insecticides allowed a large number of eggs to hatch, only 22.5, 27.5, and 12.5% of larvae survived from the eggs treated using chlorpyrifos, profenofos/lufenuron and triazophos/deltamethrin, respectively (Table 3). The larval mortality was probably because of the presence of waste products in the chorion, which may have been the point-of-contact of the *C. externa* larvae at the time of hatching. Normally, organophosphates are highly toxic to insects, because they inhibit the action of the enzyme acetylcholinesterase through its structural conformation of molecules that allow them to occupy the docking site on the enzyme via the phosphate group. The hydrolysis of the phosphorylated enzyme occurs very slowly, resulting in the accumulation of molecules of acetylcholine in the synapse, causing the insect to die by hyperexcitation of the nervous system (OMOTO, 2000). Rugno, Zanardi and Yamamoto (2015) applied insecticides on *Ceraeochrysa cubana* (Hagen, 1861) (Neuroptera: Chrysopidae) eggs and found that newly hatched larvae exposed to chlorpyrifos-contaminated chorion presented less than 60% survival.

High mortality of first instar larvae of *C. externa* from the treated eggs was observed by Bueno and Freitas (2001, 2004) and Rimoldi et al. (2008), when they applied cypermethrin (0.025 g a.i./L of water), imidacloprid (3.5, 7.0, 10.5, 14.0, 17.5 and 21.0 g a.i./100 L water), and lufenuron (2.50, 3.75, 5.0, 6.25, 7.5 and 10.0 g a.i./100 L water) and found no surviving larvae. Godoy et al. (2004) assessed the toxicity of deltamethrin (0.0125 g a.i./L of water) for this predator and showed that only 38.3% of the newly hatched larvae survived.

Apart from the first instar larvae, the other larval instars were not affected by the products tested (Table 3), thus confirming the results obtained by Godoy et al. (2004) regarding the effects of the insecticides thiacloprid, deltamethrin, lufenuron, tebufenozide, fenbutatin oxide and abamectin (0.0360, 0.0125, 0.0375, 0.1200, 0.4000 and 0.0054 g a.i./L of water, respectively) on *C. externa* eggs that none of the compounds reduced the survival of the second and third instar larvae. Similar results were observed by Vilela et al. (2010a) for the products (g a.i./L of water) spirodiclofen (0.12), fenpropathrin (0.15 and 0.3), sulfur (4.0 and 8.0) and abamectin (0.0067 and 0.0225). The survival of the larvae may have been because the molecules of the products used were degraded by the insects through various metabolic processes, by which the products were converted into nontoxic forms or even quickly eliminated from the body of the insect. Further, various enzymes and enzyme systems may be involved, such as esterases, oxidases and transferases (HEMINGWAY, 2000).

The application of insecticides had no detrimental effect on the duration of the larval instars. Additionally, the compounds showed no negative effect on the pupal stage (Table 3).

Effect of products on the first instar larvae of the predator

The products chlorpyrifos and triazophos/deltamethrin were highly toxic to the treated first instar larvae. Moreover, the use of other products decreased the larval survival rates for profenofos/lufenuron, fenpropathrin, zetacypermethrin, cartap hydrochloride and pyriproxyfen, thus indicating that the first larval stage of green lacewings, in general, is more sensitive to chemical compounds (Figure 1).

TABLE 3 - Duration (days) and survival (%) (± SE) of three larval instars and pupal stage of *Chrysoperla externa*, from eggs treated with the insecticides (n=40). Temperature of 25 ± 2°C, RH 70 ± 10% and photophase of 12 hours.

Treatment	First instar		Second instar	
	Duration**	Survival*	Duration**	Survival**
Chlorpyrifos	4.3 ± 0.21	22.5 ± 7.39 b	3.5 ± 0.19	95.0 ± 4.74
Cartap hydrochloride	4.1 ± 0.15	70.0 ± 7.07 a	3.5 ± 0.17	100.0 ± 0.00
Pyriproxyfen	4.3 ± 0.19	62.5 ± 12.9 a	3.6 ± 0.16	97.5 ± 2.37
Profenofos/Lufenuron	4.5 ± 0.23	27.5 ± 8.20 b	3.5 ± 0.24	95.0 ± 4.74
Fenpropathrin	4.1 ± 0.15	67.5 ± 4.15 a	3.7 ± 0.16	100.0 ± 0.00
Triazophos/Deltamethrin	4.6 ± 0.17	12.5 ± 4.15 b	3.0 ± 0.00	100.0 ± 0.00
Zetacypermethrin	4.6 ± 0.18	62.5 ± 4.15 a	3.4 ± 0.18	100.0 ± 0.00
Control	4.4 ± 0.17	92.5 ±4.15 a	3.6± 0.20	100.0 ± 0.00
CV (%)	4.96	24.55	5.22	4.76
Treatment	Third instar		Pupae	
	Duration**	Survival**	Duration**	Survival**
Chlorpyrifos	4.1 ± 0.13	100.0 ± 0.00	10.1 ± 0.20	97.5 ± 2.37
Cartap hydrochloride	4.2 ± 0.24	100.0 ± 0.00	10.1± 0.11	92.5 ± 3.62
Pyriproxyfen	4.6 ± 0.27	100.0 ± 0.00	10.0 ± 0.12	95.0 ± 4.74
Profenofos/Lufenuron	4.9 ± 0.19	95.0 ± 4.74	10.1 ± 0.19	97.5 ± 2.37
Fenpropathrin	4.6 ± 0.21	100.0 ± 0.00	10.0 ± 0.13	100.0 ± 0.00
Triazophos/Deltamethrin	4.6 ± 0.21	100.0 ± 0.00	10.0 ± 0.27	100.0 ± 0.00
Zetacypermethrin	4.1 ± 0.17	100.0 ± 0.00	10.0 ± 0.17	97.5 ± 1.67
Control	4.2 ± 0.22	100.0 ± 0.00	10.2 ± 0.26	97.5 ± 2.37
CV (%)	6.14	3.25	2.55	4.40

*Means followed by the same letter in the column are not statistically different according to the Scott-Knott test ($P < 0.05$).

** Not statistically difference ($P > 0.05$).

In this case, the product chlorpyrifos acted by contact and prevented the degradation of acetylcholine by acetylcholinesterase inhibition, causing neurological disorders. For pyrethroids, such as deltamethrin, fenpropathrin and zetacypermethrin, it may have been that the insecticide molecules were positioned in some units of the binding site of the sodium ion channels, which thereby stayed open for long periods, increasing the influx after an action potential and triggering death by nervous hyperexcitation (OMOTO, 2000; RIGITANO; CARVALHO, 2001).

According to Fernandes et al. (2010), penetration rate of insecticides through the integument of the insect depends on its affinity with insecticides, physiological factors and chemical composition of products. The lipophilicity of insecticides is inversely proportional to their solubility in water, therefore more lipophilic compounds may have different penetration rates. In the case of first-instar larvae, another factor that may have influenced the penetration of chemical compounds is the thickness of the cuticle (CHAPMAN, 2013). The newly hatched larvae (about 24 hours) evaluated in this study were very small and their cuticle have low thickness.

Several studies have shown the toxic effects of compounds used directly on first instar larvae. Maroufpoor et al. (2010) evaluated the toxicity of spinosad on *C. carnea* first instar larvae and observed a larval mortality rate of about 70.0% at the highest tested concentration (2500 ppm).

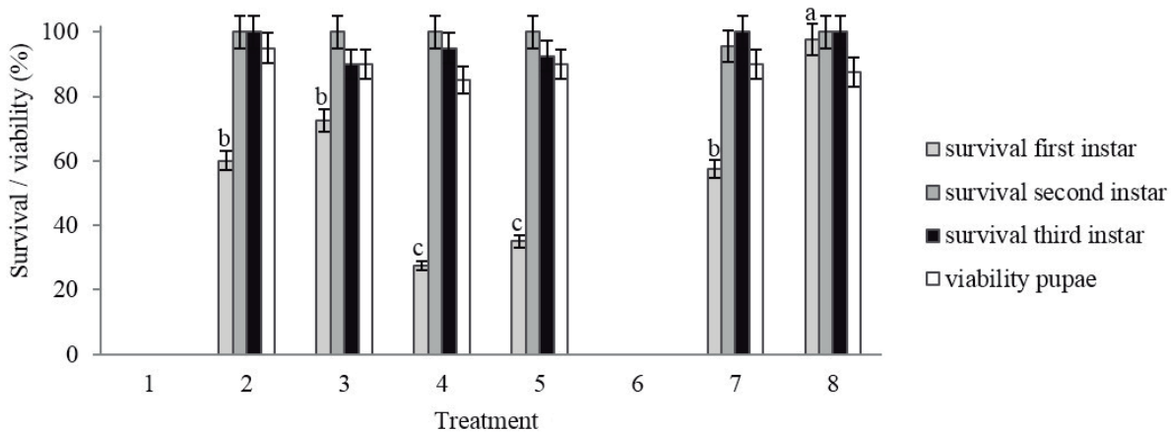

FIGURE 1 - Survival of three larval instars and viability pupal (%) (± SE) stage of *Chrysoperla externa*, from first instar larvae treated with the insecticides. Treatments: 1: chlorpyrifos; 2: cartap hydrochloride; 3: pyriproxyfen, 4: profenofos/lufenuron; 5: fenpropathrin; 6: deltamethrin/triazophos; 7: zetacypermethrin and 8: control.

The juvenile hormone analogue (pyriproxyfen) were highly harmful to *C. cubana* first instar larvae, causing 100% mortality before they reach the pupal stage (ONO et al., 2017). Silva et al. (2005) observed 100% mortality of first instar larvae of *C. externa* when using chlorpyrifos (1.2 g a.i./L of water) and beta-cyfluthrin (150 g a.i./100 L of water). Additionally, Ferreira et al. (2006) showed 100% mortality in predators treated using chlorpyrifos (0.72 g a.i./L of water). Vilela et al. (2010b) verified that treatments using fenpropathrin (0.15 and 0.3 g a.i./L of water) did not allow the survival of any *C. externa* larvae, and similar results were obtained by Moura et al. (2012) when *C. externa* larvae were treated (g a.i./L) using trichlorfon (1.5), carbaryl (1.73), fenitrothion (0.75) and methidathion (4.0).

The first instar larval duration was not reduced by treatment using all the products, and no differences were observed between treatments for the survivors of the second and third instar larvae (Figure 2). The averages were similar to those obtained for larvae of *C. externa* that fed on different prey, which were not contaminated by chemicals (AUAD et al., 2007; COSTA et al., 2002).

Both duration and survival of pupae from the treated first instar larvae were not adversely affected by the usage of the different insecticides (Figure 2).

Effect of the insecticides on the second instar predator larvae

Similarly as occurred to first instar larvae, chlorpyrifos and triazophos/deltamethrin caused 100% mortality of treated second instar larvae (Figure 3), confirming the results obtained by Silva et al. (2005) when using chlorpyrifos (1.2 g a.i./L of water) on the second instar larvae of *C. externa*. The second instar larval survival was low when they were treated using zetacypermethrin, fenpropathrin, profenofos/lufenuron and cartap hydrochloride (Figure 3), thus showing that in addition to sensitivity toward organophosphates and pyrethroids, the second instar larvae of *C. externa* also showed sensitivity to another group of chemical neurotoxic insecticides, thiocarbamates, which are acetylcholine antagonists and prevent the transmission of nerve impulses in the synapse, causing rapid paralysis of the insect and death (OMOTO, 2000).

The higher lipophilicity presented by chlorpyrifos and triazophos/deltamethrin probably favored their the penetration through the cuticle of the larvae, favoring their translocation to the site of action of the insecticides (FERNANDES et al., 2010).

Carvalho et al. (2003) observed the median survival of the second instar larvae of *C. externa* at around 43.3% and 10.0%, respectively, 96 h after the application of fenpropathrin (0.125 g a.i./100 mL of water) and trichlorfon (0.020 g a.i./100 mL of water). In their study, treatment by using chlorpyrifos ensured that no larvae survived.

Although pyriproxyfen acts as an agonist of juvenile hormone, and its action is more pronounced in the last instar of insect development, it had no effect on the treated larvae (FARIA, 2009; FERREIRA, 1999). Velloso et al. (1999) showed that pyriproxyfen (0.1 g a.i./L of water) was toxic to *C. externa*, thereby ensuring that none of the treated second instar larvae survived.

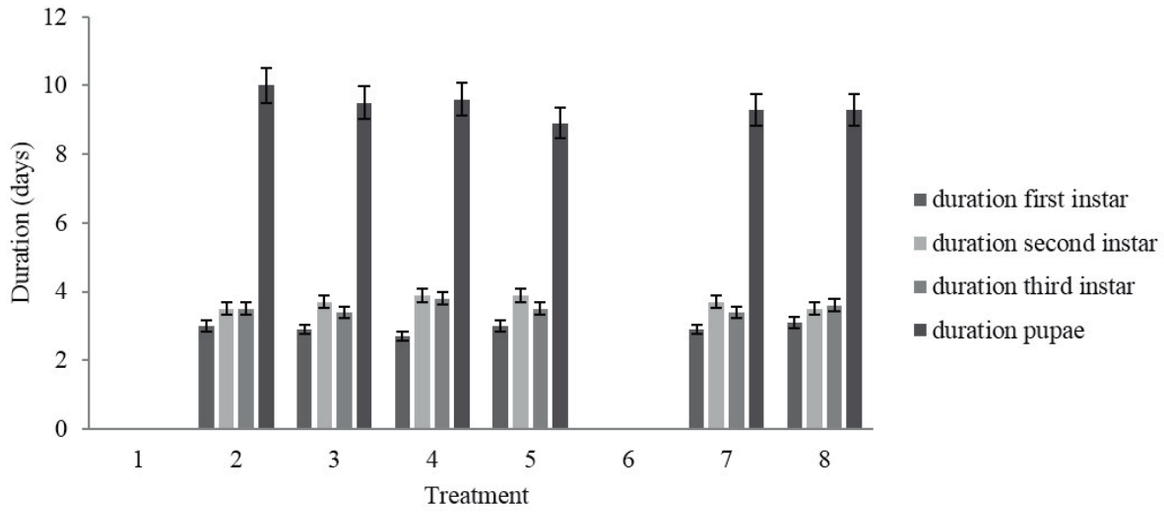

FIGURE 2 - Duration (days) of three larval instars and pupal stage of *Chrysoperla externa*, from first instar larvae treated with the pesticides. Treatments: 1: chlorpyrifos; 2: cartaphydrochloride; 3: pyriproxyfen, 4: profenofos/ lufenuron; 5: fenpropathrin; 6: deltamethrin/triazophos; 7: zetacypermethrin and 8: control.

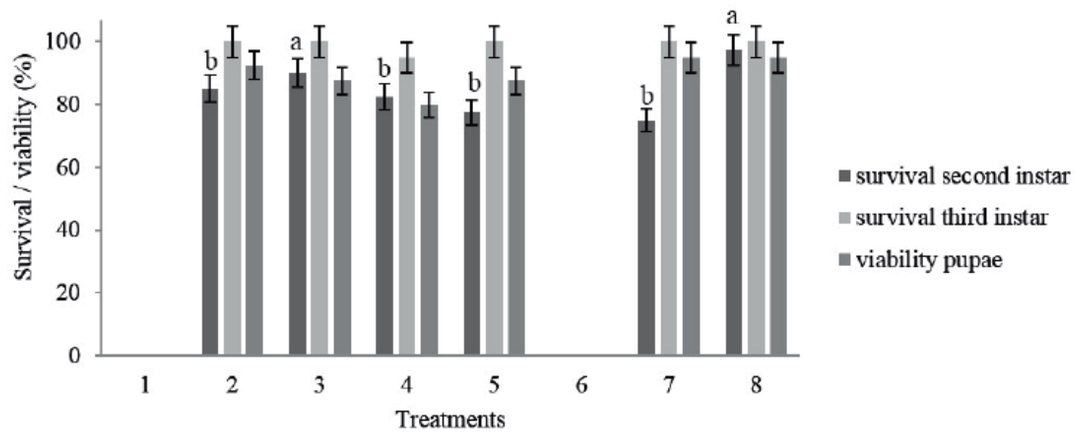

FIGURE 3 - Survival (%) (± SE) of immature stages of *Chrysoperla externa* from second instar larvae treated with the pesticides. Treatments: 1: chlorpyrifos; 2: cartap hydrochloride; 3: pyriproxyfen, 4: profenofos/lufenuron; 5: fenpropathrin; 6: triazophos/deltamethrin; 7: zetacypermethrin and 8: control.

This discrepancy can be explained by the methodology applied, since these authors sprayed compounds on the larvae that were maintained in treated Petri dishes; hence, increasing the exposure of the insects to the insecticide residues in addition to the dosage used, which was higher than that used in the present study.

Amarasekare, Shearer and Mills (2016) applied insecticides (mg a.i. L^{-1}) directly to *C. carnea* second instar larvae and found that cyantraniliprole (160.2), chlorantraniliprole (117.9), spinetoram (131.1), novaluron (388.5) and lambda -cyhalothrin (49.9) were lethal to the predator. Castilhos et al. (2017) observed, under semi-field conditions, that insecticides (% a.i.) lufenuron (0.005) proved to be harmless (class 1); deltamethrin (0.001) and malathion (0.200) were slightly harmful (class 2), and fenthion (0.05) and phosmet (0.100) moderately harmful (class 3) to second instar larvae of *C. externa*.

The surviving second instar larvae showed no significant differences in the duration of further larval instar stages. Further, the duration and survival of pupae from the treated second instar larvae were not affected by the insecticides used (Figure 4).

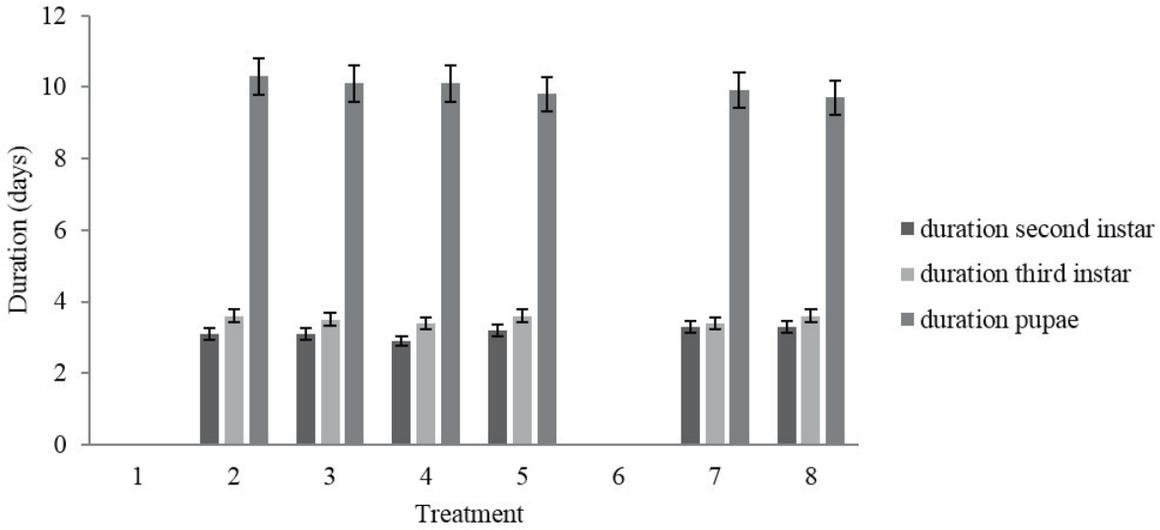

FIGURE 4 - Duration (days) of immature stages of *Chrysoperla externa* from second instar larvae treated with the insecticides. Treatments: 1: chlorpyrifos; 2: cartap hydrochloride; 3: pyriproxyfen, 4: profenofos/lufenuron; 5: fenpropathrin; 6: triazophos/deltamethrin; 7: zetacypermethrin and 8: control.

Effect of the insecticides on the third instar predator larvae

The treated third instar larvae were more tolerant to the tested insecticides, due to the survival of the insects was reduced only by treatments using triazophos and chlorpyrifos products/deltamethrin (Table 4). The same product used at the same concentration can cause different toxicity, depending on the development stage of exposed natural enemy (SOUZA et al., 2014; BUENO et al., 2017). Another factor that must be considered is that insects in advanced development stages can better support the effect of some groups of insecticides. It occurs due to increase of reserves and degradation capacity of insecticides by several metabolic processes, in which insecticide molecules are converted into non-toxic forms or even quickly eliminated from insects' body (CROFT, 1990; HEMINGWAY, 2000).

Nonetheless, Moura et al. (2011) showed 100% mortality of the third instar predator larvae to the organophosphates (g a.i./L of water), fenitrothion (0.75) and methidathion (0.4). Several factors may be associated with the discrepancies in the results such as the differences in the dosages of the insecticides used and origin of the populations of the treated green lacewings.

Regarding insect growth regulators, Bortolotti, Sbrenna and Sbrenna (2005) found that the same dose of the insecticide fenoxycarb, applied in third instar larvae of *C. carnea*, at different intervals after ecdysis (24, 48, 60 and 72 hours) had different effects. The effect on larvae survival was more severe for those treated after 60 hours of ecdysis, indicating the beginning of metamorphosis, where insects have less capacity to metabolize the insecticidal molecules. This may explain why *C. externa* larvae treated with pyriproxyfen and profenofos/lufenurom were not affected by insecticides in the present study, since they were treated with products via spraying 24 hours after ecdysis.

In general, an increased tolerance of the predator to several chemical compounds was observed during the third instar larval stage (CARVALHO et al., 2003; FERREIRA et al., 2006; GODOY et al., 2004; SILVA et al., 2005).

The duration of the third instar was not affected by treatments using any of the insecticides. Similar results were observed by Silva et al. (2005), who presented that the duration of the third instar larvae of *C. externa* after treatment using beta-cyfluthrin and pyrethroids was 3.3 days. For the duration of the pupal stage, there were no differences between treatments. Furthermore, no decrease in the rates of emergence of *C. externa* predators from the treated larvae was observed (Table 4).

TABLE 4 - Duration (days) and survival (%) (± SE) of immature stages of *Chrysoperla externa*, from third instar larvae treated with the insecticides (n=40). Temperature of 25 ± 2°C, RH 70 ± 10% and photophase of 12 hours.

Treatment	Third instar		Pupae	
	Duration**	Survival*	Duration**	Survival**
Chlorpyrifos	3.5 ± 0.13	20.0 ± 7.07 b	9.0 ± 0.12	90.0 ± 5.00
Cartap hydrochloride	4.0 ± 0.20	77.5 ± 2.16 a	9.1 ± 0.15	92.5 ± 6.50
Pyriproxyfen	4.6 ± 086	87.5 ± 4.15 a	8.8 ± 0.15	95.0 ± 4.33
Profenofos/Lufenuron	3.7 ± 0.11	82.5 ± 2.16 a	9.1 ± 0.15	97.5 ± 2.16
Fenpropathrin	3.7 ± 0.13	92.5 ± 4.15 a	8.7 ± 0.14	95.0 ± 4.33
Triazophos/Deltamethrin	4.2 ± 0.14	57.5 ± 8.93 b	9.0 ± 0.17	87.5 ± 8.20
Zetacypermethrin	4.2 ± 0.15	87.5 ± 4.15 a	8.8 ± 0.12	95.0 ± 4.33
Control	3.9 ± 0.13	95.0 ± 4.33 a	9.0 ± 0.18	97.5 ± 2.16
CV (%)	12.94	6.87	4.23	3.14

* Means followed by the same letter in the column are not statistically different according to the Scott-Knott test ($P < 0.05$).

** Not statistically difference ($P > 0.05$)

4 CONCLUSIONS

None of the tested insecticides exerted a negative effect on the duration of the embryonic period and viability of the treated eggs.

The first larval instar was more sensitive to the effects of the insecticides than those by the second and third larval instars.

The products chlorpyrifos and triazophos/deltamethrin were toxic to larvae of the first, second and third instars.

Products considered toxic to the predator should be evaluated in semi-field and field conditions to confirm their toxicity. This information is important to the conservation of *C. externa* in the coffee agroecosystem and its compatibility in IPM programs.

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