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Non-target toxicity of nine agrochemicals toward larvae and adults of two generalist predators active in peach orchards

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Abstract

Chrysoperla externa and *Coleomegilla quadrifasciata* are important biological control agents in peach orchards. However, orchard management with these predatory insects is viable only by using selective agrochemicals. The objective of this study is to evaluate the toxicity of nine agrochemicals used in peach orchards in larval and adult stages of the *C. externa* and *C. quadrifasciata* in laboratory conditions. The bioassays followed the methodologies proposed by the International Organization for Biological and Integrated Control (IOBC). Larvae and adults of *C. externa* and *C. quadrifasciata* were exposed to the dry residues of these products. Lethal and sublethal effects were evaluated in bioassays with the larval and adult stages of both predators. The agrochemicals were classified according to the IOBC guidelines. The insecticide chlorantraniliprole was harmless (class 1) to the larval stage of *C. externa* and *C. quadrifasciata*. Azadirachtin, copper 25% + calcium 10%, and deltamethrin were harmless to the adult stage of both insect species. The organophosphates fenitrothion and malathion were harmful (class 4) to both species in the larval and adult stages and should not be used in peach orchards. Therefore, this study demonstrates the importance of toxicity and the lethal and sublethal effects of these agrochemicals to better determine their compatibility with IPM in peach production.

Keywords Natural enemy · Chemical control · Biological control · *Chrysoperla externa* · *Coleomegilla quadrifasciata*

Introduction

Insect pests represent a constant challenge to peach producers because of sporadic and/or persistent occurrence of several species in orchards, causing substantial economic losses to fruit production (Nava et al. 2014). Among them, the South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) (Botton et al. 2002; Araujo et al. 2019), the Mediterranean fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Hafsi et al. 2016; Sciarretta et al. 2018), the oriental butterfly *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) (Duarte et al. 2015; Yang et al. 2016), and the corn weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) (Nava et al. 2014; Nörnberg et al. 2016) may occur in peach trees in Brazil and some of them are key peach pests in other world regions. Chemical control by spraying organophosphates and pyrethroids using a predefined schedule is the most commonly strategy used to control insect populations, due to the low cost and fast action when compared to neonicotinoids, spinosyns, benzoylureas, diacylhydrazines, diamides, and oxadiazines, especially for *A. fraterculus* and *G. molesta* (Botton et al. 2005). However, despite their efficiency these products, can cause adverse effects, including insecticide resistance, resurgence of secondary pests, and reduction or extinction of beneficial organisms populations (Desneux et al. 2007; Guedes et al. 2016; Rashidi et al. 2018), as predators (Passos et al. 2018; Rugno et al. 2018), parasitoids (Jam and Saber 2018) and pollinators (Varikou et al. 2019). In addition to lethal effects, agrochemicals may cause sublethal effects on natural enemies, including changes in insect

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physiology and behavior, insect reproduction and development, mobility and orientation, and food foraging behavior, indirectly causing insect death (Desneux et al. 2007; Rugno et al. 2018). Understanding the impact of agrochemicals is essential for the sustainability and resilience of integrated pest management (IPM).

The presence of natural enemies, such as predators and parasitoids, is essential to the success of IPM programs. Generalist predators have advantage over specialists, due to polyphagia, they can exploit food resources widely, they can survive in the agroecosystem without target pests, thus preventing their resurgence (Symondson et al. 2002). Among these species, the predatory insect *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) is the most prominent because it has a wide geographic distribution, high predatory capacity at the larval stage, high host diversity, tolerance to some insecticides, and high reproductive potential (Castilhos et al. 2011; Pasini et al. 2018). Other predatory insects relevant in this crop belong to the family Coccinellidae and predate aphids and mites (Oliveira et al. 2004). The ladybug *Coleomegilla quadrifasciata* (Schöenherr) (Coleoptera: Coccinellidae), stands out as a generalist predator, feeding on larval and adult prey, including scale insects, psyllids, whiteflies, mites, in addition to eggs and immature stages of coleopterans and lepidopterans (Lixa et al. 2010).

Non-synthetic agrochemicals based on copper, calcium, and sulfur, such as Bordeaux mixture and lime sulfur, are alternatives to the indiscriminate use of insecticides and are used to treat diseases and control pests such as scale insects and aphids (Venzon et al. 2016). The Bordeaux mixture contains lime and copper sulfate and is applied to several vegetable crops to prevent diseases. This mixture has fungicidal and bactericidal activity, repellent activity against insects, and is used in winter treatment on *Malus domestica*, *Prunus* sp., and *Vitis* sp. (Gessler et al. 2011; Venzon et al. 2016). Lime sulfur is obtained by the thermal treatment of lime and sulfur and is effective in controlling mites and scale insects (Venzon et al. 2016). Despite the proven effectiveness of these mixtures in controlling insect pests, little information is currently available on the lethal and sublethal effects of these products towards the mortality of natural enemies. In Brazil, studies involving these non-synthetic agrochemicals are limited to evaluating the mortality of the parasitoids *Telenomus remus* Nixon (Hymenoptera: Platygastridae) (Silva et al. 2016) and *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) (Silva and Bueno 2014). However, to our best knowledge, there are few studies to date evaluated the effects of these products on predatory insects (Bengochea et al. 2014; Tuelher et al. 2014; Vogelweith and Thiéry 2018). In addition to advantages such as easy application and low cost, these products are not restricted to peach production and are used worldwide in many other crops, including citrus, apple, and grapevine (Gessler et al. 2011; Venzon et al. 2016; Vogelweith and Thiéry 2018).

Several factors contribute to the toxicity of agrochemicals, including insect-specific factors associated with cuticle structure and composition and environmental factors, which may attenuate or minimize the toxic effects (Fernandes et al. 2010; Bueno et al. 2017). In this respect, the International Organization for Biological and Integrated Control (IOBC) proposes standardizing biological assays, which are initially performed under laboratory conditions and later under semi-field and field conditions to validate the results (Hassan 1988). These assays allow measuring the toxicity of insecticides with the view to integrate chemical and biological control, therefore combining the advantages of each control strategy (Garzón et al. 2015). Therefore, given the lack of data on the non-target toxicity of these products, especially non-synthetic products, additional studies are needed to determine their lethal and sublethal effects on *C. externa* and *C. quadrifasciata*. The objective of this study is to evaluate the toxicity of nine agrochemicals, including commercial and manipulated formulations used in peach orchards, to the larval and adult stages of the predatory insects *C. externa* and *C. quadrifasciata* according to the methodology proposed by IOBC.

Materials and methods

Insects

The populations of *C. externa* and *C. quadrifasciata* used in the assays were maintained under laboratory conditions (temperature, 25 ± 1 °C; relative air humidity, 70 ± 10%; photo-phase, 14 h). Larvae of *C. externa* were fed *ad libitum* on eggs of the alternative host *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), reared according to the methodology proposed by Parra (1997) and were maintained in test tubes (length, 12 cm; diameter, 5 cm). Adults were kept in acrylic cages (height, 15.5 cm; diameter, 18.5 cm), covered by a white paper, that served as bottom and cover and fed an artificial diet consisting of a mixture of 15 mL of condensed milk, two egg yolks, one egg white, 30 g of honey, 20 g of fructose, 30 g of brewer's yeast, 50 g of wheat germ, and 45 mL of distilled water (Vogt et al. 2000). Larvae of *C. quadrifasciata* were reared according to the methodology adapted from Silva et al. (2009) and fed *ad libitum* on *E. kuehniella* eggs. The adults were kept in plastic pots (height, 9 cm; diameter, 12 cm), closed with white paper, some cotton was also placed as a substrate for oviposition and were fed *E. kuehniella* eggs and honey.

Agrochemicals

For the bioassays were used nine agrochemicals recommended for Integrated Fruit Production (IFP) were used (Table 1) (NORMAS 2003), and a control treatment with

Table 1 Label information of the agrochemicals tested for their side effects on *Chrysoperla externa* and *Coleomegilla quadrifasciata*

Active ingredient (a.i.)	Technical name	Chemical group	Mode of action	Crops	Target pests	[a]D.C.P.	C[2]
Synthetic agrochemicals							
Abamectin	Vertimec 18 EC	Avermectin	Glutamate-Gated Chloride Channel (GLUCL) Allosteric Modulators.	Apple, Citrus, Grape, Strawberry Peach.	Mites	80	0.18
Azadirachtin	Neemax	Tetranortriterpenoid	Compounds of Unknown or Uncertain MOA	No restrictions for crops	Mites, Thrips, Psyllids, Aphids, Lepidoptera	1%	0.15
Chlorantraniliprole	Altacor	Anthranilamide	Ryanodine receptor modulators	Apple, Peach	Lepidoptera	14	4.90
Deltamethrin	Decis 25 EC	Pyrethroid	Sodium channel modulators	Apple, Citrus, Strawberry, Peach, Plum,	Diptera, Mites, Lepidoptera,	40	1.00
Fenitrothion	Sumithion 500 EC	Organophosphate	Acetylcholinesterase inhibitors	Apple, Peach	Diptera, Lepidoptera.	100	5.00
Malathion	Malathion 1000 EC	Organophosphate	Acetylcholinesterase inhibitors	Apple, Citrus, Peach	Diptera, Lepidoptera, Thrips, Psyllids, Aphids	150	10.00
Nonsynthetic agrochemicals							
Copper + Calcium	Bordatec (concentrate)	Inorganic	Compounds of unknown or uncertain MOA	No restrictions for crops	Fungal disease, Mites. Thrips, Psyllids, Aphids, Lepidoptera.	–	25% + 10%
Copper + Calcium	Bordeaux mixture	Inorganic	Compounds of unknown or uncertain MOA	No restrictions for crops	Fungal disease, Mites. Thrips, Psyllids, Aphids, Lepidoptera.	–	1%
Sulfur + Calcium	Lime sulfur	Inorganic	Compounds of unknown or uncertain MOA	No restrictions for crops	Fungal disease, Mites. Thrips, Psyllids, Aphids, Lepidoptera.	–	3.5 BD*

[a]D.C.P.= Dose of commercial product (g or mL.100 L^{-1}) or Percentage of Active Ingredient Concentration in the mixture mL or g 100 L^{-1} water; [2] Concentration of a.i. in the mixture, g 100 L^{-1} or %; *Baúme degrees (BD)

distilled water. These products were chosen because they are usually recommended in the production of peaches and other crops grown in temperate regions. The doses of abamectin, chlorantraniliprole, deltamethrin, fenitrothion, and malathion corresponded to the maximum dosage recommended for peach trees (MAPA 2018).

The products azadirachtin, copper 25% + calcium 10%, copper + calcium 1%, and sulfur + calcium (3.5 Ba) are included in the Norms for Organic Production of Vegetables and Animals (MAPA 1999) and were used as recommended for peach orchards (Table 1).

The non-synthetic formulations copper 25% + calcium 10%, copper + calcium 1%, and sulfur + calcium (3.5 Baume [Ba]) were manipulated and diluted immediately before spraying. Copper 25% + calcium 10% was prepared by dissolving the ingredients in 500 mL of water, and a portable pH meter (length: 18.8 cm; width: 3.8 cm; Instrusul, Esteio, RS, Brazil) was used to measure the pH in the range 7–9. Copper 1% + calcium 1% was prepared according to the recommendations of Fortes (2002) for temperate fruit trees, as follows: 30 g of copper sulfate, 30 g of lime, and 5 L of water. The ingredients were homogenized, and pH was measured using a portable pH meter until it reached the range of 8–9. Sulfur + calcium (3.5 Ba) was prepared using the methodology of Venzon et al. (2016), as follows: 100 g of sulfur, 50 g of lime, and 5 L of water. The components were homogenized and heated until a reddish-gray color was obtained. The density of the mixture was measured using a Baume hydrometer (Incoterm, Porto Alegre, RS, Brazil), and the concentration was adjusted to 3.5 Ba degrees.

Bioassays

The assays were conducted in the laboratory using the methodology established by IOBC for *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae), with modifications (Schmuck et al. 2000; Vogt et al. 2000). Spraying was performed using a 500-mL manual sprayer (Guarany Ultrajet, Itu, SP, Brazil), and spray deposition of 2.0 ± 0.2 mg cm^{-2} was measured using a precision scale (Shimadzu do Brasil Comércio Ltda, São Paulo, SP, Brazil).

Toxicity assessment on larvae

The bioassays consisted of exposing larvae of *C. externa* and *C. quadrifasciata* to dry residues of agrochemicals, which were sprayed onto glass plates (50 cm × 41 cm). The control treatment was composed by glass plates sprayed with distilled water. First-instar larvae (1–2 days old) were placed in arenas with a fine-tipped brush and remained in contact with the product residues until the emergence of adults, during this period they were daily fed *ad libitum* on *E. kuehniella* eggs.

Treatments were arranged in a completely randomized block design. Each treatment consisted of two plates with 20 arenas each, totaling 40 insects, and each insect was considered a repetition, totaling 40 larvae per experimental unit. Larval mortality and the duration of the larval stage in days (L1, L2, L3, pre-pupa, pupa, and total period) were evaluated daily. Sublethal tests were performed in the treatments in which accumulated mortality was ≤ 50%.

Toxicity assessment on adults

Spraying was performed onto glass plates (length, 14 cm; width, 14 cm). The plates were dried and served as the floor of insect cages. Each cage was composed of a methacrylate ring (diameter, 10 cm; height, 3 cm) with five holes (diameter of 1.3 cm) closed with voile-type fabric to allow ventilation. One hole was connected to a suction pump to eliminate toxic vapors, and another hole (diameter of 0.8) was used to supply water to the insects. The artificial diet was provided on the side of the cage for *C. externa*, and *E. kuehniella* eggs were left on the bottom of the cage for *C. quadrifasciata*. After preparing the cages, 1-week old adults of both species were separated by sex as females have larger body size than males (Brooks 1994; Carvalho and Souza 2000; Milléo and Meira 2012), and inserted into the cages.

Treatments were arranged in a completely randomized block design. Each treatment consisted of four cages, each containing four insect pairs (one male and one female). Each cage was considered a repetition, totaling 16 insect pairs per experimental unit. The accumulated mortality of male and female insects was evaluated after 120 h of exposure to insecticide residues (Schmuck et al. 2000; Vogt et al. 2000). Sublethal tests were performed in the treatments in which accumulated mortality was ≤ 50%.

Sublethal effects on survived adults

In addition to the lethal effects, sublethal effects on fecundity and fertility were evaluated in larvae and adults. Only treatments with a mortality rate of ≤ 50% were evaluated. To analyze reproductive parameters, five to seven couples of *C. externa* that survived previous bioassays were sedated with the of CO_2 and transferred with a forceps to acrylic cages (height, 15.5 cm; diameter, 18.5 cm) closed by two white papers, that served as bottom and cover and were fed as previously described, and five to seven couples of *C. quadrifasciata* were transferred with a fine-tipped brush to plastic pots (height, 9 cm; diameter, 12 cm), closed with white paper, fed *E. kuehniella* eggs *ad libitum* and were kept in the same climatic conditions of the insect colony. Seven days after the first egg-laying, the eggs deposited by both species were collected daily for 10 consecutive days. Counting was performed to determine the mean number of eggs per female per day. The eggs the *C. externa* and *C. quadrifasciata* were removed from the oviposition white paper and cotton, respectively, with a pair of scissors and fine-tipped brush, and incubated in cell culture plates with 96 wells (Kasvi Ltda., Pinhais, PR, Brazil) coated with transparent PVC film, to prevent cannibalism and escape, and to calculate the mean percentage of larval hatching in each treatment.

Classification of insecticide toxicity

For classifying insecticide toxicity to larvae and adults, the mortality rate was calculated in each treatment and corrected using the Schneider-Orelli formula (Püntener 1981), and the cumulative effect was determined using the formula proposed by Vogt et al. (1992).

$$E = 100\% - (100\% - M\%) \times R1 \times R2$$

where: E, cumulative effect (%); M%, mortality in the treatment corrected according to the control; R1, the ratio of the mean number of eggs laid daily by treated and untreated females; and R2, the ratio of the mean viability of eggs laid by treated and untreated females.

After calculating the cumulative effect for both larvae and adults, the products were classified as (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%) (Sterk et al. 1999).

Statistical analysis

The data regarding the duration of each larval instar of *C. externa* and *C. quadrifasciata* were analyzed using the Kruskal–Wallis test to assess significance ($p \leq 0.05$) and the Dunn test of means with Bonferroni correction at a level of significance of 5%. Data on adult mortality were analyzed for normality using the Shapiro–Wilk test, homoscedasticity was assessed using the Barlett's test, and residual independence was checked graphically. Subsequently, these data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$). Statistically significant means were compared using the Tukey's test ($p \leq 0.05$) for both insect species. To analyze the difference in the mortality of male and female insects, the data on *C. quadrifasciata* were transformed using the formula $\sqrt{x}$ to meet the ANOVA assumptions ($p \leq 0.05$), whereas the data for *C. externa* did not require transformation because they met the assumptions of the Shapiro–Wilk test, Barlett's test, and the graphical analysis of residual independence. The data with significant differences between the species using ANOVA were analyzed using Student's *t*-test. The fecundity and fertility of surviving adults in larval and adult assays were evaluated using the Shapiro–Wilk and Barlett's tests and by graphically analyzing residual independence. Data not meeting these assumptions were transformed using the formula $\sqrt{x}$. Subsequently, the data were subjected to ANOVA ($p \leq 0.05$). The statistically significant means were compared using the Tukey's test ($p \leq 0.05$).

Results

Larval development duration

Deltamethrin, fenitrothion, and malathion caused mortality of 100% of larvae of *C. externa*. Therefore, it was not possible to evaluate the larval development duration of this species. The duration of the first larval instar using sulfur + calcium was significantly longer than when the other agrochemicals were used (df = 6, H = 14.78, $p \leq 0.001$) for *C. externa*. Nonetheless, larval mortality was 100% starting from the third larval instar. Chlorantraniliprole significantly increased the overall duration of the second and third instars compared to the other products (df = 6, H = 126.15, $p \leq 0.0001$; and df = 5, H = 53.92, $p \leq 0.001$, respectively), corresponding to 4.55 and 3.50 days, respectively. The prepupal period was significantly longer using azadirachtin, chlorantraniliprole, copper 25% + calcium 10%, and copper 1% + calcium 1% than in the control treatment (df = 5, H = 46.52, $p \leq 0.001$). In general, the duration of the immature stage ranged from 17.16 to 19.27 days, which chlorantraniliprole and copper 25% + calcium 10%, showed the highest values than in the control treatment for *C. externa* (df = 5, H = 43.52, $p \leq 0.001$) (Table 2).

For *C. quadrifasciata*, the duration of the first larval instar (df = 5, H = 46.52, $p = 0.1914$) was not significantly different between the tested products, and abamectin, deltamethrin, fenitrothion, and malathion caused 100% mortality in the first few hours of exposure (Table 2). In contrast, the duration of the second larval instar using all tested products was similar to that of the control treatment (df = 4, H = 12.42, $p = 0.0145$). Azadirachtin 1% caused high mortality in this instar, and this result was not observed in the previous evaluation. The duration of the third larval instar of *C. quadrifasciata* (7.09 days) was higher using sulfur + calcium (3.5 Ba) and was significantly different from that using the control treatment (df = 4, H = 11.93, $p = 0.0179$) (Table 2). The prepupal stage was significantly longer (6.95 days) using sulfur + calcium (3.5 Ba) compared to the other treatments and the control (df = 4, H = 67.72, $p \leq 0.001$) (Table 2). The duration of the pupal stage was significantly higher with copper 25% + calcium 10% and copper 1% + calcium 1% when compared to the control treatment (df = 4, H = 25.57, $p \leq 0.001$) (Table 2). The combined duration of the larval and adult stages using sulfur + calcium (3.5 Ba) (21.25 days) was significantly longer than that using the other treatments and the control treatment (df = 4, H = 31.20, $p \leq 0.001$) (Table 2).

Adult mortality

Fenitrothion, malathion and sulfur + calcium caused 100% mortality of females (df = 9, F = 22.25, $p = \leq 0.001$) and males (df = 9, F = 17.86, $p \leq 0.001$) of *C. externa*. The mortality using abamectin, azadiracthin, chlorantraniliprole, copper 1% + calcium 1%, and deltamethrin was not significantly different from that of the control treatment. No sex-dependent mortality was recorded when testing abamectin (t = 0.94, $p = 0.540$), chlorantraniliprole (t = 1.69, $p = 0.320$), copper 1% + calcium 1% (t = 1.22, $p = 0.360$), and deltamethrin (t = 2.32, $p = 0.800$). Nonetheless, azadirachtin 1% showed higher mortality of females (25%) than males (6.25%) (t = 0.61, $p = 0.030$) (Fig. 1).

As in *C. externa*, fenitrothion and malathion caused 100% mortality of *C. quadrifasciata* in both sexes (females: df = 9, F = 25.63, $p = 0.0024$, males df = 9, F = 13.26, $p \leq 0.001$). The other agrochemicals showed female mortality rates similar to the control treatment. In contrast, in males, abamectin caused mortality above 60%, statistically different from the control treatment, similarly to the organophosphate insecticides. A significant difference in mortality rate was recorded between *C. quadrifasciata* males and females after exposed to azadirachtin (t = 0.96, $p = 0.020$), and copper + calcium (25 + 10%) (t = 0.96, $p = 0.020$), with mortality rate of 18.75 and 17.25% for females and 0% for males, respectively. However, abamectin (t = 0.53, $p = 0.494$), deltamethrin (t = 0.14, $p = 0.720$), azadirachtin, chlorantraniliprole, copper 1% + calcium 1%, and sulfur + calcium caused a predator mortality similar to the control treatment (Fig. 2).

Larval exposure

Chlorantraniliprole had no effect in the parameters, larval mortality, fecundity (df = 5, F = 8.35, $p \leq 0.001$) and fertility (df = 5, F = 7.78, $p = 0.027$) of adults, being thus classified as harmless (class 1) to *C. externa* (Table 3). Azadirachtin caused cumulative mortality of 10%, resulting in a cumulative effect of 26.16% and, hence, remained in class 1 (Table 3). Abamectin, copper 25% + calcium 10%, and copper 1% + calcium 1% were slightly harmful (class 2) to *C. externa*. These products significantly reduced fecundity of the crisopid (df = 5, F = 8.35, $p \leq 0.001$). Deltamethrin, sulfur + calcium, and organophosphates caused high mortality in the first hours of exposure and were classified as harmful (class 4) to larvae of *C. externa* (Table 3).

The treatment with chlorantraniliprole was harmless (class 1) to larvae of *C. quadrifasciata* and did not affect adult fecundity (df = 3, F = 11.38, $p \leq 0.001$) and fertility (df = 3, F = 17.19, $p = 0.0297$) (Table 3). Sulfur + calcium was considered slightly harmful (class 2) to larvae of *C. quadrifasciata* compared to the control treatment because it

Table 2 Duration in number of days (mean ± SEM) of larval instars, pre-pupal, and pupal stages and duration of larval-adult stages for *Chrysoperla externa* and *Coleomegilla quadrifasciata* after exposure of larvae to residual concentrations of agrochemicals used in peach orchards

Treatment	Duration (Days)					
	1st instar	2nd instar	3rd instar	Pre-pupal	Pupal	Larval-adult
Chrysoperla externa						
Control	2.57 ± 0.08 b	3.35 ± 0.08 cd	2.47 ± 0.09 c	2.57 ± 0.08 c	6.55 ± 0.15 a	17.55 ± 0.45 cd
Abamectin	2.02 ± 0.09 d	2.95 ± 0.13 de	3.03 ± 0.05 b	2.64 ± 0.09 bc	6.47 ± 0.09 ab	17.16 ± 0.23 d
Azadirachtin	2.57 ± 0.08 b	3.30 ± 0.10 cd	2.57 ± 0.11 c	3.50 ± 0.15 a	6.59 ± 0.10 a	18.42 ± 0.16 b
Chlorantraniliprole	2.20 ± 0.06 cd	4.55 ± 0.08 a	3.50 ± 0.11 a	3.10 ± 0.13 ab	5.92 ± 0.07 bc	19.27 ± 0.23 a
Copper + Calcium	2.60 ± 0.08 b	4.12 ± 0.09 ab	2.87 ± 0.09 bc	3.12 ± 0.14 ab	5.70 ± 0.17 c	18.55 ± 0.11 ab
Copper + Calcium[a]	2.52 ± 0.09 bc	2.52 ± 0.09 e	2.70 ± 0.12 bc	3.50 ± 0.10 a	6.60 ± 0.21 a	18.22 ± 0.22 bc
Deltamethrin	–	–	–	–	–	–
Sulfur + Calcium	3.02 ± 0.10 a	3.70 ± 0.37 bc	–	–	–	–
Fenitrothion	–	–	–	–	–	–
Malathion	–	–	–	–	–	–
Coleomegilla quadrifasciata						
Control	2.10 ± 0.06[ns]	2.51 ± 0.14 ab	5.33 ± 0.29 b	1.82 ± 0.09 b	3.60 ± 0.14 c	15.78 ± 0.367 b
Abamectin	–	–	–	–	–	–
Azadirachtin	1.70 ± 0.19	–	–	–	–	–
Chlorantraniliprole	2.05 ± 0.23	2.42 ± 0.22 ab	6.35 ± 0. 59 ab	1.49 ± 0.16 b	3.83 ± 0.13 bc	15.68 ± 0.46 b
Copper + Calcium	1.83 ± 0.14	2.07 ± 0.05 b	6.48 ± 0.22 ab	1.36 ± 0.10 b	4.30 ± 0.10 ab	15.50 ± 0.41 b
Copper + Calcium[a]	1.82 ± 0.08	3.04 ± 0.26 ab	6.62 ± 0.34 ab	1.65 ± 0.15 b	4.50 ± 0.13 a	17.09 ± 0.65 b
Deltamethrin	–	–	–	–	–	–
Sulfur + Calcium	1.70 ± 0.12	3.12 ± 0.47 a	7.09 ± 0.59 a	6.95 ± 0.59 a	3.75 ± 0.14 c	21.25 ± 0.85 a
Fenitrothion	–	–	–	–	–	–
Malathion	–	–	–	–	–	–

Means followed by the same letter in columns did not differ significantly from each other. The means were analyzed by the Kruskal–Wallis test followed by the Dunn test and Bonferroni correction at 5% probability

[a]Bordeaux misture

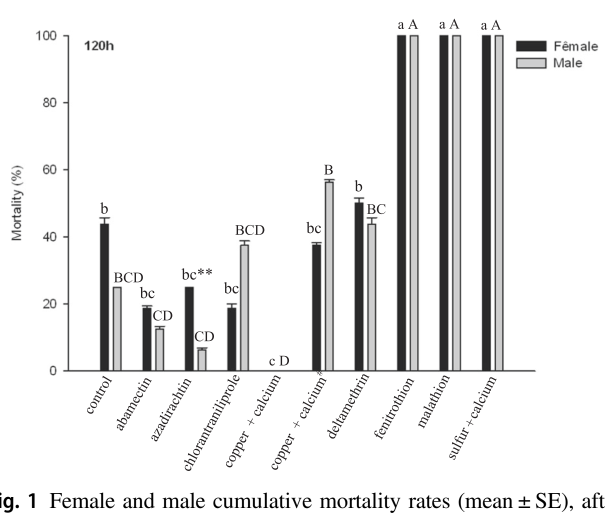

Fig. 1 Female and male cumulative mortality rates (mean ± SE), after 120 h exposure of *Chrysoperla externa* adults to residual concentrations of agrochemicals sprayed in peach orchards. For females (black bars), means followed by the same lowercase letter did not differ significantly from each other by the Tukey's test ($p < 0.05$). For males (gray bars), means followed by the same capital letter did not differ significantly from each other by the Tukey's test ($p < 0.05$). ** There were statistical differences between female and male mortality rates by the t-test ($p < 0.05$). # copper + calcium (1%), Bordeaux mixture

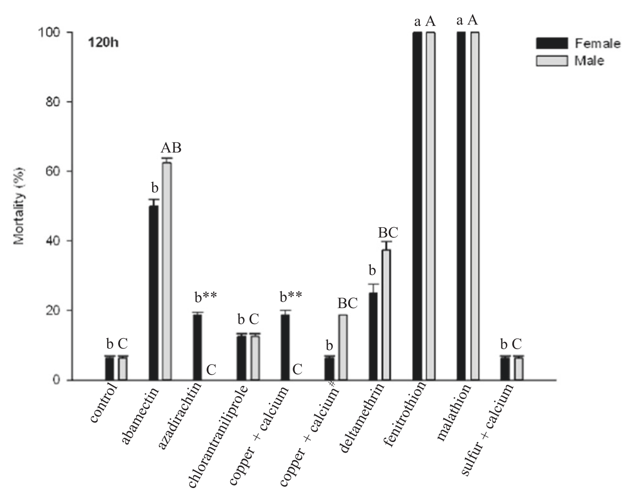

Fig. 2 Female and male cumulative mortality rates (mean ± SE), after 120 h exposure of *Coleomegilla quadrifasciata* adults to residual concentrations of agrochemicals sprayed in peach orchards. For females (black bars), means followed by the same lowercase letter did not differ significantly from each other by the Tukey's test ($p < 0.05$). For males (gray bars), means followed by the same capital letter did not differ significantly from each other by the Tukey's test ($p < 0.05$). ** There were statistical differences between female and male mortality rates by the t-test ($p < 0.05$). # copper + calcium (1%), Bordeaux mixture

Table 3 Total effect and IOBC classification after exposure of *Chrysoperla externa* and *Coleomegilla quadrifasciata* larvae to residual concentrations of agrochemicals used in peach orchards

Treatment	M (%)[a]	Fecundity (mean ± SEM)	Fertility (mean ± SEM)	E (%)[b]	C[c]
Chrysoperla externa					
Control	–	32.44 ± 3.31 a	83.33 ± 0.61 ab	–	–
Abamectin	22.50	16.64 ± 0.71 b	88.54 ± 1.08 ab	57.79	2
Azadirachtin	10.00	27.35 ± 1.38 ab	84.38 ± 1.08 ab	26.16	1
Chlorantraniliprole	0.00	34.67 ± 4.60 a	85.42 ± 0.56 ab	0.00	1
Copper + Calcium	5.00	17.64 ± 1.16 bc	94.79 ± 0.22 ab	41.24	2
Copper + Calcium[d]	0.00	16.33 ± 0.80 c	98.95 ± 0.22 a	40.23	2
Deltamethrin	100.00	–	–	100.00	4
Sulfur + Calcium	92.50	–	–	100.00	4
Fenitrothion	100.00	–	–	100.00	4
Malathion	100.00	–	–	100.00	4
Coleomegilla quadrifasciata					
Control	–	42.32 ± 8.97 a	71.88 ± 1.14 a	–	
Abamectin	100.00	–	–	100.00	4
Azadirachtin	88.89	–	–	100.00	4
Chlorantraniliprole	0.00	35.69 ± 3.83 a	69.79 ± 1.56 ab	18.10	1
Copper + Calcium	35.37	9.31 ± 4.04 b	70.83 ± 0.94 ab	85.99	3
Copper + Calcium[d]	25.93	–	–	100.00	4
Deltamethrin	100.00	–	–	100.00	4
Sulfur + Calcium	25.93	41.63 ± 4.67 a	47.92 ± 0.90 b	51.37	2
Fenitrothion	100.00	–	–	100.00	4
Malathion	100.00	–	–	100.00	4

[a]M = Mortality rate corrected by Schneider-Orelli's formula

[b]E = Total effect

[c]C = IOBC classification: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%). Means followed by the same letter in columns do not differ significantly from each other by the Tukey's test at 5% probability

[d]Bordeaux misture

reduced adult fertility (47.92 vs. 71.88%). Copper 25% + calcium 10% and copper 1% + calcium 1% were classified as moderately harmful (class 3) and harmful (class 4), respectively (Table 3). Copper 1% + calcium 1% was more toxic than copper 25% + calcium 10% because the emerging adults had morphological deformities and did not survive the 7-day experimental period, which prevented the evaluation of fecundity and fertility. The larvae exposed to copper 25% + calcium 10% did not present deformities; however, fecundity was lower in the group treated with this formulation compared to the control treatment (9.31 vs. 42.32 eggs per female per day) (Table 3) Abamectin, azadirachtin, deltamethrin, fenitrothion, and malathion were classified as toxic (class 4) because they caused high mortality of larvae of *C. quadrifasciata* (Table 3).

Adult exposure

The results of adult reproductive parameters in *C. externa* and *C. quadrifasciata* and the classification of toxicity according to the IOBC standards are shown in Table 4. Abamectin, azadirachtin, chlorantraniliprole, copper 25% + calcium 10%, and deltamethrin were harmless (class 1) to adults of *C. externa*. Copper 1% + calcium 1% and deltamethrin caused accumulated mortality of 19.05% in adults of *C. externa*, copper + calcium 1% was classified as moderately harmful (class 3) and deltamethrin harmless (class1) to adults of the crisopid. This result is because copper + calcium significantly altered reproductive parameters (df = 5, F = 6.94, $p \leq 0.001$), compared to the control treatment. The product deltamethrin despite having fecundity values lower than the control treatment did not affect the adults (df = 5, F = 6.00, $p \leq 0.001$), resulting in total effect of 27.66%, near the limit of class 1. Sulfur + calcium, fenitrothion, and malathion caused 100% mortality of adults of *C. externa* and were classified as harmful (class 4) (Table 4).

Azadirachtin, copper 25% + calcium 10%, deltamethrin, and sulfur + calcium were classified as harmless (class 1) to *C. quadrifasciata* (Table 4). Chlorantraniliprole and copper 1% + calcium 1% were considered slightly harmful (class 2) because they decreased egg fertility in *C. quadrifasciata*

Table 4 Total effect and IOBC classification after exposure of *Chrysoperla externa* and *Coleomegilla quadrifasciata* adults to residual concentrations of agrochemicals used in peach orchards

Treatment	M (%)[a]	Fecundity (mean ± SEM)	Fertility (mean ± SEM)	E[b]	C[c]
Chrysoperla externa					
Control	–	10.66 ± 1.33 abc	70.83 ± 0.94 a	–	–
Abamectin	0.00	11.54 ± 1.47 abc	88.54 ± 0.54 a	0.00	1
Azadirachtin	0.00	14.54 ± 1.86 ab	93.75 ± 0.75 a	0.00	1
Chlorantraniliprole	0.00	19.64 ± 3.42 a	70.83 ± 1.37 a	0.00	1
Copper + Calcium	0.00	11.73 ± 1.68 ab	75.00 ± 2.62 a	0.00	1
Copper + Calcium[d]	19.05	4.71 ± 0.66 c	32.29 ± 1.92 b	83.74	3
Deltamethrin	19.05	8.54 ± 1.34 bc	71.88 ± 1.67 a	27.66	1
Sulfur + Calcium	100.00	–	–	100.00	4
Fenitrothion	100.00	–	–	100.00	4
Malathion	100.00	–	–	100.00	4
Coleomegilla quadrifasciata					
Control	–	23.97 ± 0.90 ab	57.29 ± 0.65 b	–	-
Abamectin	53.33	–	–	100.00	4
Azadirachtin	3.33	19.78 ± 0.54 b	52.08 ± 1.03 b	27.44	1
Chlorantraniliprole	0.00	29.68 ± 3.47 a	21.88 ± 1.14 c	52.71	2
Copper + Calcium	3.33	24.22 ± 0.52 ab	56.25 ± 1.14 b	0.80	1
Copper + Calcium	6.67	21.18 ± 1.36 b	26.04 ± 0.41 c	59.84	2
Deltamethrin	26.67	24.48 ± 0.99 ab	60.42 ± 1.24 b	25.26	1
Sulfur + Calcium[d]	0.00	22.96 ± 1.20 ab	88.54 ± 0.54 a	0.00	1
Fenitrothion	100.00	–	–	100.00	4
Malathion	100.00	–	–	100.00	4

[a]M = rate corrected by Schneider-Orelli's formula,

[b]E = Total effect;

[c]C = IOBC classification: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%). Means followed by the same letter in columns do not differ significantly from each other by the Tukey's test at 5% probability

[d]Bordeaux misture

(df = 6, F = 25.93, $p <= 0.001$). Fenitrothion and malathion caused 100% mortality of adults in the first few hours of exposure and were classified as harmful (class 4). Abamectin was also considered harmful because it caused accumulated mortality of 53.33%, which prevented analyzing reproductive parameters, and the cumulative effect was 100% (Table 4).

Discussion

Abamectin, azadirachtin, copper 25% + calcium 10%, copper + calcium 1%, deltamethrin, and sulfur + calcium presented different degrees of toxicity to *C. externa* and *C. quadrifasciata* under laboratory conditions, corroborating studies with other predatory insect species, including *C. externa* and *Eriopis connexa* (Gemar) (Coleoptera: Coccinellidae), which had different toxicity thresholds (Rimoldi et al. 2017; Pasini et al. 2018). Moreover, significant differences were verified in terms of toxicity according to the development stage of insects (Pérez-Aguilar et al. 2018; Prabhaker et al. 2017; Pasini et al. 2018).

Abamectin presented adverse effects on *C. externa* and *C. quadrifasciata*. This finding corroborates other studies with the predatory insects *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae) (Azod et al. 2016), *Engytatus varians* (Distant) (Heteroptera: Miridae) (Pérez-Aguilar et al. 2018). Abamectin is used to control mites, insects, and nematodes, and acts as an agonist of the neurotransmitter gamma-aminobutyric acid. The active ingredient binds to nerve cells, preventing the transport of chlorine ions, consequently causing death (Azod et al. 2016). Therefore, as abamectin can negatively affect *C. externa* and *C. quadrifasciata*, its use should be considered with caution in peach IPM programmes.

Azadirachtin was harmless (class 1) to adults of *C. externa* and *C. quadrifasciata*. However, the toxicity of this agrochemical was different between the larvae of the two species, being harmless (class 1) to *C. externa*, causing larval mortality of 26.16%, but harmful (class 4) to *C. quadrifasciata*, causing 100% larval mortality. This product acts as a growth regulator, adjusting the peaks of ecdysteroid, a hormone that regulates molting (Marco et al. 1990). Similar results were found by Zanuncio et al. (2016), whereby the mortality rate of the third-, fourth-, and fifth-instar nymphs of *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) increased as the concentration of azadirachtin increased, without significant effect on adults at the same concentrations used for nymphs. Other studies observed a sublethal effect of an azadirachtin-based formulation, which reduced the longevity of the parasitoid *Bracon nigricans* (Szépligeti) (Hymenoptera: Braconidae) (Biondi et al. 2013). Therefore, azadirachtin has variable effectiveness against natural enemies, with either a selective or non-selective activity.

Chlorantraniliprole was harmless to both development stages of *C. externa* and the larval stage of *C. quadrifasciata*. This product acts on ryanodine receptors of the muscle of insects, causing rapid death via muscular dysfunction and paralysis (Hannig et al. 2009). Chlorantraniliprole is recognized as a selective insecticide for non-target organisms. Studies reported the low toxicity of this compound to the predatory insect species *Harmonia axyridis* (Pallas) and *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) (Cabrera et al. 2018), *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Biondi et al. 2012), *Podisus nigrispinus* (Dallas), *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) (Castro et al. 2013). Moreover, this chemical did not show negative effects on the parasitoid *B. nigricans* survival and fertility, causing negative interaction only at higher temperatures such as 35 and 40 °C (Abbes et al. 2015), which agree with the results of our study. Chlorantraniliprole was classified as moderately harmful to the adult stage of *C. quadrifasciata* because it reduced egg hatchability in coccinellids. Oliveira et al. (2019) reported that chlorantraniliprole had a sublethal effect on larvae of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), evidenced by a reduced development cycle, lower body weight of males in the F1 generation, and lower survival and fertility of treated adults, as observed in our study.

Agrochemicals based on copper and calcium had different toxicity to predatory insects. Copper 25% + calcium 10% and Copper + calcium 1% were slightly harmful (class 2) to *C. externa* larvae, already for adults only copper + calcium 1% was moderately harmful (class 3). However, for *C. quadrifasciata* copper + calcium 1% was moderately harmful to the larval stage and toxic to the adult stage. Copper at high concentrations may damage the DNA structure and is considered toxic to arthropods (Bernabò et al. 2017). Other studies indicate that Copper has negative effects on beneficial organisms, such as *Apis mellifera* Linnaeus (Hymenoptera: Apidae) causing high larval mortality after seven days of exposure and slowed larval development, caused reduction in pre-pupal and pupal weights, decreased the survival rate of both larvae and foragers (Di et al. 2016), high mortality of predator *Halmus chalybeus* (Boisduval) (Coleoptera: Coccinellidae) (Lo 2004), and parasitoid *T. remus* (Silva et al. 2016). Few studies evaluated the lethal and principally sublethal effects of these compounds, highlighting the importance of the present study.

Deltamethrin was classified as harmful to larvae and harmless to adults of both predators. According to Garzón et al. (2015), this insecticide caused mortality of 52.38% in larvae of *C. carnea* and a reduction of 13.89% in the emergence of adults, when the application of insecticide was realized in the pupal stage and therefore it was classified as slightly harmful (class 2), with a cumulative effect of 47.29%. In contrast, other insecticides, as chlorpyrifos caused 100% mortality of females of *C. externa* in coffee crops and was classified as harmful (class 4) (Torres et al. 2013). The lambda-cyhalothrin and beta-cypermethrin are toxic to larvae of *Chrysoperla genanigra* Freitas (Neuroptera: Chrysopidae) (Silva et al. 2017). These results indicate that the genus *Chrysoperla* is sensitive to pyrethroids, and in our study, these products also were harmful to the larval stage. The toxicity of deltamethrin to adult stage can be explained by the repellent effect of pyrethroids to arthropods and may also be due to the low exposure of insects to the active ingredient (Benamú et al. 2013). Resistance to insecticides may also be associated with reduced toxicity. *E. connexa* populations were resistant to lambda-cyhalothrin in cotton crops, presenting higher LD_{50} and DL_{90}, because of hereditary resistance and metabolic detoxification of insecticide molecules (Rodrigues et al. 2013).

Sulfur + calcium (lime sulfur) was more toxic to *C. externa* than to *C. quadrifasciata*. This product was classified as harmful (class 4) to both stages of the chrysopid, slightly harmful (class 2) to coccinellid larvae, and harmless (class 1) to coccinellid adults. The sulfur gas (H_2S) and sulfur dioxide (SO_2) released by lime sulfur inhibit the respiratory chain (Abbott 1945; IRAC 2018). Tuelher et al. (2014) have shown that the toxicity of sulfur + calcium to *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) is dose-dependent and, in coffee crops, doses higher than the recommended (20–40 mL/L) may cause a drastic reduction in this insect population. These compounds when used at low doses can cause sublethal effects, negatively affecting the reproductive capacity of *O. laevigatus* (Biondi et al. 2012), *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) (Zappalà et al. 2012; Madbouni; et al. 2017), because they can act as oviposition repellent or as an egg dryer (Zappalà et al. 2012; Biondi et al. 2012). This result corroborates with the present study, where significant differences were found in the toxicity between the predators.

The organophosphates fenitrothion and malathion are highly toxic to natural enemies (Rugno et al. 2018). These products were harmful to larvae and adults of both *C. externa* and *C. quadrifasciata*. Organophosphates act by irreversibly inhibiting the enzyme acetylcholinesterase, which leads to the accumulation of acetylcholine at the synaptic endings in the nervous system, causing insect death (Bacci et al. 2007). Our results were similar to those obtained by Rugno et al. (2018), whereby malathion was toxic to larvae and adults of *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae).

The evaluation of mortality for each sex is necessary because, in addition to primary sex differences, females are directly responsible for oviposition and perpetuation of the species (Carvalho and Souza 2000). Therefore, toxicity tests that address sex differences are vital to the success of IPM programs. Mortality was higher in females of *C. externa* exposed to azadirachtin than in males. Similarly, mortality was higher in females of *C. quadrifasciata* treated with azadirachtin and copper 10% + calcium 25%. Castilhos et al. (2011) reported difference in mortality between males and females using commercial insecticides recommended for peach production. Nonetheless, mortality was relatively higher in males. In this study, the higher mortality of females may be related to the greater body surface area exposed to the product because of their larger body size (Carvalho and Souza 2000).

The toxicity of insecticides may vary between species of natural enemies and between the stages of development. Therefore, the sex and species differences in the mortality rate in the control treatment may be due to these reasons and to the insect strain used (Carvalho et al. 2001). The factors related to this variability include the rate of insecticide penetration through the tegument and the rate of metabolism (Bueno et al. 2017). Once penetration rate is associated with the lipophilicity of a product and with cuticle thickness and chemical composition, factors related to the cuticle vary with the development stage of insects and then promote different selectivity results for a compound (Fernandes et al. 2010; Bueno et al. 2017). Structural factors of the cuticle vary among species and influence insecticide penetration, and genera with hydrophobic cuticles because of the higher amount of lipids have a higher penetration rate and may be more susceptible to insecticides (Carvalho et al. 2001; Fernandes et al. 2010; Bueno et al. 2017). Furthermore, variances in biochemical processes of molting can also cause a difference in susceptibility between species of the same family (Cabrera et al. 2018). In addition, the tolerance of predatory insects to insecticides may be linked to the higher rate of metabolism of chemical compounds and changes in the site of action (Guedes et al. 2016). The production of monooxygenases in cytochrome P450 is the primary mechanism of insecticide degradation. These enzymes degrade lipophilic compounds, transforming them into primary metabolites for later elimination (Brattsten et al. 1986; Bueno et al. 2017). Future studies may help understand the factors associated with toxicity to *C. externa* and *C. quadrifasciata*, which are essential control agents in the IPM of peach orchards.

It is worth emphasizing that the present results were obtained under laboratory conditions, with maximum exposure of larvae and adults to the insecticides. For this reason, the larvae were more susceptible to the analyzed products than adults. Moreover, the mortality of larvae was higher than that of adults when both developmental stages were exposed to the same concentration of the active ingredient. In the 18 treatments used in larval bioassays (nine agrochemicals for *C. externa* and nine for *C. quadrifasciata*), 10 treatments were classified as harmful (class 4), and three were classified as harmless (class 1). In contrast, 18 treatments used in adult bioassays, six treatments were classified as class 4, and nine were considered harmless.

Further testing is required in semi-field and field conditions using products classified as moderately harmful (class 3) or harmful (class 4) because toxic effects can be attenuated by the mobility of predatory insects and environmental conditions (Hassan 1988). It should be noted that the results of this study are relevant to peach cultivation since effective insecticides should be prioritized in IPM programs, thus contributing to the biological control performed by *C. externa* and *C. quadrifasciata* in peach orchards.

Our results suggest that chlorantraniliprole is more indicated to peach production because the product is harmless to the larval stage of both predatory insect species; moreover, was longer during the larval-adult stage of *C. externa*, and this characteristic is crucial because this insect has predatory behavior only in the larval stage. Lime sulfur should be used sparingly in peach orchards, especially in the presence of larvae, because they are more sensitive to this product than adults. The results on the impact of lime sulfur in the two insect species were conflicting because this product caused high mortality of both stages of *C. externa*. In *C. quadrifasciata*, the product increased the larval-adult stages but did not cause high mortality. The organophosphates fenitrothion and malathion caused high mortality of both insect species and, therefore, should not be used in peach orchards.

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Author contributions FSA, ADG and DEN conceived research. FSA, MR and RAP kept insects rearing. FSA, MR, RAP and JBP conducted experiments. FSA analyzed data. FSA, MR and RAP wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The authors agree with the publication of the manuscript in this form.

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