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Research Article

Insecticide selectivity to *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) under extended laboratory conditions¹

Willian Yoshio Sanomia², Fabricio Fagundes Pereira², Ivana Fernandes da Silva³

ABSTRACT

Bioassays to evaluate the selectivity of pesticides to beneficial organisms are important tools to discriminate products compatible with biological pest control programs. This study aimed to evaluate the effects of the main insecticides used in the soybean crop on the egg parasitoid *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae). The tested treatments (active ingredients) were: methomyl, lambda-cyhalothrin + thiamethoxam, beta-cyfluthrin + imidacloprid, novaluron and teflubenzuron, in the highest doses indicated by the manufacturer for the soybean crop, and a control treatment (water). The evaluations enabled to calculate the mortality and parasitism capacity of adults exposed to the pesticides, in addition to the sex ratio of their descendants. Methomyl and lambda-cyhalothrin + thiamethoxam were classified as Class 4 (harmful), beta-cyfluthrin + imidacloprid as Class 3 (moderately harmful), and novaluron and teflubenzuron as Class 1 (harmless). The products classified as Class 3 and Class 4 should, as much as possible, be replaced by insecticides selective to *O. submetallicus*.

KEYWORD: Pesticide, egg parasitoids, integrated pest management, neotropical brown stink bug.

RESUMO

Seletividade de inseticidas a *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) em condições ampliadas de laboratório

Testes de avaliação da seletividade de pesticidas a organismos benéficos são importantes ferramentas para discriminar produtos compatíveis com programas de controle biológico de pragas. Objetivou-se avaliar os efeitos dos principais inseticidas utilizados na cultura da soja sobre o parasitoide de ovos *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae). Os tratamentos (ingredientes ativos) testados foram: metomil, lambda-cialotrina + tiametoxam, beta-ciflutrina + imidacloprid, novaluron e teflubenzuron, nas maiores dosagens indicadas pelo fabricante para a cultura da soja, e um tratamento testemunha (água). As avaliações permitiram calcular a mortalidade e capacidade de parasitismo de adultos expostos aos pesticidas, além da razão sexual dos seus descendentes. Metomil e lambda-cialotrina + tiametoxam foram classificados como Classe 4 (prejudicial), beta-ciflutrina + imidacloprid como Classe 3 (moderadamente prejudicial) e novaluron e teflubenzuron como Classe 1 (inócuo). Os produtos classificados como Classe 3 e Classe 4 devem, na medida do possível, ser substituídos por inseticidas seletivos a *O. submetallicus*.

PALAVRAS-CHAVE: Pesticida, parasitoides de ovos, manejo integrado de pragas, percevejo-marrom-da-soja.

INTRODUCTION

Ooencyrtus submetallicus (Howard) (Hymenoptera: Encyrtidae) is an endoparasitoid that reproduces through thelytoky parthenogenesis, originating only diploid female individuals (Espinosa et al. 2016). Therefore, this insect has a great potential to be used in biological control programs, because it parasitizes eggs of Pentatomidae bugs such as *Nezara viridula* (Linnaeus) (Wilson & Woolcock 1960), *Piezodorus guildinii* (Westwood) (Corrêa-Ferreira &

Moscardi 1995), *Edessa meditabunda* (Fabricius) (Golin et al. 2011), *Chinavia pengue* (Rolston) and *Euschistus heros* (Fabricius) (Ferreira 2016). *E. heros* is currently the main species of phytophagous stink bug that causes relevant damage in the soybean crop [*Glycine max* (L.)] (Tuelher et al. 2018).

Chemical control is the most widely used tactic to control these insects in the soybean crop, when it approaches the level of economic damage. Thus, studies on the lethal and sublethal effects of pesticides on biological control agents, such as

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O. submetallicus, are needed, since the use of non-selective products is a major factor that compromises the effectiveness of beneficial organisms in the field.

In Brazil, no information is available yet about the impact of the main phytosanitary products on this species and other parasitoids. In contrast, the European Union requires laboratory bioassays for insecticides and their effects on natural enemies, as an integral part of the registration process for a phytosanitary product (Desneux et al. 2007), thus indicating the degree of toxicity for natural enemies of these products used in crops, in the context of integrated pest management.

These selectivity studies are applied to classify insecticides tested on natural enemy species (Mills et al. 2016). Generally, initial tests are conducted in the laboratory and, depending on the obtained results, bioassays are carried out in semi-field or field tests (Hassan 1992, Hassan 1997, Vogt et al. 2000). The results demonstrate if there is direct mortality from natural enemies or if exposure to the insecticide can affect the physiological characteristics (development, fertility, longevity and sex ratio) and behavior (mobility, orientation, lodging and oviposition) of the control agent (Saber & Abedi 2013).

Therefore, this study aimed to evaluate the side effects of some of the main active ingredients used in the soybean crop, in order to control caterpillars and stinkbugs, on adults of *O. submetallicus*, under extended laboratory conditions.

MATERIAL AND METHODS

The experiment was conducted at the laboratory of insect biological control of the Universidade Federal da Grande Dourados (Dourados, Mato Grosso do Sul state, Brazil), from March to May 2018.

The ASPECLE (Evaluation of Pesticide Selectivity in Extended Laboratory Conditions) system (Miranda 2010) was adapted from the International Organization for Biological Control (IOBC) standard model for studies with egg parasitoids with the objective of carrying out selectivity tests under laboratory conditions, as well as evaluating the product safety for adults of *O. submetallicus*. As treatments, the main active ingredients, selected due to their efficiency in controlling lepidopteran pests and phytophagous stinkbugs registered for use in the soybean crop, in Brazil (Agrofit 2019), were one carbamate, two inhibitors of chitin synthesis (benzoylphenylureas) and two neonicotinoids mixed with pyrethroids (Table 1).

The selectivity cages were made of glass cylinders (Borosilicate, Laborglas™, São Paulo, SP, Brazil) (3.50 cm in diameter × 25.00 cm in length). The extremity of the cylinder was sealed with two styrofoam stoppers, one of which had a central perforation of approximately 0.50 cm in diameter to connect the ventilation system hose and allow gas exchange (Figure 1).

This ventilation system consisted of a vacuum pump aspirator/compressor (Mod 089/Cal, Fanem™, São Paulo, SP, Brazil) to suck toxic gases from inside the cage with the aid of a 2.20-cm-diameter central tube (Polypropylene Copolymer Random - PPR, Amanco™, São Paulo, SP, Brazil), responsible for distributing the vacuum pump suction between the cages. In addition, a hose with 1.00 cm in diameter × 100.00 cm in length was used to connect the vacuum pump to the central tube, and several hoses with 0.50 cm in diameter × 13.00 cm in length to connect the cages to the central tube.

The styrofoam stoppers used to seal the tubes were coated with black plastic, to prevent parasitoids from being attracted to the white color of

Table 1. Test substances (treatments) evaluated for adults of *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) identified by active ingredient, commercial product (CP), company, concentration (C), commercial dosage (D), group or chemical subgroup at IRAC¹ (*MoA*) and formulation.

Active ingredient	CP	Company	C	D	<i>MoA</i>	Formulation ²
Lambda-cyhalothrin + thiamethoxam	Engeo Pleno	Syngenta Proteção de Cultivos Ltda.	141 + 106	0.20	3A; 4A	CS
Beta-cyfluthrin + imidacloprid	Connect	Bayer S.A.	100 + 12.50	1.00	3A; 4A	CS
Methomyl	Bazuka 216 SL	Rotam do Brasil Produtos Agrícolas Ltda.	383.50 + 216	1.99	1A	SC
Novalurom	Rimon 100 EC	Adama Brasil S.A.	100	0.07	15	EC
Teflubenzuron	Nomolt 150	BASF S.A.	150	0.15	15	CS

¹ Insecticide Resistance Action Committee (IRAC 2019); ² CS: concentrated suspension; SC: soluble concentrate; EC: emulsifiable concentrate.

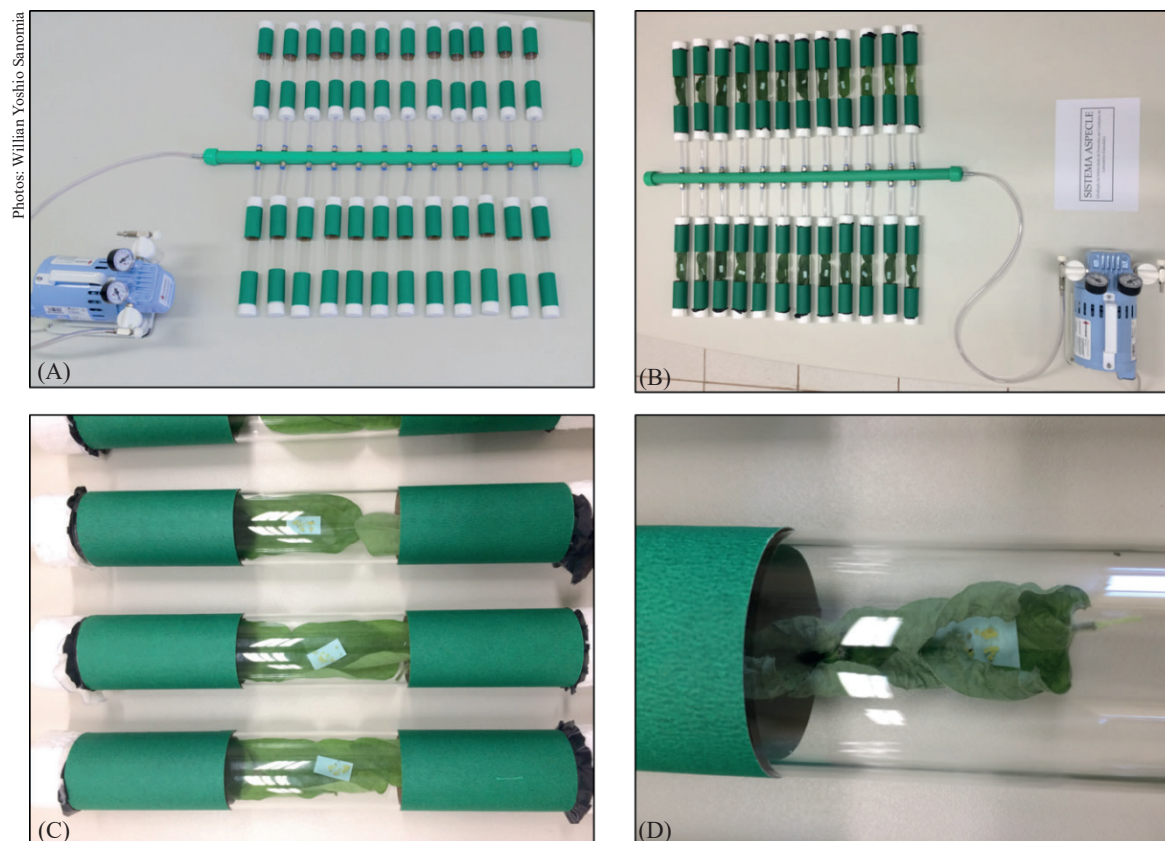


Figure 1. Scheme of the ASPECLE (Evaluation of Pesticide Selectivity in Extended Laboratory Conditions) system: A-B) cages after 1 h of the system operation; C) detail of the selectivity cage containing substrate (soybean leaves), egg carton of *Euschistus heros* and female of *Ooencyrtus submetallicus*; D) cage after 14 h of system operation.

the styrofoam. Green cardboard cylinders (3.60 cm in diameter \times 8.00 cm in length) were made and overlaid on the extremity of the cage, producing a shadow inside, to encourage the parasitoid to remain in the center of the cage, place where the light travels and where the soybean leaves used as substrate were deposited, in addition to egg carton of the host *E. heros* (Figure 1).

The compression sprayer (GuaranyTM, Itu, SP, Brazil) was used to spray the egg cartons and soybean leaves at the maximum dose recommended by the manufacturer of each insecticide for the crop. The soybean leaves used in the experiment were collected at the beginning of the plant flowering (stage R1) (Fehr & Caviness 1977). During this period, the plants had a larger leaf area, which allowed a better coverage of the internal surface of the cage. After the spraying, the leaves were packed in trays, labeled, kept until turn dry and later introduced into the selectivity cages. Sky-blue cartons (3.00 cm \times 3.00 cm) containing 30 *E. heros* eggs, glued with

gum arabic (20 % of water), were also sprayed with insecticides and water, and then packed in Petri dishes to dry and be introduced into the selectivity cages, together with the soybean leaves and five females of *O. submetallicus*.

The bioassay was developed in an air-conditioned room, with temperature of 25 ± 2 °C, relative humidity of 70 ± 10 % and photophase of 14 h. The cages were disconnected after 24 h and the cartons containing the parasitized eggs were placed in Petri dishes and kept in an air-conditioned room until the evaluation of adult mortality from parasitoids; corrected mortality [Cm (%) = (control - treatment)/(100 - control) \times 100] (Abbott 1925); number of parasitized eggs (identified by the dark color of the eggs); percentage of parasitism [(number of dark eggs/total number of eggs) \times 100]; emergence percentage [(number of eggs with hole/number of dark eggs) \times 100]; sex ratio [$\Sigma \text{♀} / \Sigma (\text{♀} + \text{♂})$]; and parasitism reduction {R = [1 - (P/p)] \times 100, where R is the percentage of reduction in the

parasitoid's parasitism capacity, P is the average of each treatment, and p the average of the control treatment} (Rocha & Carvalho 2004).

The design was completely randomized, with six treatments (active ingredients) and 12 replications each (cages). The insecticide Bazuka™ 216 SL (methomyl) was used as a positive control, due to its toxicity pattern (class). In addition, a treatment consisting of only water was used as a negative control.

The results were submitted to analysis of variance (Anova) and the means were compared by the Tukey test ($p \leq 0.05$), using the statistical software SASM-AGRI (Canteri et al. 2001). The data for the number of parasitized eggs, parasitism, emergence and sex ratio were submitted to the Shapiro-Wilk normality test (Shapiro & Wilk 1965) and, subsequently, to the analysis of variance (Anova). The insecticides were classified according to the norms standardized by the IOBC, under extended laboratory conditions: Class 1 - harmless ($< 25\%$); Class 2 - slightly harmful ($25-50\%$); Class 3 - moderately harmful ($51-75\%$); and Class 4 - harmful ($> 75\%$) (Sterk et al. 1999).

Both the females of the parasitoid, as well as the eggs of the host *E. heros*, used in the bioassay were obtained from the LECOBIO stock creations, kept in an air-conditioned room with temperature of $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ of relative air humidity and 12-h photophase.

RESULTS AND DISCUSSION

The highest adult mortality was caused by methomyl, while teflubenzuron presented an innocuousness similar to the control (Table 2).

Insecticides that belong to the chemical group of organophosphates and carbamates, such as methomyl, are known to cause high and rapid mortality (Turchen et al. 2015, Ramos et al. 2018), and are generally considered to be broad spectrum compounds that have a low selectivity to natural enemies (Fukuto 1990, Hemingway et al. 1993).

The toxicity exerted by these two chemical groups is related to their low molecular masses (Bacci et al. 2007), which allow an easy penetration into the insect cuticle (Stock & Holloway 1993) and a high mortality rate, as observed affecting the behavior of parasitic wasps of *Copidosoma truncatellum* (Dalman) (Hymenoptera: Encyrtidae), when exposed to nine insecticides containing methomyl, which caused 100 % of mortality (Ramos et al. 2018).

Results on the application of organophosphate insecticides (chlorpyrifos, fenitrothion, phoxim, profenofos and triazophos) and carbamates (carbosulfan, carbaryl, isoprocarb, metolcarb and promecarb) were obtained by Wang et al. (2012), and these products were responsible for causing the highest mortality rates in adults of *Trichogramma nubilale* (Ertle & Davis) (Hymenoptera: Trichogrammatidae). Conversely, products from the chemical group of benzoylphenylureas (chlorfluazuron, fufenozide, hexaflumuron and tebufenozide) caused the lowest percentages of mortality to the parasitoid, fact corroborated by the results obtained with the same chemical groups in this study.

The number of *E. heros* eggs parasitized by *O. submetallicus* was negatively affected by the insecticides methomyl and lambda-cyhalothrin + thiamethoxam and beta-cyfluthrin + imidacloprid, when compared to treatments with benzoylphenylureas or the control (Table 3). This was already expected,

Table 2. Average percentage (\pm standard error) of mortality, corrected mortality and parasitism reduction in females of *Ooencyrtus submetallicus* submitted to the selectivity test under extended laboratory conditions and the allocation of phytosanitary product within the IOBC classification.

Treatments	Mortality (%)	Corrected mortality ²	Parasitism reduction (%) ³	Toxicity class ⁴
Control	1.67 ± 1.67 a ¹	-	-	-
Lambda-cyhalothrin + thiamethoxam	91.67 ± 4.58 b	91.52	80.37	4
Beta-cyfluthrin + imidacloprid	81.67 ± 4.58 b	81.35	74.25	3
Methomyl	96.67 ± 2.25 b	96.61	86.09	4
Novaluron	3.33 ± 2.25 a	1.68	23.80	1
Teflubenzuron	1.67 ± 1.67 a	-	24.68	1
CV (%)	32.43			

¹ Means (\pm standard error) followed by the same letter in the column do not differ statistically from each other by the Tukey test ($p \leq 0.05$); ² Corrected mortality (%) = $(\text{control} - \text{treatment}) / (\text{control} - \text{control}) \times 100$ (Abbott 1925); ³ Reduction in parasitism, when compared to the control (Rocha & Carvalho 2004); ⁴ Classes: 1 - harmless ($< 25\%$); 2 - slightly harmful ($25-50\%$); 3 - moderately harmful ($51-75\%$); 4 - harmful ($> 75\%$) (Sterk et al. 1999).

Table 3. Means (\pm standard error) of the biological characteristics of the individual *Ooencyrtus submetallicus* (Hymenoptera: Encyrtidae) submitted to the selectivity test under extended laboratory conditions.

Treatments	Number of parasitized eggs (n = 30) ²	Parasitism (%)	Emergence (%)
Control	18.00 \pm 1.19 a ¹	61.38 \pm 3.97 a ¹	94.12 \pm 2.80 a ¹
Lambda-cyhalothrin + thiamethoxam	4.00 \pm 0.89 b	13.05 \pm 2.97 b	62.89 \pm 11.28 ab
Beta-cyfluthrin + imidacloprid	5.00 \pm 0.66 b	18.05 \pm 2.26 b	51.06 \pm 10.04 bc
Methomyl	3.00 \pm 0.58 b	9.44 \pm 1.92 b	26.38 \pm 10.22 c
Novaluron	15.00 \pm 1.05 a	48.88 \pm 4.41 a	90.94 \pm 3.25 a
Teflubenzuron	14.00 \pm 1.05 a	48.33 \pm 3.49 a	94.40 \pm 1.83 a
CV (%)	34.26	34.26	38.05

¹ Means (\pm standard error) followed by the same letter in the column do not differ statistically from each other by the Tukey test ($p \leq 0.05$); ² number of evaluated eggs.

since the insecticides of the carbamate and pyrethroid chemical groups are toxic to other egg parasitoids, such as *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) (Rocha & Carvalho 2004), *Telenomus podisi* (Ashmead) (Turchen et al. 2015) and *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) (Lowenstein et al. 2019).

Parasitism was also affected by the active ingredients methomyl, lambda-cyhalothrin + thiamethoxam and beta-cyfluthrin + imidacloprid, while novaluron and teflubenzuron were harmless and similar to the control (Table 3). Methomyl and lambda-cyhalothrin + thiamethoxam were classified as Class 4 (harmful) and beta-cyfluthrin + imidacloprid as Class 3 (moderately harmful), while novaluron and teflubenzuron proved to be Class 1 (harmless to parasitoids) (Table 3). The safety of the insecticides teflubenzuron, triflumuron, tebufenozide, novaluron and methoxyfenozide was observed by Stecca et al. (2018), who demonstrated that the *T. podisi* parasitism in *E. heros* eggs was similar to the control, corroborating the results obtained in this study. Insecticides from the benzoylphenylurea chemical group are considered to regulate the insect growth, specifically focused on their target insects (Pazini et al. 2016, Pazini et al. 2017), what may explain the innocuous action of these insecticides on the parasitoid. These insecticides act on the exoskeleton (Sparks & Nauen 2015), inhibiting the chitin synthesis (Stecca et al. 2018), and mainly on immature stages, during the process of insect molting, and, thus, rarely reach adult parasitoids (Bastos et al. 2006).

The emergence of parasitoids was drastically affected by the methomyl product, if compared to the other treatments (Table 3). This may be related to the high neurotoxic action of the product (IRAC 2019), and this effect caused by neurotoxic insecticides was already expected, due to their mode of action on insects.

In some studies, neurotoxic insecticides were harmful to parasitoids, reducing their emergence rate, due to the synergistic action of insecticides with pyrethroid + neonicotinoid mixtures (Turchen et al. 2015); but, in some species, these insecticides had a less harmful action, allowing the emergence of parasitoids (Feltrin-Campos et al. 2018). Knowledge about the action of each neurotoxic insecticide, for each natural enemy species, is essential for a subsequent implementation in biological control programs.

The sex ratio of the *O. submetallicus* parasitoids in *E. heros* eggs was not affected by insecticides, with only female offsprings obtained in all treatments. Bayram et al. (2010) observed that pyrethroids (cyfluthrin and deltamethrin) also did not affect the sex ratio in *Telenomus busseolae* (Gahan) (Hymenoptera: Scelionidae). On the other hand, lufenuron, abamectin, acephate and spenvalerate in *T. pretiosum* negatively affected their sex ratio, in relation to the control (Rocha & Carvalho 2004). However, it is worth noting that the main factor that affects the sex ratio of *O. submetallicus* is the temperature condition, considering the critical temperature of 85 °F (29.44 °C) (Wilson & Woolcock 1960). Therefore, the biological characteristics of this species allow the use of selective products without changing their sex ratio, regardless of the toxicity class of the applied insecticide.

The reduction in parasitism was ranked with IOBC standards, depending on the results obtained from each insecticide. The egg parasitoids *T. podisi* and *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) submitted to the selectivity test by Zantedeschi et al. (2018), with the neurotoxic insecticides imidacloprid + beta-cyfluthrin and lambda-cyhalothrin + thiamethoxam, were classified as Class 2 (slightly harmful to *T. podisi*) and Class 3 (moderately harmful to *T. basalis*). The insecticides

diflubenzuron and lufenuron were described as Class 1 (harmless to *T. podisi* and *T. basalis*) (Zantedeschi et al. 2018), while flufenoxuron and methoxyphenenoid were harmless to *Telenomus remus* Nixon (Hymenoptera: Platygasteridae) (Carmo et al. 2010); thus, these insecticides may be recommended for biological control programs.

This is the first study to determine, under extended laboratory conditions, which insecticides have a selective action against the *O. submetallicus* egg parasitoid. However, tests in field conditions are still necessary, because insecticides may have less impact due to their degradation, or because natural enemies retreat to shelters and avoid locations with applications (Lowenstein et al. 2019). With this knowledge, biological control programs can be conducted without compromising the activity of this parasitoid in controlling pest insect populations. In addition to evaluating the selectivity of the insecticide, it is possible to determine how each insecticide influences biological characteristics.

CONCLUSIONS

1. The active ingredients novaluron and teflubenzuron were classified as Class 1 (harmless to the parasitoid *O. submetallicus*); beta-cyfluthrin + imidacloprid as Class 3 (moderately harmful); and lambda-cyhalothrin + thiamethoxam and methomyl as Class 4 (harmful), under extended laboratory conditions;
2. Insecticides with a selective action may contribute to the survival of *O. submetallicus* and other natural enemies, regulating populations of key pests and resulting in a more sustainable management in the soybean crop;
3. Products classified as Class 3 and Class 4 should, as far as possible, be replaced by other insecticides that are more selective to natural enemies in the soybean crop.

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