ISSN: 2230-9926

*Available online at http://www.journalijdr.com*

IJDR

*International Journal of Development Research*
*Vol. 10, Issue, 01, pp. 33293-33302, January, 2020*

RESEARCH ARTICLE OPEN ACCESS

# EFFECTS OF INSECTICIDES AND PHYTOINSECTICIDES ON THE EGG PARASITOID *Telenomus podisi* ASHMEAD (HYMENOPTERA: PLATYGASTRIDAE)

***[1,7]Jaqueline S. de Souza, [2,8]Luan R. Obem, [3,8]Angélica Massarolli, [4]Bruna Magda Favetti, [5,8]Mônica Josene B. Pereira and [6,8]Alessandra Regina Butnariu**

[1]Bióloga, Mestre em Ambiente e Sistemas de Produção Agrícola; [2]Graduando do Curso de Agronomia; [3]Doutora, Professora do Curso de Ciências Biológicas; [4]Bióloga, Doutora em Agronomia (Proteção de Plantas); [5]Doutora, Professora do Curso de Agronomia; [6]Doutora, Professora do Curso de Ciências Biológicas; [7]Centro Universitário de Sinop – UNIFASIPE, R. Carine, 11, Res. Florença, Sinop – MT - Brasil. CEP: 78550-000.[8]Universidade do Estado de Mato Grosso Campus Universitário de Tangará da Serra. Rodovia MT – 358, Km 07, Jardim Aeroporto, Tangará da Serra – MT – Brasil. CEP: 78300-000.

**ARTICLE INFO**

***Article History:***
Received 29th October, 2019
Received in revised form
07th November, 2019
Accepted 11th December, 2019
Published online 31st January, 2020

***Key Words:***

Euschistus heros; Brown stink bug; Selectivity; Soybean.

***Corresponding author: Jaqueline S. de Souza**

**ABSTRACT**

The brown stink bug *Euschistus heros* Fabrícius (Hemiptera: Pentatomidae) is one of the main pests of the soybean crop. Aiming at the population reduction of this insect, an alternative to chemical control is the association of selective products with biological control through egg parasitoids, such as *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae). The objective of this study was to evaluate the lethal and sublethal effect of insecticides and phytoinsecticides on adult and immature stages of the parasitoid in pre and post parasitism bioassays and sublethal effects. The treatments consisted: Water, Methanol (controls),*Annona crassiflora* extract,Neem oil, Lufenuron, Thiamethoxam+Lambda-cyhalothrin, Imidacloprid and Acephate. A reduction in parasitism rate of 68% and 30% was observed for pre-parasitism treatments with imidacloprid+beta-cyfluthrin and acephate, respectively, both classified as slightly harmful according to IOBC. The emergence of parasitoids was mainly affected when treated with *A. crassiflora*, tiametoxan+lambda-cyhalothrin and imidacloprid+beta-cyfluthrin with means below 10%. In the post-parasitism bioassay, *A. crassiflora* was moderately harmful, reducing 82% of emergence. Among sublethal effects, sexual ratio values below 0.5 indicated absence of females in the treatment with lufenuron and alternating absence and presence of females in the treatment with acephate.



**Citation:** ***Jaqueline S. de Souza, Luan R. Obem, Angélica Massarolli, Bruna Magda Favetti, Mônica Josene B. Pereira and Alessandra Regina Butnariu.*** **2020.** "Effects of insecticides and phytoinsecticides on the egg parasitoid telenomus podisi Ashmead (hymenoptera: platygastridae)", *International Journal of Development Research*, 10, (01), 33293-33302.

## INTRODUCTION

With the modernization of agriculture, pesticides have been commonly used to reduce pest damage, but concerns of long-term adverse consequences for the environment have been reported (Gaboardi Junior 2013). The Brazilian Cerrado is one of the biomes where soybeans have adapted well and the area planted has expanded. Among the states covered by the Cerrado, Mato Grosso has the highest crop yields, generating development for the region (Freitas 2011). One of the main soybean insect pests is the brown stink bug *Euschistus heros* Fabrícius (Hemiptera: Pentatomidae) that feeds directly on the beans and can reach high population levels, consequently decreasing yields (Corrêa-Ferreira and Azevedo 2002, Silva *et al*. 2012). To control this insect, neurotoxic pesticides containing pyrethroids, organophosphates, carbamates, and neonicotinoids, are commonly used (Agrofit 2016). One alternative to insecticides for the control of phytophagous insects is the use of natural enemies, such as the egg parasitoid *Telenomus podisi* (Ashmead) (Hymenoptera: Platygastridae), which affects the embryonic development of the pest, causing its death and preventing damage to the crop (Corrêa-Ferreira and Moscardi 1996). Several authors have confirmed the potential of parasitoids in the control of *E. heros*, thus avoiding the excessive use of chemical products (Hirose 1986, Pacheco and Corrêa-Ferreira 1998, Foerster and Avanci 1999). The

genera *Trissolcus* and *Telenomus* (Hymenoptera: Platygastridae) that naturally occur in the state of Mato Grosso (Godoy *et al*. 2005, Laumann *et al*. 2010, Golin *et al*. 2011). The combination of chemical and biological pest control with native or introduced natural enemies can reduce pesticide use, resulting in less impact to the environment and savings to farmers. The use of *T.podisi* has produced positive results in the management of *E. heros* (Czepak *et al*. 2005, Godoy *et al*. 2013, Golin 2014). Studies on the selectivity of insecticides are therefore essential for the conservation of natural enemies and the environment. The maintenance of biological control agents of insect pests is adversely affected by incompatible pesticides. Thus, insecticides need to be effective in control of the target insect and have low toxicity and residual activity to natural enemies, contributing to the sustainability of the crop (Stecca 2015). Plant oils and extracts have also been successfully used in pest control (Krinski *et al*. 2014; Massarolli *et al*. 2016; Massarolli *et al*. 2017) and their association with other control methods, such as biological control, may be a viable strategy for the reduction of pest insect populations, as it is less harmful to agroecosystems (Cavalcante *et al*. 2006). However, its effects on natural enemies should be evaluated. The lethal and sublethal effects of synthetic and botanical insecticides on *T. podisi* was investigated by several authors (Koppel *et al*. 2011, Smaniotto *et al*. 2013, Silva and Bueno 2014, Golin 2014, Turchen *et al*. 2015). Bueno *et al*. (2008) that concluded that immature stages of parasitoids are more resistant than adults when treated with neurotoxic insecticides, although sublethal effects have been reported on their offspring. Further studies, however, are needed on sublethal effects on parasitoids, as decreased longevity and fecundity have been reported (Desneux *et al*. 2007, Lim and Mahmoud 2008, Bayram *et al*. 2010). Studies on the selectivity of commercial insecticides and phytoinsecticides to parasitoid insects are still incipient but extremely relevant. Brazil is a major producer of soybeans, and more sustainable methods of Integrated Pest Management (IPM) can increase yields and assist in the conservation of biodiversity. Thus, this study was aimed at evaluating the lethal and sublethal effects of insecticides and phytoinsecticides on adult and immature stages of the parasitoid *T. podisi*.

## MATERIAL AND METHODS

**Insect collection:** To establish a laboratory rearing, specimens of the egg parasitoid *T. podisi* and the brown stink bug *E. heros* were obtained in soybean fields in Tangará da Serra, Mato Grosso, Brazil. To obtain parasitoids of pentatomid eggs, egg masses of the brown stink bug were randomly collected in soybean fields. Egg masses were taken to the laboratory and placed on Petri dishes until parasitoid emergence. Newly emerged nymphs were removed to avoid predation of the remaining eggs and raised in the laboratory. Nymphs and adults of *E. heros* were collected in the field with the aid of a beat cloth and transported in clear 5L plastic containers to the laboratory.

**Parasitoid rearing:** Wasps were reared according to the protocol adapted from Peres and Corrêa-Ferreira (2004), in 15 ml test tubes with a cotton plug, fed a thin layer of honey placed on the tube wall with the aid of a fine-bristled brush. Mass rearing of wasps consisted of periodically offering *E. heros* eggs of up to 24 hours of age. After being parasitized, eggs were maintained in a BOD chamber at a temperature of 26°C ± 2°C, 12 hour photophase, and 70% ± 10% relative humidity. Parasitized eggs were kept in test tubes and three drops of water were provided for humidity, which was monitored daily until parasitoids emerged.

***Euschistus heros*rearing:** Eggs, nymphs, and adults of the brown stink bug collected in the field were used to establish a rearing facility at the Laboratory of Entomology of the State University of Mato Grosso, Tangará da Serra. Insects were maintained at 26ºC ± 2°C and 14 hour photophase in adapted cages with an oviposition substrate consisted of strips of cotton fabric, following Corrêa-Ferreira and Oliveira (1982). Insects were fed a diet of bean pods, peanuts, and soybeans, and cotton moistened with water.

**Bioassays:** Bioassays consisted of eight completely randomized groups conducted with 15 replicates each (Table 1). The treatments consisted of four synthetic insecticides chosen because they were commonly used in soybean crops, two phytoinseticides, and two control groups. One of the groups was used as a control for the Anona extract and consisted of 10% methanol (Massarolli *et al*. 2016), while water was used as the second control group. Insecticides were diluted according to the manufacturer's recommendation for the minimum doses, given the controlled conditions and greater exposure in the laboratory (Table 1). The insecticide lufenuron is not used in the chemical control of the brown stink bug, but during the harvest it is used to control caterpillars in the colonization phase of the brown stink bug population and of *T. podisi*. Therefore, this pesticide was evaluated because of the phases where the two pests are present in the crop and, and consequently the possibility of parasitoid exposure to the product. The *A. crassiflora* extract was obtained following Massarolli *et al*. (2016). The concentration was determined based on the study by Turchen *et al*. (2016) that reported a minimum concentration of 0.5% to control *E. heros*. The bioassays were carried out in a BOD incubator under the same conditions of temperature and photophase described for parasitoid rearing. Unhatched eggs were dissected to confirm the presence of parasites according to the methodology described by Turchen *et al*. (2015). For the pre and post-parasitism bioassays, the percentage decrease in parasitism and parasitoid emergence, respectively, were calculated as: E (%) = (1 – Vtrat/Vcontr) x 100, where E is the reduction of viability of parasitism or emergence of parasitoids, Vtrat is the mean of parasitized eggs (or emerged parasitoids) observed in each treatment, and Vcontr is the mean of parasitized eggs (or emerged parasitoids) observed in the control group (Smaniotto *et al*. 2013). The products were classified based on their toxicity to parasitoids using the scale proposed by Hassan (1992) and Manzoni *et al*. (2007) and following the standards determined by IOBC (Table 2).

**Pre-parasitism bioassay**: To evaluate the toxicity to eggs, *E. heros* egg masses of up to 24 hours of age, containing 10 eggs each, were used in treatments (n = 150 eggs per treatment). Eggs were placed in a voile bag, secured by an elastic at the ends, and immersed in treatment solutions for five seconds with the aid of forceps. After drying at room temperature, eggs were placed in test tubes and offered to one couple, with a female previously mated at one day of age. Test tubes then were stored in climate chamber at a temperature of 26°C (± 2°C) and 12 hour photophase. After 24 hours, the couples were removed and the eggs, stored until the emergence ofparasitoids. The numbers of parasitized eggs and emerged wasps were recorded (Turchen *et al*. 2016).

**Table 1. Commercial name, active ingredient, chemical group, mode of action, and dosage used in the treatments**

| Treatments | | Commercial Name | Active Ingredient | Chemical Group | Mode of Action[1] | Dose[2] |
|---|---|---|---|---|---|---|
| T1 | Water (Control 1) | - | - | - | - | 100 ml |
| T2 | Solubilizer (Control 2) | Methanol P.A. | Methyl Alcohol | Methanol | Compound with unknown or uncertain mode of action | 20 ml |
| T3 | Phyto insecticide | Non commercial | Raw *Annona crassiflora* extract | Undetermined | Compound with unknown or uncertain mode of action | 0.50 ml |
| T4 | Phyto insecticide | Neen Oil | Azadirachtin | Azadirachtin | Compound with unknown or uncertain mode of action | 0.50 ml |
| T5 | Insecticide | Match®EC | Lufenuron | Benzoylurea | Chitin synthesis inhibitor | 0.75 ml |
| T6 | Insecticide | Engeo™ Pleno | Tiametoxan + Lambda- cyhalothrin | Neonecotinoid + Pyrethroid | Nicotinic acetylcholine receptor competitive modulators + Sodium channel modulator | 0.10 ml |
| T7 | Insecticide | Conect®SC | Imidacloprid + Beta-cyfluthrin | Neonecotinoid + Pyrethroid | Nicotinic acetylcholine receptor competitive modulators + Sodium channel modulator | 0.50 ml |
| T8 | Insecticide | Perito 970 SG | Acephate | Organophosphate | Acetylcholinesterase inhibitor | 0.375 gr |

[1] Mode of Action according to the Insecticide Resistance Action Committee (IRAC) available at: www.irac-br.org.br; [2] Quantity of commercial product diluted in 100 ml of water to be used in bioassays.

**Table 2. Classification of the selectivity of chemical insecticides to natural enemies according to the International Organization of Biological Control (IOBC)**

| Classification | Reduction in the population of natural enemies (%) | Classes |
|---|---|---|
| Harmless | E <30 % | 1 |
| Slightly harmful | 30 ≤ E ≤ 79% | 2 |
| Moderately harmful | 80 ≤ E ≤ 99% | 3 |
| Harmful | E > 99% | 4 |

**Table 3. Percentage reduction of parasitism viability E (%) and toxicity class of compounds on eggs treated before parasitism by *Telenomus podisi* of according to the International Organization of Biological Control (IOBC)**

| Treatments | | E% | Classificação[1] |
|---|---|---|---|
| T1 | Water | -- | -- |
| T2 | Solubilizer | -- | -- |
| T3 | Anona | 25 | 1 |
| T4 | Azadiratchtin | 16 | 1 |
| T5 | Lufenuron | 8 | 1 |
| T6 | Tiametoxan + Lambda- cyhalothrin | 24 | 1 |
| T7 | Imidacloprid + Beta-cyfluthrin | 68 | 2 |
| T8 | Acephate | 30 | 2 |

[1]Class 1 - harmless (E<30%), class 2 - sightly harmful (30 ≤ E ≤ 79%), class 3 - moderately harmful (80≤ E≤99%), class 4 – harmful (E>99%).

**Sublethal effect bioassay:** The reproductive capacity and the behavioral and/or morphophysiological changes of wasps born in the pre-parasitism bioassay were evaluated. For 10 days, 10 *E. heros* eggs were offered daily to a previously mated *T.podisi* female (Pacheco and Corrêa-Ferreira 1998, Turchen *et al*. 2015). Every 24 hours, eggs were removed and stored in Petri dishes, where they remained until the emergence of wasps. The number of parasitized eggs was also recorded as well as of emerged parasitoids and possible behavioral and/or morphophysiological changes in the offspring.

**Post-parasitism bioassay**: To evaluate wasp emergence in treated eggs after parasitism at the different stages of parasitoid development: egg-larva (one day), larva (five days), and pupa (nine days after parasitism), egg masses containing 10 eggs were immersed in the treatment solutions following the same procedures and evaluations described in the pre-parasitism bioassay.

**Statistical analysis:** The results were examined with an analysis of variance (ANOVA) and when necessary, tested for normality with the Shapiro-Wilk test. Means were compared with the Scott-Knott clustering algorithm with significance set at 5% using the statistical program RStudio (R Core Team 2010) with the package ScottKnott (Jelihovschi *et al*. 2014).

## RESULTS AND DISCUSSION

**Pre-parasitism:** Eggs treated with imidacloprid + beta-cyfluthrin (T7) and acephate (T8) had the largest reduction in parasitism. These compounds are classified as slightly harmful according to IOBC, whereas *A. crassiflora* (T3), thiamethoxan + lambda-cyhalothrin (T6), azadirachtin (T4), and lufenuron (T5) are classified as harmless and induced lower levels of parasitism in this order (Table 3). Many factors are associated to the process of parasitism, such as visual and olfactory stimuli, which can be modified by some compounds, resulting in rejection or repellency, thus reducing oviposition (Pacheco and Corrêa-Ferreira 1998, Abudulai and Shepard 2003, Smaniotto *et al*. 2013, Turchen *et al*. 2015). Lufenuron (T5) did not affect parasitism, as it did not differ statistically from the controls. This might be explained by the selectivity of compounds belonging to the group of growth regulators (chitin synthesis inhibitors) to natural enemies (Carmo *et al*. 2010). In egg parasitoids, adults are little affected, as their mode of action is directed at larvae, acting on chitinases, which are crucial at this stage of development. Growth regulators alter these enzymes, preventing the formation of the exoskeleton, also part of the composition of the epidermis and cuticle (Obrycki *et al*. 1986, Carvalho *et al*. 1994).

**Table 4. Mean (± standard deviation) of parasitized eggs, emerged parasitoids, sex ratio, and live non-emergent parasitoids of *Euschistus heros* eggs treated before parasitism by *Telenomus podisi***

| Treatment | | Parasitized eggs | Emerged Parasitoids | Sex Ratio | Live Non-Emerged Parasitoids |
|---|---|---|---|---|---|
| T1 | Water | 9.6 ± 0.5a[1] | 9.6 ± 0.5a | 0.78 ± 0.0a | 0.0 ± 0.0c |
| T2 | Solubilizer | 9.5 ± 0.6a | 9.3 ± 0.7a | 0.83 ± 0.1a | 0.0 ± 0.0c |
| T3 | Anona | 7.3 ± 1.3b | 0.1 ± 0.3d | 0.06 ± 0.3b | 0.9 ± 1.5c |
| T4 | Azadiratchtin | 8.1 ± 2.5b | 6.6 ± 2.3b | 0.87 ± 0.2a | 0.5 ± 0.9c |
| T5 | Lufenuron | 8.9 ± 1.3a | 3.9 ± 2.1c | 0.69 ± 0.3a | 2.5 ± 1.5b |
| T6 | Tiametoxan+L.c. | 7.3 ± 2.3b | 0.4 ± 0.8d | 0.22 ± 0.4b | 4.2 ± 2.1a |
| T7 | Imidacloprid+B.c. | 3.1 ± 2.0c | 1.0 ± 1.7d | 0.31 ± 0.4b | 1.0 ± 1.1c |
| T8 | Acephate | 6.7 ± 3.3b | 4.8 ± 3.2c | 0.71 ± 0.3a | 0.8 ± 1.0c |
| | *p* value | $2.9^{-15}$ | $2.0^{-16}$ | $1.8^{-15}$ | $2.0^{-16}$ |

[1]Means followed by different letters in the column are significantly different according to the Scott-Knott test and significance set at 5% ($p<0.05$).

**Table 5. Number of surviving pairs of *Telenomus podisi* of the pre-parasitism bioassay and mean (± standard deviation) of parasitized *Euschistus heros* eggs during 10 days**

| Treat. | DAY 1 | | DAY 2 | | DAY 3 | | DAY 4 | | DAY 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N[1] | Parasitized Eggs | N | Parasitized Eggs | N | Parasitized Eggs | N | Parasitized Eggs | N | Parasitized Eggs |
| T1 | 15 | 9.4 ± 0.8a[2] | 15 | 9.8 ± 0.4a | 15 | 9.7 ± 0.6a | 15 | 9.6 ± 0.5a | 15 | 9.3 ± 0.8a |
| T2 | 15 | 9.0 ± 0.6a | 15 | 9.0 ± 0.7a | 15 | 9.1 ± 0.6a | 15 | 9.2 ± 0.5a | 15 | 8.4 ± 2.4a |
| T4 | 7 | 8.2 ± 1.4b | 7 | 7.0 ± 2.1b | 7 | 7.8 ± 2.5b | 6 | 9.2 ± 1.1a | 7 | 6.8 ± 3.2b |
| T5 | 15 | 9.8 ± 0.4a | 15 | 9.4 ± 0.8a | 15 | 8.1 ± 1.3b | 15 | 8.7 ± 1.2b | 15 | 5.5 ± 2.1b |
| T7 | 3 | 5.0 ± 2.6d | 0 | - | 0 | - | 0 | - | 0 | - |
| T8 | 11 | 6.3 ± 2.6c | 11 | 4.0 ± 4.2c | 10 | 2.50 ±4.0c | 3 | 7.7 ± 0.6c | 3 | 8.3 ± 0.5a |
| p value | | $5.0^{-09}$ | | $2.2^{-09}$ | | $1.9^{-11}$ | | $1.9^{-03}$ | | $1.2^{-04}$ |

| Trat. | DAY 6 | | DAY 7 | | DAY 8 | | DAY 9 | | DAY | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N[1] | Parasitized Eggs | N | Parasitized Eggs | N | Parasitized Eggs | N | Parasitized Eggs | N | Parasitized Eggs |
| T1 | 15 | 9.4 ± 0.5a | 14 | 8.0 ± 0.8b | 14 | 7.6 ± 1.7a | 14 | 7.1 ± 0.9b | 12 | 7.4 ± 1.4b |
| T2 | 15 | 7.9 ± 3.3a | 13 | 9.4 ± 0.9a | 13 | 9.1 ± 0.8a | 12 | 8.4 ± 0.9a | 12 | 8.0 ± 0.8b |
| T4 | 7 | 8.4 ± 2.6a | 6 | 8.5 ± 1.4b | 7 | 7.8 ± 2.5a | 6 | 8.3 ± 1.5a | 7 | 7.2 ± 1.1b |
| T5 | 15 | 7.2 ± 1.9a | 13 | 5.3 ± 2.3d | 13 | 6.8 ± 2.5a | 13 | 6.9 ± 1.0b | 0 | - |
| T7 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |
| T8 | 3 | 7.0 ± 3.0a | 3 | 7.0 ± 1.0c | 3 | 8.0 ± 1.0a | 3 | 8.0 ± 1.0a | 2 | 9.5 ± 0.7a |
| p value | | $1.1^{-01}$ | | $1.6^{-07}$ | | $5.6^{-02}$ | | $3.0^{-03}$ | | $5.8^{-02}$ |

[1]N = Number of surviving pairs. [2] Means followed by different letters in the column are significantly different of according to the Scott-Knott test and significance set at 5% ($p<0.05$).

The reduction in parasitism rates observed in the treatments with imidacloprid + beta-cyfluthrin (T7) and acephate (T8) can be explained by the toxicity of these products, which affects females at the moment of oviposition, causing their death shortly afterwards, thus reducing the number of parasitized eggs. Carmo *et al*. (2010) observed a 42.72% reduction in parasitism rates by *Trichogrammapretiosum* Riley (Hymenoptera: Trichogrammatidae) when treated with imidacloprid + beta-cyfluthrin. Few studies have examined these changes but neurotoxic insecticides are known to cause disturbances in nerve transmissions of females depending on the dose, altering their behavior during egg parasitism, such as sensory and motor perception (Garcia 2011). The physico-chemical alterations of eggs also compromise the acceptance of the host by female parasitoids, as well as the repellency caused by the products (Beserra and Parra 2005). Pyrethroids and organophosphate insecticides have neurotoxic activity, producing a knockdown effect by blocking nerve impulses. Since the detection of host eggs is dependent on these impulses, insecticides can affect this mode of action (Werdin González *et al*. 2013), as indicated by the reduction in parasitism rates caused by these types of products reported by several authors. Carvalho *et al*. (1999) reported that the pyrethroid lambda-cyhalothrin reduced in 60% the parasitism rate of *T. pretiosum* in eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae). Vieira *et al*. (2012) classified this product as toxic to *Telenomus remus* Nixon (Hymenoptera: Platygastridae). On the other hand, Panizzi *et al*. (2016) found that *T. podisi* was more tolerant than *T. pretiosum* to insecticides used in rice fields, but lambda-cyhalothrin +thiamethoxam was not selective to parasitoids, reducing in 100% the rate of parasitism. According to Rocha and Carvalho (2004), acephate reduced the parasitism rate of *T. pretiosum* by 80% and was classified as class 3 (moderately harmful), unlike the reduction of 30% (class 2 - slightly harmful) found in the present study. However, toxicity may vary according to species, exposure time, sex, and parasitoid size (Foerster 2002). Phytoinsecticides also affected the ability to parasitize, as reported by Luckmann *et al*. (2014), indicating that some natural products are not selective and can reduce parasitism rates by *T. pretiosum*. Smaniotto (2011) also found that when *E. heros*eggs were treated with azadirachtin, no repellence effect was observed based on parasitism rates by *T. podisi* in a choice test. However, in a no-choice test, parasitism reduction was observed. In the present study, azadirachtin was harmless, unlike the results obtained by Gonçalves-Gervasio and Vendramim (2004), where a reduction in parasitism rates by *T. pretiosum*was observed when *A. Kuehniella* host eggs were sprayed with a 10% aqueous extract. Oliveira *et al*. (2003) also obtained similar results and reported a reduction in parasitsm by *T. pretiosum* following the application of neem extract based emulsifiable concentrate on *A. kuehniella* eggs. Neem extracts have several non-selective effects on insects, including repellency, behavioral changes, disruption in the processes of ecdysis, feeding, and oviposition (Mordue (Luntz) and Nisbet 2000). Differences found in our study may be due to non-standardization of the concentration of active ingredients in the different brands, thus, resulting in differences in selectivity (Silva and Bueno 2014).

**Table 6. Mean (± standard deviation) of emerged parasitoids and sex ratio of eggs parasitized by surviving pairs of *Telenomus podisi* of the pre-parasitism bioassay during 10 days**

| Treat. | DAY 1 | | DAY 2 | | DAY 3 | | DAY 4 | | DAY 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E.P.[1] | S.R.[2] | E.P. | S.R. | E.P. | S.R. | E.P. | S.R. | E.P. | S.R. |
| T1 | 9.4 ± 0.8a[3] | 0.7 ± 0.1a | 9.7 ± 0.4a | 0.7 ± 0.1a | 9.6 ± 0.6a | 0.7 ± 0.1a | 9.6 ± 0.5a | 0.8 ± 0.1a | 9.1 ± 0.9a | 0.7 ± 0.1a |
| T2 | 8.8 ± 0.7a | 0.8 ± 0.1a | 8.8 ± 0.9b | 0.8 ± 0.1a | 9.1 ± 0.6a | 0.8 ± 0.1a | 8.9 ± 0.7a | 0.8 ± 0.1a | 8.8 ± 0.7a | 0.8 ± 0.1a |
| T4 | 6.8 ± 1.3b | 0.6 ± 0.3b | 6.5 ± 1.5c | 0.6 ± 0.1b | 6.3 ± 2.2b | 0.7 ± 0.2a | 7.5 ± 1.0b | 0.8 ± 0.1a | 5.3 ± 2.3b | 0.7 ± 0.1a |
| T5 | 7.6 ± 2.1b | 0.2 ± 0.3d | 8.0 ± 1.5b | 0.1 ± 0.2c | 7.0 ± 1.6b | 0.2 ± 0.3b | 6.4 ± 2.0c | 0.1 ± 0.2b | 5.1 ± 2.5b | 0.1 ± 0.2b |
| T7 | 3.6 ± 2.1d | 0.5 ± 0.5b | - | - | - | - | - | - | - | - |
| T8 | 5.4 ± 2.4c | 0.4 ± 0.3c | 6.3 ± 2.2c | 0.6 ± 0.3b | 4.7 ± 3.1c | 0.70 ± 0.2a | 5.0 ± 1.7d | 0.0 ± 0.0b | 6.0 ± 2.0b | 0.0 ± 0.0c |
| Valor p | $7.4^{-09}$ | $1.7^{-08}$ | $5.1^{-07}$ | $1.4^{-13}$ | $1.0^{-08}$ | $1.3^{-09}$ | $3.4^{-09}$ | $2.0^{-16}$ | $3.2^{-08}$ | $2.0^{-16}$ |

| Treat. | DAY 6 | | DAY 7 | | DAY 8 | | DAY 9 | | DAY 10 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E.P. | S.R. | E.P. | S.R. | E.P. | S.R. | E.P. | S.R. | E.P. | S.R. |
| T1 | 9.0 ± 0.3a | 0.8 ± 0.1a | 7.7 ± 0.8a | 0.7 ± 0.1a | 7.3 ± 1.2b | 0.7 ± 0.1a | 6.9 ± 1.1b | 0.7 ± 0.2a | 6.6 ± 1.3b | 0.7 ± 0.1b |
| T2 | 9.0 ± 0.8a | 0.8 ± 0.1a | 9.0 ± 0.9a | 0.8 ± 0.1a | 8.8 ± 1.1a | 0.8 ± 0.1a | 8.1 ± 0.9a | 0.7 ± 0.1a | 7.8 ± 0.7a | 0.8 ± 0.1a |
| T4 | 6.1 ± 2.2b | 0.6 ± 0.3b | 6.0 ± 2.7b | 0.5 ± 0.4b | 5.7 ± 2.1c | 0.7 ± 0.2a | 7.1 ± 1.8b | 0.7 ± 0.1a | 6.3 ± 0.7b | 0.8 ± 0.1a |
| T5 | 5.3 ± 2.1b | 0.2 ± 0.3c | 3.9 ± 2.7c | 0.2 ± 0.4c | 5.8 ± 1.9c | 0.3 ± 0.4c | 6.0 ± 1.3c | 0.3 ± 0.4b | - | - |
| T7 | - | - | - | - | - | - | - | - | - | - |
| T8 | 5.0 ± 3.4b | 0.1 ± 0.1d | 4.6 ± 1.5c | 0.54 ± 0.5b | 6.6 ± 2.52b | 0.5 ± 0.4b | 6.3 ± 1.5c | 0.7 ± 0.1a | 8.5 ± 0.7a | 0.7 ± 0.0a |
| Valor p | $3.0^{-08}$ | $1.1^{-10}$ | $1.1^{-07}$ | $2.0^{-04}$ | $1.9^{-04}$ | $1.4^{-05}$ | $3.0^{-03}$ | $4.5^{-05}$ | $4.0^{-03}$ | $2.3^{-02}$ |

[1] E.P. = Emerged Parasitoids; [2] S.R. = Sex Ratio; [3] Mean followed by different letters in the column are significantly different according to the Scott-Knott test and significance set at 5% ($p<0.05$).

Regarding parasitoid emergence, all evaluated products differed from control groups (T1 and T2) and, although lufenuron (T5) did not impair parasitism, it adversely interfered in wasp development, causing a significant reduction in parasitoid emergence (Table 4). The treatments with *A. crassiflora* (T3), tiametoxan + lambda-cyhalothrin (T6), and imidacloprid + beta-cyfluthrin (T7) induced the greatest reduction in wasp emergence (Table 4). In addition to acetogenins, *A. crassiflora* extract contains other secondary compounds toxic to insects, such as tannins, alkaloids, and lectins, with different bioactivities that might cause high mortality in wasps throughout their embryonic development and thus a very harmful treatment (Coelho *et al*. 2007, Silva 2010). This is in contrary to the described in the literature that phytoinsecticides are less harmful to natural enemies (Moreira *et al*. 2006). Pyrethroids, present in the composition of thiamethoxan + lambda-cyhalothrin (T6) and imidacloprid + beta-cyfluthrin (T7), are known to be highly toxic to insects. In a study by Koppel *et al*. (2011) on pyrethroid lambda-cyhalothrin, the same compound present in the composition of T6, mortality of *T. podisi* was higher than 90%, also affecting its development.

Oliveira *et al*. (2013) assessed the emergence, parasitism, and survival rates of *T. galloi* on *Diatraea saccharalis* (Fabricius) eggs treated with tiametoxam + lambda-cyhalothrin at the maximum dose and found a 100% reduction for all variables, classifying it as harmful. In the present study, the minimum dose indicated by the manufacturer was used, not reaching the toxicity level for class 4, possibly due to differences in dosages. The egg chorion has openings in areas, such as aeropyles, hydropyles, and micropyles, that play a role in the exchange of gas, water, and other compounds, and despite its rigid texture that acts as a barrier, these products may be able penetrate, especially if we consider possible physico-chemical changes in the egg (Gallo *et al*. 2002; Beserra and Parra 2005). During parasitism after the treatment of eggs, contamination may have occurred during the insertion of the ovipositor into the host egg, killing parasitoids or affecting their development, due to the exposure to residues of insecticides and phyto-insecticides (Pak and Ostman 1982). The reduced emergence observed in some treatments may be associated to the direct action of these products, with lethal effects resulting in disruptions and malformations of organs. Another factor that may have contributed to the low emergency rate is product composition, since some components can contribute to the penetration of the active ingredient in the egg chorion (Desneux *et al*. 2007, Youssef *et al*. 2004).In the assessment of the number of emerged individuals, no signs of emergence were observed in eggs treated with *A. crassiflora* (T3), thiamethoxan + lambda-cyhalothrin (T6), and imidacloprid + beta-cyfluthrin (T7), suggesting a penetration of these products inside the egg. Regarding the treatment with acephate (T8), signs of emergence were observed, although unsuccessful, indicating that wasps attempted to leave the eggs. This suggests that residues on the surface of the chorion may have contaminated wasps while trying to break the eggs with their mandible (Figure 1).

Source: The author

**Figure 1. Dead parasitoids that attempted to leave eggs treated with acephate before parasitism**

The sex ratio obtained for the treatments with *A. crassiflora* (T3), tiametoxan + lambda-cyhalothrin (T6), and imidacloprid + beta-cyfluthrin (T7) differed statistically from the obtained for the other treatments, with values under 0.5, indicating a very low number of females (Table 4). The mean of live non-emerged parasitoids represents parasitoids that were alive as eggs desiccated. Among treatments, tiametoxan + lambda-cyhalothrin (T6) had the highest mean, differing from the other

treatments (Table 4). It should be pointed out that under laboratory conditions, eggs were subjected to maximum exposure to the products in a controlled environment prior to parasitism. Thus the toxicity of many of the products tested and classified as non-selective may be reduced in the field, due to factors such as degradation caused by light, humidity, and temperature (Rocha and Carvalho 2004). Also, egg masses may not be exposed to the insecticide during spraying as they are often laid on the abaxial surfaces of leaves.

**Sublethal effects associated to the pre-parasitism bioassay**: The number of eggs parasitized by *T. podisi* females that emerged in the pre-parasitism bioassay was constant in the control group until the fifth day, and some treatments during the first five days did not differ from the control groups (Table 5). However, starting on the sixth day, more treatments did not differ from control. Treatments with *A. crassiflora* (T3) and thiamethoxan + lambda-cyhalothrin (T6) were not included in the analysis due to insufficient number of surviving couples (Table 5).

Corrêa-Ferreira 1998). Values equal to 1.0 and 0 indicate absence of males and females, respectively (Navarro 1998). Some studies have demonstrate changes in parasitoid sex ratio due to exposure to insecticides.

Rosenheim and Hoy 1988 reported a bias toward males in the offspring of the parasitoid *Aphytismelinus* (Hymenoptera: Aphelinidae) when exposed to chlorpiryfos. Rocha and Carvalho (2004) observed that abamectin, acephate, and esfenvalerate caused alterations in the sex ratio of the parasitoid *T. pretiosum,* similar to the observed by Celestino *et al*. (2014) in *Blaptostethus pallescens* Poppius (Hemiptera: Anthocoridae) with the phytosiniticide azadirachtin. Significant differences in F2 were found by Moura *et al*. (2005), as a result of treatment with imidacloprid, with values of 0.2 for *T. pretiosum*, while tiametoxam did not affect sex ratio, with a mean of 0.7, indicating differences in susceptibility among species (Table 6). The treatments with imidacloprid + beta-cyfluthrin (T7) and acephate (T8) had variable results, with days with absence of females and days

**Table 7. Percentage of reduction of emergency viability E (%) and toxicity class of treatments on developmental stages (Egg-Larva: 1 day; Larva: 5 days; Pupa: 9 day) of *Telenomus podisi***

| | Treatment | Egg-Larva (1 day) | | Larva (5 days) | | Pupa (9 days) | |
|---|---|---|---|---|---|---|---|
| | | E (%) | Class | E (%) | Class | E (%) | Class |
| T1 | Water | - | - | - | - | - | - |
| T2 | Solubilize | 3 | 1 | 2.1 | 1 | 2.2 | 1 |
| T3 | Anona | 82 | 3 | 52.0 | 2 | 77.0 | 2 |
| T4 | Azadiratchtin | 48 | 2 | 28.5 | 1 | 39.5 | 2 |
| T5 | Lufenuron | 16 | 1 | 35.5 | 2 | 13.5 | 1 |
| T6 | Tiametoxan+L.c. | 59 | 2 | 79.5 | 2 | 28.0 | 1 |
| T7 | Imidacloprid+B.c. | 38 | 2 | 60.0 | 2 | 22.0 | 1 |
| T8 | Acephate | 32 | 2 | 38.5 | 2 | 7.0 | 1 |

[1]Class 1 - harmless (E<30%), class 2 - slightly harmful (30 ≤ E ≤ 79%), class 3 - moderately harmful (80≤ E≤99%), class 4 – harmful (E>99%).

**Table 8. Percentage of emerged parasitoids, mean (± standard deviation) of sex ratio and live non-emerged parasitoids of *Euschistus heros* eggs treated at day one, five, and nine after parasitism by *Telenomus podisi***

| Treatment | | 1 D.A.P.[1] | | | 5 D.A.P. | | | 9 D.A.P. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | E.P.[2] | S.R.[3] | L.N.E.P.[4] | PE | S.R. | L.N.E.P. | PE | S.R. | L.N.E.P. |
| T1 | Water | 100.0a[5] | 0.7 ± 0.1a | 0.0 ± 0.0c | 100.0a | 0.7 ± 0.1a | 0.0 ± 0.0c | 99.3a | 0.8 ± 0.1a | 0.0 ± 0.0b |
| T2 | Solubilize | 97.3a | 0.8 ± 0.1a | 0.0 ± 0.0c | 96.6a | 0.8 ± 0.1a | 0.0 ± 0.0c | 91.8a | 0.7 ± 0.2a | 0.0 ± 0.0b |
| T3 | Anona | 20.0d | 0.6 ± 0.7b | 1.4 ± 1.6b | 62.8b | 0.8 ± 0.1a | 1.5 ± 1.3b | 87.8a | 0.7 ± 0.1a | 0.3 ± 0.4a |
| T4 | Azadiratchtin | 67.3b | 0.7 ± 0.2a | 0.0 ± 0.0c | 14.1d | 0.4 ± 0.4b | 1.8 ± 1.3b | 67.7b | 0.7 ± 0.1a | 0.5 ± 0.7a |
| T5 | Lufenuron | 83.0b | 0.8 ± 0.1a | 0.1 ± 0.5c | 48.0c | 0.7 ± 0.1a | 2.8 ± 1.5a | 22.2c | 0.6 ± 0.0a | 0.5 ± 0.6a |
| T6 | Tiametoxan+L.c. | 41.5c | 0.5 ± 0.4b | 2.1 ± 1.2a | 46.0c | 0.8 ± 0.1a | 2.4 ± 1.8a | 74.5b | 0.8 ± 0.1a | 0.7 ± 1.0a |
| T7 | Imidacloprid+B.c. | 75.6b | 0.6 ± 3.4b | 0.4 ± 0.7c | 65.4b | 0.7 ± 0.1a | 0.0 ± 0.0c | 83.2a | 0.8 ± 0.1a | 0.0 ± 0.0b |
| T8 | Acephate | 78.8b | 0.7 ± 0.2a | 0.5 ± 1.1c | 72.0b | 0.8 ± 0.2a | 0.0 ± 0.0c | 61.0b | 0.8 ± 0.1a | 0.0 ± 0.0b |
| *p* Value | | $2.0^{-16}$ | $3.4^{-02}$ | $2.1^{-11}$ | $2.0^{-16}$ | $1.3^{-07}$ | $2.0^{-16}$ | $2.0^{-16}$ | 0.0 | $7.7^{-04}$ |

[1] D.A.P = Day after Parasitism; [2] E.P. = Emerged Parasitoids; [3] S.R. = Sex Ratio; [4] L.N.E.P. = Live Non-Emerged Parasitoids; [5] Means followed by different letters in the column are significantly different according to the Scott-Knott test and significance set at 5% (p<0.05).

In *E. heros* eggs, *T. podisi* females can lay eggs since their first day of life. Pacheco and Corrêa-Ferreira (1998) reported that most eggs were laidduring the first 10 days, with a decrease in reproductive capacity after this period. The means of emerged parasitoids differed from those of the control groups, except for the treatment with acephate (T8), which on some days did not differ from controls (Table 6). The sex ratio obtained for treatment with lufenuron (T5) was lower than 0.5, while for acephate (T8) the ratio oscillated throughout the days, with presence and absence of females. The remaining treatments had values above 0.5 (Table 6), with results that allow mating and stabilization of the parasitoid population. According Flanders (1939), sexual ratio in hymenoptera is biased toward females, approximately 0.7 under normal conditions. The sex ratio of *T. podisi* in *E. heros* eggs under normal conditions has been reported to vary between 0.6 and 0.8 (Pacheco and

with values greater than 0.55, reaching 0.75 for acephate (T8) and allowing mating, suggesting changes in female behavior. The sex ratio for the treatment with azadirachtin (T4) and the control groups was higher than 0.5, which allows greater offspring stability (Table 6). According to Vinson (1997), the sex of progeny of egg parasitoids is determined during egg laying by factors such as physico-chemical characteristics of the host egg, environmental alterations that can cause females to lay more eggs that will give rise to males. The penetration of the products in the egg chorion can also affect sex ratio, killing females as they are require more nutrients (Smaniotto *et al*. 2013). The production of males is know to require less energetic investment, compared to that of females, with sexual allocation being a strategy of wasps when under unfavorable conditions (Flanders 1939). In hymenopterans, haplodiploidy is the mechanism of sex determination, as the control of the

sperm stored in the spermathecae can alter egg fertilization, with fertilized eggs originating females and males developing from unfertilized eggs (Flanders 1965). This could explain the greater number of males obtained, in which the depletion of spermatozoa might have resulted in unfertilized eggs. The absence of females prevents the maintenance and stability of the parasitoid population, as they are responsible for parasitism. On the other hand the absence of males is also detrimental, as it prevents mating and leads to arrhenotokous parthenogenesis, which gives rise to males, making biological control unfeasible (Garcia 2011). Rocha and Carvalho (2004) reported that treatment with lufenuron and acephate resulted in changes in sex ratio (0.5), with in a higher percentage of males in *T. pretiosum.* However, in the present study, no alterations in sex ratio were observed in the treatment with imidacloprid. The variations in sex ratio observed in the present study demonstrate reproductive changes in females (F1) with transgenerational consequences, which in the field affects population growth and establishment of wasps, thus hindering the control of *E. heros* (Turchen *et al*. 2015). The main biological alterations resulted from sublethal effects of insecticides and phytosiniticides are associated with fecundity, longevity, and sexual ratio, while behavioral effects are characterized by changes in the search for host egg search by parasitoids, feeding, physiological conditions, fecundity, emergence index, and developmental period (Foerster 2002). The sublethal alterations observed indicate the need for further studies to examine the effects of the products used in crops to create strategies to minimize them and develop compatible methods of chemical and biological pest control.

**Post-parasitism**: The emergence of parasitoids was affected at all stages of development, when compared to control groups, thus not classified as harmless (Table 7). Even after parasitism, the contact of the insecticide with eggs can affect wasp emergence (Manzoni *et al*. 2007, Vieira *et al*. 2012, Turchen *et al*. 2015). Parasitoid emergence varied among different developmental stages (egg-larva, larva, and pupa-imago). For treated eggs at day one and five after parasitism, results obtained for treatments differed from those of the control groups (Table 8). For treated eggs when wasps were in the pupal phase (9 days), treatments with lufenuron (T5) and acefate (T8) did not differ from those of the control groups (Table 8). During the egg-larva period and the larval stage, parasitoids are more vulnerable, possibly due to the penetration of the product through the micropyle of the chorion, contaminating the embryo and leading to death in the embryonic phase, due to the longer exposure to the period (Carvalho *et al*. 2001). The treatment with thiamethoxan + lambda-cyhalothrin (T6) resulted in a greater reduction in the emergence of parasitoids when compared to other insecticides, being classified as slightly harmful in the egg-larva and larval stages (Table 7). In the pupal stage, this treatment was harmless, although a decrease was observed, suggesting a higher resistance of pupae (Table 8) and corroborating the observed in *T. pretiosum* by Carmo *et al*. (2010). Evaluating the effect of insecticides on *T. pretiosum* on eggs of *A. kuehniella*, Vieira *et al*. (2012) reported that seven days after parasitism (pupal stage), acephate was harmless, while beta-cyfluthrin + imidacloprid and lambda-cyhalothrin + thiamethoxam were moderately harmful and harmful, respectively, unlike the obtained in our study. In a bioassay conducted by Koppel *et al*. (2011), treatment with insecticides belonging to the organophosphorus, pyrethroid, and neonecotinoid chemical groups resulted in a mortality rate above 90% of the immature stages of *T. podisi* in *Euschistusservus* (Say) eggs. The growth regulator lufenuron (T5) was inocuous to *T. podisi* in the egg-larva and pupal phases according to Golin (2014), as also found in the present study. When tested on *T. remus*, lufenuron did not affect treated eggs after parasitism (Carmo *et al*. 2009). However, in *Trichogramma galloi* Zucchi, the same product was extremely toxic one day after parasitism (Cônsoli *et al*. 2001). Treatment with *A. crassiflora* extract (T3) adversely affected the emergence of *T. podisi* in all developmental stages and was the only treatment classified as moderately harmful, with reduction rates ranging between 51 and 82% depending on the stage (Tables 7 and 8). On the other hand, Turchen *et al*. (2014) reported that the extract was selective to the egg parasitoid *Trissolcus urichi* Crawford (Hymenoptera: Platygastridae), regardless of developmental stage.These differences may be due to structure of the egg host (Cônsoli *et al*. 1999) and parasitoid species and reinforce the need for studies with several biological agents, both in embryonic and adult stages. The low emergence rate found for eggs treated with *A. crassiflora* may be due to its ovicidal effect. Several authors have reported an insecticidal activity of compounds present in the various species of *Annona* (Bermejo *et al*. 2005, Trindade *et al*. 2011, Krinski *et al*. 2014, Krinski and Massaroli 2014). However, no studies have examined the ovicidal activity on egg parasitoids.

Differences in the treatments with *A. crassiflora*can be attributed to chemical variations of the extract, due to solvents, solubilizers, storage, dosages, as well as other insecticides and phytosiniticides used in the cited studies and the characteristics of each species. According to our results, susceptibility of wasps to insecticides and phytoinsecticides depends on their developmental state. The higher susceptility during the pupal stage might be due to more exposure to residues in treated eggs, as a result of the proximity between emergence and treatment. In addition, the selectivity to the immature stages depends on intrinsic properties of each species and products used (Foerster 2002). During the embryonic development of *T. podisi* in the field, chances of contact with pesticides are lower, as they does not require feeding and egg masses are mostly laid on the abaxial surface of leaves, contributing for the protection of parasitoids (Panizzi *et al*. 2012, Castilhos *et al*. 2014). Some results obtained would hinder the stability of the parasitoid in the field and would not be compatible with the release of *T. podisi*. Thus studies in semi-field and field conditions are recommended, in order to understand how environmental and behavioral variables can contribute to the selectivity and maintenance of wasps. Consideration should also be given to differences in products, such as the use of insecticides with doses recommended for other crops, indicating a need examine different dosages and mixtures of commonly used active ingredients, as they may affect parasitoid differently (Antigo 2013). A reduction in parasitism rate of 68% and 30% was observed for pre-parasitism treatments with imidacloprid + beta-cyfluthrin and acephateapplied, respectively, both classified as slightly harmful according to IOBC. The emergence of parasitoids was mainly affected when treated with *A. crassiflora*, tiametoxan + lambda-cyhalothrin and imidacloprid + beta-cyfluthrin, with means below 10%. In the post-parasitism bioassay, *A. crassiflora* was moderately harmful, reducing 82% of emergence. Among sublethal effects, sexual ratio values below 0.5 indicated absence of females in the treatment with

lufenuron and alternating absence and presence of females in the treatment with acephate.

**Acknowledgments**

We thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by postgraduate scholarships awarded to the authors. The Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPEMAT) by financial aid to research (Universal Edit Nº 003-2014, Process Nº. 163079/2014).

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