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Selectivity of *Annona* (Annonaceae) extract on egg parasitoid *Trissolcus urichi* (Hymenoptera: Platygastridae)

Selectividad del extracto de *Annona* (Annonaceae) a parasitoide de huevos *Trissolcus urichi* (Hymenoptera: Platygastridae)

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Abstract: The effect of the *Annona crassiflora* (Annonaceae) extract was evaluated on the egg parasitoid *Trissolcus urichi* (Hymenoptera: Platygastridae), natural enemy of *Euschistus heros* (Heteroptera: Pentatomidae), major pest of soybean in Brazil. For this two bioassays were conducted with extract concentrations 0.5%, 1.0%, 2.0% and 4.0% and controls (distilled water and DMSO). In the first bioassay, unparasitized eggs of *E. heros* were treated with the extract of *A. crassiflora* and offered to *T. urichi* females. In second bioassay, parasitized eggs were treated in three stages in order to achieve different development stages of *T. urichi* (egg, larvae and pupae). The variables evaluated were the number of eggs parasitized and parasitoids emerged. The percentage of parasitism was calculated and classified according to IOBC. The extract of *A. crassiflora* reduced parasitism in concentrations higher than 0.5% when applied on unparasitized eggs and when applied on parasitized eggs, it did not affected the developmental stages of the parasitoid indicating the selectivity of the extract on the development of the parasitoid. This is an important result to be considered in a field test, useful as for decisions on the moment of application and the release of the wasps.

Key words: Biological control. *Euschistus heros*. Microhymenopterans. Phytoinsecticides. Annonaceae.

Resumen: Se evaluó el efecto del extracto de *Annona crassiflora* sobre el parasitoide de huevos *Trissolcus urichi*, enemigo natural de *Euschistus heros*, principal plaga de la soya en Brasil. Para esto, se realizaron dos bioensayos utilizando concentraciones de 0,5%, 1,0%, 2,0% y 4,0% y como controles agua y dimetilsulfóxido (10%). En el primer bioensayo, huevos de *E. heros* no parasitados fueron sumergidos en el extracto de *A. crassiflora* y ofrecidos a las hembras de *T. urichi*. En el segundo bioensayo, huevos previamente parasitados fueron sometidos a tratamientos en tres diferentes edades de *T. urichi* (huevo, larva y pupa). Las variables evaluadas fueron el número de huevos parasitados y parasitoides emergidos. El porcentaje de reducción del parasitismo fue calculado y clasificado de acuerdo con la IOBC. El extracto de *A. crassiflora* aplicado antes de ser ofrecido a las hembras redujo el parasitismo en concentraciones superiores al 0,5%, y cuando aplicado después del parasitismo no interfirió en las etapas de desarrollo del parasitoide. Este resultado puede ser útil tanto para una prueba de campo como para la toma de decisiones sobre las fechas de aplicación y liberación de las avispas.

Palabras clave: Control biológico. *Euschistus heros*. Microhymenóptero. Fitoinsecticida. Annonaceae.

Introduction

The neotropical brown stink bug, *Euschistus heros* (Fabricius, 1798) (Heteroptera: Pentatomidae) is an insect pest of economic importance to the soybean crop in Brazil (Panizzi and Slansky Jr. 1985; Panizzi *et al.* 2000), especially in the state of Mato Grosso (MT), due to its abundance and the damage to crops that can cause losses of up to 30% in soybean plantations (Corrêa-Ferreira and Azevedo 2002; Vivan and Degrande 2011). Insecticides are extensively used to control this stink bug, but the frequent use of this practice has caused problems such as residues in food, selection of resistant populations, pest resurgence, reduction of natural enemies and damage to human health (Roel *et al.* 2000; Carmo *et al.* 2010; Belo *et al.* 2012).

An alternative to the use of insecticides, that can reduce negative impacts on the environment, is the implementation of control tactics (Kogan 1998; Bueno *et al.* 2011). However, to integrate management of *E. heros*, it is necessary to know the products that are effective for its control, with minimal impact on non-target species. A strategy for the control of stink bugs is the use of plant extracts with potential insecticides, such as species of *Annona* which are rich in acetogenins with insecticide activity confirmed for *Tibraca limbativentris* Stål, 1860 (Hemiptera: Pentatomidae) (Krinski and Massaroli 2014); *Dichelops melacanthus* Dallas, 1851 (Hemiptera: Pentatomidae) (Souza *et al.* 2007) and *E. heros* (Silva *et al.* 2013).

Another alternative is the adoption of biological control given that, in central Brazil, microhymenopterous parasitoids of the family Platygastridae have been reported in eggs of stink bugs, being the genera *Telenomus* and *Trissolcus* the most abundant (Medeiros *et al.* 1998; Laumann *et al.* 2010; Golin *et al.* 2011; Favetti *et al.* 2013). These parasitoids are effective control agents because they act directly on the embryonic development of the host, preventing the formation of nymphs (Corrêa-Ferreira and Moscardi 1996; Corrêa-Ferreira and Peres 2003).

In this context, the use of more control strategies, associated or not, are important for plant defense in agroecosystems. For

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Cavalcante *et al.* (2006), the combination of plant extracts with other control methods, such as biological control, is a viable alternative. Researchers have reported selectivity of phytoinsecticides on egg parasitoids of *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Platygastridae) using essential oils (Werdin-González *et al.* 2013) and plant extracts (Abudulai and Shepard 2003), as well as in *Telenomus podisi* Ashmead, 1893 (Hymenoptera: Platygastridae), using products of natural origin (Smaniotto *et al.* 2013). However, studies of this nature are scarce.

Nevertheless, phytoinsecticides can be considered promising products as they are effective in the control of stink bugs (Silva *et al.* 2013; Krinski and Massaroli 2014) and show less toxicity on natural enemies and minor residual effect on the environment (Werdin-González *et al.* 2013). Therefore, we carried out this research to evaluate the selectivity of the extract of *Annona crassiflora* Mart, 1841 (Annonaceae) on *Trissolcus urichi* Crawford, 1913 (Hymenoptera: Platygastridae) in *E. heros* eggs.

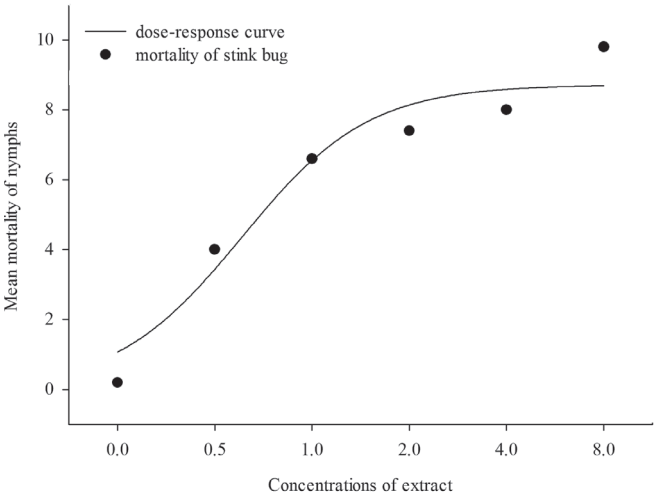

Figure 1. Dose-response curve on nymphs of the *Euschistus heros* showing physiological selectivity of the extract of *A. crassiflora*.

Material and methods

Insects. The eggs used in the bioassays were obtained from the colony of *E. heros* adults, established in the laboratory of Entomology, maintained in cages with dimensions of 40 x 40 x 60 cm, temperature of 26 °C ± 2 °C; 70% ± 10% relative humidity and 12 hours photoperiod. The colony of *T. urichi* were maintained on glass Petri dishes lined with paper and maintained at 25 °C ± 2 °C; 70% ± 10% HR and 12 hours photoperiod. Droplets of honey were offered to adult parasitoids as food and, later, eggs were offered to parasitoids for 24 hours.

Extract. The extract was prepared with mature fruit of *A. crassiflora* collected in the Cerrado field in Deciolândia, state of Mato Grosso. The extract was prepared according to the method of Costa *et al.* (2012), where the fruits are depulped and the seeds placed to dry in an incubator with forced ventilation, for 72 hours at 40 ºC. The dried seeds were triturated to obtain vegetable powder. This powder was added to methanol solvent in the proportion of 500 g in 1500 ml and left to percolate for seven days. After this period, the solution was filtered and taken to a rotary evaporator to evaporate the solvent and obtain the crude extract of *A. crassiflora.*

Previously, a bioassay was conducted with nymphs of *E. heros* to determine the toxicity of the extract of *A. crassiflora* on the stink bug. These bioassays were conducted using a completely randomized design at concentrations of 0.5%, 1.0%, 2.0%, 4.0%, and 8.0% of *A. crassiflora* extract, with 10 replications per treatment. Five nymphs of 3rd instar were used per repetition (n = 50 nymph treatments^{-1}). Posteriorly, the mortality of the insects was evaluated to determine the dose-response curve (Fig. 1). Concentrations between 0.5% and 4.0% showed efficiency in the mortality of the stink bug, directing the selection of concentrations for the bioassay parasitoids.

Bioassays with egg parasitoids were conducted in a completely randomized design with six treatments at concentrations of 0.5%, 1.0%, 2.0% and 4.0%, using distilled water and DMSO 10% as controls. The variables evaluated were the number of parasitized eggs and number of parasitoids emerged.

Bioassay 1 – Effect of the extract of *A. crassiflora* on the parasitism and emergence of *T. urichi*. In this bioassay, unparasitized eggs of *E. heros* were treated with the extract of *A. crassiflora* and then offered to *T. urichi* females. The egg mass containing 10 eggs with up to 24 hours of age were immersed to the treatment concentrations for ten seconds. Ten replicates were assessed for each concentration. After immersion, the eggs were maintained on a glass surface to dry for 30 minutes. Then, the egg masses were individually placed in Petri dishes lined with paper and offered to female parasitoids. The Petri dishes were maintained in a BOD incubator at 25 ºC ± 2 ºC, 70% ± 10% RH and 12 hours photoperiod, using the methodology of Lim and Mahmoud (2008), adapted for *T. urichi*.

After 24 hours of parasitism, females were removed and the eggs maintained until emergence of parasitoids. The eggs from which wasps did not emerge were dissected under stereomicroscope in saline solution (0.9%) for confirmation of parasitism.

Bioassay 2 – Effect of the extract of *A. crassiflora* on the development of *T. urichi*. In this bioassay, egg mass parasitized for *T. urichi* received the treatments mentioned above and for each day five replicates were assessed, containing nine eggs per repetition. The parasitized egg mass was immersed for 10 seconds in the extract of *A. crassiflora* in different stages of wasp development: one day (egg-larvae), five days (larvae-pupae) and nine days (pupae - adult), using the methodology of Lim and Mahmoud (2008) adapted for *T. urichi*. The stages of development of the parasitoid were determined using egg dissection according to the description in Volkoff and Colazza (1992).

After immersion, the eggs were maintained on glass surface to dry for 30 minutes. Then, the egg masses were individually placed in Petri dishes lined with paper and offered to one-day old female parasitoids in a BOD incubator at 25 ºC ± 2 ºC, 70% ± 10% HR and 12 hours photoperiod.

Statistical analysis. Data of parasitism and emergence were submitted to an analysis of variance (ANOVA) and, when significant, the means were compared using the Tukey test

(P > 0.05). The mean of parasitism was used to calculate the percentage of reduction of parasitism (P%) using the equation,

$$P(\%) = \left(1 - \frac{Pt}{Pc}\right) \times 100$$

being Pt the mean parasitism in the treatment and Pc the mean parasitism in the control (Carmo *et al.* 2010). Subsequently, the reduction percentages of parasitism were classified according to the International Organization for Biological Control (IOBC): class 1, harmless (P < 30%); class 2, slightly harmful (30% ≤ P ≤ 79%); Class 3, moderately harmful (80% ≤ P ≤ 99%); and class 4, harmful (P > 99%) (Hassan and Degrande 1996).

To calculate the oviposition deterrence of the egg parasitoid, we used the following formula:

$$D\% = \left(\frac{NC - NT}{NC + NT}\right) \times 100$$

adapted from Obeng-Ofori (1995), where D% is the average percentage of deterrence; NC is the number of eggs in the treatment with distilled water; and NT is the number of eggs in each treatment with extract. The following classification was assigned: Deterrent D > 0 and Neutral D < 0.

Results

Bioassay 1 – Effect of the extract of *A. crassiflora* on the parasitism and emergence of *T. urichi*. The results indicated a significant difference between treatments in the variables parasitized eggs (F = 18.06, df = 5, P < 0.05) and parasitoids emerged (F = 15.25, df = 5, P < 0.05). The immersion of the eggs of *E. heros* at 0.5% of the extract showed 85% of parasitism by *T. urichi* and of these 89% emerged, indicating that this concentration had no effect on oviposition and emergence of the parasitoid. The concentrations 1.0%, 2.0%, and 4.0% showed significant reduction in the number of eggs parasitized with parasitism percentages of 34%, 12% and 12%, respectively (Fig. 2).

The calculation of the percentage of oviposition deterrence in concentrations of 0.5%, 1.0%, 2.0% and 4.0% showed the following results: -1.2%, 41.9%, 74.7% and 74.7%, respectively. These results indicate that, with the exception of concentration 0.5%, which showed no deterrence, the other concentrations inhibited oviposition of the wasp. According to the IOBC classification in relation to reduced parasitism concentration, the concentration of 0.5% was harmless to the parasitoid (class 1), 1.0% was considered slightly harmful to the parasitoid (class 2), and concentrations of 2.0% and 4.0 % were moderately harmful to the parasitoid (class 3).

Bioassay 2 – Effect of the extract of *A. crassiflora* on the development of *T. urichi.* The extract applied on the immature stages of the parasitoid was selective because, independent of the development stage, no significant difference was observed in the emergence of *T. urichi*: egg-larvae (F = 0.76, df = 5, P < 0.05), larvae-pupae (F = 0.43, df = 5, P > 0.05) and pupae-adults (F = 2.36, df = 5, P > 0.05). This is evidence that eggs treated with extract of *A. crassiflora* do not cause the death of the parasitoid during the immature stages (Table 1).

Discussion

It was observed that 0.5% concentration of the extract of *A. crassiflora* was selective for the parasitoid *T. urichi* because it did not interfere in the parasitism and emergence of wasps. This may be associated to the low concentration of the extract and the rapid degradation of the active compound of this solution. These results are similar to those found by Abudulai and Shepard (2003), testing based insecticide for azaradiractin (Neemix™) on the parasitoid *T. basalis* in eggs of *Nezara viridula* (L., 1758) (Heteroptera: Pentatomidae), with parasitism of 89% and emergence of 74% of *T. basalis*. The results observed by Mitchell *et al.* (2004) that verified 84.7% parasitism of eggs of *Clavigralla scutellaris* (Westwood, 1842) (Hemiptera: Coreidae) by *Gryon fulviventris* (Crawford, 1912) (Hymenoptera: Scelionidae) were also similar, characterizing the selectivity of neem extract for this wasp.

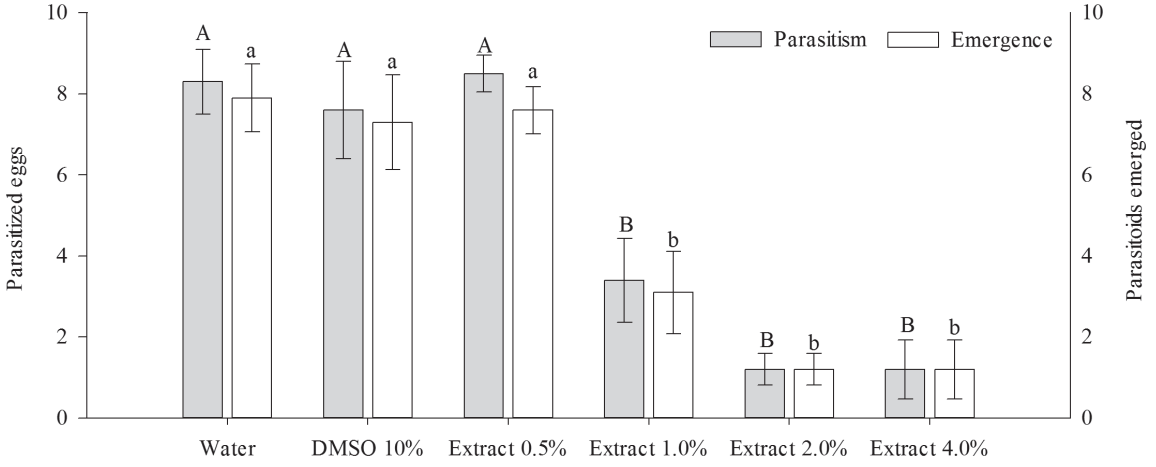

Figure 2. Parasitism and emergence of *Trissolcus urichi* in eggs of *Euschistus heros* treated before parasitism with *Annona crassiflora* extract. Gray bars (± standard error) followed by the same letter (uppercase) and white bars (± standard error) followed by the same letter (lowercase) do not differ by Tukey test (P < 0.05).

Table 3. Parasitism (P) and emergence (E) of *Trissolcus urichi* in eggs of *Euschistus heros* treated after parasitism with *Annona crassiflora* extract.

Treatments	Development stage (days after parasitism)					
	Egg-larvae (1 day)		Larvae-Pupae (5 days)		Pupae-adults (9 days)	
	P*ns	E*ns	P*ns	E*ns	P*ns	E*ns
Water	6.6 ± 0.93	6.0 ± 1.30	7.2 ± 0.97	5.8 ± 1.07	8.2 ± 0.20	7.2 ± 0.86
DMSO 10%	7.2 ± 0.74	6.6 ± 0.69	5.4 ± 0.87	4.6 ± 1.21	6.4 ± 0.68	5.6 ± 0.87
Extract 0.5%	8.0 ± 0.45	7.0 ± 0.55	5.4 ± 1.50	5.4 ± 0.87	7.8 ± 0.20	6.6 ± 0.68
Extract 1.0%	7.2 ± 0.67	5.8 ± 0.58	4.2 ± 1.07	3.8 ± 0.73	7.4 ± 0.40	5.8 ± 0.58
Extract 2.0%	8.0 ± 0.63	7.4 ± 0.40	5.2 ± 0.58	4.6 ± 0.51	6.4 ± 0.40	4.0 ± 0.71
Extract 4.0%	6.2 ± 0.86	5.6 ± 1.03	7.0 ± 0.32	4.4 ± 1.21	7.8 ± 0.37	4.8 ± 0.80
F	0.99	0.76	1.43	0.43	3.52	2.36

*ns no differ statistically (P > 0.05) according to Test F;

In contrast fenitrothion (organophosphate) decreased by 82% the parasitism of *Trissolcus nigripedius* Nakagawa, 1900 (Hymenoptera: Scelionidae) on eggs of *Dolycoris baccarum* L., 1758 (Heteroptera: Pentatomidae) (Lim and Mahmoud 2008), differing from the results observed for *T. urichi* due to high toxicity of fenitrothion to the parasitoid, showing that products of plant origin (phytoinsecticides) may be safer to natural enemies in relation to insecticides (Werdin-González *et al.* 2013).

According to the IOBC classification, the results indicate that concentrations of 1%, 2% and 4% reduced parasitism of *T. urichi*. This behavior was observed by Smaniotto (2011) with alternative insecticides on *T. podisi* in eggs of *E. heros*, with parasitism rates being less than 28%. It was also observed that the extract of *A. crassiflora* inhibits oviposition (deterrence) at concentrations above 0.5%, probably due to alteration of the chemical perception of the host by the parasitoid, interfering with oviposition behavior, reducing the ability to search and of antennation movement during the selection of the host (Foester 2002; Desneux *et al*. 2007).

In bioassay 2, the results indicate physiological selectivity of the extract of *A. crassiflora*, because this presents efficient control of *E. heros* (Fig. 1), at the same time showing low toxicity to the development of *T. urichi* (Table 1). We believe that this selectivity is related to the process of penetration and absorption of the extract (Foerster 2002), since the chorion of the egg of *E. heros* impeded the penetration of the extract of *A. crassiflora*, not affecting the development of the embryo of *T. urichi*. This protection mechanism of the chorion was observed in eggs of various insect orders (Smith and Salkeld 1996), which has significant influence on the susceptibility of eggs, as it constitutes a barrier that protects the embryo-host or even immature stages (egg, larvae and pupae) from the parasitoid (García 2011).

Divergent results were found by Lim and Mahmoud (2008) in eggs parasitized by *T. nigripedius* immersed in fenitrothion, significant reduction being observed in the rate of emergence when the application occurred on the 4th and 6th day after parasitism. Possibly, the low rate of emergence is related to the behavior of the parasitoid. As the wasp feeds on the chorion to emerge it is probably contaminated by contact or ingestion, since this product is highly toxic and has higher residue compared to phytoinsecticides that are less toxic and rapidly degrades (Werdin-González *et al.* 2013).

This research provides important information for the integrated management of *E. heros*, because it shows that the extract of *A. crassiflora* can be used in areas colonized with *T. urichi*, since offspring will be protected inside the egg, favoring the recolonization of the field. It is also a strategy for the producer to reduce the population of nymphs and adults that are not controlled by the wasps.

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