Original Article

POTENTIAL PHYTOINSECTICIDE OF *Annona mucosa* (JACQ) (Annonaceae) IN THE CONTROL OF BROWN STINK BUG

POTENCIAL FITOINSETICIDA DE Annona mucosa *(JACQ) (Annonaceae) NO CONTROLE DO PERCEVEJO-MARROM*

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ABSTRACT: Plant extract have been recognized as important natural source of insecticide. Herein, we assessed the toxicity of leaves-extract and seeds-extract of *Annona mucosa* (Annonaceae) against brown stink bug, *Euschistus heros* (F.) (Hemiptera: Pentatomidae), a soybean pest in Neotropical America. For this, bioassays with nymphs and adults were conducted using the concentrations of 5, 10, 20, 40 and 80 mg.mL^{-1} of leaves and seeds extract of *A. mucosa* and DMSO (10%), as control. Ten replicates were conducted by treatment, with five nymphs (3rd instar) by replicate (bioassay with nymphs) and one couple of adults (< 24h of old) by replicate (bioassay with adults). The number of nymphs death (mortality) and survival of adults were assessed. For adults the fecundity (number of eggs) and fertility (number of nymphs hatched) of females also were assessed. The nymphs bioassay has promising results in seeds extract, when compared to leaves extract. The mortality of nymphs treated with seeds extract was dependent-concentration, with increased in mortality in concentrations > 5.0mg.mL^{-1}, while nymphs treated with leaves extract do not exhibit difference between treatments. In adults the survival was reduced, when were treated with seed extract of *A. mucosa* at concentrations higher than 20mg.mL^{-1}, with reduction up to 92 days in contrast to control. A drastic reduction in the fecundity and fertility was observed, which highlight deleterious action of seed extract against reproductive potential of *E. heros*. This is the first report about toxicity *A. mucosa* (leaves and seed) on *E. heros*, and our results suggest that seeds extract of *A. mucosa* is promising for control of nymphs and adults of brown stink bug.

KEYWORDS: Botanical insecticides. *Euschistus heros*. Toxicity. Nymphs. Adults.

INTRODUCTION

The Neotropical brown stink bug, *Euschistus heros* (F.) (Hemiptera: Pentatomidae) is pest in soybean crop (PANIZZI et al., 2012). The damage caused by this insect are directly in the soybean pods, which reduces the yield and grain quality (commercial product) (PANIZZI et al., 2012; SILVA et al., 2012). In Mato Grosso, the state with greater soybean production is reported that *E. heros* can cause damage of up to 30% in production (VIVAN; DEGRANDE, 2011).

Insecticides are generally used for control of arthropod pests, mainly due the efficiency of these products. However, these are often used incorrectly, which can contribute to selection of resistant populations of insects (SOSA-GOMES and SILVA 2010) and suppression of natural enemies, such as egg parasitoids (TURCHEN et al., 2016). In contrast, plants with insecticidal properties have been investigated as a strategy for pest control, with particular emphasis on substances of secondary metabolism, which are known to act as repellents, deterrents (oviposition or feeding) or may even be lethal to herbivorous insects (MITHOFER and BOLAND, 2012). Furthermore, they have different modes of action (EL-WAKEIL, 2013), which makes them interesting for researchers aiming to develop new insecticidal molecules.

In this context, Annonaceae family has been investigated to insect control (BERMEJO et al., 2005; CASTILLO-SÁNCHEZ et al., 2010; AMINIMOGHADAMFAROUJ et al., 2011; COSTA et al., 2014; KRINSKI et al., 2014) with promising results in the control of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) pest in maize crop (BLESSING et al., 2010), *Sitophilus zeamais* Mots (Coleoptera: Curculionidae) pest of stored grain (RIBEIRO et al., 2013), *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae) cotton pest (COLOM et al., 2008) and also against *E. heros*, soybean pest (SILVA et al., 2013; TURCHEN et al., 2014).

In Annonaceae family the specie *Annona mucosa* (Jacq) have biologically active compounds, such as acetogenins, alkaloids, amides and lignans (CHEN et al., 1996; CHAVEZ et al., 1999; ESTRADA-REYES et al., 2002; LIAW et al.,

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2003), which makes it interesting to search for substances with insecticidal properties for the development of new insecticidal molecules. However, few studies have been conducted with this species to evaluate its potential in control of insect pests. Therefore, we used as a model nymphs and adults of *E. heros* to assess the insecticide potential of *A. mucosa*.

MATERIAL AND METHODS

Insect populations and extract vegetal:

The populations of *E. heros* were collected in soybean fields and reared in controlled environmental conditions at 26ºC ± 2ºC temperature, 70% ± 10% relative humidity, and L12:D12 photoperiod, the same conditions in which the bioassays were carried out. The brown stink bugs were reared in natural diet, as described elsewhere (SILVA et al., 2008). The leaves and seed of *A. mucosa* were collected in Tangará da Serra, Mato Grosso (14° 29'S and 57°54'W), in December (2013) and January (2014). These plants were identified and deposited in the *Tangará herbarium* (TANG) (voucher number: 964).

For the preparation of the extract, the leaves and seed were dried in a drying oven with forced air circulation for 96 hours at 37°C, following the methodology of Prista et al. (1981). After drying, the leaves and seed were ground to a vegetable powder using a knife mill with a sieve with an aperture of 1 and 2mm of diameter, respectively. This powder was added to the solvent (methyl alcohol) at a ratio of 500g to 1500mL and allowed to percolate for seven days. Thereafter, the mixture (solvent + powder) was filtered and the solvent was evaporated by rotative evaporator to obtain the crude extract of leaves and seeds of *A. mucosa*.

Stink bug bioassay

Nymphs and adult bioassays were conducted in a completely randomized design at concentrations 0, 5, 10, 20, 40 and 80 $mg.mL^{-1}$ of the crude extract of leaves and seed of *A. mucosa*, with 10 replications/treatment. For the bioassay with nymphs, five 3rd instar individuals were used per repetition (n=50 nymphs/treatment), and for the bioassay with adults, one pair of *E. heros* up to 24 hours old was used per repetition (n=20 adults/treatment). All bioassays were conducted following the methodology described by Piton et al. (2014). The nymphs and adults were treated by topic application of lμL and 5μL treatments, respectively. After treatment the insects were kept in plastic pots (145mL) with the opening sealed with organza. The pots were lined with filter paper and green-bean pods (*Phaseolus vulgaris*) were used for feeding the insects (COSTA et al., 1998) and changed every two days. The number of dead nymphs (15 days) and survival, fecundity (number of eggs/female) and fertility (number of eggs viable/female) of adults until death were assessed.

Statistical analysis

Normality and homoscedasticity of the data obtained were checked using Shapiro-Wilk and Bartlett tests, respectively. The number of nymph dead (mortality), number of eggs (fecundity) and number of nymphs hatched (fertility) were subjected to analyses of deviance and general linear model (GLM) with *Poisson* distribution and *log* as function link. Significant treatments were compared by *contrasts* ($P < 0.05$). The survival of adults over time were subjected to survival analyses using Kaplan-Meier (Gehan-Breslow) estimators and the survival curves compared using Holm-Sidak's method ($P < 0.05$), always using the R software version. 3.1.1 (R-CORE TEAM, 2014).

RESULTS

Nymphs bioassay:

The mortality of the nymphs of *E. heros* was different between extracts (leaves or seeds) ($\chi^2 = 219.39$; df. = 1,118; $P < 0.001$), with greater mortality in the seeds extract (4.1 ± 1.75a), when compared to the leaves extract (0.36 ± 0.63b). In seeds extract of *A. mucosa* the nymphs mortality was dependent of concentrations ($\chi^2 = 66.98$; df. = 5,54; $P < 0.001$), with mortality higher 90% of nymphs at concentrations of 5 $mg.mL^{-1}$ and 80 $mg.mL^{-1}$, which differ of control (< 10% nymphs death) (see gray bars - Figure 1). Contrasting results were observed for nymph treated with leaf extract, since that do not have difference between treatments ($\chi^2 = 2.06$; df. = 5,54; $P = 0.84$), which indicated inactivity of leaves extract against *E. heros* nymphs (see white bars - Figure 1).

Adults bioassay:

The longevity of *E. heros* adults was affected, when applied the seed extract of *A. mucosa* (Kaplan-Meier = 59.747; df. =5; $P > 0.001$). The concentrations 5 and 10$mg.mL^{-1}$ not exhibit reduction in longevity of adults. However, the concentrations of 20, 40 and 80$mg.mL^{-1}$ significantly differ, with reduction up to 92 days in survival of adults, when compared to control. In addition, seeds extract also can affected fecundity ($\chi^2 = 4386.4$; df. = 5,54; $P < 0.001$) and fertility ($\chi^2 = 5749.1$; df. = 5,54; $P < 0.001$) of female. The

fecundity varied from 72.70 to 7.10 eggs/female at concentrations 10 to 80mg.mL^{-1}, which indicate a drastic reduction (97.32% of eggs) in the egg laying of the insect, when contrasted with control. Similar results were observed in fertility, with 100% of unviable eggs at concentrations 40 and 80 mg.mL^{-1}. These results highlight deleterious action of seed extract against reproductive potential of *E. heros* (Table 1).

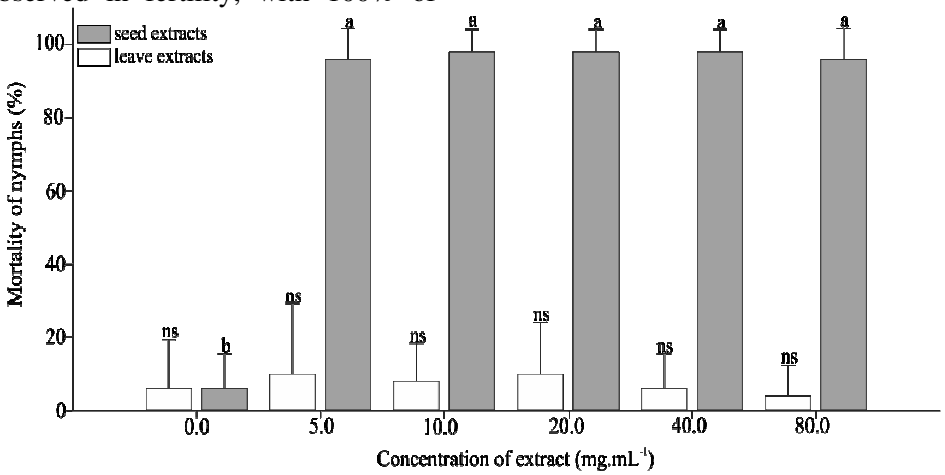

Figure 1. Mean of nymphs of *Euschistus heros* dead, when treated with seed extract (gray bar) and leaves extract (white bars) of *Annona mucosa*. [ns] no significant difference between treatments by analysis of deviance ($P < 0.05$). Same letters indicate no differences between concentrations by contrast analysis ($P > 0.05$)

Table 1. Longevity (days), fecundity (number of eggs) and fertility (number of nymphs hatched) of *Euschistus heros* adults, when treated with seed extract of *Annona mucosa*

Concentrations	Longevity [1]	Fecundity [2]	Fertility [2]
0.0 mg.mL^{-1}	122.93 ± 3.42 a	262.60 ± 165.92 a	191.20 ± 116.35 a
5.0 mg.mL^{-1}	148.98 ± 3.67 a	112.20 ± 146.80 a	69.90 ± 126.68 b
10.0 mg.mL^{-1}	106.06 ± 3.66 a	72.70 ± 138.99 b	0.50 ± 1.08 c
20.0 mg.mL^{-1}	80.83 ± 3.47 b	42.80 ± 87.15 b	10.70 ± 26.75 c
40.0 mg.mL^{-1}	91.25 ± 3.59 b	30.70 ± 65.24 b	-
80.0 mg.mL^{-1}	30.20 ± 2.63 c	07.10 ± 22.45 b	-

[1]Median (± Standard error) followed by the same letter in the column do not differ by multiple comparison of Holm-Sidak test ($P > 0.05$). [2]Means (± Standard error) followed by the same letter in the column do not differ by *contrast* ($P < 0.05$).

DISCUSSION

Botanical insecticides have demonstrated efficiency against pentatomid pests, as example Neem (*Azadiractina indica* Meliaceae) with lethal effect (mortality) and sublethal (i.e., behaviors, food deterrence, low fecundity and fertility) against *Oebalus poecilus* Dallas (Hemiptera: Pentatomidae) rice pest (PINHEIRO; QUINTELA, 2010), *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (ABUDULAI et al., 2003; RIBA et al., 2003; SINGHA et al., 2007) and *E. heros* (SILVA et al., 2013). Similarly, the specie *A. mucosa* has promising results as phytoinsecticide for control of Neotropical brown stink bug (*E. heros*).

In this research, the seeds extract was more toxic for *E. heros* when compared to leaves extract of *A. mucosa*. It is likely that leaves and seeds extract of *A. mucosa* showing variation in their chemical composition. This hypothesis is consistent with Ribeiro et al. (2013) that had higher insect mortality (*S. zeamais*) in the *A. mucosa* seed extract compared to leaves and branches extracts. Nevertheless, further investigation is needed to confirm this hypothesis. In nymphs bioassay seeds extract was highly toxic for nymphs of *E. heros*. This can be clearly seen, since a low lethal concentration (5 mg.mL^{-1}) is required to cause the high death of the stink bug.

For some species of Annonaceae there insecticidal action reports (BERMEJO et al., 2005; CASTILLO-SÁNCHEZ et al., 2010; AMINIMOGHADAMFAROUJ et al., 2011; COSTA et al., 2014; KRINSKI et al., 2014),

although few studies have also shown promising results in the control of Hemiptera. For example, Parra-Henao et al. (2007) reported mortality of 56% of nymphs of *Rhodinius prolixus* Stal (Hemiptera: Reduviidae) and *Rhodinius pallescens* Barber (Hemiptera: Reduviidae) with extract of *Annona muricata* (Annonaceae) (70 mg mL^{-1}). Carneiro et al. (2013) reported to *Rhodinius neglectus* Lent (Hemiptera: Reduviidae) nymphs mortality of 80.0 and 93.3% (100 and 200 mg ml^{-1}) with seeds extract of *Annona coriacea* (Annonaceae).

In Pentatomidae the lethal effect of Annonaceae was reported against *Dichelops melacanthus* Dallas (Hemiptera: Pentatomidae) with mortality of 96% nymphs treated with seeds extract of *A. coriacea* (SOUZA et al., 2007), also have reported of seeds extract of *A. coriacea* and *Annona crassiflora* (Annonaceae) against adults and nymphs of *E. heros* with (SILVA et al., 2013; TURCHEN et al., 2014) and *A. mucosa* and *A. crassiflora* against *Tibraca limbativentris* Stal, 1860 (Hemiptera: Pentatomidae) (KRINSKI; MASSAROLI, 2014). It believed that the toxic effect of Annonaceae against pentatomids can be related to acetogenins, as suggested by Isman (2006); Parra-Henão et al. (2007); Aminimoghadamfarouj et al. (2011), Carneiro et al. (2013), Ribeiro et al. (2013); Silva et al. (2013), Krinski and Massaroli (2014) and Turchen et al. (2014), however, tests for identification and isolation of this substance are needed to confirm this hypothesis.

For adults, decreases in the survival rate was observed, as well as an effect on fertility and fecundity of females, when treated with seeds extract. The drastic reduction in survival, as seen in concentrations of 20 at 80mg.mL^{-1}, may consequently reduce the damage caused by the stink bug in soybean crops, besides preventing the increase in the number of individuals in the next generation. Although concentrations lower than 10mg.mL^{-1} do not reduced survival of adults, a significant reduction in fecundity and/or fertility of females was observed (Table 1), indicating a sublethal effect on physiological traits of *E. heros*, when treated with seeds extract of *A. mucosa*. The reduction in longevity and inhibition of oviposition of females of Pentatomidae are also reported in *N. viridula*, when treated with neem-based products containing compounds rich in azadirachtin (95%) (RIBA et al., 2003) and of *E. heros*, when treated with leaves extract of *P. aduncum* (PITON et al., 2014). We believe that the toxic effect of *A. mucosa* against nymphs and adults of *E. heros*, is likely associated with the presence of the bioactive compounds, such as acetogenins, however, in this research was used crude extract (miscellaneous of compounds), thus the possibility of synergistic action between compounds cannot be discarded, as suggested by Nerio et al (2010), therefore, further investigation is needed to confirm this hypothesis.

In summary, *A. mucosa* seed extract shows promising results for control of nymphs and adults of *E. heros*, because has lethal on nymphs and adults, as well as showed sublethal effect on the reproductive potential of females. In addition, other advantages in *Annona*-extract is physiological selectivity, as suggested by Turchen et al. (2014), since that is toxic against *E. heros,* at the same time exhibit low toxicity to the development of egg parasitoid as *Trissolcus urichi* (Hymenoptera: Platygastridae). This information encourages studies to search for control alternatives and highlights the need for research to isolate and identify the active compounds, aiming to prospect new biopesticides to be incorporated into the management of pests.

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RESUMO: Extratos de plantas tem sido reconhecidos como um importante recurso natural de inseticidas. Nesta pesquisa avaliou-se a toxicidade do extrato de folhas e de sementes de *Annona mucosa* (Annonaceae) sobre o percevejo marrom, *Euschistus heros* (F.) (Hemiptera: Pentatomidae), uma praga-chave na cultura da soja na America Neotropical. Para tanto, bioensaios com ninfas e adultos foram conduzidos usando as concentrações de 5, 10, 20, 40 e 80 mg.mL^{-1} do extrato de folhas e sementes de *A. mucosa* e DMSO (10%), como controle. Dez repetições foram conduzidas por tratamento, com cinco ninfas (3° instar) por repetição (bioensaio com ninfas) e um casal de adultos (< 24h de idade) por repetição (bioensaio com adultos). Foram avaliados o número de ninfas mortas (mortalidade) e a sobrevivência dos adultos. Para fêmeas também foi avaliada a fecundidade (número de ovos) e fertilidade (número de ninfas eclodidas). O extrato de semente de *A. mucosa* apresentou resultados promissores na mortalidade de ninfas de *E. heros*, quando comparado ao extrato de folhas. A mortalidade de ninfas tratadas com extrato de semente foi dependente da concentração, com aumento da mortalidade em concentrações > 5,0mg.mL^{-1}, enquanto as ninfas tratadas com extrato de folhas não apresentaram diferença entre os tratamentos. A sobrevivência dos adultos foi reduzida, quando estes foram tratados com

extrato de semente de *A. mucosa* em concentrações maiores que 20mg.mL^{-1}, com redução de até 92 dias, em contraste ao controle. Foi observada uma drástica redução na fecundidade e fertilidade, o que destaca a ação deletéria do extrato de sementes sobre o potencial reprodutivo de *E. heros*. Este é o primeiro registro sobre a toxicidade de *A. mucosa* (folhas e sementes) sobre *E. heros* e os resultados sugerem que o extrato de semente de *A. mucosa* é promissor para o controle de ninfas e adultos do percevejo marrom.

PALAVRAS-CHAVE: Inseticida botânico. *Euschistus heros*. Toxicidade. Ninfas. Adultos.

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