Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v35i4.16597

Effect of *Anacardium humile* St. Hill (Anacardiaceae) Aqueous Extract on *Mahanarva fimbriolata* (Stal, 1854) (Hemiptera: Cercopidae)

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ABSTRACT. The study of plant-derived substances for the control of insect pests is desirable in the attempt to discover less toxic insecticides that are safe for the environment. Indeed, extracts from the cashew of the savannah, *Anacardium humile*, have shown insecticidal activities against certain insects. The sugarcane root spittlebug *Mahanarva fimbriolata* is considered an important pest of sugarcane, causing severe damage and significant yield reductions. The aim of this study was to evaluate the effect of the aqueous extract of *A. humile* (0.05, 0.4 and 1.0%) on *M. fimbriolata*. The application of the aqueous extract of *Anacardium humile* resulted in 53.1% nymphal mortality at a concentration of 1.0%, which was significantly higher than that observed in the negative control. The nymphal period and longevity of *M. fimbriolata*, however, were not affected by the aqueous *A. humile* extract.

Keywords: insecta, insecticidal plants, plant active ingredients, sugarcane pests.

Efeito do extrato aquoso de *Anacardium humile* St Hill (Anacardiaceae) sobre *Mahanarva fimbriolata* (Stal, 1854) (Hemiptera: Cercopidae)

RESUMO. O estudo com substâncias derivadas de plantas com potencial no controle de pragas justifica-se pela necessidade de se identificar compostos menos agressivos ao meio ambiente. Extratos do cajuzinho-do-cerrado, *Anacardium humile* têm mostrado potencial como inseticida sobre alguns insetos. A cigarrinha-das-raízes, *Mahanarva fimbriolata* é considerada praga importante na cultura da cana-de-açúcar, ocasionando danos severos e sensível redução na produção. O objetivo deste trabalho foi avaliar o efeito do extrato aquoso de *Anacardium humile*, nas concentrações 0,05; 0,4 e 1,0%, sobre *M. fimbriolata*, em cana-de-açúcar. A ação do extrato aquoso resultou em mortalidade ninfal de 53,1% na concentração 1,0%, valor significativamente superior aquele verificado na testemunha negativa. O período ninfal e longevidade de *M. fimbriolata*, no entanto, não foram afetados pelo extrato aquoso.

Palavras-chave: insecta, plantas inseticidas, princípios ativos vegetais, pragas da cana-de-açúcar.

Introduction

Brazil is the world's largest sugar cane producer, and the sugar and ethanol produced supply both the domestic and international needs (ÚNICA, 2012). In the last few years the State of Mato Grosso do Sul, Brazil, has increased its sugar cane plantation areas. However, this crop is attacked by numerous insect species, including the sugarcane root spittlebug *Mahanarva fimbriolata* (Stål, 1854), an insect that causes considerable damage to both sugar cane plants and pastures (GALLO et al., 2002). The management and cultural practices in sugar cane fields have been modified, aiming for higher productivity with a reduced impact on the environment (ALMEIDA et al., 2003). The main modification in the sugarcane management system is with regard to the harvesting process: instead of the traditional burning prior to manual harvest, growers have been forced to avoid it, performing only the mechanical harvest.

Although desirable, this new harvesting process, termed "greencane", results in a significant pest problem, as it creates a habitat that is conducive to spittlebug development. Without burning, the greencane harvesting process favors the buildup of a straw thatch layer on the soil surface. Such litter creates a dark and humid microclimate, stimulating the emission of surface roots and providing an ideal habitat for the development of spittlebug nymphs (DINARDO-MIRANDA et al., 2004, GARCIA et al., 2007a and b).

The proximity of large areas of sugarcane to the fragile Pantanal biome in Mato Grosso do Sul State is worrisome because there is a great risk of contaminating both the water and soil due to the common use of fertilizer and biocides in sugarcane production. However, the use of substances of plant origin that present insecticidal activity provides a sustainable alternative to control insect pests in agriculture (RATAN, 2010).

Anacardium humile St. Hill, a common species of plant in the Pantanal and Cerrado, is widely used by the local populations for medicinal purposes (ALMEIDA et al., 2003). This species of plant has also been the subject of studies conducted by research groups in search of compounds with insecticidal properties (ANDRADE FILHO et al., 2010; PORTO et al., 2008).

The purpose of this work was to evaluate the effect of an aqueous extract of *A. humile* leaves on the survival and biology of the sugarcane root spittlebug.

Material and methods

The bioassays were conducted in the Entomology Laboratory of Tropical Forage Plants, at Embrapa Beef Cattle Research Center, from December 2008 to June 2009 using the susceptible sugarcane cultivar RB86-7515. The mean temperature and relative humidity in the laboratory were 24.9 ± 1.4°C and 54.8 ± 11.3%.

The nymphs

To obtain the nymphs for conducting the trial, eggs of the spittlebug *M. fimbriolata* were obtained according to methodology described by Valério (1993). After being removed from the oviposition substrate (agar-water) and superficially sterilized with Clorox solution, the eggs were kept in Petri dishes in an incubator at 27°C until the nymphs hatched.

The treatments

The aqueous extract of *A. humile* leaves was prepared following the methodology cited by Andrade Filho et al. (2010). The extract was diluted in water to three concentrations (0.05, 0.4 and 1.0%). The insecticide Thiamethoxam (Actara® 250 WG) was used for the positive control, and distilled water only was used for the negative control. Each experimental unit consisted of a plastic pot with a lid that had a central circular opening for fitting and hanging a smaller plastic pot containing a sugarcane seedling. The bottom of this seedling pot was cut to expose the three-month-old sugarcane plant roots. After the application of the plant extracts to these roots, six newly hatched spittlebug nymphs were introduced into the unit. The outer pot was covered with opaque plastic sheeting to ensure darkness, a condition that is optimal for both the roots and nymphs. There were ten replications for each treatment and control.

Nymphal development

The duration of nymphal period was estimated through daily observations, as based on the emergence of *M. fimbriolata* adults in each treatment. The adults showing malformations were recorded and photographed.

Longevity of females and males

The newly emerged adults were maintained in treatment-identified cages containing a sugarcane plant. Through daily observations, the female and male longevities were obtained by recording and removing the dead insects from the cages.

Fecundity

To determine the total number of eggs laid per female in each treatment, the eggs laid throughout the life span of each female were collected and counted at various time intervals.

Egg viability

For each treatment, ten samples of twenty eggs were maintained in Petri dishes in a climatic chamber at 27°C. All of the hatched nymphs were recorded through daily observations. For the statistical analysis, the data were transformed to a percentage of the viable and nonviable eggs.

Statistical analysis

The experimental design was completely randomized. The factors in the aqueous extract treatment consisted of three concentration levels (0.05, 0.4 and 1.0%) using spray applications. Ten replicates were used per treatment. Each experimental unit was infested with six newly hatched nymphs. The data analysis was performed using the Sanest program, applying an F test by ANOVA. The means were compared using the Tukey test ($p < 0.05$), and the results were expressed as the mean ± standard error of the mean. The results were transformed into the square root ($x + 0.5$) for the statistical analysis of the variables fertility and egg viability.

Results and discussion

Nymphal mortality

The mortality caused by the aqueous extracts of *A. humile* ranged from 23.2 at the 0.05% concentration after 5 days of treatment to 53.1 at the

1.0% concentration 15 and 20 days after the application (Table 1). In contrast, the nymphal mortality rate ranged from 14.7 to 21.3% for the negative control (distilled water). These percentages were higher than the natural mortality of 6% recorded by Garcia et al. (2006a and b).

Table 1. Mortality (%) ± standard error of *Mahanarva fimbriolata* nymphs in sugarcane, at the 5, 10, 15 and 20th day after the application of aqueous extract of *Anacardium humile* in different concentrations. Temperature: 24.9 ± 1.4°C, RH 54.8 ± 11.3%.

Treatment	5th Day	10th Day	15th Day	20th Day
0.05%	23.2 ± 7.54 bc[1]	24.9 ± 7.56 bc	29.8 ± 7.78 bc	29.8 ± 7.78 bc
0.4%	31.3 ± 7.64 bc	32.9 ± 8.24 bc	32.9 ± 8.24 bc	32.9 ± 8.24 bc
1.0%	48.1 ± 9.44 b	51.5 ± 10.08 b	53.1 ± 10.18 b	53.1 ± 10.18 b
Negative control[2]	14.7 ± 4.61 c	17.9 ± 3.88 c	21.3 ± 3.55 c	21.3 ± 3.55c
Positive control[3]	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
C.V. (%)	48,8	48,3	46,6	46,6

[1]Means followed by same letter in the column are not different by tukey test ($p < 0.05$). [2]Distilled water. [3]Thiametoxam.

Nymphal mortality rate increased with the rise in the aqueous extract concentration, although only at the highest concentration (1%) a significant difference in relation to negative control was observed. Andrade Filho et al. (2010) reported that the same extract, in concentrations ranging from 0.05 to 2.0% presented insecticidal activity to the whitefly nymph of *Bemisia tuberculata* (Bondar, 1923). Porto et al. (2008) however, found no toxic effect of aqueous extract of *A. humile* in concentrations from 0.0125 to 1% on *Aedes aegypti* (Linnaeus, 1762).

Nymphal development and the longevity of males and females

Data regarding the duration of nymphal period in the different concentrations are presented in Table 2. No statistical difference was observed among treatments and the negative control, whose average nymphal period was of 35.2 days. Such data agree with the findings of Garcia et al. (2006b). This author registered a nymphal period of 37 days when nymphs of *M. fimbriolata* was reared on sugar cane seedlings, at 25°C.

Table 2. Nymphal period (days), longevity (days) of males and females of the root spittlebug, *Mahanarva fimbriolata* in sugarcane, treated with aqueous extract of *Anacardium humile* in different concentrations. Temperature: 24.9 ± 1.4°C, RH 54.8 ± 11.3%.

Treatment	Nymphal Period[1]	Longevity	
		Males	Females
0.05%	34.0 ± 0.43a	13.8 ± 0.74 a	17.3 ±2.18 a
0.4%	34.6 ± 0.38 a	13.6 ± 1.82 a	12.1 ± 1.71 a
1.0%	34.8 ± 0.57 a	15.2 ± 2.56 a	17,6 ± 2.59 a
Negative control[2]	35.2 ± 0.43 a	13.1 ± 1.71 a	15.2 ± 1.33 a
C.V. (%)	7.5	35.8	32,2

[1]Means followed by same letter in the column are not different by tukey test ($p < 0.05$). [2]Distilled water.

There were no significant differences for the longevity of the males and females among all of the tested concentrations and the negative control: the average lifespan for the males was 13 days and 15 days for the females (Table 2). Similar results were reported by Garcia et al. (2006b), with an average longevity of 17.2 days for *M. fimbriolata* reared under conditions similar to those described in the present work. These researchers also noted that there was a reduction in the spittlebug longevity, regardless of the sex, in relation to the control. It was reported that the males exposed to neem *Azadirachta indica* products and aqueous extracts showed longevity reductions of approximately 50%, whereas the reductions were a slightly higher for females (55-60%).

Fecundity, viability and number of fertile females

The average number of eggs per female in the negative control was 165.4 (Table 3). Studying the same insect species at a similar temperature, Garcia et al. (2006b) reported an average of 342.1 eggs per female, though different sugarcane varieties were used. Similar data were found by Gallo et al. (2002), who stated that each female can produce, on average, 340 eggs during her lifetime. In the present study, the lowest tested aqueous extract concentration (0.05%) did not alter the number of eggs laid by each female (145.7) compared to the negative control (165.4) in which only a water spray was used. However, the females from the nymphs treated with the aqueous *A. humile* extract at concentrations of 0.4 and 1% produced 70.7 and 78.1% fewer eggs, respectively, compared to both, the negative control and the 0.05% concentration. A similar result was observed by Schmutterer (1992) when measuring the effect of a neem extract, verifying that the fecundity of Hemiptera was strongly influenced by the neem extract.

Although all ten of the females used in the negative control laid eggs, this was not true for the tested aqueous extract concentrations. When the concentrations of 0.05, 0.4 and 1.0% were used, eight, six and five females laid eggs, respectively. This finding suggests that the *A. humile* aqueous extract impaired the *M. fimbriolata* female fecundity.

Regarding the viability of the obtained eggs, only 40.5% of the eggs were viable in the negative control. Conversely, Garcia et al. (2006b) found that 81% of the eggs were viable. Such a difference could be explained by considering that the authors used different sugarcane varieties than that used in the present study. However, another more probable explanation is that the present work was conducted during the final phase of the infestation period, a time when females tend to oviposit increasing proportions of diapausing eggs.

Table 3. Fecundity (average number) and egg viability (%) ± standard error of the root spittlebug, *Mahanarva fimbriolata*, in sugarcane after application of aqueous extract of *Anacardium humile* in different concentrations. Temperature: 24.9 ± 1.4°C, RH 54.8 ± 11.3%.

Treatment	Fecundity	Viability (%)	Fertile females[1]
0.05%	145.7 ± 38.5 ab[2]	22.0 ± 10.4 ab	8
0.4%	70.7 ± 44.0 b	54.5 ± 9.0 a	6
1.0%	78.1 ± 45.6 b	4.5 ± 1.3 b	5
Negative control[3]	165.4 ± 14.8 a	40.5 ± 12.0 a	10
C.V. (%)	61.4	80.0	

[1]Initial number of couples. [2]Means followed by same letter in the column are not different by tukey test ($p < 0.05$). [3]Distilled water.

A very low egg viability (4.5%), however, was observed at the 1.0% concentration, confirming an important effect of the *A. humile* aqueous extract on the biology of *M. fimbriolata*. Possible effects on both the female fecundity and viability of the eggs suggest a possible cumulative effect on the *M. fimbriolata* females originating from nymphs reared on *A. humile* aqueous extract-treated plants. Evaluating the effect of an aqueous extract of *Azadirachta indica* A. Juss (neem), Garcia et al. (2006a) found an 80% reduction in egg fertility plus morphological and physiological changes in approximately 10% of the eggs of this spittlebug. This species (*A. indica*) of the Meliaceae family contains active triterpenoids ingredients; the Anacardiaceae *A. humile*, also contains theses ingredients and, may cause similar effects. Specifically, the aqueous extract of *A. humile* leaves contains tannins, reducing sugars and saponins. Saponins are complex molecules of high molecular weight that consist of triterpenes or steroids (ANDRADE FILHO et al., 2010). Moreover, Correia et al. (2006) indicated that flavonoids, phenolic lipids and triterpenes are the main substances in several species of the Anacardiaceae family. Although in very low numbers, the emergence of deformed teneral *M. fimbriolata* adults was observed in all three of the *A. humile* aqueous extract concentrations, but not in the negative control. Of the 60 nymphs in each treatment, the numbers of malformed individuals were two, one and one, respectively, for the 1, 0.4 and 0.05% concentrations.

Conclusion

The aqueous extract of *A. humile* sprayed onto the roots of sugarcane caused reductions in the *M. fimbriolata* nymphal survival rate and additional detrimental effects such as decreased fecundity and egg viability. Further investigations of the chemical composition of the extract could determine the active compound acting as an insecticide and could help direct the synthesis of molecules for the control of insect pests.

Acknowledgements

To Pantanal Research Center (Portuguese acronym CPP), National Institute for Science and Technology in Wetlands (INAU), National Council for Scientific and Technological Development (CNPq), Ministry of Science and Technology (MCT), Foundation of Teaching, Science and Technology Development of Mato Grosso do Sul State (Fundect/MS), Embrapa Beef Cattle Research Center (Cnpgc).

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Received on March 30, 2012.
Accepted on July 28, 2012.