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Insecticidal activity of plant extracts on *Dysmicoccus brevipes* in pineapple

Atividade inseticida de extratos vegetais em *Dysmicoccus brevipes* sobre abacaxi

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ABSTRACT

Dysmicoccus brevipes is an insect that can cause many losses in the pineapple crop. Thus, it is necessary to study alternative methods to chemical control. The objective of this study was evaluate the insecticidal activity of extracts of *Azadiractha indica* and *Cyperus iria* on *D. brevipes* in pineapple. Tests were carried out on: colonization of *D. brevipes* and growth of pineapple plants; mortality of this insect by the application of extracts directly and by residual contact, with five repetitions in completely randomized design. Each repetition was composed by one plant to test of colonization of *D. brevipes* and growth of pineapple plants, and a Petri dish with ten female nymphs of 2nd instar to evaluate the mortality of *D. brevipes* by application of extracts directly and by residual contact. The treatments tested were: 1) control 1 (water); 2) control 2 (insecticide Evidence® WG, imidacloprid 700 g kg^{-1}); 3 and 4) aqueous extract of *A. indica* at 5% and 10%, respectively; 5 and 6) aqueous extract of *C. iria* at 5% and 10%, respectively. The data were submitted to analysis of variance and Scott-Knott's test at the 5% level of significance. It was found that extracts *A. indica* 10% and *C. iria* 5% and 10% sprayed once in pineapple plants may adversely affect *D. brevipes* population, not interfering with pineapple growth. Therefore, these extracts have a potential to be used in Integrated Pest Management alone or associated with other control methods.

Keywords: mealybug, neem, *Cyperus iria*, *Ananas comosus*

RESUMO

Dysmicoccus brevipes é um inseto que pode causar perdas na cultura do abacaxizeiro. Assim, é necessário o estudo de medidas alternativas ao controle químico. O objetivo neste estudo foi avaliar a atividade inseticida de extratos de *Azadiractha indica* e *Cyperus iria* sobre *D. brevipes* em abacaxizeiro. Realizaram-se testes de: colonização de *D. brevipes* e crescimento das plantas de abacaxizeiro; mortalidade deste inseto pela aplicação direta dos extratos e por contato residual, com cinco repetições em delineamento inteiramente casualizado. Cada repetição foi composta por uma planta para o teste de colonização de *D. brevipes* e crescimento das plantas, e uma placa de Petri com dez ninfas fêmeas de segundo instar para os testes de mortalidade de *D. brevipes* por aplicação direta dos extratos e por contato residual. Os tratamentos foram: 1) testemunha 1 (água); 2) testemunha 2 (inseticida Evidence® WG, imidacloprid 700 g kg^{-1}); 3) e 4) extrato aquoso de *A. indica* a 5% e 10%, respectivamente; 5) e 6) extrato aquoso de *C. iria* a 5% e 10%, respectivamente. Os dados coletados foram submetidos à análise de variância e teste de comparação de médias de Scott-Knott, com nível de 5% de significância. Verificou-se que os extratos *A. indica* a 10% e *C. iria* a 5% e 10% pulverizados uma única vez em plantas de abacaxizeiro afetam negativamente a população de *D. brevipes*, não interferindo no crescimento inicial das plantas de abacaxizeiro, tendo, portanto, um potencial para serem utilizados no Manejo Integrado de Pragas, isolados ou associados a outros métodos de controle.

Palavras-chave: cochonilha-algodão, nim, *Cyperus iria*, *Ananas comosus*

INTRODUCTION

Pineapple (*Ananas comosus* L. Merril) (Bromeliaceae) (Versieux and Wendt, 2007) is a monocotyledonous, herbaceous and perennial angiosperm that can reach 1.5 m in height (Ferreira *et al*., 2011). It is called king of colonial fruits for having crown and great commercial acceptance. The origin center is South America and Brazil is one of the major centers of genetic diversity in addition to being the world's third largest producer (Crestani *et al*., 2010; FAO, 2017). In Amazonas State, Brazil, pineapple has economic and social importance for local family farming. The pineapple in this region is known to be quite sweet due to its low acidity.

The main obstacles to its development are the damages caused by the action of the pineapple mealybug, *Dysmicoccus brevipes* (Cockerell 1893) (Hemiptera: Pseudococcidae). This insect transmits a disease known as pineapple mealybug wilt caused by the virus Pineapple Mealybug Wilt-Associated Virus (PMWaV), which is widespread in all countries where the pineapple is grown, and can survive in more than 30 host plants (Silva *et al*., 1968).

The warm and humid weather are most favorable to the development of *D. brevipes* (Giacomelli, 1969; Choairy, 1992). The mealybug lives in colonies and are commonly found sucking sap in the roots and armpits of the leaves, but can also be found on fruits, on top of leaves, crowns and seedlings (Sanches and Matos, 1999). During vegetative development, plants infested by mealybug show slow growth, and reduction of leaf number and root length (Lim, 1972). This pest prevents normal fruiting, causes plants weakening by sap sucking, which can lead to plant death. In addition, mealybugs can inoculate Pineapple Mealybug Wilt-Associated Vírus (PMWaV) (genus Ampelovirus; family Closteroviridae), currently considered a virus complex of the PMWaV-1, PMWaV-2, PMWaV-3, PMWaV-4 and PMWaV-5 (Gunasinghe and German, 1986; Ullman *et al*., 1989; Gambley *et al*., 2008).

Currently, chemical control is the most used for pest control. However, some studies showed that plant extracts cause direct mortality, acute toxicity, repellency, inhibition of feeding, sterility, interference with development and modification of arthropod behavior (Menezes, 2005; Coitinho *et al*., 2006; Costa *et al*., 2010; Silva *et al*., 2010), and so it can be alternative crop protectants. Azadirachtin is one of the most promising insecticidal compounds extracted from leaves, fruits and, most importantly, the seeds of neem plant (*Azadiractha indica* A. Juss). This compound is a limonoid that interferes negatively in growth regulation, stopping the molting process, causing death of the insect in the young phase, larvae or nymph, and in the pupal stage; it acts directly on the juvenile hormone balance of several insect pests such as soybean caterpillar, fruit flies, cotton aphid, oriental moth, whiteflies and leafminer (Singh *et al*., 2008; Almeida *et al*., 2010; Bernardi *et al*., 2010; Mordue *et al*., 2010; Janini *et al*., 2011; Alvarenga *et al*., 2012; Andrade *et al*., 2012; Yildirim and Baspinar, 2012). In the same way, "junquinho", *Cyperus iria* Linnaeus (Cyperaceae), has been studied because it has some secondary metabolism compounds that interfere in physiological processes of the insects, and are analogues of the juvenile hormone (Bede *et al*., 2001). This species is a common weed in Brazil (Lorenzi, 2008).

Thus, the objective of this study was to evaluate the effects of *Azadiractha indica* and *Cyperus iria* extracts on *Dysmicoccus brevipes* in pineapple.

MATERIAL AND METHODS

The experiments were conducted in greenhouse (colonization test) and phytosanitary laboratory (mortality tests) of the Institute of Education, Agriculture and Environment (IEAA), Federal University of Amazonas (UFAM), municipality of Humaitá, southern of the Amazonas State.

Capture and rearing of Dysmicoccus brevipes

Adults of *D. brevipes* were obtained in pineapple crop at Humaitá and kept in laboratory ambient conditions. The mealybugs were kept in pumpkins (*Cucurbita maxima* Duchesne), 'Moranga' type, cv. Cabotcha, in plastic trays. To increase *D. brevipes* population new pumpkins were put together with mealybugs colonized pumpkins.

Obtaining plant extracts

The *Cyperus iria* plants used in the experiments were collected from properties located next to Madeira River, and *A. indica* leaves were collected in an urban property in the city of Humaitá, Amazonas State. To obtain the extracts aerial part and root of *C. iria* and leaves of *A. indica* were used. *C. iria* plants were collected at the beginning of flowering. The plants were packed in plastic bags, separated, and immediately transported to the UFAM laboratory where they were washed in running water.

The *C. iria* and *A. indica* plants collected were exposed to solar irradiation until completely dried (constant weight). After drying, they were ground by an industrial blender to obtain a very fine powder, which were stored separately by species and kept in hermetically sealed glass jars.

To obtain the aqueous extracts, 10 g of powder of each plant species was diluted in 100 ml of distilled water to obtain 10% concentration (w/v) extract. This remained at 4 °C for 24 hours for extraction. After this period, the extracts were filtered through filter paper and were used in the experiment in this concentration (10%) and 5% obtained by a 50% dilution.

Colonization of D. brevipes and pineapple plants growth

This experiment was carried out under greenhouse conditions. Pineapple seedlings, cv. Pérola, were obtained from local crops: plants of approximately 30 cm, free from mealybug infestation and Pineapple wilt virus infection were selected and later transplanted to pots (10 L). The substrate used was a mixture of soil, sand and organic matter (3: 2: 1). Soil chemical analysis and the necessary corrections for the pineapple crop were carried out, according to Ribeiro *et al*. (1999). It was used one pineapple per pot, which composes the repetition.

The treatments tested were: 1) control 1 (water); 2) control 2 (Insecticide Evidence 700 WG, a.i. imidacloprid 700g kg^{-1}) at the concentration of 0,3 g L^{-1}; 3 and 4) aqueous extract of *A. indica* at 5% and 10% concentrations; 5 and 6) aqueous extract of *C. iria* at 5% and 10% concentrations.

The solutions (extracts, insecticide or water) were sprayed 100 days after pineapple seedlings had been transplanted to the pots, using 30 ml per plant. After 48 hours, infestation was carried out with 20 female nymphs per plant of 2nd and 3rd instar of *D. brevipes* collected from the rearing with the aid of a fine brush. After the infestation, the plants were placed in individualized cages. It was used five repetitions for each treatment. Each repetition consisted in one pineapple plant. The plants were completely randomized after treatments application.

When at least one plant was weakened by the mealybug attack with yellowed leaves and rolled down, compared to the others without symptoms, event that happened 50 days after the infestation, all the plants were removed. The plants were packed individually in plastic bags identified by treatment and repetition and taken to the laboratory to evaluate the number of mealybugs.

The plant height was measured by the distance between the stem base and the end of the longer length leaf. The weight of green matter of the root, of the aerial part and leaves D (younger leaf among adult leaves with higher metabolic activity) were also considered.

Mortality of Dysmicoccus brevipes by application of extracts directly on the insects

The treatments tested were the same of colonization of *D. brevipes* and pineapple plants growth tests. Five repetitions were used. Ten females nymphs of 2nd instar into a Petri dish composed each repetition. The Petri dishes were completely randomized.

The plant extracts were added of spreader-stickers in the ratio of 10 μl to 100 ml of solution.

It was applied 10 μl of the solutions previously mentioned, directly on the back of each insect with a micropipette and then placed in a 9 cm diameter Petri dish. In the treatment with only water (Control 1), 10 μl of distilled water was applied and in the insecticide treatment (Control 2) was applied 10 μl of the imidacloprid solution on the back of each insect.

Observations were performed 24, 48 and 72 hours after the extract application to evaluate the insect mortality (those insects that did not respond to the touch of the brush were considered dead).

Mortality by residual contact

The treatments tested were the same of the above assays (colonization of *D. brevipes* and pineapple plants growth tests).

In the laboratory, immediately after obtained the extracts of *C. iria* and *A. indica* at both concentrations, a spreader-sticker (mineral oil, 756 g L^{-1}) was added in the ratio of 10 µl to 100 ml of solution to each extract. Solutions were sprayed into Petri dishes (9 cm diameter) containing filter paper, by hand sprayer with 2 L capacity and working pressure at 0.2 to 0.3 MPa. The spray jet was applied until runoff. It was waited 40 min to the filter paper dry naturally. Subsequently, the insects were transferred to these Petri dishes. Mortality assessment was performed at 24, 48 and 72 h (those insects that did not respond to the touch of the brush were considered dead). Each repetition was a Petri dish with ten female nymphs of 2nd instar. Five repetitions were used. The Petri dishes were completely randomized after treatments application.

Statistical analysis

ANOVA assumptions were checked and the mealybugs number data (colonization) transformed by $(x+1)^{0,5}$. Data were submitted to variance analysis tests and Scott-Knott test at 5% probability using the statistical software SISVAR 5.6 (Ferreira, 2014).

RESULTS AND DISCUSSION

Colonization of D. brevipes and pineapple plants development

There was no significant difference between the treatments for the parameters plant height, green shoot weight (GSW), and green weight of leaf D (GWL). However, it was found significant difference in the colonization of pineapple plants by *D. brevipes* (Table 1).

In the colonization assay it was found that the number of insects present in plants treated with *A. indica* 5% concentration was not different from to the control with water. However, there was a reduction on the insect number when *A. indica* at 10%, *C. iria* at 5% and 10% and the reference insecticide were applied, without significant difference among these treatments (Table 1). This reduction in insect number was probably due to the ingestion of insect growth inhibiting substances, possibly present in the applied extracts and in the sap of pineapple plants, which inhibit the development of insects, affecting growth *D. brevipes* population. Similarly, Verson *et al.* (2007) verified that the application of neem extract reduced the growth population of *Myzus persicae*.

Sahito *et al.* (2011) studying the effects of neem on cotton mealybugs (*Phenacoccus solenopsis* Tinsley) observed that neem seed oil affected the survival

Table 1 - Colonization of *Dysmicoccus brevipes* and growth of pineapple (cv. 'Pérola') (mean and standard error) submitted to *Azadirachta indica* and *Cyperus iria* aqueous extracts at different concentrations

Treatments	Plant height* (cm)	GSW** (g)	GWL*** (g)	Colonization* (number of mealybugs)
C1	80,4 ± 2,02 a	0,436 ± 0,018 a	0,035 ± 0,0016 a	32,0 ± 10,54 b
C2	80,4 ± 2,01 a	0,464 ± 0,032 a	0,036 ± 0,0019 a	0,0 ± 0,00 a
A. indica 5%	83,8 ± 2,62 a	0,492 ± 0,032 a	0,038 ± 0,0020 a	44,4 ± 13,05 b
A. indica 10%	78,4 ± 1,66 a	0,407 ± 0,018 a	0,032 ± 0,0012 a	10,2 ± 5,75 a
C. iria 5%	79,2 ± 1,77 a	0,485 ± 0,024 a	0,037 ± 0,0012 a	21,0 ± 10,71 a
C. iria 10%	82,2 ± 2,06 a	0,452 ± 0,026 a	0,035 ± 0,0027 a	16,4 ± 7,50 a
C.V. (%)	5,66	12,58	11,64	-

* Means followed by the same letter, did not differ by Scott-Knott test (P <0.05); ** GSW = Green shoot weight per plant; *** GWL = Green weight of leaf D; C1 = Control 1 (water); C2 = Control 2 (insecticide)

of this insect. Mamoon-ur-Rashid *et al*. (2012) also verified neem effect on biology and survival of *P. solenopsis*.

The azadirachtin disrupts the ecdysis processes, which may affect feeding and reproduction, causes larvae death and growth retardation or lethal physiological deformations (Kumar *et al*., 2008). Souza *et al*. (2015) found that the growth rate of *Aphis gossypii* decreased when they applied neem to discs of watermelon leaves to which the insects were exposed. Okomu *et al*. (2007) observed that few adults of *Anopheles gambiae* were able to emerge and adult lifetime was also reduced after treatment of larvae with 0.2% neem oil, evidencing that azadirachtin is capable of causing sublethal effects. Possibly, this occurs by the action of azadirachtin present in neem oil, which affects the *corpus cardiacus* of the insect that controls the secretion of other glands and, at the end, different hormones. Because metamorphosis requires a perfect synchronization of several hormones to be successful, it eventually causes deformed adult insects.

An explanation for the reduction of the *D. brevipes* when treated with *C. iria* extract is that, according to Bede *et al*. (2001) in these plants there are compounds of the secondary metabolism that interfere in the physiological processes of the insects, and are analogues of the juvenile hormone. These authors verified that the juvenile hormone III (JH III) and its precursor, methyl-farnesoate, active in the control of some species, were identified in 'junquinho', *Cyperus iria*. Other studies are necessary to verify if there is repellent effect of these compounds on *D. brevipes*, thus affecting the rate of colonization.

A study of Toong *et al*. (1988), evaluating the effect of *C. iria* in insects isolated a similar compound to JH III, with morphogenetic effect in several insects. The locust *Melanoplus sanguinipes* (Fabricius 1789) (Orthoptera: Acrididae) reared in the laboratory using *C. iria* as host, presented 90% of the adults with wing deformation. Furthermore, the excess of juvenile hormone made the females sterile.

Mortality of D. brevipes by application of extracts directly and by residual contact

There was no significant difference between the treatments that received direct application of extracts of *C. iria* and *A. indica* in the two concentrations tested (5 and 10%) and water in the three evaluated periods (24, 48 and 72h), except for *A. indica* at 72h that presented an intermediate mortality, independent of the concentration (Table 2). However, there was a significant difference between all the extract treatments and the insecticide, which caused the highest mortality in all the evaluation periods.

The direct application of neem extracts at 5% and 10% concentrations did not cause immediate mortality on *D. brevipes*. This effect was only observed at 72 hours after the contact. Also, Bharathi and Muthukrishnan (2017) observed mortality of *P. solenopsis* greater than 70%, 72 h after application of solution with 5% neem seed extract.

Badshah *et al*. (2015) found that the neem seed aqueous extract at 3% concentration applied to *P. solenopis* caused 38% mortality after 3 days of exposure and, after 7 days, this value reached 61%. In this same work, neem was also applied using the n-hexane solvent. In this case, the mortality was 81% and 100% after 3 and 7 days of exposure, respectively.

Gonçalves-Gervásio and Vendramim (2007) verified, in tomato, 100% mortality of the tomato moth, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) when neem extract at 5% concentration

Table 2 - Mortality (%) (mean and standard error) of *Dysmicoccus brevipes* submitted to *Azadirachta indica* and *Cyperus iria* aqueous extracts at different concentrations by direct application

Treatment	Period (hours)		
	24*	48*	72*
Control 1 (water)	0,0 ± 0,00 b	0,0 ± 0,00 b	2,0 ± 2,00 c
Control 2 (insecticide)	50,0 ± 7,07 a	50,0 ± 7,07 a	50,0 ± 8,60 a
A. indica 5%	2,0 ± 2,00 b	10,0 ± 4,47 b	14,0 ± 4,00 b
A. indica 10%	0,0 ± 0,00 b	4,0 ± 2,45 b	12,0 ± 5,83 b
C. iria 5%	2,0 ± 2,00 b	2,0 ± 2,00 b	4,0 ± 4,00 c
C. iria 10%	2,0 ± 2,00 b	2,0 ± 2,00 b	0,0 ± 0,00 c

* Means followed by the same letter, did not differ by Scott-Knott test (P <0.05).

was applied. However, these researchers applied again the treatment after 7 days and that was not done in the present study. These results indicate that the action of neem extract compounds can be active only for a short period. Future trials are needed to verify the mortality rate of this mealybug for a longer evaluation time and with more than one application.

When treatments were applied on paper surface, there was no significant difference between treatments of *C. iria* and *A. indica* extracts at the two concentrations tested (5 and 10%) and the control 1 (water) at 24, 48 and 72h. However, the mortality with *A. indica* extract, at both concentrations, at 72h was not statistically different to control 2 (insecticide) and higher than the others (Table 3).

Table 3 - Mortality (%) (mean and standard error) of *Dysmicoccus brevipes* submitted to *Azadirachta indica* and *Cyperus iria* aqueous extracts at different concentrations by surface contamination

Treatments	Period (hours)		
	24*	48*	72*
Control 1 (water)	0,0 ± 0,00 b	0,0 ± 0,00 b	6,0 ± 4,00 b
Control 2 (insecticide)	40,0 ± 15,17 a	42,0 ± 15,62 a	42,0 ± 15,62 a
A. indica 5%	2,0 ± 2,00 b	8,0 ± 3,74 b	16,0 ± 4,00 a
A. indica 10%	6,0 ± 2,45 b	12,0 ± 2,00 b	18,0 ± 3,74 a
C. iria 5%	4,0 ± 2,45 b	4,0 ± 2,45 b	8,0 ± 3,74 b
C. iria 10%	0,0 ± 0,00 b	4,0 ± 2,45 b	6,0 ± 2,45 b

* Means followed by the same letter, are not statistically different by Scott-Knott test (P <0.05).

Capps *et al.* (2010) in order to control *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae) using *C. iria* powder at three concentrations (1, 2 and 5%) during 30, 60, 90, 120 and 150 days, found greater repellency when using root powder and aerial parts of the plant at 5% concentration, repelling up to 87% of insects. Adult mortality was 77% when the grains were treated with root powder at 5% concentration, having the other treatments inferior efficiencies. They also verified that the treatment of the grains with *C. iria* powder was more efficient to control *S. oryzae* than the extracts applied to paper.

The tests carried out to evaluate the mortality of *D. brevipes* showed the insecticidal activity of neem extracts at 5 and 10% concentrations in contact with the insects in a 72 h period. These results are in agreement with those obtained by Marcomini *et al.* (2009) that verified a higher mortality rate of *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidade) as the concentration of neem was increased, reaching 87% when this oil was applied at 10% concentration. Alves *et al.* (2012) found that neem, in the form of a pie, reduced the feeding activity and oviposition of mealworms (*Alphitobius diaperinus*) (Panzer) (Coleoptera: Tenebrionidae).

According to Costa *et al.* (2016), the application of an aqueous extract of neem seeds caused more than 90% mortality on larvae and pupae of *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae) in melon. Also, Giongo *et al.* (2016) observed that some nanoformulations with neem caused mortality and sublethal effects; in addition, they presented antifeedant effect for short time. Similarly, Formentini *et al.* (2016) that observed 80% mortality on *Gyropsylla spegazzaniana* (Lizer e Trelles) (Hemiptera: Psyllidae) nymphs in mate seedlings after spraying with neem oil. Pinto *et al.* (2013) found that the effects of neem application on cotton aphids were similar to that of thiamethoxam 72 h after exposure to treatment. However, in a study by Alves *et al.* (2012), neem pie had no effect on mortality of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae).

CONCLUSIONS

The extracts *Azadiractha indica* 10% and *Cyperus iria* of 5% and 10% concentrations sprayed once in pineapple plants may adversely affect the population of *Dysmicoccus brevipes*, not interfering in the normal development of the pineapple crop in the early stages of growth, thus having a potential to be used in Integrated Pest Management alone or associated with other control methods.

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