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Insecticidal activity of *Azadirachta indica* A. Juss. extracts on *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae)

Kleydejany Lima de Lemos[1], Raimunda Nonata Santos de Lemos[2]*, Fabíola Rodrigues Medeiros[2], Ester Azevedo da Silva[2], Mário Luiz Ribeiro Mesquita[3] and José Ribamar Gusmão Araujo[2]

[1]Federal Institute of Education, Science and Technology of Maranhão, São Luis, Brazil.
[2]Graduate Program in Agroecology, Maranhão State University 65055-098. C.P. 09. São Luís, Maranhão State, Brazil.
[3]Maranhão State University Campus Bacabal, Brazil.

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The use of natural products with insecticidal action is a control method compatible with integrated pest management, since it reduces the impacts caused to human health and the environment. This study aimed to assess the effect of *Azadirachta indica* A. Juss. (Meliaceae) leaf and seed aqueous extracts, on citrus blackfly (*Aleurocanthus woglumi* Ashby). *Citrus* x *latifolia* Tanaka ex Q. Jiménez leaves infested with blackfly nymphs were immersed for five seconds in different *A. indica* leaf and seed extract concentrations (1.0, 2.5, 5.0 and 10 w/v). Distillate water was used as control. The assessments were performed at 3, 6 and 9 days after leaf immersion, by counting dead insect number. The *A. indica* leaf and seed aqueous extracts were efficient in the mortality of *A. woglumi* nymphs, representing an alternative method to control this pest.

Key words: Insecticidal activity, *Azadirachta indica* extracts, *Aleurocanthus woglumi* Ashby

INTRODUCTION

The citrus blackfly (*Aleurocanthus woglumi* Ashby) is an important citrus pest. It is an insect whose feeding activity occurs through mouthparts of the piercing-sucking type that causes damage when feeding on phloem parts from citrus plants either in immature or adult phases (Oliveira et al., 2001). Direct damages are caused by nymphs and adults by means of continuous leaf nutrient suction which results in plant weakness, wilting, and in some cases, plant death. Indirect damages arise from sooty mold which is a symptom resulting from fungus growth on *A. woglumi* nymph exudates over leaves and fruits which impair leaf respiration and photosynthesis (Raga et al., 2013). Citrus black fly may cause 20-80% citrus yield losses, thus affecting fruit production and exportations. About 5 to 10 nymphs per square centimeter are enough to reduce the nitrogen level below the 2.2% needed for

*Corresponding author. E-mail: rlemos@cca.uema.br.

orange fruit growth. Research carried out in Mexico showed that more than 90% citrus fruit yield reduction occurs when infestations exceed 5 to 7 nymphs/cm²/leaf. When severe attack occurs in young plants or in plants at seedling stage, may result in plant death (Summy et al., 1983; Nguyen and Hamon, 1993; Oliveira et al., 2001).

Because of its widespread geographical presence in Brazil, citrus black fly is no longer considered a quarantine pest for internal market. Despite the economic and social importance for Brazilian citrus production, research for alternative control methods to control *A. woglumi* is rare.

In order to minimize the problems caused by pesticides, such as environmental pollution, effects on non-target organisms and emergence of resistant organisms, alternatives are being investigated, including the use of bioactive plants by extracts and oils (Lovatto et al., 2012). The ovicidal effect of eucalyptus (*Eucalyptus globulus* Labill.), garlic (*Allium sativum* L.), sesame (*Sesamum indicum* L.), castor bean (*Ricinus communis* L.) and carnation (*Dianthus caryophyllus* L.) oils for the control of *A. woglumi* was studied by Vieira et al. (2013). These authors observed that the concentrations of the tested oils decreased the egg viability. They also noted that commercial eucalyptus and garlic oils reduced the hatch *A. woglumi* nymphs.

Azadirachta indica A. Juss.(Meliaceae), popularly known as neem, is the most studied and used plant species due to its high efficacy and very low toxicity to humans (Martinez, 2002). In recent decades, *A. indica* has been extensively studied because it contains terpenoids with powerful insecticidal activity (Schumutterer, 1990). One of the most active terpenoids in neem seeds is azadirachtin, which is used in commercial formulations and its effects include repellency, feeding deterrence, power repellant, growth disruption, interference with metamorphosis, sterility and anatomical abnormalities (Simmonds et al., 1992).

Despite the derivatives from *A. indica* potential on pest management, little is known regarding their action against the citrus black fly. Thus, this study aimed to evaluate the activity of *A. indica* leaf and seed aqueous extracts on the *A. woglumi* nymph mortality, in laboratory conditions.

MATERIALS AND METHODS

This research was carried out at the Entomology Laboratory of the Agronomic Biotechnology Unity of the Maranhão State University in São Luís, state of Maranhão, northeastern Brazil.

Neem leaf and seeds were collected in the Maranhão State University campus, an *A. indica* plant 10 years age and the exsiccate was deposited in the Rosa Mochel Herbarium at the Maranhão State University. After collection the leaves and seeds were immediately dried in a chamber with forced air circulation at 45 °C for 72 h and subsequently ground in a knife mill to obtain a fine powder for the aqueous extract preparation. The leaf and seed extracts were prepared by adding respectively 1.0, 2.5, 5.0 and 10.0 g of dried powder in 100 mL of distillate water. They were manually stirred until reaching complete homogenization. Suspensions were kept at rest for 24 h and thereafter they were filtered to eliminate solid particles and sprayed over the *A. woglumi* nymphs.

Due to difficulties in the establishment of citrus black fly colony in the laboratory, we used leaves of Tahiti lime (*Citrus latifolia* T.) infested by *A. woglumi*. The leaves were collected, from an orchard located in the municipality of São Luís (02º54'30" S and 44º12'51" W).

Leaves de *C. latifolia* infested by *A. woglumi* nymphs were collected at random in the middle part of the trees. Abscission was performed by means of a pruning shears, close to the branch insertion. Thirty nymphs the second and third instars were selected at random in each leaf. The excess was removed with the aid of a stylus. Nymphs were identified by means of visual analysis with a stereo microscope. The nymphs of 2nd and 3rd stages are oval, with dark Brown or Black color with thorns all over the body. The third instar differs from the second only by the size of the nymphs, which measure 0.87 mm in length and 0.74 mm in width with more visible thorns.

Two bioassays were performed: (1) direct immersion of the leaves in the neem leaf extracts; (2) direct immersion of the leaves in neem seed extracts. In both experiments the concentrations were: 1.0, 2.5, 5.0 and 10.0% (w / v). Distillated water was used as control treatment. The experiments were conducted in completely randomized design with five treatments and ten replications. The experimental plot consisted of an infested leaf of Tahiti lime and an average of 30 nymphs.

After immersion, the leaves were disposed on outdoor paper towels to remove excess surface moisture. Thereafter, the leaves were transferred to Petri dishes containing filter paper, which were periodically moistened to maintain leaf turgor and to avoid nymphs death by lack of food and dryness. The petiole was involved in cotton moistened with distillated water, which was humidified daily. The plates were covered with plastic film, perforated and incubated in an acclimatized-controlled chamber maintained at temperature (26 ± 1°C, relative humidity of 70 ± 10% and photophase of 12 h).

The insect mortality was evaluated by counting of dry nymphs at 3, 6 and 9 days after the immersion of leaves in the extracts.

Data were subjected to analysis of variance (ANOVA) and the values compared by the Nemenyi nonparametric test at 5% probability. Analyses were performed using SAS System software, version 8.2 (SAS, 2001).

RESULTS

All tested concentrations of *A. indica* leaf and seed aqueous extracts were toxic to *A. woglumi* nymphs at 6 and 9 days after leaf immersion (DAI) (Table 1).

Nymphs treated with the *A. indica* leaf extracts at 3 (DAI), in the concentrations of 5 and 10% (w/v) caused different mortality when compared to control.

In the bioassay with the *A. indica* seed extract (Table 2) at 3 DAI treatments 5 and 10% concentrations caused higher nymph mortality, differing from the other treatments. At 6 and 9 DAI, the concentrations of 2.5, 5.0 and 10.0% caused significant nymph mortality (higher than 98%).

DISCUSSION

The *A. indica* leaf and seed aqueous extracts at 5.0 and 10% (w/v) concentrations caused nymph mortality above

Table 1. Nymph mortality (%) of *Aleurocanthus woglumi* treated with *Azadirachta indica* leaf extracts (temperature = 26ºC; relative humidity = 80%; fotophase of 12 h).

Concentration (%)	Nymph mortality (%) (EP[1])		
	3 DAI [2]	6 DAI	9 DAI
1.0	42.20 (11.55)[ab]	82.63 (8.12)[a]	92.98 (4.92)[a]
2.5	46.63 (8.00)[ab]	86.97 (6.58)[a]	97.58 (1.46)[a]
5.0	67.68 (9.87)[a]	85.73 (9.94)[a]	98.00 (2.00)[a]
10.0	81.20 (6.35)[a]	100.00 (0.00)[a]	100.00 (0.00)[a]
Control	13.87 (3.66)[b]	38.68 (3.89)[b]	55.62 (6.46)[b]
CV (%)	69.14	37.14	23.05

Means in each column followed by the same letter are not significantly different ($p< 0.05$) using the Nemenyi test. [1] EP: standard error; [2] DAI: days after immersion.

Table 2. Nymph mortality (%) of *Aleurocanthus woglumi* treated with *Azadirachta indica* seed extract (temperature = 26ºC; relative humidity = 80%; fotoperíodo of 12 h).

Concentration (%)	Nymph mortality (%) (EP[1])		
	3 DAI [2]	6 DAI	9 DAI
1.0	29.48(3.77)[b]	77.56(5.43)[ab]	94.74(2.85)[ab]
2.5	53.04(7.17)[b]	95.77(2.38)[a]	99.12(0.88)[a]
5.0	83.06(6.07)[a]	95.00(3.56)[a]	98.63(1.37)[a]
10,0	86.64(5.63)[a]	99.00(1.00)[a]	100.00(0.00)[a]
0.00	15.08(3.66)[b]	41.68(3.89)[b]	59.30(8.37)[b]
CV (%)	63.42	31.06	22.08

Means in each column followed by the same letter are not significantly different ($p< 0.05$) using the Nemenyi test. [1] EP: stand; [2] DAI: days after immersion.

80%, and therefore can be recommended for use in the citrus integrated pest management programs.

Although information about the nymph mortality of *A. woglumi* by *A. indica* extracts are scarce, Silva et al. (2012) assessing the effect of soybean (*Glycine max* (L.) Merr.), corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and sunflower (*Helianthus annuus* L.) commercial oils and *A. indica* root extract on nymphs of black fly, found that higher oil concentrations (0.5, 1.0 and 1.5%) caused 100% nymph mortality.

Research conducted by Bezerra-Silva et al. (2010), to assess the effect of organic extracts of *A. indica*, *Melia azedarach* L., *Toona ciliata* M. Roem. and *Trichilia pallida* Sw. on eggs and nymphs of *Bemisia tabaci* biotype B, found greater nymphicidal activity in treatments with ethanolic extracts of *M. azedarach* branches (97.56%) and *A. indica* branches and leaves (95.45 and 92.94% respectively). Lima et al. (2011) evaluated the effectiveness of ethanolic extracts of *Ipomoea carnea* subsp., *R. communis*, *Mascagnia rigida* Griseb, *Argemone mexicana* L. and neem oil (*A. indica*), for control of *B. tabaci* biotype B and found that the *Ipomoea carnea* subsp. *fistulosa* and neem oil treatments were the most efficient on the nymph control, showing nymph mortality of 72.41 and 67.26%, respectively.

Thus, in both bioassays of this study it was found that the highest nymph mortality occurs with 6 and 9 DAI, corroborating the findings of Martinez (2002) who found that the larger active ingredient peaks derived from the *A. indica* products, which translocate into the plant, take place five days after the treatment.

Bleicher et al. (2007) evaluated the effect of *A. indica* leaf aqueous extracts and an azadirachtin-based formulation on nymphs of *B. tabaci*. The authors confirmed that azadirachtin and *A. indica* leaf aqueous extracts significantly reduced the average number of live nymphs and did not differ from the standard buprofezin insecticide action.

According to Costa et al. (2004), each plant species may have a variation in the content of active ingredients, depending on the plant stage, used plant structure, and the soil and climate conditions under which plants grow. The *A. indica* seeds and leaves contain terpenoids with powerful insecticidal activity. The leaf active terpenoids include nimbin, deacetylnimbin and thionemon (Simmononds et al., 1992). Thus, in both bioassays in this study, it was found that the *A. indica* leaf and seed extracts effect on *A. woglumi* adversely affect insect development, suggesting that the active ingredients are present in different plant structures, although with varying

concentrations.

According to Coudriet et al. (1985), the sensitivity of nymphs may be related to plant extract action on the neuro-endocrine system that regulates the ecdysteroid or anti-ecdysteroid production. These results are similar to those found by Natarajan and Sundaramurthy (1990), in assessing the nymphal mortality caused by *A. indica* oil (0.5 to 1%) in whitefly (*B. tabaci* biotype B). The authors found that only 14.3 and 13.0% of nymphs reached the adult stage, of which 51.3 and 56.8%, respectively, showed abnormalities and disorders characteristics of the insects endocrine system, according to Saxena and Khan (1985), while in the controls (monocotrophos 0.08% and water), 84.3 and 94.0% of nymphs reached adulthood, with abnormality rates of 7.0 and 2.8% respectively.

Given the high availability and ease of growing of *A. indica* plants, the preparation and use of aqueous extracts may be feasible and efficient to control the citrus black fly. However, more research is needed, such as comparative testing of commercial formulations with the plant extracts, assessment of lethal and sublethal effects of these products on natural enemies, and the development of field work, that are of fundamental importance, because the product action may be altered by environmental conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Bezerra-Silva GCD, Vendramim JD, Silva MA, Dias CTS (2010). Efeito de extratos orgânicos de Meliaceae sobre *Bemisia tabaci* (Gennadius) biotipo B em tomateiro. Arq. Inst. Biol. 77(3):477-485.

Bleicher E, Gonçalves MEC, Silva L (2007). Efeito de derivados de nim aplicados por pulverização sobre a mosca-branca em meloeiro. Hortic. Bras. 25(1):110-113.

Costa ELN, Silva RFP, Fiúza LM (2004). Efeitos, aplicações e limitações de extratos de plantas inseticidas. Acta Biol. Leopold. 26(2):173-185.

Coudriet DL, Prabhaker N, Meyerdirk DE (1985). Sweerpotato whitefly (Homoptera: Aleyrodidae): Effects of nem-seed extract on oviposition and immature stages. Environ. Entomol.14(6):776-779.

Lima BMFV, Moreira OTM, Pinto HCS (2011). Avaliação de extratos vegetais no controle de mosca branca em tomate. Rev. Caat. 24(4):36-42.

Lovatto PB, Martinez EA, Mauch CR, Shiedek G (2012). A utilização de espécie *Melia azedarach* L (Meliaceae) como alternativa à produção de insumos ecológicos na região sul do Brasil. Rev. Bras. Agroec. 7(2):137-149.

Martinez SS (2002). O nim *Azadirachta indica*: natureza, usos múltiplos, produção**.** Londrina: IAPAR. 142 p.

Natarajan K, Sundaramurthy VT (1990). Effect of neem oil on cotton whitefly (*Bemisia tabaci*). India J. Agric. Sci. 60(4):290-291.

Nguyen R, Hamon AB (1993). Citrus blackfly, *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae). DPI Entomology Circular, Gainesville, n. 360.

Oliveira MRV, Silva CCA, Navia D (2001). Mosca-negra-dos-citros *Aleurocanthus woglumi*: Alerta quarentenário. Brasília: Ministério de Agricultura, Pecuária e Abastecimento, 12 p.

Raga A, Imperato R, Maia WJMS (2013). Mosca Negra dos Citros. Citrus Res. Technol. 34(2):57-63.

SAS System, the. Version 8.2. Cary: SAS Institute (2001) 6 CD-ROM. Windows 98.

Saxena RC, Khan ZR (1985). Effect of neem oil on survival of *Nilaparvata lugens* (Homoptera: Delphacidae) and on grassy stunt and ragged stunt vírus transmission. J. Econ. Entomol. 78(3):647-651.

Schumutterer H (1990). Properties and potential of natural pesticides from the neem tree, (*Azadirachta indica*). Ann. Rew. Entomol. 35:271-297.

Silva JG, Batista JL, Silva JG, Brito CH (2012). Use of vegetable oils in the control of the citrus black fly, *Aleurocanthus woglumi* (Hemiptera: Aleyrodidae). Rev. Colomb. Entomol. 38(2):182-186.

Simmononds MSJ, Evans HC, Blaney WM (1992). Pesticides for the year 2000: mycochemicals and botanicals. In: Aziz A, Kadir SA, Barlow HS (Ed.) Pest management and the environment in 2000. Wallingford: CAB International. pp. 127-164.

Summy KR, Gilstrap WG, Hart JM, Caballero L (1983). Biological control of citrus blackfly (Homoptera: Aleyrodidae) in Texas. Environ. Entomol.12:782-786.

Vieira DL, Souza GMM, Oliveira R, Barbosa VO, Batista JL, Pereira WE (2013). Aplicação de óleos comerciais no controle ovicida de *Aleurocanthus woglumi* Asbhy. Biosci. J. 29(5):1126-1129.