

# Methanolic extract of the root bark of *Mimosa tenuiflora* (Willd.) Poiret to control *Aleurocanthus woglumi* ASHBY (Hemiptera: Aleyrodidae) †

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† Presented at the 1st International Electronic Conference on Entomology (IECE 2021), 1–15 July 2021;

Available online: <https://iece.sciforum.net/>.

**Abstract:** The black fly, *Aleurocanthus woglumi* Ashby, is an important pest of citrus in Brazil causing damage that leads to significant economic losses. In this work, we sought to test extracts of *Mimosa tenuiflora*, a typical plant of the Brazilian caatinga, as an alternative for use in controlling the pest. We used extracts at concentrations of 3, 5, 7 and 10 mg L<sup>-1</sup>. The nymphs were exposed by immersing the leaf in the solution and kept in controlled conditions of temperature, humidity and photoperiod for evaluation of mortality. The extract at 5 mg L<sup>-1</sup> provided the highest mortality over the days of evaluation. The species showed promise to be further investigated for use in citrus blackfly control.

**Keywords:** Vegetal Extract; Pest Control; Citrus black fly

**Citation:** Souza, D. S.; Cruz, L. S.; Pereira, R. C. Methanolic extract of the root bark of *Mimosa tenuiflora* (Willd.) Poiret to control *Aleurocanthus woglumi* ASHBY (Hemiptera: Aleyrodidae), in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10411

Published: 30 June 2021

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## 1. Introduction

The black fly (*Aleurocanthus woglumi* Ashby) is an important pest of citrus. It is an insect whose feeding activity occurs through mouthparts of the chipper-sucker type that cause damage when they feed on the contents of the phloem of citrus plants, both in the immature and adult stages [1]. The continuous sucking of leaf nutrients results in weakness, wilting and, in some cases, plant death. Indirect damage arises from sooty mold, which is a symptom resulting from the growth of the fungus in the nymph exudation of *A. woglumi* on leaves and fruits, which impair leaf respiration and photosynthesis [2]. Due to its wide geographic presence in Brazil, the citrus blackfly is no longer considered a quarantine pest for the domestic market.

Despite the economic and social importance of Brazilian citrus production, research on alternative control methods for the control of *A. woglumi* is rare. To minimize the problems caused by pesticides, such as environmental pollution, effects on non-target organisms and the emergence of resistant organisms, alternatives are being researched, including the use of bioactive plants by extracts and oils [3].

The use of plant extracts in agriculture presents itself as a viable and efficient alternative in pest control, since a large number of synthetic insecticide chemical molecules have been molded from organic substances from plant species. As an alternative and sustainable pest control tool, it consists in the identification of new bioactive botanical compounds that are compatible with pest management programs [4], have specific spectrum and mode of action, and are easy to process and apply [5,6].

As an example, there are carbamates analogous to physostigmine, a substance present in the seed of *Physostigma venenosum*. (Fabaceae) [7,8], pyrethroids analogous to pyrethrin, present in the flowers of *Chrysanthemum cinerariaefolium* (Asteraceae) [9], and reanodin, coming from *Ryania speciosa* (Flacourtiaceae) [10,5].

The insecticidal molecules found in plants come mainly from the synthesis of secondary metabolites of plants, present in families such as Asteraceae, Fabaceae, Malvaceae and Meliaceae [11,12,6], being verified, in recent years, an increasing increase in the identification of plant species with insecticidal potential. The main secondary metabolites present in species of Fabaceae: terpenes, flavonoids, alkaloids and tannins. Besides high concentrations of protease and trypsin inhibitors [13].

In the Fabaceae family we find the species *Mimosa tenuiflora* (Willd) Poiret, an abundant plant in the semi-arid northeastern region of Brazil, popularly known as Jurema preta. It is widespread in the northeastern states of Brazil, from Piauí to Bahia, in northern Venezuela and Colombia and dry valleys in southern Mexico, Honduras and El Salvador [14,15].

The chemical composition of *M. tenuiflora* has attracted considerable interest, mainly due to the presence of indolic alkaloids and tannins [16]. In Brazil, however, studies on this species are mostly limited to the forage importance of its leaves and, more recently, the tannin potential of its stem bark, with few studies on its chemical constituents [17]. No studies have been reported on its use in insect control.

Thus, this work aimed to evaluate the activity of the methanolic extract of *M. tenuiflora* root bark on the mortality of *A. woglumi* nymphs, under laboratory conditions.

## 2. Methodology

The work was conducted in the Entomology Laboratory of the Federal University of the Recôncavo da Bahia, in Cruz das Almas.

### 2.1. Insects

Nymphs between the second and third instar were collected in orange orchards of Embrapa Cassava and Tropical Fruits in the city of Cruz das Almas, state of Bahia, Brazil. After collection, the leaves were taken to the laboratory and cleaned with de-ethylated water for further use in the experiment.

### 2.2. Obtaining the methanolic extract

*Mimosa tenuiflora* root shells were collected in the experimental field of the Federal University of the Recôncavo da Bahia, from plants approximately one and a half years old. To gain access to the roots the plants were tumbled with a tractor attached to a blade. The barks were dried in a forced air circulation oven at 40° C for a period of 5 days, and then ground in a Willey type knife mill. The powder obtained was weighed and transferred to Erlenmeyers and submitted to the cold extraction technique, with 6 macerations using methanol (MeOH) as solvent. The extract was filtered through a paper filter and placed to concentrate in a ro-taevaporator at 60° C, dried, and then transferred to labeled vials. Subsequently diluted to the working concentrations.

### 2.3. Mortality Bioassay

After dilution of the extract at concentrations of 3, 5, 7, and 10 mg L<sup>-1</sup>, field-collected leaves containing 30 nymphs were immersed in the extract for 5 seconds and placed under absorbent paper so that excess extract was absorbed. The leaves were then placed in a B.O.D. oven at 26°C and 70±10% RH. The experiment was evaluated every 72 hours until 216 hours after installation (9 days). Nymphs with withered or dry appearance were considered dead. The design adopted was entirely randomized, consisting of 5 treatments (four concentrations and control) and 10 replicates, with each replicate represented by one leaf containing 30 nymphs.

### 2.4. Statistical Analysis

All data obtained were analyzed in R statistical software, and in cases where the ANOVA F-test was significant, the means were compared by Tukey's test at 5% significance. Lethal concentrations were determined by means of Probit analysis.

### 3. Results and Discussion

Concentrations above 5 mg L<sup>-1</sup> provided the highest mortalities, which did not differ statistically from treatments where 7 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup> of the extract were applied, as can be seen in table 1 below.

**Table 1.** Concentration-response data of *M. tenuiflora* extract on *A. woglumi*

Concentrations (mg L <sup>-1</sup> )	Number of Insects (n)	Number of Fatalities (death)
0	300	88 c
3	300	168 b
5	300	255 a
7	300	215 a
10	300	252 a

Equal letters in the columns do not differ statistically using the t-test

When we analyzed the percentage of mortality for the days after the application of treatments, we observed that at 3 days after the application, the concentrations of 7 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup> of the extract provided the highest percentages of mortality (Table 2), however, at 9 days after the application, the treatment with 5 mg L<sup>-1</sup> of the extract provided mortality of 56% of the population tested (Table 2). This result can be explained by a greater persistence of the extract at lower concentrations on the insect. According to Costa et al. [18], each plant species may have a variation in the content of active ingredients, depending on the stage of the plant, the structure of the plant used and the soil and climate conditions under which the plants grow.

These results corroborate those found by Lemos et al. [19], who when testing *Azadirachta indica* extracts on *Aleurocanthus woglumi* observed that nymphs treated with the leaf extracts at 3 days after immersion (DAI), at concentrations of 5 and 10% (weight/volume) caused different mortality when compared to the control.

Figure 1 shows the differences between the survival curves of the control and the treatments. It can be seen that nymph survival was reduced with the use of *Mimosa tenuiflora* root bark extracts. With respect to mean survival, the concentrations of 5 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup> did not differ, which is observed by the overlapping of the curves in the graph, and that these concentrations were those that provided more abrupt falls in survival until 216 hours after the application of treatments. The application of the extract at 5 mg L<sup>-1</sup> proved to be as efficient as that of 10 mg L<sup>-1</sup>, making the use of the extract at the lower concentration more sustainable.

**Table 2.** Percent mortality of the tested population for the concentrations-day after immersion in *M. tenuiflora* extract.

Concentrations (mg L <sup>-1</sup> )	Days after immersion		
	3	6	9
0	3%	18%	8%
3	8%	15%	33%
5	10%	18%	56%
7	18%	21%	32%
10	18%	14%	53%

Equal letters in the columns do not differ statistically using the t-test

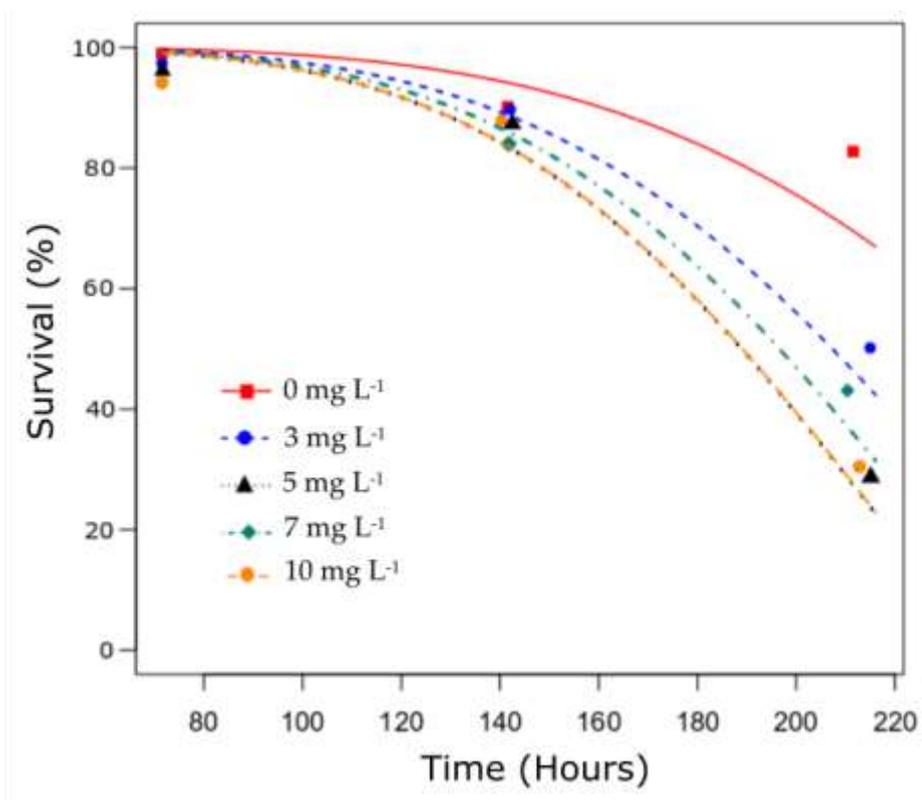


Figure 1. Survival curve of *A. woglumi* nymphs treated with *M. tenuiflora* root extract.

The high percentages of mortality of *A. woglumi* after exposure to *M. tenuiflora* extract allowed the use of Probit analysis. It was determined that the lethal concentration to kill 50% of the population (CL50) of *A. woglumi* was 0.630 mg L<sup>-1</sup> and the CL90 corresponding to 1.540 mg L<sup>-1</sup> (Table 3), the low chi-squared values in the bioassays indicate a homogeneity of the test population.

**Table 3.** Lethal concentrations of *M. tenuiflora* root bark extract on *A. woglumi*.

N	Inclination (±CI)	CL50 (IC95) mg L <sup>-1</sup>	CL90 (IC95) mg L <sup>-1</sup>	X <sup>2</sup>	DF	p-value
1500	1,43 ± 0,71	0,630 (9,58.10 <sup>-1</sup> ; 1,27)	1,540 (5,02.10 <sup>-1</sup> ; 2,37)	36,43	3	0,99

N: Number of insects used; CI: Confidence interval; X<sup>2</sup>: Chi-square; DF: Degree of freedom.

#### 4. Conclusion

The methanolic extract of *M. tenuiflora* root bark applied via immersion (direct contact) caused significant mortality of *A. woglumi* nymphs in a concentration-dependent manner (LC50 = 0.630 mg L<sup>-1</sup> of extract). The extract at 5 mg L<sup>-1</sup> provided the highest mortality of nymphs. However, further studies on the chemical composition of this plant with a focus on insecticidal molecules are still needed.

**Author Contributions:** Conception, D.S.S., L.S.C., and R.C.P.; methodology, D.S.S.; research, D.S.S. and L.S.C.; data curation, D.S.S.; preparation of draft essay, D.S.S. and L.S.C.; proofreading and editing, D.S.S. and R.C.P.; project administration, D.S.S.; acquisition of funding, R.C.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** State Research Support Foundation of Bahia

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** The authors thank Embrapa Cassava and Tropical Fruits for the support in obtaining the specimens in the field.

**Conflicts of Interest:** The authors declare no conflict of interest

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