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Bioactivity and phenolic composition of extracts of noni (*Morinda citrifolia* L., Rubiaceae) in tomato moth (*Tuta absoluta* Meyrick, 1917) (Lepidoptera: Gelechiidae)

Rubens Pessoa de Barros[1*], Ligia Sampaio Reis[2], João Gomes da Costa[3], Amanda Lima Cunha[4], Isabelle Cristina Santos Magalhães[4], Claudio Galdino da Silva[4], Aldenir Feitosa dos Santos[5,9], Jhonatan David Santos das Neves[6], Adriana Guimarães Duarte[7], Gabriela Souto Vieira de Mello[8], Johnnatan Duarte de Freitas[8], Jonas dos Santos Sousa[8] and Simone Paes Bastos Franco[8]

[1]Department of Biological Sciences, State University of Alagoas, Campus I, Brazil.
[2]Plant Protection at the Agricultural Sciences, Federal University of Alagoas, Maceió, Brazil.
[3]Embrapa Coastal Tracks, Rio Largo-AL, Brazil.
[4]Biological Sciences and Chemistry, State University of Alagoas, Arapiraca-AL, Brazil.
[5]Department of Chemistry, Universidade Estadual de Alagoas, Brazil.
[6]Institute of Land and Agrarian Reform of Alagoas, Brazil.
[7]Plant Protection, Agrarian Sciences Center, CECA/UFAL, Brazil.
[8]Federal Institute of Alagoas, IFAL, Brazil.
[9]Department of Chemistry, Centro de Estudos Superiores de Maceió Brazil.

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We evaluated the bioactivity of *Morinda citrifolia* L., Rubiaceae on *Tuta absoluta* (Lepidoptera: Gelechiidae) and quantified the phenolic components. Ethanolic extracts of leaves and fruits were used in five concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 mg / L). The leaves of the plants were immersed in the solution (10 s) and placed in contact with five caterpillars of the same instar in Petri dishes. The caterpillars were kept under controlled conditions, temperature of 25 ± 1 ° C, and relative humidity of 65 ± 10%, photophase of 12 h. The experimental design was completely randomized with five concentrations, five replicates with two different extracts, the control being distilled water. The phenolic composition of the extracts was determined by high-performance liquid chromatography (HPLC) based on the retention times using the standard external method. Leaf extracts and fruits of *M. citrifolia* had bioinseticidal activity against caterpillars (leaves at 0.02 mg / L gave 100% mortality and fruits at 0.03 mg / L gave 46.08% mortality). The greater efficiency of leaves can be attributed to their high percentage of phenolic compounds and flavonoids. Eleven phenolic compounds were identified and quantified in extracts of leaves and fruits that showed significant bioactivity.

Key words: Plant bioinsecticides, secondary metabolism, plant protection, damages, olericulture.

INTRODUCTION

The tomato (*Solanum Licopersycum* L., Solanaceae) adapts well to subtropical and temperate climate. Tomato

crop is infested by various pests that can substantially alter their productivity and quality. Of all the insect pests, the tomato moth *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) has been infesting the tomato crop in Brazil since 1980 (Carvalho and Borgoni, 2006; Araújo et al., 2013).

Farmers have attempted to control this insect pest with insecticides. Though these are effective management options, but insecticides carry the risk of environmental degradation. In addition, as use of these chemicals became routine, their efficacy against *T. absoluta* decreased (Silvério et al., 2009; Lebdi-Grissa et al., 2010). Several researchers have reported alternatives to the use of conventional pesticides, including plant extracts as bioinsecticides or mass capture with sexual pheromones that attract males to traps where they are killed (Hassan and Alzaidi, 2009; Moreno et al., 2011, Tomé et al., 2013; Cocco et al., 2013). The bioinseticidal botanical families include Asteraceae, Meliaceae, Rutaceae, Annonaceae, Lamiaceae and Canellaceae (Zabel et al., 2002, Pereira et al., 2002, Tamm, 2004). *Morinda citrofolia* L., from the Rubiaceae family, popularly known as noni, has been used since antiquity for the treatment and prevention of various diseases in humans and animals. This species is well adapted to the various regions of cultivation in Brazil (Navarro-Silva et al., 2009). Among the chemicals found in *M. citrifolia* fruits, phenolic compounds such as anthraquinones (Deng et al., 2007) are found in trace quantities (Lin et al., 2007). Reyes et al. (2011) reported that ethanolic and aqueous extracts of the fruit contained free quinones, steroids and flavonoids. Studies have tested the bioactivity of extracts of *M. citrifolia* against Diptera such as *Drosophila sechellia* (Tsacas and Baechli, 1981) (Diptera: Drosophilidae). The plant's fruit contains secondary defense compounds, primarily octanoic acid that are lethal to most other flies of the same genus (Morales et al.; 2010; Kovedan et al., 2012; Silva et al., 2015; López et al., 2017). The bioactivity of noni extracts (*M. citrifolia*) has chemical similarities with other natural products in terms of bioinsecticidal activity. The hypothesis raised is that the phenolic compounds of this plant have bioinsecticidal properties. Therefore, the objective of this work was to evaluate the leaf and fruit ethanolic extracts in the control of the tomato moth, in addition to identifying and quantifying its phenolic compounds, to assist in the integrated pest management.

MATERIALS AND METHODS

Study location

The experiment was carried out in the Research Laboratory of the State University of Alagoas, Campus I in Arapiraca-AL. Tomato plants were cultivated in a greenhouse from February to June 2016. The insect pest *T. absoluta* was raised in a Styrofoam cage in a Laboratory of the State University of Alagoas. The production of ethanolic extracts from *M. citrifolia* and the bioassay with the tomato moth caterpillars were carried out in subsequent phases.

Tomato cultivation

For maintenance of the caterpillars, we transplanted tomato seedlings in five-liter pots with organic management soil and placed them in a greenhouse where they were monitored weekly. The first and second instar caterpillars of *T. absoluta* were collected from tomato varieties in crops in the rural region of Alagoas.

Raising the insects in cages

The immature stages 1 and 2 moth were placed in Styrofoam cages of 50 cm long × 40 wide × 30 cm high and maintained until adulthood. After population stabilization, the next generation was used in the bioassays. The caterpillars were kept under controlled conditions, with a temperature 25±1°C, relative humidity of 65 ± 10%, and photophase 12 h. In each cage, the plant leaves were conditioned for oviposition by *T. absoluta*.

Obtaining plant ethanolic extracts

In order to obtain the extracts, the leaves and fruits of *M. citrifolia* were dried in an air circulation oven at 60°C for 72 h and were ground in a knife mill. Then, leaf extract was macerated in ethanol: 2,500 g of vegetable powder (leaves or fruit) was placed in 3,500 mL of absolute ethyl alcohol for seventy-two hours, with filtration and alcohol replacement done every 24 h. The dry ethanolic extracts of leaves and fruit of *M. citrifolia* were obtained by rotational evaporation of the extracting liquid.

Bioassay for evaluation of insecticidal activity

Five concentrations of vegetable ethanol extracts such as 0.01, 0.02, 0.03, 0.04, 0.05 mg/L were used. Tomato leaves were previously immersed in the extract for 10 s and dried under laboratory conditions; five caterpillars of the same instar were placed on each leaf and in five petri dishes. The experimental design was completely randomized with five concentrations, five replicates with two different extracts and distilled water as the control. The Petri dishes were kept on benches in the laboratory at room temperature of 24-28°C. Mortality index (MI) was evaluated every 24, 48 and 72 h, and dead insects were counted in the Petri plates using the hair of a watercolor brush, as described by Sun-Shepard and Schneider-Orelli (1947) adapted by Puntener (1981) and used by Silva et al. (2015) in a similar study. The results were analyzed by the Dunnett test at 5% probability using an alternative bilateral hypothesis. Dunnett (1955) pioneered the concept that when a control is present, comparisons of preliminary interest may be comparisons of each new treatment with control through the GENES Program (Cruz, 2006).

*Corresponding author. E-mail: pessoa.rubens@gmail.com.

Quantification of total phenols

The method for the determination of total phenols consisted of the reaction of the constituent acids of the Folin-Ciocalteau reagent and phenolic or non-phenolic compounds. The Folin-Ciocalteau reagent is composed of phosphomolybdic and phosphotungstic acids, in this solution, molybdenum is in the oxidation state +6 with a yellowish coloration; in the presence of phenolic compounds, it undergoes a reduction reaction and passes to a +5 oxidation state (Rezende, 2010). With the reduction reaction there is the formation of blue-colored molybdenum-tungsten complexes. This reaction occurs in alkaline medium, specifically in the presence of Na_2CO_3 (Rezende, 2010). The total phenol content was quantified by the method described by Freitas et al. (2014) with some adaptations. To create the gallic acid calibration curve, 0.04 g of gallic acid was weighed into 8 mL of MeOH (stock solution). Dilutions (Gallic acid test solutions) were then prepared at concentrations of 0.15, 0.1, 0.05, 0.025, 0.01 and 0.005 mg/mL.

The following procedures were performed from the dilutions of gallic acid (in triplicate for each concentration): into an amber glass we added 100 µL of gallic acid test solution, 500 µL of Folin-Ciocalteau reagent and 1 µL of distilled H_2O and then vortexed for one minute. Subsequently, 2 mL of 15% sodium carbonate was added and stirred for another 30 s in the vortex. Subsequently, the solution was filled into a 10 mL volumetric flask. The solution was incubated in the dark for two hours. Absorbance readings were obtained using a UV-VIS spectrophotometer with a wavelength of 750 nm. To obtain the test solution of the vegetable sample, 0.005 g of each vegetable ethanolic extract was diluted in 5 mL of MeOH. Then, a 0.075 mL aliquot of this solution was added to 0.425 mL MeOH. To perform the test, the same procedure was performed as for gallic acid, replacing the test solution of gallic acid with the test solution of the vegetable ethanolic extract. To obtain the blank control solution, a solution of 100 µL of MeOH, 500 µL of the Folin-Ciocalteau reagent and 1 mL of distilled H_2O was prepared. The procedure described for gallic acid was repeated, replacing the gallic acid test solution with the blank control solution. The absorbances were read in a UV-VIS spectrophotometer with a wavelength of 750 nm. Before any reading, the blank was used to reset the spectrophotometer.

Quantification of flavonoids

The method for the quantification of flavonoids consisted of preparing the calibration curve of quercetin, where 1 mg of quercetin was weighed and diluted in 1 ml of MeOH. Dilutions were then carried out at concentrations of 0.03, 0.025, 0.020, 0.015, 0.01, 0.005, 0.0025 and 0.00125 mg/mL. Subsequently, solutions were made of the extract, where 1 mg of the extract was weighed and diluted in 1 mL of MeOH. After preparation of the test solution, the solutions (in wells in triplicate) were prepared for reading with 200 µl of the test solution of the plant sample and 100 µl of 2% aluminum chloride methanolic solution. The solution was prepared for the blank (in triplicate) with 200 µl of MeOH and 100 µl of 2% methanolic chloride of aluminum chloride. Thereafter, the well plate was placed in the dark for 30 min. Then, the reading was performed in UV-VIS spectrophotometer at 420 nm. The flavonoid content was determined by interpolating the mean absorbance of the samples against the quercetin calibration curve and expressed in mg QE (quercetin equivalent) per gram of ethanolic extract.

Separation, identification and quantification of phenolic compounds

The determination of the phenolic compounds was performed using high-performance liquid chromatography (HPLC). The equipment used was a Shimadzu HPLC, equipped with four high pressure pumps, model LC-20AT, degasser model DGU-20A 5R, interface model CBM-20A, automatic injector model SIL-20A HT and detector model SPD-20A. The chromatographic column employed in both analyses was an Agilent-Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 µm).

Table 1. Mobile phase gradient.

Time (min.)	A%	B%
0	93	7
10	84	16
30	75	25
38	50	50
50	30	70
54	25	75
58	15	85
66	93	7

The standards used for chromatographic analysis of the phenolic compounds were gallic acid, catechol, vanillic acid, salicylic acid, vanillin, syringaldehyde, coumaric acid, chlorogenic acid, coumarin, rutin, quercetin, kaempferol and caffeic acid. All standards were purchased from Sigma-Aldrich or AcrosOrganics. All solvents used for chromatography were of analytical grade; methane was from Panreac, formic acid was from Dynamic and ultrapure water was obtained from a Milli-Q system. For the standards, stock solutions with a concentration of 40 mg/L in water/alcohol at 30%/70% were prepared. The method used for quantification was that of external standardization. For the construction of analytical curves, dilutions of an intermediate solution were carried out containing a mixture of all the standards. This was obtained by diluting the stock solutions previously prepared. In this intermediate solution, all standards were at a concentration of 10 mg/L. The 1% formic acid solution in milli-Q water (Solvent A) and methanol (Solvent B) were used as the mobile phases for the elution of the tested compounds.

Plant samples and standards were eluted according to the gradient (Table 1) with a total run of 66 min. The wavelength used was 290 nm at 33°C, flow rate of 0.6 mL/min and injection volume of 20 µL. Samples and standards were filtered on 0.45 µm polyethylene membranes (Milipore) and were injected directly into the chromatographic system. Each injection was performed three times in the HPLC system, in order to obtain the mean concentrations and retention times. Thus, the identity of the analytes was confirmed by the retention time, and the profile of the peaks of the sample was compared to the patterns.

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RESULTS AND DISCUSSION

Bioassay for evaluation of insecticidal activity

The data in Tables 2 and 3 refer to the average obtained by the Dunnett test at 5% probability, as an alternative bilateral hypothesis shown to be effective in relation to the control. The hypothesis was that both the ethanolic extract of the leaf and the fruit in the concentrations 0.01 to 0.05 mg/L would provoke a mortality effect in *T. absoluta* in a similar way. There was a higher mortality of *T. absoluta* with the leaf extracts at the concentration of 0.02 mg/L than in the control, with 88.04% mortality

Table 2. Insecticidal activity of the ethanol extract of *M. citrifolia* leaf.

	Mortality (%)	Mortality difference with control (%)
Control (H_2O)	11.46	-
**MC 0.01 mg/L	79.96	68.05*
MC 0.02 mg/L	100.00	88.04*
MC 0.03 mg/L	86.64	75.18*
MC 0.04 mg/L	86.64	75.18*
MC 0.05 mg/L	79.96	68.05*
C.V. (%)	23.96	

*The Dunnett test was applied at a 5% probability level (bilateral). **MC – *M. citrifolia*.

Table 3. Insecticidal activity of the ethanol extract of *M. citrifolia* fruit.

	Mortality (%)	Mortality difference with control (%)
Control (H_2O)	11.46	-
**MC 0.01 mg/L	35.12	23.66*
MC 0.02 mg/L	33.50	22.04*
MC 0.03 mg/L	46.08	34.62*
MC 0.04 mg/L	36.62	25.16*
MC 0.05 mg/L	35.92	24.46*
C.V. (%)	9.00	

*The Dunnett test was applied at a 5% probability level (bilateral). **MC – *M. citrifolia*

(Table 2). In the fruit extracts, the highest mortality was observed at the concentration of 0.03 mg/L, and the difference from the control was 34.62% (Table 3). To the best of our knowledge, this study was the first to show *M. citrifolia* bioinsecticidal activity against insects of agricultural interest, microlepidoptera such as *T. absoluta*. To date, there have been no reports on the bioactivity of this plant in relation to caterpillars of this order of insects. In the difference of the means of the treatments with the control, we observed that the results were better than the control, both for the leaf extracts and for the fruit extracts in terms of insect mortality.

Dunnett (1955) pioneered the concept that when a control is present, comparisons of preliminary interest may be the comparisons of each new treatment with the control. In this study, the control group was water (H_2O). When multiple comparisons are performed with a control, the primary interest parameters are the differences between each new mean treatment and the mean of the control, that is, the hypotheses are tested. In this test, a level of 5% significance is considered, thus, the hypothesis of equality between the mean concentrations of the bioactivity of the extracts of *M. citrifolia* on the mortality of *T. absoluta.*

The bioactivity of *M. citrifolia* has been demonstrated in Diptera such as *Drosophila sechellia* (Tsacas and Baechli, 1981) (Diptera: Drosophilidae), a species of fruit fly. Unlike other species of this genus that are generalists, *D. sechellia* evolved to be specialist for the host plant *M. citrifolia*. This is interesting because the plant's fruit contains secondary defense compounds, especially octanoic acid that is lethal to most other flies of the same genus (López et al., 2017). Morales et al. (2010) tested several plant extracts on larvae of the dengue mosquito *Aedes aegypti* (Diptera: Culicidae). They obtained significant results using the *M. citrifolia* L. ethanolic extract at 300 mg/L, with a mortality of 98% of the larvae. The neurotoxic action was attributed to octanoic acid, the main ingredient of noni oil, also known as caprylic acid, a potential larvicide.

According to Silva et al. (2015), aqueous extracts of *M. citrifolia* applied to newly hatched larvae of fruit flies *Ceratitis capitata* (Wiedmann, 1824) (Diptera: Tephritidae), presented control efficiency (E%) of 10.8% and larval mortality of 18%. Kovendan et al. (2012) also confirmed the insecticidal activity of *M. citrifolia*, reporting that there were significant results with leaf extracts of this plant with the promotion of mortality of mosquito larvae of the order Diptera and family Culicidae: *Anopheles stephensi* (Liston, 1901), *Culex quinquefasciatus* (Say 1823), and *Aedes aegypti* (Linnaeus, 1762).

Quantification of total phenols and flavonoids

In the determination of the total phenols, the method was

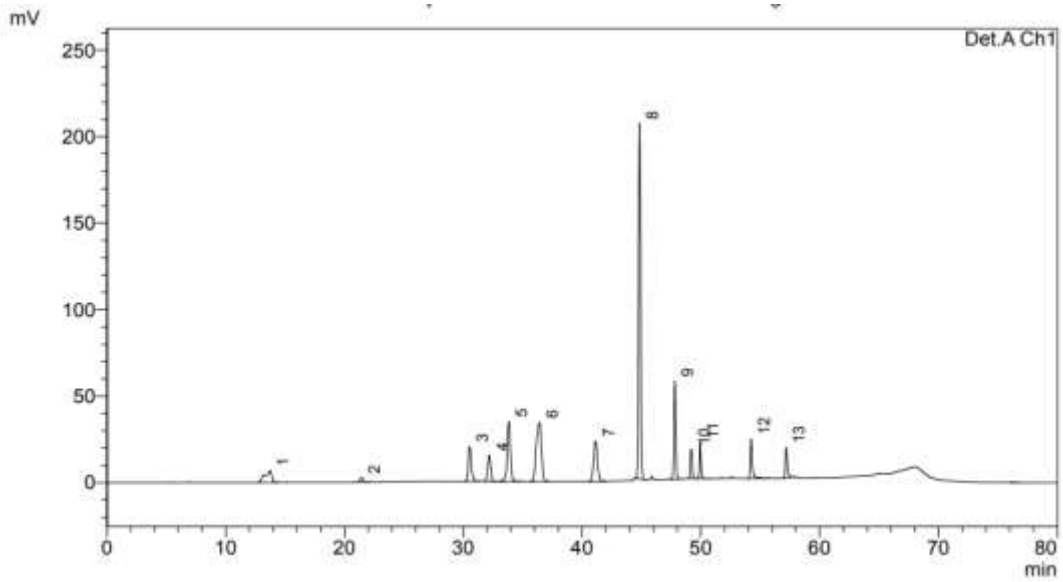

Figure 1. Chromatogram of the polyphenols solution with spectrophotometric detection of 290 nm. Identification of peaks: 1 = Gallic acid; 2 = Catechol, 3 = Chlorogenic acid, 4 = Vanillic acid, 5 = Caffeic acid, 6 = Vanillin, 7 = Seringaldehyde, 8 = Coumaric acid, 9 = Coumarin, 10 = Salicylic acid, 11 = Rutin, 12 = Quercetin; 13 = Kaempferol.

followed by determination of the calibration curve of synthetic standard Gallic acid (y=7.701x + 0.0131 and R^2=0.9785). We quantified the total phenols in all the extracts. The leaf of *M. citrifolia* had the highest phenol content with 1.094 g of GAE/g of crude extract compared to the extract of the fruit that had a ratio of 0.497 g GAE/g of the crude extract, compatible with its particular antioxidant potential. For the quantification of flavonoids, the method of determining the calibration curve of the quercetin standard was followed (y=32.262x + 0.595 and R^2 = 0.9157), allowing the quantification of the flavonoids of the ethanol extract of *M. citrifolia*. The leaf had the highest concentration of flavonoids (60.23 mg EQ/g of the crude extract) and the fruit had 12.20 mg EQ/g of the crude extract. The mean absorbance of the phenols and flavonoids in the fruit and leaf were different. We observed that the leaves of *M. citrifolia* had a higher percentage of phenolic compounds and of flavonoids, chemical constituents often mentioned in the literature for their bioactivity. These chemical characteristics may be related to higher bioactivity for *T. absoluta* mortality presented by the leaves of *M. citrifolia*.

According to Chan-Blanco et al. (2006), the most abundant bioactive compounds in *M. citrifolia* were phenolics, including damnacanthal, scopoletin, morindone and rubiadin, with rutin and scopoletin as major components and damanacantal with demonstrated anti-carcinogenic properties. In reports of phenolic compounds with insecticidal bioactivity of *M. citrifolia* on dipterans, nanoparticles synthesized from root extracts were used, with significant results against *Aedes aegypti* larvae (Suman et al., 2015). Many phenolic compounds have antioxidant, anticarcinogenic, antimutagenic and anti-inflammatory activities. However, most interest is focused on antioxidant activity, and the attributed physiological and pharmacological functions originate in this activity (Thani et al., 2010).

Zin et al. (2002) demonstrated high levels of antioxidant activity in *M. citrifolia* extracts when using methanolic extracts of roots, fruits and leaves. Faria et al. (2014) measured the presence of various phytochemical compounds, including tannins, flavonoids, conjugated anthraquinones, saponins, coumarins and alkaloids and suggested pharmacological and functional functions. Studies demonstrate current interest in the study of phenolic compounds, primarily due to the antioxidant potential of these substances in terms of sequestering free radicals that are harmful to human health (Alves et al., 2007; Neves et al., 2008).

Costa et al. (2013) carried out studies with *M. citrifolia* using the seed, peel and pulp, and demonstrated the antioxidant capabilities of this plant. The bioactive compounds found in plants are phenolic substances with diverse functions including plant growth, sensory properties, seed germination processes, and defense against pests and oxidative damage (Liu, 2007).

Phenolic compounds identified and quantified

In this study, 11 phenolic compounds were detected in the fruit and leaf extracts of *M. citrifolia*. Figure 1 shows the chromatogram with spectrophotometric detection at 290 nm. The phenolic profile of the plant samples is very

Table 4. Concentrations of phenolic compounds in leaves and fruits of *M. citrifolia.*

Compound	Retention time (min.)	Concentration (mg / L)	
		Leaf extract	Fruit extract
Gallic acid	-	-	-
Catechol	20.915	31.853	8.370
Chlorogenic acid	29.951	14.690	-
Vanillic acid	31.540	16.250	22.921
Caffeic acid,	33.010	3.561	3.901
Vanillin	35.887	2.263	-
Seringaldehyde	-	-	-
Coumaric acid	44.259	12.611	24.508
Coumarin,	47.595	3.573	1.898
Salicylic acid	48.826	24.282	1.431
Rutin	49.708	89.1063	21.1252
Quercetin	53.858	24.767	16.515
Kaempferol	56.699	8.595	1.539

similar except for the detection of chlorogenic acid and vanillin patterns found only in the extract of the fruits of *M. citrifolia*. The other compounds were detected in both the fruit and leaf extracts. The concentrations of the identified products were calculated from standard curves generated with commercial products chromatographed under identical conditions (Table 4). The bioactivity of *M. citrifolia* on the mortality of caterpillars can be attributed to phenolic compounds that were found in greater quantity in leaf extracts than in fruit extracts. Some of these phenolic compounds are distinguished terms of insect control in bioassays.

Bendassolli et al. (2010) reported mortality of ants of the genus *Atta*, with extracts of Anarcadiaceae. The results obtained with methyl gallate and quercetin were significant, with quercetin resulting in mortality of 50% and Gallic acid resulting in 50% mortality only at the end of the experiment. In the present study was this effect was found at concentrations 24.767 mg/L in the leaf and in the fruit 16.515 mg/L. Rutin had a bioinseticidal effect on caterpillars (*Anticarsia gemmatalis*as) that was potentiated when the caterpillars fed on mixed diets with rutin. In that study, the rutin was present in the leaf at 89.1063 mg/L and in the fruit at 21.1252 mg/L (Wang et al., 2002; Céspedes et al., 2004; Scott et al. 2010). Villaño et al. (2006) found that phenolic acids (syringic acid, vanillic acid and p-coumaric acid) protected plants against insects. Saponins have been shown to be substances in plant secondary metabolism that protect from insects as well. Peyser et al. (2017) suggested that octanoic acid and hexanoic acid were the toxins that mediated the effects of *M. citrifolia*, and that octanoic acid was a neurotoxin as well.

Conclusion

The bioinseticidal action of *M. citrifolia* was efficient against the tomato moth (*T. absoluta*), especially the ethanolic extract of its leaves. The effect of *M. citrifolia* leaves in terms of bioinseticidal activity may be related to their chemical composition, evidenced here by the quantification of phenolic compounds and flavonoids.
The mortality data from the tomato moth resulting from the bioactivity of *M. citrifolia* extracts and their chemical compositions suggest that they can be used to control the tomato moth.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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