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RESEARCH ARTICLE

Chemical composition and aphidicidal properties of castor-bean leaves against *Rhopalosiphum maidis* and *Sipha flava* (Hemiptera: Aphididae)

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ABSTRACT

Plant extracts are a great source of molecules with insecticidal activity and are used to control pests in various crop production systems. In this study, we evaluated the aphidicidal properties in vitro of the *n*-hexane extract of leaves of castor-bean (*Ricinus communis* L.; Euphorbiaceae) at different concentrations (0.5, 1.0, 2.5, 5.0 and 10 kg m^{-3}) against apterous adults of corn leaf aphid (*Rhopalosiphum maidis* Fitch) and the yellow sugarcane aphid (*Sipha flava* Forbes). The observed effects were dose-dependent, and the highest concentration evaluated (10 kg m^{-3}) caused 82% mortality in *R. maidis* and 79% in *S. flava*. To identify chemical compounds, present in the extract we used mass gas chromatography, identifying 16 compounds. Palmitic acid was the major compound, with 45.34% abundance. The data obtained indicate the existence of insecticidal compounds in the castor-bean *n*-hexane extract, which could be used as an alternative bioinsecticide in the integrated management of these two aphid species.

Key words: Aphids, castor-bean, GC-MS, palmitic acid, pest, *Ricinus communis*.

INTRODUCTION

Aphids are insects that belong to the Aphididae family, Hemiptera order, about 4700 species are known in the world (Remaudière and Remaudière, 1997). Of these, about 450 species of crop plants have been recorded, about 100 species have been classified as pests of crops (Blackman and Eastop, 2000). Aphids are agricultural pests of temperate regions and cause both direct damages to crops as phloem feeders, and indirect damage by transmitting pathogenic viruses; however, aphids are interesting organisms in evolutionary biology and ecology, developing life cycles complexes that involve sexual and asexual reproduction, as well as the development of multiple different phenotypes that are derived from environmental stimuli (Vilcinskas, 2016).

The corn leaf aphid, *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae), is a small to medium aphid with an elongated body of 1.5 to 2.7 mm, its head and body are olive green to bluish green (Peña-Martínez et al., 2017). Originally an Asian species, but it is now distributed worldwide, especially in warm tropical and subtropical regions (Blackman and Eastop, 2000). *Rhopalosiphum maidis* is found more frequently in corn (*Zea mays* L., Poaceae) crops than other aphids because it has greater resistance to the chemical defenses of the plant,

such as benzoxazinoids, which protect against a large number of herbivores and pathogens (Chen et al., 2019). When this aphid ingests the sap of leaves, it transmits the *Sugarcane mosaic virus*, which reduces the yield and quality of maize (Xie et al., 2014). Severe infestations of this aphid on corn plants can cause serious economic losses (Kuo et al., 2006).

The yellow sugarcane aphid *Sipha flava* Forbes (Hemiptera: Aphididae) is native to North America, and occurs in temperate and subtropical regions (January et al., 2020). *Sipha flava* is an aphid that commonly feeds on the sap of sugarcane plants (*Saccharum officinarum* L., Poaceae). It is 1.3-2.0 mm long, has numerous long hairs, and has dark transverse markings on the back (Machado et al., 2012). It is widely distributed in South, Central, and North America, in addition to various Caribbean regions, mainly feeding on important crops including sugarcane, sorghum, wheat, barley, rye (Hentz and Nuessly, 2004). *Sipha flava* frequently attacks young sugarcane plants; it first feeds on the underside of immature leaves which produces yellowing/redness, which can lead to early aging and chlorosis. Like *R. maidis*, *S. flava* is an important vector of *Sugarcane mosaic virus* (Wilson, 2019).

In Mexico, farmers usually control the populations of these aphids with synthetic neonicotinoid type chemical insecticides (Tejeda-Reyes et al., 2017). Synthetic chemical insecticides are usually effective for the control of aphids, but their use is questioned because they cause damage to the environment and health problems in humans (Müller et al., 2005). Faced with such a panorama, there is a general interest in the search for natural alternatives for the control of pest insects. Plant extracts are generally considered a more environmentally- and health-friendly product than chemical insecticides (Isman, 2008). Castor-bean (*Ricinus communis* L.; Euphorbiaceae) is a plant species probably originated in Africa and has been used by humans since the ancient Egyptian, Roman, and Greek civilizations (Worbs et al., 2011) and is currently cultivated for oil production in various parts of the world. This plant grows in tropical and subtropical areas, it is fast developing and can grow up to 6 m or more in height. Its leaves are green or reddish, 30-60 cm in diameter, and contain lobes with toothed segments. The stems are of varied coloration. The flowers are monoecious and measure approximately 30-60 mm. The fruit is a spiny capsule with three cells, containing seeds that vary in size and color but are generally oval and between 8-18 mm long and 4-12 mm wide (Jena and Gupta, 2012). Phytochemical studies have identified and isolated a great diversity of natural products from various parts of *R. communis*, such as alkaloids, terpenoids, flavonoids, benzoic acid derivatives, coumarins, tocopherols, and fatty acids (Ribeiro et al., 2016). Furthermore, its main alkaloid, ricinin, has been isolated from the leaves of *R. communis* (El-Naggar et al., 2019). Ricinoleic acid has been identified as the main component in the seeds in *R. communis* (Sturtevant et al., 2019). Flavonoids such as quercetin have also been identified from leaves of *R. communis* (Singh et al., 2009).

The main objective of this study was to determine the aphidicidal properties of the *n*-hexane extract of *R. communis* leaves against adults of *R. maidis* and *S. flava* and identify the main chemical components of the *n*-hexane leaf extract using gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Plant material

Castor-bean (*Ricinus communis* L.) leaves were collected in March 2020 (Voucher 34874) in Yautepec (18°49´19.7" N, 99°05´36.6" W), Morelos, Mexico. The taxonomic determination of the plant material was carried out by Gabriel Flores Franco, curator of the HUMO-Universidad Autónoma del Estado de Morelos (UAEM) herbarium. The leaves were dried in the shade at room temperature, and 150 g were macerated with *n*-hexane. The extraction was carried out for 3 d in triplicate using 5 L solvent for each 1 kg dry leaves. The solvent was completely removed using a rotary evaporator (205, BÜCHI Labortechnik AG, Flawil, Switzerland), and the extract was subsequently dried under vacuum.

Preparation of insect culture

Apterous adults of corn leaf aphid (*Rhopalosiphum maidis* Fitch) and yellow sugarcane aphid (*Sipha flava* Forbes) were collected from corn and sugarcane crops, respectively, and were identified using a field guide (Peña-Martínez et al., 2017). The aphids were transported to a greenhouse at the Centro de Investigación en Biodiversidad y Conservación de UAEM, Cuernavaca, Morelos. Aphids identified as *R. maidis* were established on ~ 80 cm tall hybrid corn plants planted in plastic pots. Aphids identified as *S. flava* were established on ~ 60 cm tall sugarcane plants planted in plastic pots. For both aphid species, the environmental conditions were

24 ± 2 °C ambient temperature, 60% relative humidity and a 12:12 h light:darkness cycle. The aphids were supplied with new plants every 20 d to maintain the brood.

In vitro bioassays

The aphidicidal effect of the *n*-hexane extract of *R. communis* leaves was independently evaluated against the apterous adults of *R. maidis* and *S. flava*. Using a camel hair brush, 10 specimens of *R. maidis* or *S. flava* were placed into a 30 cm^3 plastic Petri dishes containing 5×5 cm leaves of corn and sugarcane, respectively. To maintain humidity in the Petri dish, a sterile filter paper was placed and 1 mL sterile distilled water was added. The prepared extracts were previously homogenized with water and 0.2% solution Tween 20, and 0.15 mL was applied using an airbrush (Aero-35, Truper, Estado de México, México). We used a randomized experimental design, with two replicates of each of the five concentrations evaluated (0.5, 1.0, 2.5, 5.0 and 10 kg m^{-3}), for a total of 10 replicates. The mortality of the treatments was evaluated 24, 48, and 72 h after treatment application. The chemical insecticide imidacloprid (350 g L^{-1} Confial, Monterrey, Nuevo León, Mexico) at the dose recommended by the manufacturer (1% = 10 kg m^{-3}) was used as the positive control, and a 0.2% Tween 20 solution was used as the negative control.

Gas chromatography-mass spectrometry (GC-MS)

The *n*-hexane extract from *R. communis* leaves was analyzed using a gas chromatography system coupled with a mass spectrometer (Agilent Technology, Santa Clara, California, USA). Mass spectrometry was by electronic impact and the equipment has an MSD detector and an Agilent 5973 N ionic source at 230 °C. The system was equipped with automatic injection. The chromatographic column was a 30 m × 0.25 mm HP-5MS column with a 0.25 µm film thickness. The initial temperature of the column was 40 °C, which was increased to 250 °C for 10 min and then at 10 °C per 5 min until reaching 285 °C for 20 min. The helium gas flow was 1 mL min^{-1} and the total execution time was 20 min. The diluted sample (10 µL extract in 1.0 mL solvent) was injected (2 mL) in divided mode at 250 °C. The spectra were obtained at an ionization voltage (e/m) of 1918 with a 40-450 m/z range of mass analyzed. The compounds in the extracts were identified and authenticated using their mass spectra compared to NIST Mass Spectral Library version 1.7 A (National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

Statistics analysis

Mortality data were arcsine transformed before statistical analysis in a completely randomized design using an initial ANOVA test within a randomized study design. The comparison of means was performed using the Tukey test at a significance level of $P < 0.05$. The data were subjected to regression analysis to calculate the mean lethal concentration (LC_{50}) by means of a Probit analysis, in the statistical program SAS 9.0 (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Figure 1 shows the cumulative mortality results of *R. maidis* adults treated with the extract of *n*-hexane from *R. communis* leaves, at different concentrations and times. The treatments showed aphidicidal effects by 24 h; at this time, 10 kg m^{-3} concentration presented the highest mortality (45%). At 48 h, there was an increase in the mortality of *R. maidis* in all treatments, but 10 kg m^{-3} continued to have the strongest effect (57%). The highest percentages of mortality were observed at 72 h, still at the 10 kg m^{-3} concentration (82%). The positive control (imidacloprid) had 100% aphid mortality by 24 h; while the negative control Tween 20, only showed a mortality of 4% at 72 h. No treatment was significantly similar to the positive control ($F = 108.21$; $P < 0.0001$).

The aphidicidal effect of the extract of *n*-hexane from leaves of *R. communis* against adults of *S. flava* is shown in Figure 2. By 24 h, the highest mortality was observed with 10 kg m^{-3} (48%), at 48 h aphid mortality increased in all treatments, and again 10 kg m^{-3} obtained the highest mortality rate (61%). The mortality of *S. flava* continued to increase by 72 h after applying the treatments, still with highest mortality with the 10 kg m^{-3}, with 79% mortality. The positive control (imidacloprid) eliminated 100% of aphids in 24 h, while the negative control (Tween 20) had only 2% mortality by 72 h. No treatment was significantly similar to the positive control ($F = 74.00$; $P < 0.0001$).

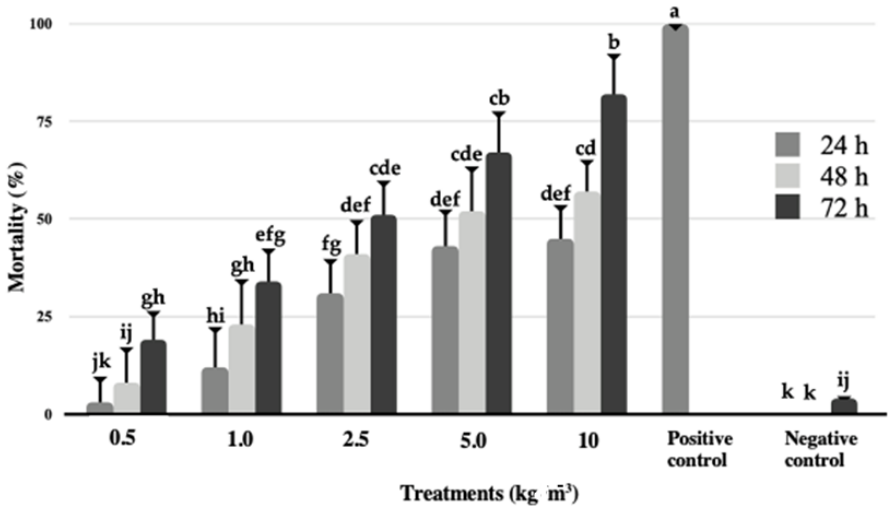

Figure 1. Percentage mortality (± SD) of *Rhopalosiphum maidis* in contact bioassays of *Ricinus communis* extract at various concentrations, compared to controls. Means with different letters among treatments are significantly different, Tukey $P \leq 0.05$ (LC_{50} = 2733 ppm in 72 h).

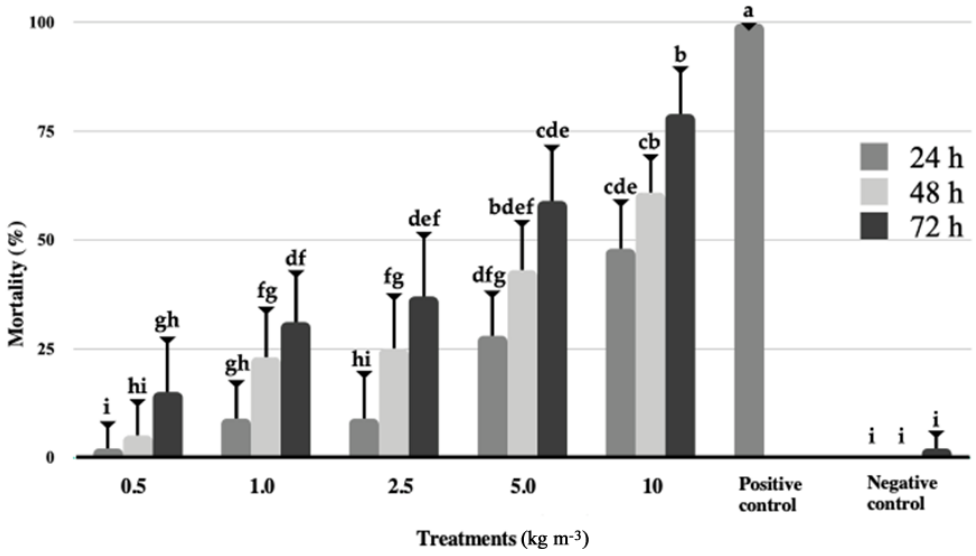

Figure 2. Percentage mortality (± SD) of *Sipha flava* in contact bioassays of *Ricinus communis* extract at various concentrations, compared to controls. The means with different letters for each treatment are significantly different, Tukey $P \leq 0.05$ (LC_{50} = 3888 ppm in 72 h).

The *n*-hexane extract of *R. communis* leaves used in this research was shown to be effective against *R. maidis* and *S. flava*. Most studies show that low-polarity extracts are toxic against aphids, given that the cuticle of aphids is mainly composed of alkyl esters, methyl esters of fatty acids, triacylglycerols, and free fatty acids (Brey et al., 1985). The aphidicidal activity of *n*-hexane extract may be due to this chemical affinity. In recent literature, the aphidicidal activity of *R. communis* against *R. maidis* or *S. flava* is not reported, but the insecticidal effect of extracts of various parts of *R. communis* against other species of aphids is reported. For example, the *n*-hexane extract from *R. communis* leaves exhibited an insecticidal effect against the sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae) of 96% at 10 kg m^{-3} in 72 h (Sotelo-Leyva et al., 2020). In our research, the *n*-hexane extract from *R. communis* leaves, at the same concentration (10 kg m^{-3}) and at the same time (72 h), presented similar mortalities of 82% and 79% in *R. maidis* and *S. flava*, respectively. The seeds of *R. communis* are also effective against aphids. The *n*-hexane seed extract eliminated 100% of *M. sacchari* at 10 kg m^{-3} in 48 h (Salinas-Sánchez et al., 2021). The petroleum ether extract of *R. communis* seeds against the mustard aphid *Lipaphis erysimi* Kalt. (Hemiptera: Aphididae), resulted in 100% and 75% mortality in 48 h, respectively, at concentrations of 10 and 5 kg m^{-3} (Arya et al., 2014). In our work, lower mortalities are reported with the same concentrations and times. This may be because a greater number of low polarity compounds are found in the seeds than in leaves (Al-Shahib and Marshall, 2003). In another investigation of the insecticidal activity of the methanol extract of *R. communis* leaves against *Myzus persicae* Sulzer (Aphididae: Hemiptera), the extract eliminated 67.4% of the aphids at a concentration of 3.2 kg m^{-3} in 72 h (Madanat et al., 2016). Recent literature has revealed that extracts of high polarity are less effective compared to those of low polarity, due to the low chemical affinity that exists with the cuticle of aphids (Do-Ik et al., 2005; Pavela et al., 2009; Dang et al., 2010).

The objective of this section was to identify the low-polarity compounds contained in the *n*-hexane extract of *R. communis*, which could be involved in the extract's aphidicidal effect. The gas-mass chromatography analysis identified 16 compounds (Table 1), which represent 95.71% of the total number of identified compounds. Palmitic acid was identified as the main chemical compound, with 45.34% (Figure 3), followed by lupeol (8.64%) and stigmasterol (6.61%).

Table 1. Chemical composition of the *n*-hexane extract from *Ricinus communis* leaves.

Nr	Constituents	Retention time	Abundance
		min	%
1	2(4*H*)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	14.72	2.94
2	Myristic acid	17.19	3.05
3	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo [4.1.0] hept-1-yl)-propenyl ester	17.44	2.18
4	6,10,14-Trimethyl-2-pentadecan-2-one	18.06	3.21
5	Pentanoic acid	18.24	0.97
6	Palmitic acid	19.39	45.34
7	7-Methyl-*Z*-tetradecen-1-ol acetate	20.43	3.32
8	Phytol	20.69	2.16
9	4,8,12,16-Tetramethylheptadecan-4-olide	22.90	1.91
10	Hexanedioic acid, bis (2-ethylhexyl) ester	23.30	1.22
11	Pyrrolidine butanoic acid	29.89	4.48
12	Vitamin E	33.45	5.45
13	Stigmasterol	35.59	6.61
14	ɤ-Sitosterol	36.73	2.91
15	β-Amyrin	37.39	4.45
16	Lupeol	38.49	8.64
	Total		95.71

The chemical composition of *R. communis* has been extensively studied, but little of this research has focused on identifying chemical compounds related to aphidicidal activity. In one investigation, chemical compounds such as palmitic acid (14.38%), lupeol (7.70%), phytol (5.45%), ɤ-sitosterol (4.01%), stigmasterol (2.61%), vitamin E (1.96%), and β-amyrin (1.34%), were shown to have larvicidal activity against vector mosquitoes (Sogan et al., 2018). Here, we identified these same chemical compounds in the *n*-hexane extract of *R. communis* leaves in similar percentages: palmitic acid (45.34%), lupeol (8.64%), phytol (1.22%), ɤ-sitosterol (2.91%), stigmasterol (6.61%), vitamin E (5.45%) and β-amyrin (4.45%). Polyphenolic compounds have also been identified in *R. communis* (gallic acid, proto-catechuic acid, syringic acid, chlorogenic acid, gentisic acid, and *p*-coumaric acid), and the extract presented aphidicidal activity against *Aphis spiraecola* Patch (Hemiptera: Aphididae) (Boualem et al., 2017). In another study, myristic and stearic acids were isolated from an active fraction of *R. communis* leaves, and this fraction showed bioactivity against *M. sacchari* (Sotelo-Leyva et al., 2020). In this work, we also identified myristic acid as a minor component in the *R. communis n*-hexane extract. Extracts of *R. communis* seeds, which have also shown an important aphidicidal effect against *M. sacchari*, were found to contain squalene as the main component in the extract (Salinas-Sánchez et al., 2021). Here, however, we did not identify squalene as a component of *R. communis* leaf extract.

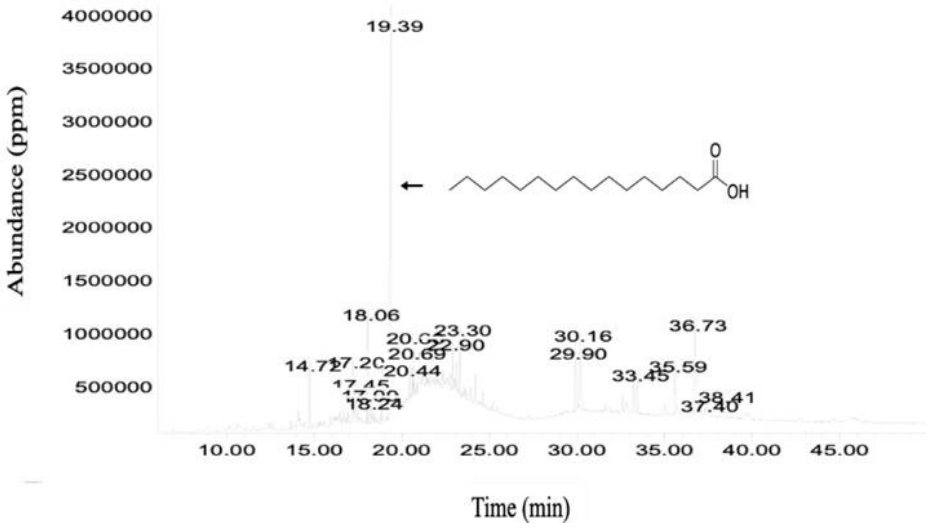

Figure 3. Gas chromatogram and chemical structure of palmitic acid, identified as the major compound in the *n*-hexane extract of *Ricinus communis* leaves.

In our research, palmitic acid was the majority compound in *R. communis* leaf extract. Recent literature does not report the insecticidal effect of palmitic acid against any species of aphid. The literature reports the effect of palmitic acid against other insects of agricultural importance, for example, in a study of the extract of *n*-hexane from the leaves of *R. communis* showed that it is toxic against *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and the GC-MS study revealed the presence of palmitic acid (13.01%) in the extract (Ramos-López et al., 2012). In another investigation, fractions of *R. communis* leaf extracts were evaluated against *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae), the active fraction demonstrated an insecticidal effect, and the chemical study revealed that palmitic acid was the main compound in the active fraction, with 81.0% abundance (Bigi et al., 2004).

CONCLUSIONS

The *n*-hexane extract of *Ricinus communis* had very effective insecticidal activity against *Rhopalosiphum maidis* and *Sipha flava*. The extract contains a complex mixture of chemical components, so it is difficult to attribute the insecticidal effect to particular active ingredients. It is possible that the minority compounds could be involved

in synergistic interactions with the active compound. It is important to isolate the molecules responsible for the activity in order to continue to develop botanical insecticides that are environmentally and health-friendly to replace toxic chemical insecticides in the future.

Author contributions

Conceptualization: C.S-L., G.P-C. Methodology: E.T.H. Software, G.H-S., D.O.S-S. Validation: C.S-L., G.P-C. Formal analysis: C.S-L. Investigation: L.A-M., D.O.S-S., G.H-S. Data curation: N.N-T. Writing-original draft: C.S-L., G.P-C. Writing-review & editing: B.L. Visualization: C.S-L. Supervision: G.P-C. Project administration: B.L. All co-authors reviewed the final version and approved the manuscript before submission.

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