Insecticidal compounds in *Ricinus communis* L. (Euphorbiaceae) to control *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae)

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**Abstract**

The sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), is recognized as an important pest of sorghum cultivation. The use of natural products in the form of botanical extracts represents an alternative for its control. In this investigation, we evaluated the insecticidal activity of hexanic, acetic, and methanolic extracts of leaves, fruits, and roots of *Ricinus communis* L. (Euphorbiaceae). These were applied in contact bioassays at different concentrations to control apterous adults of *M. sacchari*. We found that the chemical components of lower polarity contained in the hexane extract of leaves (RcLH) produced the best biological effect, with 96% mortality at 72 h. Thin layer chromatography allowed fractions of this extract to be grouped into 7 categories (F1–F7) based on their chemical content. The F3 category produced 90% mortality at 10,000 ppm at 72 h in contact bioassays. The ¹H and ¹³C nuclear magnetic resonance analysis in addition to the gas chromatography-mass spectrometry of F3 revealed the presence of myristic and stearic acid. Our results showed that the hexanic extracts of *R. communis* and their fatty acids may be an alternative for the development of new insecticides, constituting a better option in terms of effectiveness and lower toxicity compared with the synthetic products currently on the market used for their control.

**Key Words:** hexane extracts; aphids; myristic acid; stearic acid

**Resumen**

El pulgón de la caña de azúcar *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), es reconocido como una plaga importante para el cultivo de sorgo. El uso de productos naturales en forma de extractos botánicos representa una alternativa para su control. En esta investigación, evaluamos la actividad insecticida de los extractos hexánicos, acéticos, y metánolicos de hojas, frutos y raíces de *Ricinus communis* L. (Euphorbiaceae). Estos se aplicaron en bioensayos de contacto a diferentes concentraciones contra adultos ápteros de *M. sacchari*. Descubrimos que los componentes químicos de menor polaridad contenidos en el extracto de hojas de hexano (RcLH) producían el mejor efecto biológico, con una mortalidad del 96% a las 72 horas. La cromatografía en capa fina permitió que las fracciones de este extracto se agruparan en siete categorías (F1–F7) en función de su contenido químico. F3 produjo 90% de mortalidad a 10.000 ppm a las 72 horas en bioensayos de contacto. El análisis de resonancia magnética nuclear (RMN) ¹H y ¹³C, además de la cromatografía de gases acoplado a espectrometría de masas (CG-MS) de F3 reveló la presencia de ácido mirísico y esteárico. Nuestros resultados mostraron que los extractos hexánicos de *R. communis* y sus ácidos grasos pueden ser una alternativa para el desarrollo de nuevos insecticidas, constituyendo una mejor opción en términos de efectividad y menor toxicidad en comparación con los productos sintéticos actualmente en el mercado.

**Palabras Clave:** extractos de hexano; áfidos; ácido mirísico; ácido esteárico

Worldwide the USA and Mexico are the most important producers of sorghum (*Sorghum bicolor* [L.] Moench; Poaceae) (FIRA 2016). Historically, this ancient commodity is one of the most important cereals for animal feed (Chuck-Hernández et al. 2011). *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae) is an economically important insect in many areas of Africa, Asia, Australia, and the Far East, but it is a severe sorghum pest in the USA and Mexico (Singh et al. 2004).

In 1970, *M. sacchari* entered the USA as a pest of sugar cane (Schenck & Lehrer 2000). By 2013, outbreaks of this pest were found in sorghum crops causing economic losses in North America (Arm-
strong et al. 2015). Although the buds of sugar cane can be infested with M. sacchari, no significant damage occurs to that crop (Medina et al. 2017). However, damage to sorghum by this aphid is considerably more serious. About 20 US states and sorghum-producing areas in Mexico have suffered severe damage since the introduction of this pest in 2013 (Bowling et al. 2016; Harris-Shultz et al. 2017). According to Nibouche et al. (2018) the change of host from sorghum to sugarcane may be due to a change in host preference, or the arrival of a new biotype of yellow aphid specializing in sorghum.

In Mexico, chemical insecticides such as neonicotinoids have been used to control this aphid (Tejeda-Reyes et al. 2017). The negative effects of these chemical compounds on human health have been reported (Müller et al. 2005), and they have been implicated in bee colony collapse (Medrzycki et al. 2003). In view of this situation, it is necessary to search for new alternatives for the control of agronomic pests, such as those from botanical extracts that are generally more environmentally friendly and less damaging to the health of humans as well as other non-target organisms (Isman 2008).

A plant species of possible interest for use to control M. sacchari is Ricinus communis L. (Euphorbiaceae), commonly known as “higuerrilla.” This plant is native to Africa, although some authors suggest that it may have originated from India or China (Worbs et al. 2011). Currently, this plant species is widely distributed worldwide and has many industrial, ornamental, and medicinal applications (Rana et al. 2012). To date, a total of 83 compounds have been isolated from various parts of R. communis, including alkaloids, terpenoids, flavonoids, benzoic acid derivatives, coumarins, tocopherols, and fatty acids. These compounds have demonstrated cytotoxic, insecticidal, anti-inflammatory, antioxidative properties, and anti-asthmatic properties, among others (Ribeiro et al. 2016). We report here our investigations with polarity extracts of derivatives, coumarins, tocopherols, and fatty acids. These compounds R. communis is industrially, ornamental, and medicinal applications (Rana et al. 2012). To this plant species is widely distributed worldwide and has many in-
tality between 5,000 and 10,000 ppm was not significantly different during this period of time. At 10,000 ppm, hexane leaf mortality was not significantly different from that of hexane root and fruit, and the positive control, imidacloprid, at 72 h. At 48 h, hexane leaf at 5,000 and 10,000 ppm also showed no significant difference compared with the positive control, exhibiting mortalities of 70 and 94%, respectively. In the same way, hexane fruit and root at 10,000 ppm were not significantly different from the positive control at 48 h.

All acetone extracts were less effective compared with hexane extracts (Table 2). At 10,000 ppm, acetone leaf and root had the highest aphid mortality (56%), and were significantly lower than the positive control. On the other hand, it was observed that the percentage of aphid mortality (56%), and were significantly lower than the positive control at 48 h. The yellow aphid *M. sacchari* is an invasive species and the most important pest associated with sorghum cultivation. This insect has caused economic losses to farmers in the USA and Mexico, the top producers worldwide of this crop. The use of insecticides of synthetic origin represents the most effective option for their control because they are broad spectrum. The search for botanical alternatives for the control of *M. sacchari* is necessary because the use of bioinsecticides represents a more friendly option for the environment while minimizing the adverse impact to non-target organisms. Plants can defend themselves against the attack of aphids by producing secondary metabolites that can be used to control these insects. *Ricinus communis* is an interesting plant species with documented insecticidal activity (Rana et al. 2012). However, a literature search revealed little to no information regarding the use addition, the presence of a carbonyl confirmed the presence of these acids. Gas chromatography-mass spectrometry analysis yielded the following data: tetradecanoic (myristic) acid (6.8 min, 228 uma [M] for \( C_{14}H_{28}O \)) and octadecanoic (stearic) acid (8.4 min, m / z 284 uma [M + H]) for \( C_{18}H_{36}O \).

### Discussion

The yellow aphid *M. sacchari* is an invasive species and the most important pest associated with sorghum cultivation. This insect has caused economic losses to farmers in the USA and Mexico, the top producers worldwide of this crop. The use of insecticides of synthetic origin represents the most effective option for their control because they are broad spectrum. The search for botanical alternatives for the control of *M. sacchari* is necessary because the use of bioinsecticides represents a more friendly option for the environment while minimizing the adverse impact to non-target organisms. Plants can defend themselves against the attack of aphids by producing secondary metabolites that can be used to control these insects. *Ricinus communis* is an interesting plant species with documented insecticidal activity (Rana et al. 2012). However, a literature search revealed little to no information regarding the use

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Table 1. Mean percent mortality (± SD) of apterous *Melanaphis sacchari* from contact bioassays of various hexane extracts of *Ricinus communis* plant parts.

| Treatments               | Concentration (ppm) | 24 h         | 48 h         | 72 h         |
|--------------------------|---------------------|--------------|--------------|--------------|
| *n*-hexane extract – leaf| 10,000              | 60 ± 2.0\(^a\) | 94 ± 1.3\(^a\) | 96 ± 0.8\(^a\) |
|                          | 5,000               | 50 ± 1.6\(^a\) | 70 ± 1.2\(^a\) | 74 ± 1.1\(^a\) |
|                          | 2,500               | 20 ± 0.5\(^a\) | 38 ± 1.2\(^a\) | 42 ± 1.6\(^a\) |
| *n*-hexane extract – fruit| 10,000             | 66 ± 1.6\(^a\) | 78 ± 1.7\(^a\) | 84 ± 1.5\(^a\) |
|                          | 5,000               | 22 ± 2.3\(^a\) | 32 ± 1.6\(^a\) | 44 ± 0.8\(^a\) |
|                          | 2,500               | 26 ± 1.6\(^a\) | 42 ± 2.3\(^a\) | 42 ± 2.3\(^a\) |
| *n*-hexane extract – root| 10,000              | 76 ± 1.5\(^a\) | 86 ± 1.6\(^a\) | 88 ± 1.3\(^a\) |
|                          | 5,000               | 32 ± 1.6\(^a\) | 46 ± 2.3\(^a\) | 60 ± 2.4\(^a\) |
|                          | 2,500               | 28 ± 1.7\(^a\) | 36 ± 2.0\(^a\) | 54 ± 1.6\(^a\) |
| Tween 20 (negative control) | 2,000              | 0\(^a\)       | 0\(^a\)       | 6 ± 0.5\(^a\) |
| Imidacloprid (positive control) | 10,000             | 100\(^a\)    | 100\(^a\)    | 100\(^a\)    |

Means in each column with different letters were significantly different, Tukey multiple comparison test (\( P \leq 0.05 \)).

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Table 2. Mean percent mortality (± SD) of apterous *M. sacchari* from contact bioassays of various acetone extracts of *Ricinus communis* plant parts.

| Treatments               | Concentration (ppm) | 24 h         | 48 h         | 72 h         |
|--------------------------|---------------------|--------------|--------------|--------------|
| acetone extract – leaf    | 10,000              | 32 ± 1.0\(^a\) | 46 ± 1.8\(^a\) | 56 ± 1.4\(^a\) |
|                          | 5,000               | 26 ± 1.3\(^a\) | 38 ± 1.6\(^a\) | 42 ± 1.3\(^a\) |
|                          | 2,500               | 10 ± 1.0\(^a\) | 14 ± 0.5\(^a\) | 26 ± 0.8\(^a\) |
| acetone extract – fruit   | 10,000              | 28 ± 1.0\(^a\) | 34 ± 1.6\(^a\) | 46 ± 1.6\(^a\) |
|                          | 5,000               | 8 ± 0.8\(^a\)  | 18 ± 0.8\(^a\) | 26 ± 1.1\(^a\) |
|                          | 2,500               | 4 ± 0.5\(^a\)  | 16 ± 0.8\(^a\) | 22 ± 0.4\(^a\) |
| acetone extract – root    | 10,000              | 40 ± 1.7\(^a\) | 52 ± 1.7\(^a\) | 56 ± 1.8\(^a\) |
|                          | 5,000               | 16 ± 1.1\(^a\) | 40 ± 1.8\(^a\) | 50 ± 1.2\(^a\) |
|                          | 2,500               | 16 ± 0.5\(^a\) | 24 ± 1.3\(^a\) | 36 ± 1.6\(^a\) |
| Tween 20 (negative control) | 2,000              | 0\(^a\)       | 0\(^a\)       | 6 ± 0.5\(^a\) |
| Imidacloprid (positive control) | 10,000             | 100\(^a\)    | 100\(^a\)    | 100\(^a\)    |

Means in each column with different letters were significantly different, Tukey multiple comparison test (\( P \leq 0.05 \)).
of extracts from this species for control of *M. sacchari*. Thus, to our knowledge, this is the first report on this subject. In our study, it was shown that hexane extracts from different parts of *R. communis* resulted in the highest mortality, compared with other extracts that were bioassayed with rates of 88 to 96% mortality occurring at 10,000 ppm, 78 to 94% at 5,000 ppm, and 60 to 76% at 2,500 ppm at 72 h. This is consistent with previous reports of other plant species for control of aphids. For example, Ateyyat and Abu-Darwish (2009) evaluated hexane bark extracts of *Rhamnus dispermus* (Ehrenb. ex Boiss.) (Rhamnaceae) to control the aphid *Pterochloroides persicae* (Cholodkovsky) (Hemiptera: Aphididae), reporting 40% mortality at 10,000 ppm in 72 h. Moreover, results of our investigation during the 48-h time period revealed insecticidal activity with ranges from 78 to 94% at 10,000 ppm, 32 to 70% at 5,000 ppm, and 38 to 42% at 2,500 ppm. This is similar to previous studies, such as that of Arya et al. (2014), who tested the oil ether extracts of *R. communis* seeds to control the aphid *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae) with concentrations of 10,000 ppm and 5,000 ppm causing 100% and 75% mortality, respectively, in 48 h. Hewage et al. (1997) reported that the hexane extract of *Pleiospermium alatum* (Wight & Arn.) Swingle (Rutaceae) produced 90% mortality at 4,000 ppm at 48 h for the aphid *Aphis craccivora* (Koch) (Hemiptera: Aphididae). Another investigation, conducted by Rodriguez et al. (2012), demonstrated that oil ether extracts from *Picrasma crenata* (Vell.) Engl. (Simaroubaceae) produced 75% mortality at 6,000 ppm at 48 h in *Myzus persicae* Sulzer (Hemiptera: Aphididae).

Our studies showed that the insecticidal action of hexane extracts 24 h after application of the treatments exerted an insecticidal effect, with mortality rates of 60 to 76% at 10,000 ppm and 22 to 50% at 5,000 ppm. These rates were similar to those published by Singh et al. (1988) to control the mustard aphid, *L. erysimi*, where hexane extracts from seeds of *Azadirachta indica* (A. Juss) (Meliaceae) produced 48% mortality at 5,000 ppm in 24 h. In addition, an investigation by Nia et al. (2015) showed that the *Artemisia herba-alba* (Asso) (Asteraceae) petroleum ether extract produced 40% mortality at 10,000 ppm in 24 h for the aphid *M. persicae*.

### Table 3. Mean percent mortality (± SD) of apterous *Melanaphis sacchari* from contact bioassays of various methanolic extracts of *Ricinus communis* plant parts.

| Treatments                  | Concentration (ppm) | 24 h       | 48 h       | 72 h       |
|-----------------------------|---------------------|------------|------------|------------|
| methanol extract – leaf     | 10,000              | 40 ± 2.9a  | 46 ± 3.0a  | 54 ± 2.0a  |
|                            | 5,000               | 28 ± 0.8a  | 40 ± 1.5a  | 50 ± 1.0a  |
|                            | 2,500               | 20 ± 2.3a  | 34 ± 1.1a  | 46 ± 1.1a  |
| methanol extract – fruit    | 10,000              | 4 ± 0.8a   | 6 ± 0.8a   | 22 ± 1.6a  |
|                            | 5,000               | 0 ± 0      | 4 ± 0.5    | 20 ± 1.0a  |
|                            | 2,500               | 0 ± 0      | 4 ± 0.8    | 16 ± 0.8a  |
| methanol extract – root     | 10,000              | 20 ± 0.7a  | 32 ± 0.8a  | 44 ± 0.8a  |
|                            | 5,000               | 12 ± 1.3a  | 18 ± 0.8a  | 40 ± 1.0a  |
|                            | 2,500               | 10 ± 0.7a  | 12 ± 0.8a  | 22 ± 1.3a  |
| Tween 20 (negative control)| 2,000               | 0          | 0          | 6 ± 0.5    |
| Imidacloprid (positive control)| 10,000             | 100        | 100        | 100        |

Means in each column with different letters were significantly different, Tukey multiple comparison test (P ≤ 0.05).

![Graph](image-url)  
**Fig. 1.** Mean percent mortality (± SD) of apterous *Melanaphis sacchari* from contact bioassays of various hexane leaf fractions and concentrations of *Ricinus communis* at 72 h compared with imidacloprid as a positive control. Means with different letters for each fraction were significantly different, Tukey’s multiple comparison test (P ≤ 0.05).
Considering that the cuticle of aphids is mainly composed of alkyl esters, the methyl esters of fatty acids, triacylglycerides, and free fatty acids (Brey et al. 1985), it is possible that the effectiveness of hexane extracts is due to the chemical affinity of low-polarity compounds present in the extract with the aphid cuticle. In contrast, the acetone and methanol extracts produced low mortality rates due to low affinity toward the fatty body of *M. sacchari*.

Our bio-directed study led us to the identification of 2 major fatty acids (myristic acid and stearic acid) which were present in the F3 fraction of hexane leaf. This mixture possessed the highest mortality (90%) at 10,000 ppm in 72 h. These 2 fatty acids had been identified previously in *R. communis* (Bigi et al. 2004; Ramos-López et al. 2012). We are not aware of reports on the insecticidal activity of these 2 fatty acids for control of aphids. In conclusion, hexane extracts were found to possess very effective insecticides for control of *M. sacchari*. *Ricinus communis* may represent a plausible avenue in the development of novel biorationale products for agronomic crop pest control for control of aphids.

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**References Cited**

Armstrong JS, Rooney WL, Peterson GC, Villanueva RT, Brewer MJ, Sekula-Ortiz D. 2015. Sugarcane aphid (Hemiptera: Aphididae): host range and sorghum resistance including cross-resistance from greenbug sources. Journal of Economic Entomology 108: 576–582.

Arya H, Sing BR, Singh K. 2014. Insecticidal activity of petroleum ether extract of castor seeds against mustard aphid *Lipaphis erysimi* Kaltenbach. Advances in Bioresearch 5: 165–168.

Ateyyat MA, Abu-Darwish MS. 2009. Short communication. Insecticidal activity of different extracts of *Rhamnus dispermus* (Rhamnaceae) against peach trunk aphid, *Pterochloroides persicae* (Homoptera: Lachnidae). Spanish Journal of Agricultural Research 7: 160–164.

Bigi MA, Torkomian LV, de Groote TCS, Hebling MJ, Bueno OC, Pagnocca FC, Fernandes JB, Vieira PC, da Silva MF. 2004. Activity of *Ricinus communis* (Euphorbiaceae) and ricinine against the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) and the symbiotic fungus *Leucoagaricus gongylodius*. Pest Management Science 60: 933–938.

Bowling RD, Brewer MJ, Kerns DL, Gordy JN, Seiter NE, Elliott NM, Buntin GD, Way MO, Royer TA, Biles S, Maxson E. 2016. Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. Journal of Integrated Pest Management 7: 1–13.

Brey TP, Chopaque H, Lesourd M, Castex H, Roucachet J, Latge JP. 1985. Ultrastructure and chemical composition of the outer layers of the cuticle of the pea aphid *Acyrthosiphon pisum* (Harris). Comparative Biochemistry and Physiology 82: 401–411.

Chuck-Hernández C, Pérez-Carrillo E, Heredia-Olea E, Serna-Saldivar S. 2011. El sorgo un cultivo multifacético para la producción de bioetanol en México: tecnologías, avances y áreas de oportunidad. Revista Mexicana de Ingeniería Química 10: 529–549.

FIRA – Fideicomisos Instituidos en Relacion con la Agricultura. 2016. Panorama agroalimentario del sorgo. Dirección de Investigación y Evaluación Económica y Sectorial. Morelia, Michoacán, México.

Harriss-Shultz K, Ni X, Wadi PA, Wang X, Wang H, Huang F, Flanders K, Seiter N, Kerns D, Meagher R, Xue Q, Reising D, Buntin D, Cuevas HE, Brewer MJ, Yang X. 2017. Microsatellite markers reveal a predominant sugarcane aphid (*Homoptera: Aphididae*) clone is found on sorghum in seven states and one territory of the USA. Crop Science 57: 2064–2072.

Hewage CM, Bandara KANP, Karunarathne V, Bandara BM. Wijesundara DSA. 1997. Insecticidal activity of some medicinal plants of Sri Lanka. Journal of the National Science Foundation of Sri Lanka 25: 141–150.

Isman MB. 2008. Botanical insecticides: for richer, for poorer. Pest Management Science 64: 8–11.

Medina RF, Armstrong SJ, Harrison K. 2017. Genetic population structure of sugarcane aphid, *Melanaphis sacchari*, in sorghum, sugarcane, and Johnson grass in the continental USA. Entomologia Experimentalis et Applicata 162: 358–365.

Medrzycki P, Montanari R, Bortoliotti L, Sabatini AG, Maini E, Porrini C. 2003. Effects of imidacloprid administered in sub-lethal doses on honey bee behavior. Laboratory tests. Bulletin of Insectology 56: 59–62.

Müller N, Faria X, Facchini LA, Gastal A, Tomasi E. 2005. Pesticides and respiratory symptoms among farmers. Revista de Saúde Pública 39: 973–981.

Nia B, Frah N, Azou I. 2015. Insecticidal activity of three plants extracts against *Myzus persicae* (Sulzer, 1776) and their phytochemical screening. Acta Agriculturae Slovenica 2: 261–267.

Nibouche S, Costet L, Holt JR, Jacobson A, Pekarick A, Sadeyen J, Armstrong JS, Peterson GC, McLaren N, Medina RF. 2018. Invasion of sorghum in the Americas by a new sugarcane aphid (*Melanaphis sacchari*) superclone. PLoS ONE 13: 1–15.

Ramos-López MA, González-Chávez MM, Cárdenas-Ortega NC, Zavala-Sánchez MA, Pérez S. 2012. Activity of the main fatty acid components of the hexane leaf extract of *Ricinus communis* against *Spodoptera frugiperda*. African Journal of Biotechnology 11: 4274–4278.

Rana M, Dhamija H, Prashar B, Sharma S. 2012. *Ricinus communis* L.-A. Review. PharmaTech 4: 1706–1711.

Ribeiro PR, de Castro RD, Fernández LG. 2016. Constituents of the oilseed crop *Ricinus communis* and their pharmacological activities: a review. Industrial Crops and Products 91: 358–376.

Rodríguez S, Regonat M, Carrizo P, Meilán J, Wagner M, Gurni A. 2012. Activity of nonpolar extracts from *Picrasma crenata* (Simaroubaceae) against *Myzus persicae* (Homoptera: Aphididae). Revista Latinoamericana de Química 39: 113–120.

SAS. 2002. SAS System for Windows, vers. 9.0. SAS Institute, Cary, North Carolina, USA.

Schenck, AR, Lehrer AT. 2000. Factors affecting the transmission and spread of sugarcane yellow leaf virus. Plant Disease 84: 1085–1088.

Singh BU, Padmaja PG, Seetharama N. 2004. Biology and management of the sugarcane aphid, *Melanopis sacchari* (Zehntner) (Homoptera: Aphididae) in sorghum: a review. Crop Protection 23: 739–755.

Singh RP, Devakumar C, Dhingra S. 1988. Activity of neem (*Azadirachta indica* A. Juss.) seed kernel extracts against the mustard aphid, *Lipaphis erysimi*. Phytoparasitica 16: 225–230.

Tejeda-Reyes MA, Díaz-Nájera JF, Rodríguez-Maciel JC, Vargas-Hernández M, Sollis-Aguilar JF, Ayvar-Serna S, Flores-Yañez JA. 2017. Evaluación en campo de insecticidas sobre *Melanaphis sacchari* (Zehntner) en sorgo. Southwestern Entomologist 42: 545–550.

Wors S, Köhler K, Pauly D, Avondet MA, Schaer M, Dorner MB, Dorner B. 2011. *Ricinus communis* intoxications in human and veterinary medicine - a summary of real cases. Toxins 3: 1332–1372.