Insecticidal Compounds in Ricinus communis L. (Euphorbiaceae) to Control Melanaphis sacchari Zehntner (Hemiptera: Aphididae)

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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, acetonic, 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 ^{1}H and ^{13}C 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, acetónicos, y metanólicos 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) ^{1}H y ^{13}C, además de la cromatografía de gases acoplado a espectrometría de masas (CG-MS) de F3 reveló la presencia de ácido miristico 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 miristico; 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-

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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 sugar cane to sorghum 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 "higuerilla." 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, antioxidant activity, and anti-asthmatic properties, among others (Ribeiro et al. 2016). We report here our investigations with polarity extracts of leaves, fruits, and roots of *R. communis* for their insecticidal effectiveness against *M. sacchari*, and to identify active compounds responsible for their bioactivity.

Materials and Methods

PREPARATION AND ANALYSIS OF PLANT EXTRACTS

Leaves, fruits, and roots of *R. communis* were collected in the community of Yautepec, Morelos, Mexico (18.8248611°N, 99.0963333°W) in Jan 2018. A specimen of the plant was identified by M.C. Gabriel Flores Franco, specialist taxonomist, at the Herbarium of the Centro de Investigación en Biodiversidad y Conservación, at the Universidad Autónoma del Estado de Morelos, México, where it was deposited under catalog number 34874.

Prior to extraction, 100 g of leaves, 50 g of fruit, and 150 g of *R. communis* roots were dried outdoors in the shade for 15 d. Each plant part was macerated consecutively in 500 mL of 3 solvents of ascending polarity (*n*-hexane, acetone, and methanol) for 72 h. This process was conducted in triplicate. The solvent was removed by distillation under reduced pressure using a rotary evaporator (Buchi 205, Flawil, Switzerland). Extracts obtained were named depending on the solvent and part used: *n*-hexane extracts: leaf, fruit, and root; acetone extracts: leaf, fruit, and root; methanol extracts: leaf, fruit, and root.

The *n*-hexane leaf extract showed a higher mortality of aphids (96%), compared with the extracts of acetone (56%) and methanol (54%), and was further investigated to determine the bioactive components. Seven g were fractionated in a glass column previously packed with 60 g of silica gel 70–230 mesh (Merck KGaA, Darmstadt, Germany). A gradient of *n*-hexane and ethyl acetate was used as the mobile phase, starting with 100% *n*-hexane and ending with 100% of the solvent of higher polarity, collecting 10 mL samples at each gradient. This process was monitored by thin layer chromatography. Thin layer chromatography was used to obtain fractions based on chemical content into 7 categories (F1–F7): F1 (2.4 g, 95:5), F2 (0.69 g, 90:10), F3 (1.5 g, 85:15), F4 (0.64 g, 80:20), F5 (0.13 g, 50:50), F6 (0.16 g, 30:70), and F7 (0.19 g, 100% ethyl acetate), then subsequently evaluated in contact bioassays described later.

The most active fraction (F3, 1.5 g) isolated from *n*-hexane extract: leaf was subjected to a column chromatographic fractionation packed with silica gel (25 g, 70–230 mesh, Merck KGaA, Darmstadt, Germany). A gradient of *n*-hexane and ethyl acetate was used as the mobile phase, starting with 100% *n*-hexane and ending with 100% ethyl acetate. A total of 433 mg of a fraction obtained from F3 was separated in a reverse phase column packed with silica gel (1 g, RP-18, 40–63 µm, Merck KGaA, Darmstadt, Germany) with water and acetonitrile as the mobile phase. Analysis of F3 using thin layer chromatography revealed, with Komarovsky reagent, the presence of 2 major products: myristic acid (25.4 mg) and stearic acid (26.3 mg). These were subsequently identified by nuclear magnetic resonance spectra recorded on a Bruker Advance III HD-600 at 600 MHz for ^{1}H nuclear magnetic resonance and ^{13}C nuclear magnetic resonance in $CDCl_3$. The mass-coupled gas chromatograph data was obtained using an Agilent 5975C equipped with a long-duration ion multiplier triple-axis detector in electronic impact mode.

BIOASSAYS

Laboratory colonized adult *M. sacchari* maintained in a greenhouse at Centro de Investigación en Biodiversidad y Conservación, at the Universidad Autónoma del Estado de Morelos, México were used in this study. Insects were maintained at the optimum temperature for insect reproduction of 25 ± 2 °C and fed hybrid sorghum variety M550 (Majestic Seeds Co., Hodges, South Carolina, USA).

Contact bioassays consisted of placing a 5 × 5 cm sorghum leaf into a 30 cm^3 plastic Petri dish with filter paper on the bottom. One mL of distilled water was added to prevent the sorghum leaf from drying out. Ten *M. sacchari* were placed on a leaf using a camel hair brush. Ten µL of Tween 20 (0.2%) was added to each of the *n*-hexane and acetone extracts to allow them to be homogenized in 2 mL of water. A standard airbrush (Truper® Aero-35, Estado de México, México) was used to spray 0.15 mL of each mixture at concentrations of 2,500, 5,000, and 10,000 ppm onto the aphids. Two replicates were carried out with 5 repetitions per extract concentration in a completely randomized design. Percent aphid mortality was recorded at 24, 48, and 72 h after treatment. The synthetic chemical insecticide Confial® (active ingredient: 1% imidacloprid) and Tween 20 (0.2%) were used as controls.

STATISTICS

Mean percent mortality data were arcsine transformed prior to statistical analysis using an initial ANOVA test within a randomized study design. Post-hoc comparison of means was carried out using the Tukey test at ($P \leq 0.05$) using SAS 9.0 (SAS 2002). Untransformed means are presented.

Results

The yield of *R. communis* leaves, fruit, and root obtained from the extracts were as follows: *n*-hexane leaf (8%), fruit (2%), and root (0.5%); acetone leaf (2%), fruit (4%), root (0.9%); methanol leaf (16%), fruit (4%), and root (3.8%). In general, hexane extracts provided the highest mortality (96%) at 10,000 ppm at 72 h (Table 1). Aphid mor-

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

		% Mean mortality		
Treatments	Concentration (ppm)	24 h	48 h	72 h
n-hexane extract – leaf	10,000	60 ± 2.0^{bcd}	94 ± 1.3^{a}	96 ± 0.8^{a}
	5,000	50 ± 1.0^{bcd}	70 ± 1.2^{abc}	74 ± 1.1^{abcd}
	2,500	20 ± 0.4^{ed}	38 ± 1.2^{cd}	42 ± 1.4^{bcde}
n-hexane extract – fruit	10,000	66 ± 1.6^{bc}	78 ± 1.7^{ab}	84 ± 1.5^{abc}
	5,000	22 ± 1.9^{de}	32 ± 1.6^{de}	44 ± 0.8^{de}
	2,500	26 ± 1.6^{cd}	42 ± 2.3^{cd}	42 ± 2.3^{e}
n-hexane extract – root	10,000	76 ± 1.5^{b}	86 ± 1.6^{a}	88 ± 1.3^{ab}
	5,000	32 ± 2.3^{cd}	46 ± 2.3^{bcd}	60 ± 2.4^{bcde}
	2,500	28 ± 1.7^{bcd}	36 ± 2.0^{bcd}	54 ± 1.6^{cde}
Tween 20 (negative control)	2,000	0^{e}	0^{e}	6 ± 0.5^{f}
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 ($P \leq 0.05$).

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 mortality at 48 h of the 3 treatments was below 50%, with the exception of acetone root at 10,000 ppm, which had a mortality of 52%. Statistically, no treatment was as effective as the positive control (imidacloprid). Methanolic extracts of *R. communis* were also less effective than the hexane and acetone extracts (Table 3).

Of all the fractions evaluated, F3 was statistically equal at all concentrations in effectiveness compared with imidacloprid (Fig. 1). The F3 category consisted of myristic and stearic acids. The ^{1}H nuclear magnetic resonance analysis of this fraction showed 4 multiple signals of δ 0.89 to 2.30 ppm, characteristic of fatty acids, and was confirmed with ^{13}C nuclear magnetic resonance spectra by δ signals 13.82 to 43.81. In 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 $C_{14}H_{28}O_2$) and octadecanoic (stearic) acid (8.4 min, m / z 284 uma $[M + H]^{+}$ $C_{18}H_{36}O_2$).

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

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

		% Mortality ± SD		
Treatments	Concentration (ppm)	24 h	48 h	72 h
acetone extract – leaf	10,000	32 ± 1.0^{cb}	46 ± 1.8^{cb}	56 ± 1.4^{b}
	5,000	26 ± 1.3^{cbd}	38 ± 1.6^{cbd}	42 ± 1.3^{cb}
	2,500	10 ± 1.0^{fed}	14 ± 0.5^{ed}	26 ± 0.8^{cd}
acetone extract – fruit	10,000	28 ± 1.0^{cbd}	34 ± 1.6^{cbd}	46 ± 1.6^{cb}
	5,000	8 ± 0.8^{fed}	18 ± 0.8^{ed}	26 ± 1.1^{cd}
	2,500	4 ± 0.5^{fe}	16 ± 0.8^{ed}	22 ± 0.4^{cd}
acetone extract – root	10,000	40 ± 0.7^{b}	52 ± 1.7^{b}	56 ± 1.8^{b}
	5,000	16 ± 1.1^{fced}	40 ± 1^{cbd}	50 ± 1.2^{b}
	2,500	16 ± 0.5^{fced}	24 ± 1.3^{cde}	36 ± 1.6^{cb}
Tween 20 (negative control)	2,000	0^{f}	0^{e}	6 ± 0.5^{d}
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 ($P \leq 0.05$).

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

		% Mortality ± SD		
Treatments	Concentration (ppm)	24 h	48 h	72 h
methanol extract – leaf	10,000	40 ± 2.9^{b}	46 ± 3.0^{b}	54 ± 2.0^{b}
	5,000	28 ± 0.8^{bc}	40 ± 1.5^{bc}	50 ± 1.0^{bc}
	2,500	20 ± 2.3^{bc}	34 ± 1.1^{bcd}	46 ± 1.1^{bcd}
methanol extract – fruit	10,000	4 ± 0.8^{bc}	6 ± 0.8^{ef}	22 ± 1.6^{def}
	5,000	0 ± 0^{c}	4 ± 0.5^{f}	20 ± 1.0^{def}
	2,500	0 ± 0^{c}	4 ± 0.8^{f}	16 ± 0.8^{ef}
methanol extract – root	10,000	20 ± 0.7^{bc}	32 ± 0.8^{bcde}	44 ± 0.8^{bcd}
	5,000	12 ± 1.3^{bc}	18 ± 0.8^{cdef}	40 ± 1^{bcde}
	2,500	10 ± 0.7^{bc}	12 ± 0.8^{def}	22 ± 1.3^{def}
Tween 20 (negative control)	2,000	0^{c}	0^{f}	6 ± 0.5^{f}
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 Rodríguez 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.*

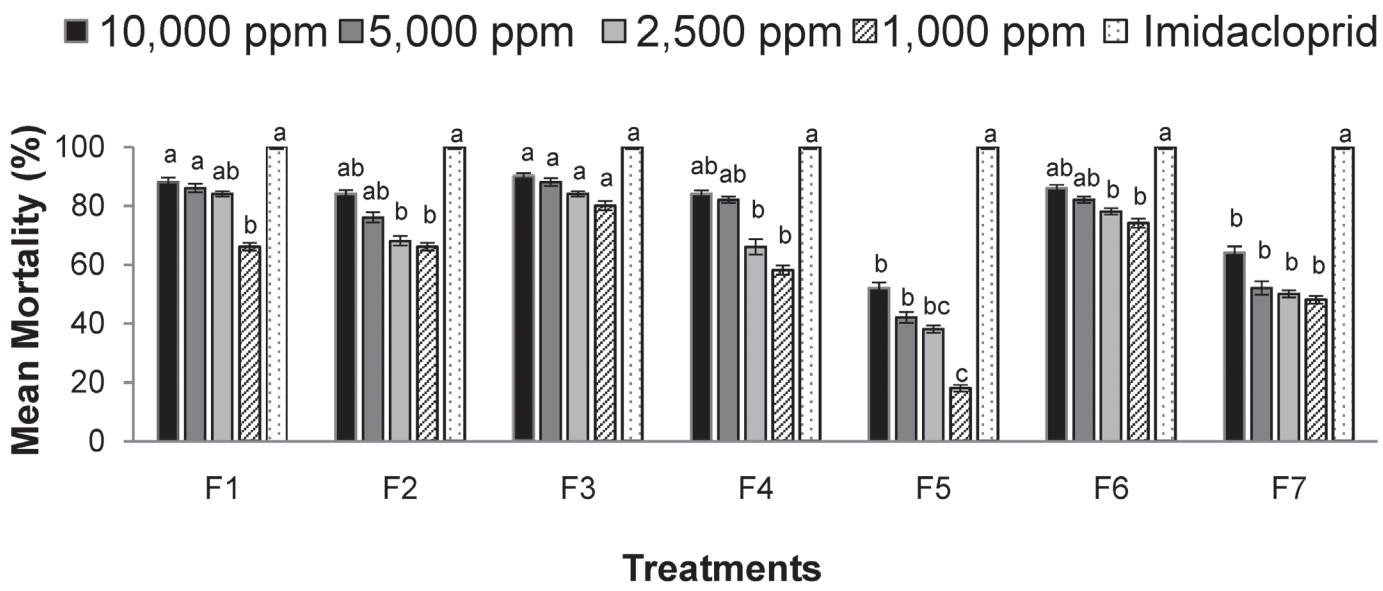

Fig. 1. Mean percent mortality (± SD) of apterous *Melanaphis sacchari* from contact bioassays of various hexane leaf fractions and concentrations of *Ricinus communis* at 72 h compared with imidacloprid as a positive control. Means with different letters for each fraction were significantly different, Tukey's multiple comparison test ($P \leq 0.05$).

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-Lopez 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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