Insecticidal and Nematicidal Effects of *Agave tequilana* Juice against *Bemisia tabaci* and *Panagrellus redivivus*

Author(s): Luis Alfredo Herbert-Doctor , Mario Saavedra-Aguilar , María Luisa Villarreal , Alexandre Cardoso-Taketa , and Odón Vite-Vallejo

Source: Southwestern Entomologist, 41(1):27-40.
Published By: Society of Southwestern Entomologists
DOI: http://dx.doi.org/10.3958/059.041.0105
URL: http://www.bioone.org/doi/full/10.3958/059.041.0105

Insecticidal and Nematicidal Effects of *Agave tequilana* Juice against *Bemisia tabaci*[1] and *Panagrellus redivivus*[2]

Luis Alfredo Herbert-Doctor[3], Mario Saavedra-Aguilar[4], María Luisa Villarreal[3], Alexandre Cardoso-Taketa[3]*, and Odón Vite-Vallejo[4]*

Abstract. There is much interest in the economic use of *Agave tequilana* Weber leaves to control major agro-industrial pests, because an estimated 741,000 tons of leaves are wasted each year as a byproduct of the tequila industry. Hexane and ethyl acetate extracts and the insecticidal activity of juice from the leaves of *A. tequilana* were evaluated against silverleaf whitefly, *Bemisia tabaci* (Gennadius), and nematicidal action against *Panagrellus redivivus* L. Undiluted juice killed 31%, hexane extract diluted to 4% killed 100%, and the check Herald®, a pyrethroid, at a concentration of 0.8% killed 100% of whiteflies. Juice diluted to 12%, ethyl acetate extract at 0.4%, and the check Vydate-L®, a carbamate, at 0.8% killed 100% of *P. redivivus* nematodes. Juice from *A. tequilana* leaves contained alkaloids, flavonoids, saponins, tannins, triterpenes, and/or steroids that might have killed silverleaf whitefly and *P. redivivus*. The results demonstrated that juice from leaves of *A. tequilana*, which are a byproduct of the tequila beverage industry, are a potential insecticide and nematicide. Agave juice is a biological alternative to organophosphate and carbamate insecticides that cause pest resistance, environmental pollution, and health problems.

Resumen. Existe un gran interés en el aprovechamiento económico de las hojas de *Agave tequilana* Weber en el control de plagas de impacto agro-industrial importante, ya que se estima que anualmente 741,000 toneladas de hojas son desperdiciadas como subproducto de la industria tequilera. En este estudio se describen las actividades insecticida del jugo obtenido de las hojas de *Agave tequilana* contra *Bemisia tabaci* (Gennadius) y nematicida contra *Panagrellus redivivus* L., así como actividades de sus extractos hexano y acetato de etilo. El jugo sin diluir, al evaluarse como insecticida causó la muerte de 31% de *B. tabaci*. El extracto hexánico, a una dilución del 4%, presentó un porcentaje de mortalidad del 100% contra *B. tabaci*. Respecto a la actividad nematicida contra *P. redivivus*, el jugo diluido al 12% logró una mortalidad del 100%. El extracto acetato de etilo al 0.4% provocó la muerte de 100% de los nematodos. El tamiz fitoquímico del jugo obtenido de las hojas de *A. tequilana* reveló la presencia de alcaloides, flavonoides, taninos, saponinas, triterpenos, y esteroides. Los resultados demostraron que el

[1](Hemiptera: Aleyrodidae)
[2](Rhabditida: Panagrolaimidae)
[3]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos, 62209, México.
[4]Promotora Técnica Industrial, Calle 56 Sur, Manzana 1, Lote 13, Civac, Jiutepec, Morelos, 62578, México.
*Corresponding authors: ataketa@uaem.mx, odon.vite@ultraquimia.com

jugo de las hojas de *A. tequilana*, un desecho industrial de la industria tequilera, puede ser utilizado tanto como insecticida, como nematicida biológico, constituyendo una alternativa frente a los productos químicos que actualmente se encuentran en el mercado.

Introduction

Silverleaf whitefly, *Bemicia tabaci* (Gennadius) (Family: Aleyrodidae), is a phytopathogenic insect considered one of the most invasive pest species in the world. The whitefly causes annual losses of vegetable and grain crops for human consumption, as well as ornamentals (Lowe et al. 2000, Latournerie-Moreno et al. 2014). The insect lives on the underside of leaves where the female lays eggs. The larvae pass through four stages before reaching the adult stage. The life cycle takes 2 to 3 weeks in warm weather and as long as 3 months when weather is cold (Ortega 2008). The insect vectors different pathogenic viruses, e.g., *Begomovirus* and *Crinivirus* (Morales 2007, Marubayashi et al. 2013). Both the transmission vectors and the viruses can cause yellowing of the plants, arrested growth, and sooty mold from fungus (Ortega 2008). Extensive use of insecticides has resulted in increased insect resistance (Roditakis et al. 2009). Use of botanical insecticides formulated from plant extracts with different types of secondary metabolites is an alternative way for fighting insects by killing or controlling growth and reproduction (Ortega 2008).

Panagrellus redivivus L. (Family: Panagrolaimidae) is a free-living nematode, which is ovoviviparous and its eggs continue to develop in the uterus of the juvenile stage (Ricci et al. 2003). An advantage of *P. redivivus* is that it uses grain and flour as sources of carbon and energy to grow quickly in tight spaces (De Lara et al. 2003). Because of its size, fast growth, short life cycle, fertility, and easy handling, the nematode is a model for research in various scientific fields such as biomonitoring wastewater, soil, genetics, biochemistry, environmental toxicology, and agriculture (Samoiloff et al. 1983, Samoiloff 1987, Ongley et al. 1988, De Lara et al. 2003). *P. redivivus* can be used in bioassays evaluating plant extracts or compounds for nematicidal action because global parasitism of plants by nematodes results in an annual loss of $125 billion in agriculture (Chitwood 2003). In the United Kingdom, the potato crop suffers annual loss of 9% of production and $67 million because of potato cyst nematodes *Globodera pallida* and *G. rostochiensis* present in 64% of potato-growing land (DEFRA 2015).

Carbamate and organophosphorus insecticides are used to control parasitic nematodes in crops (Silvestre and Cabaret 2014). However, the insecticides can induce resistance to nematicides. Because of chemical stability and persistence in the environment, the pesticides accumulate in the food chain, exceeding maximum residue limits allowed in different kinds of crops (Ogah and Coker 2012). Indiscriminant use and mishandling of synthetic pesticides in agriculture can cause environmental pollution and problems with human health. Widespread use of broad-spectrum pesticides failed to control agricultural pests, mainly because of increasing resistance of organisms (Pérez et al. 2013). Nematode resistance to carbamate and organophosphorus insecticides can increase 10- to 70-fold (Glazer et al. 1997).

An alternative to synthetic pesticides is use of bioinsecticides from plant and microorganism secondary metabolites that possess potent insecticidal, larvicidal, and antifeedant properties (Pineda et al. 2007, Abdelgaleil et al. 2008,

Perumalsamy et al. 2010, Romanelli et al. 2010) but have low toxicity to birds, mammals, and humans (Pineda et al. 2007). The use of biological nematicides has increased the production of agricultural crops affected by parasitic nematodes. One example is application of nematophagous fungi as biological control for parasitic nematodes of various plants (De Lara et al. 2003). For example, formulas of *Paecilomyces lilacinus* have been approved for banana and coffee plantations in many countries (Collange et al. 2011).

Approximately 293 species in nine genera comprise the Agavaceae family (Good-Avila et al. 2006). The most important genus is *Agave* that has 166 species. An agave plant is a monocot and monocarpic and has leaves arranged in a rosette (Cházaro-Basañez et al. 2006, Good-Avila et al. 2006). Agave plants grow in arid and semi-arid areas of North America and Mexico and in the Caribbean Islands, Colombia, and Venezuela (Rodríguez-Garay 2004). Agave is used mostly for production of alcoholic beverages and natural fibers, but also for steroidal compounds (Ruvalcaba-Ruiz and Rodríguez-Garay 2002).

Blue agave, *Agave tequilana* Weber, is one of the most important economic agaves in Mexico. According to Mexican Official Standard NOM-006-SCFI-2005, the species is the only one that can be used to produce the alcoholic drink tequila in its area of origin that includes the states of Guanajuato, Jalisco, Michoacán, Nayarit, and Tamaulipas (Iñiguez-Covarrubias et al. 2001). In 2014, the tequila industry processed 741,000 tons of agave into 226.5 million liters of tequila (CRT 2014). Cutting the leaves, a process known as “jima”, results in industrial waste byproduct. However, juice of the leaves contains various secondary metabolites of medical and agricultural interest (Meuser et al. 2009, Uribe et al. 2009).

Interest in using plants of the *Agave* genus for biological control of pests has increased. Aqueous extracts of *Agave americana* have biopesticidal activity against the mosquito *Culex quinquefasciatus* and the juice from the leaves had molluscicidal activity against the gastropod *Furcraea andina* (Iannacone et al. 2013). Ovicidal and anthelmintic *in vivo* activity against larvae of different stages were attributed to extracts of *Agave sisalana* (Botura et al. 2011).

Because agriculture is fundamental to human society, this research focused on searching for new alternatives, especially from extracts from agro-industrial byproducts that might replace some chemicals for pest control in crops. Because different species of agave have a broad spectrum of activity against insects and parasites, the byproduct from leaves of blue agave after tequila is produced might be a potential source of biopesticides. The objective of the study was to evaluate insecticidal and nematicidal activities of organic extracts from juice of leaves of *A. tequilana* against silverleaf whitefly and *P. redivivus*, respectively.

Materials and Methods

A. tequilana leaves were collected from cultivars about 8 years old in December 2014 at Tlajotla, Miacatlán, Morelos, Mexico (18° 50'1.24" N, 99° 24' 10.22" W). The leaves (1,148) weighed 780 kg. After harvested, they were washed with water to remove soil residue. The leaves were cut in half and pressed in an extractor (Weijin JZJ-280, China). The juice was filtered by using cotton to remove macroscopic plant tissue particles and refrigerated at 4°C for later use.

Extracts from the leaves of agave were obtained by liquid-liquid partition extraction using hexane, and then ethyl acetate. The extraction volume was 200 ml of solvent per liter of juice, for three times. The organic and aqueous phases were

concentrated separately on a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany) to obtain crude extracts with yields calculated in percentages of dry weight.

To evaluate insecticidal activity of the juice and organic extracts against silverleaf whitefly, 50 ± 5 whiteflies were trapped in a Pasteur pipette. The whiteflies were collected from the underside of leaves of tomato plants in a greenhouse. Carbon dioxide was used to lightly anesthetize the whiteflies inside the Pasteur pipettes to prevent their escape. The whiteflies were placed into a plastic Petri dish containing a leaf of the plant *Piper auritum* (as food for the whiteflies) and a wet filter paper, both cut the same size as the dish. The juice was tested without dilution, and organic extracts at 1, 2, 4, and 100% concentration were evaluated. Commercial Herald®, a pyrethroid of phenpropatrine at 375 g per liter, was used as a positive check, and distilled water was used as a negative check. Experiments were done in quadruplicate. A spray tower 28 cm tall was used to simulate spraying conditions in the field. A sample of 5 ml divided into two was sprinkled at 16 to 20 psi over the whiteflies. The leaves containing the tested sample and the whiteflies were placed face down on a flat surface to simulate *in situ* conditions in a field. Insecticidal activity was evaluated at 24 hours. The percentage of mortality (% M) was calculated by the number of dead / total number of organisms * 100.

To evaluate nematicidal activity of juice and extracts, *P. redivivus* nematodes were reproduced to obtain the second growth stage (J2) following the technique by Pica (2008). The extracts were tested in 96-well flat-bottom plates, with 10 J2 nematodes per well. Leaf juice at 3, 6, 12, and 100% and ethyl acetate crude extract at 0.1, 0.2, 0.3, and 0.4% were assayed. To each well, 100 µl of sample was added. Vydate-L® (S-methyl N'N'-dimethyl-N-[(methyl-carbamoyl)oxy]-1-thiooxamimidate) (oxamyl) was used as a positive check and ethanol/water solution at 1:3 (mixture where the extracts were dissolved) was used as a negative check. The plates were incubated at 26°C for 24 hours while constantly stirred at 160 rpm in an orbital shaker (MAXQ 4450, Thermo Scientific). After incubation, the number killed in each experiment was determined and mortality rate (% M) was calculated.

Phytochemical methods were used to identify groups of secondary metabolites in juice of *A. tequilana* leaves. Alkaloids, volatile coumarins, flavonoids, saponins, tannins, triterpenes, and steroids as well as free anthracene derivatives were identified (Harborne 1973, Stahl 1973, Lock 1994, Wagner and Bladt 1996).

Dragendorff, Mayer, and Wagner reagents were used to determine the presence of alkaloids. Dragendorff´s solution (a) was prepared with 106 mg of bismuth nitrate and 1.25 g of tartaric acid dissolved in 5 ml of water. For potassium iodide solution (b), 1 g of iodine was dissolved in 2.5 ml of water. Equal volumes of 2.5 ml of fresh preparation (a) and (b) solutions were mixed. Alkaloids were detected by placing 1.5 ml of *A. tequilana* leaf juice and 100 µl of Dragendorff´s reagent into a test tube. Mayer´s reagent was composed of (a): 680 mg of mercuric chloride dissolved in 30 ml of water and (b): 2.5 g of potassium iodide dissolved in 5 ml of water. Both solutions were combined and diluted with water to 50 ml. The assay was done by placing 1.5 ml of juice into a test tube and adding 100 µl of Mayer´s reagent down the tube walls. Wagner´s reagent was prepared using 635 mg of iodine and 1 g of potassium iodide dissolved in 50 ml of water. Into a test tube containing 1.5 ml of juice, 100 µl of Wagner´s reagent were added. Precipitate and change in color generated by the reagents might indicate alkaloids, and the observed colors could be red to orange or white to cream or brown.

Two milliliters of juice from *A. tequilana* leaves were placed into a test tube to test for volatile coumarins. The tube aperture was covered with filter paper impregnated with 1N NaOH solution. The test tube was placed into a water bath at 100°C for 10 minutes. The filter paper was observed under ultraviolet light to determine the presence of yellow-colored fluorescence indicative of coumarins.

To test for flavonoids, 2 ml of juice were placed into a test tube to which a small piece of magnesium band (5 x 5 mm) and 100 μl of 36% HCl were added. A color change after 24 hours indicated flavonoids.

To determine tannins the following solutions were prepared: (a) 20% ferric chloride solution prepared with 2.5 g of ferric chloride and dissolved in 25 ml of distilled water; (b) 1% gelatin solution was obtained from 125 mg gelatin (Merck) dissolved in 12.5 ml distilled water; (c) to obtain gelatin and salt solution, 500 mg of gelatin and 5 g of sodium chloride were dissolved in 50 ml of distilled water; (d) 10% saline solution consisted of 1.25 g of sodium chloride dissolved in 12.5 ml distilled water. To determine tannins, 1.5 ml of *A. tequilana* leaf juice was placed into four test tubes to which 100 μl of each prepared solution were added. The presence of a precipitate in solutions (a), (b), and (c) indicated a positive result, while the presence of a precipitate in solution (d) showed a negative result.

To test for saponins, 5 ml of *A. tequilana* leaf juice were added in a tube placed into a water bath for boiling. After cool, the tube was shaken vigorously to form persistent foam that indicated saponins.

To test for triterpenes and/or steroids, (a) Liebermann-Burchard reagent was prepared with 2.5 ml of acetic anhydride and 2.5 ml of concentrated sulfuric acid added to 25 ml of absolute ethanol. The solution was handled in an ice bath; (b) Salkowski reagent consisted of concentrated sulfuric acid. Into two test tubes, 5 ml of juice were added. To each tube, 5 ml of chloroform were placed, and the tubes were shaken. The tubes were allowed to rest to separate the organic from the aqueous phase. Liebermann-Burchard and Salkowski solutions were added individually to each tube containing the organic phase. Precipitate and a change in color of the sample indicated triterpenes and/or steroids.

To test for anthracene derivatives, 5 ml of *A. tequilana* leaf juice was placed into two tubes to which 5 ml of chloroform were added. The tubes were shaken and after separation of the organic phase, the aqueous phase was removed. One milliliter of 5% NaOH was added to one tube and 1 ml of 5% magnesium acetate was added to the second. A change in color indicated anthraquinones.

Tukey's test was used to analyze results of the bioassay against silverleaf whitefly and *P. redivivus* (SAS 1979). The significance of the values was $p < 0.05$.

Results

Pressing the 780 kg of *A. tequilana* leaves yielded 102 liters of juice. Extractions with hexane and ethyl acetate yielded 20.2 and 4.8 g, respectively.

Undiluted fresh juice of leaves of *A. tequilana* (100% juice) killed 31% of the whiteflies (Fig. 1). The positive check Herald® killed 97%. The negative check (water) killed 3%. Percentages of mortality for the three treatments were statistically different at $p < 0.05$.

Considering that hexane crude extract of *A. tequilana* juice killed 100% of the *B. tabaci* (data not shown), different dilutions of the extract were tested to check potency. Fig. 2 shows the insecticidal action of the hexane extract at concentrations of 1, 2, and 4% that killed 69.6, 91.0, and 94.0%. The positive check

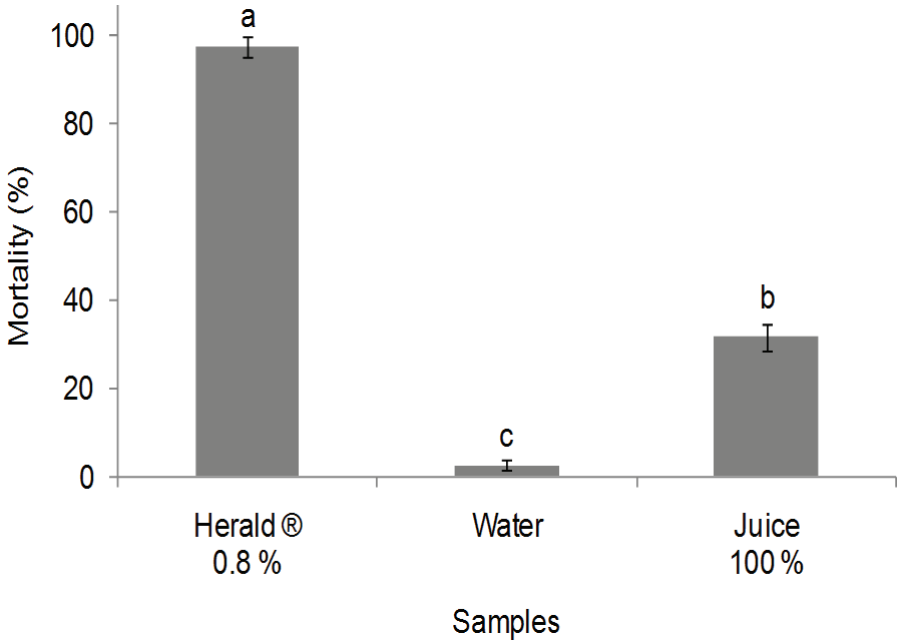

Fig. 1. Percentages of *B. tabaci* killed by *A. tequilana* juice (Tukey, $p < 0.05$).

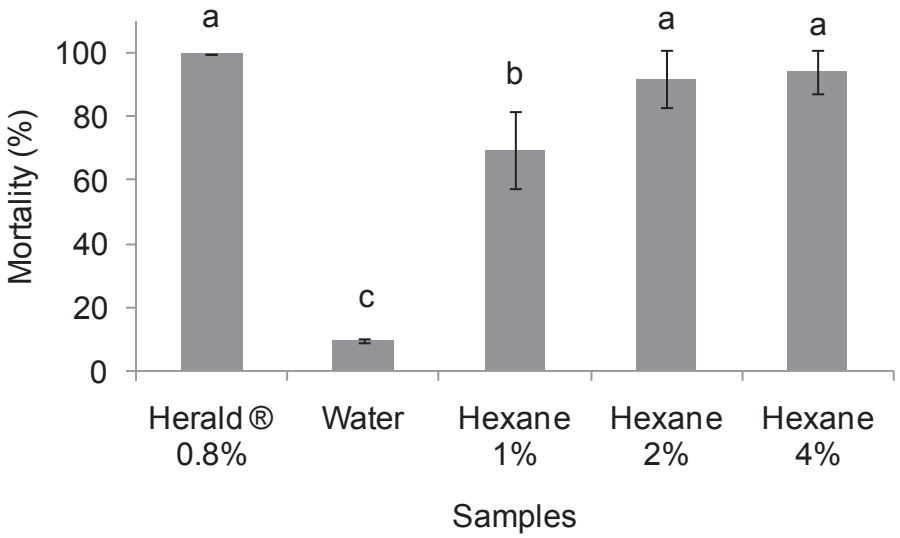

Fig. 2. Percentages of *B. tabaci* killed by hexane extracts at 1, 2, and 4%, and check (Tukey, $p < 0.05$).

Herald® killed 100%, while the negative check (water) caused 10% to die. Hexane extracts at 2 and 4% were not statistically different than the positive check.

Fresh juice without dilution killed 100% of *P. redivivus* (Fig. 3). Juice concentrations of 3, 6 and 12% killed 62, 82.3, and 100%, respectively. The positive check Vydate-L® at 0.8% concentration killed 100%. The negative check water did not cause any death. Death from juice at 12% concentration was not statistically different from that of the positive check; both killed 100% of *P. redivivus*.

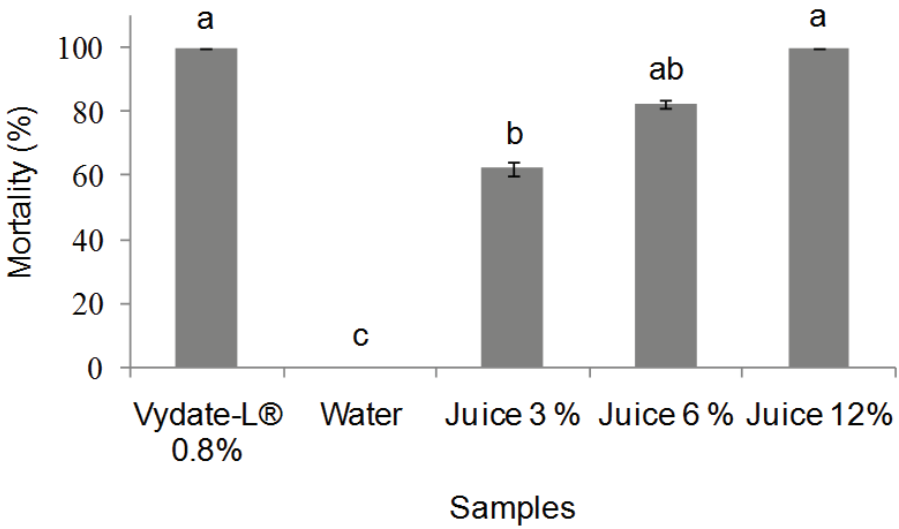

Fig. 3. Percentages of *P. redivivus* killed by *A. tequilana* juice diluted to 3, 6, and 12%, and checks (Tukey, $p < 0.05$).

Ethyl acetate crude extract from the juice of *A. tequilana* killed 100% of the nematodes (Fig. 4). Extract concentrations of 0.1, 0.2, 0.3, and 0.4% killed 12.5, 45.0, 90.0, and 100%, respectively. Ethyl acetate extracts of 0.3 and 0.4% were not significantly different than the positive check.

Different groups of secondary metabolites in *A. tequilana* juice were identified as alkaloids, flavonoids, tannins, triterpenes, and/or steroids (Table 1). Coumarins and anthracene derivatives were not identified in the juice.

Discussion

Juice from *A. tequilana* leaves killed only 31% and is not recommended for controlling silverleaf whiteflies. However, hexane extract from juice was very effective, killing 90% at concentrations of 2-4% (20-40 mg/ml). This mortality rate was comparable to that of the positive check Herald®. Aqueous extract of *A. americana* leaves and bark at 50% concentration (500 mg/ml) killed 100% of cotton aphids, *Aphis gossypii*, after 36 hours of exposure (Fuertes et al. 2010).

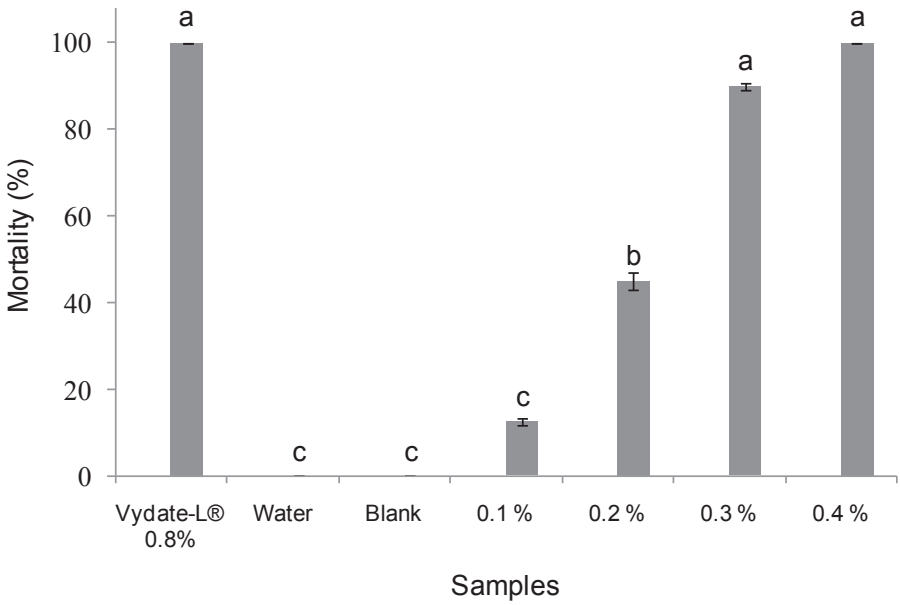

Fig. 4. Percentages of *P. redivivus* nematodes killed by ethyl acetate extracts from juice of *A. tequilana* leaves diluted to 0.1, 0.2, 0.3, and 0.4% (Tukey, $p < 0.05$).

Table 1. Secondary Metabolite Groups in Juice of *A. tequilana* Leaves

Group of metabolite	Assay	Result	Observed reaction
Alkaloid	Wagner reagent	+++	Color change to brown
	Mayer reagent	+++	Color change to yellow and precipitation
	Dragendorff reagent	-	-
Coumarin	Fluorescence test on filter paper	-	-
Flavonoid	Addition of Mg and HCl	+++	Color change to yellow, red color after 24 hours
Tannin	Ferric chloride	++	Brown halo
	Gelatin solution	-	-
	Gelatin and saline solution	-	-
	Saline solution	-	-
Saponin	Foam formation	+++	Persistent foam formation
Triterpene and steroid	Liebermann-Burchard reagent	++	Precipitate
	Salkoswski reagent	++	Precipitate with change in color (orange)
Anthracene derivative	5% NaOH	-	-
	0.5% Mg acetate	-	-

(-) not detected, (+) weak positive test, (++) positive test, (+++) strong positive test

Hexane extract from *A. tequilana* juice was at least 10 times less concentrated than *A. americana* extract and killed the same number of insects. *A. tequilana* was an effective bioinsecticide against silverleaf whitefly, an insect pest of cotton, potatoes, peppers, eggplants, and other cultivars (Ortega 2008). Fresh juice of *A. tequilana* was very effective against *P. redivivus*, killing 100% of nematodes 24 hours after exposure. Juice at 12% concentration was equally as effective. Ferreira-Domingues et al. (2010) evaluated the effect of juice from leaves of *A. sisalana* against gastrointestinal nematodes of goats, showing *in vitro* and *in vivo* effectiveness to kill 95% of the nematode *Haemonchus* spp. Aqueous extracts from *A. sisalana* leaves at a concentration of 0.12 mg/l inhibited 100% of larval development of the nematode *Haemonchus contortus*, a gastrointestinal parasite of sheep (Silveira et al. 2012). This study showed that 0.4% (4 mg/ml) concentration of ethyl acetate extract from juice of *A. tequilana* killed 100% of the nematodes. This was greater than the action of 0.8% concentration of nematicide Vydate-L® that killed 100% after 24 hours of exposure.

Juice from *A. tequilana* leaves was positive for alkaloids, flavonoids, tannins, triterpenes, and/or steroids. Háuad-Marroquin et al. (2010) identified flavonoids and anthrones in *A. tequilana* leaves. Juice of *A. sisalana* leaves contained the secondary metabolites: alkaloids, anthocyanins, anthraquinones, cardiac glycosides, coumarins, emodins, flavonoids, reduced sugars, saponins, steroids, tannins, and terpenoids (Chigodi et al. 2013).

Generally, species in a genus share the same chemical group of secondary metabolites that might vary by species, geographical condition, seasonality, ontogenetic development, and other factors. Flavonoids and saponins usually are the metabolites in the agave species most described as having insecticidal and nematicidal effects. For example, extract from juice of *A. sisalana* leaves killed a nematode of goat, and a semi-purified sample containing saponins at 0.25% (2.5 mg/ml) concentration killed 100% (Botura et al. 2013).

The antifeedant action of saponins in Dioscoreaceae against *Acromyrmex octospinosus* ants is well documented (Chaieb 2005). Saponins from the plant *Barbarea vulgaris* decreased feeding of larval diamondback moth, *Plutella xylostella*, that affects important crops such as broccoli, cabbage, and cauliflower (Shinoda et al. 2002). Because several saponins have been isolated from Agave species including *A. attenuata*, *A. americana*, *A. lechugilla*, *A. salmiana*, *A. brittoniana*, *A. shrevei*, and *A. durangensis*, the plants could be considered potential candidates from which to develop insecticides (Da Silva et al. 2002, Da Silva and Parente 2005, Hernández et al. 2005, Yang et al. 2006, Zamora et al. 2010, González-Valdez et al. 2012). The present work identified flavonoids in juice of *A. tequilana* leaves and also secondary metabolites with insecticidal effect. Flavonoids isolated from aqueous extracts of leaves of *Ricinus communis* displayed insecticide, ovicidal, and ovipositional deterrent action against the coleopteran *Callosobruchus chinensis* (Upasani et al. 2003).

Extracts containing flavonoids can act as nematicides, as do those in leaf ethanolic extracts of the species *Tithonia diversifolia* active against the nematode *Pratylenchus*, the main problem of citrus in Brazil (Slomp et al. 2009). Different flavonoids have been identified in butanol extract of *A. sisalana* (Chen et al. 2009). Three homoisoflavanones were isolated from an acetone extract of pineapple and leaves of *A. tequilana* (Morales-Serna et al. 2010) that should be further investigated for nematicidal effect.

This study established a scientific base for the use of *A. tequilana* juice as a potent nematicide. This has already been demonstrated in several species of agave, comparable to mortality by certain nematicides.

Purified compounds and fractions, as well as total extracts from plants and microorganisms have been commercialized for pest control (Pineda et al. 2007, EPA 2015). Bioactive chemicals derived from *A. tequilana* need to be standardized. Based on the amount of *A. tequilana* processed and yield of juice per leaf, approximately 94 million liters of juice are wasted each year in Mexico. Hexane and acetyl acetate extracts have advantages over juice, such as increased chemical stability, more potency as a pesticide, and less space needed for storage. Development of efficient technologies using environmentally friendly approaches, e. g., supercritical fluid extraction, might allow *A. tequilana* to be cost competitive and reliable compared with chemicals currently available for pest control.

In conclusion, *A. tequilana* juice had low insecticidal activity against silverleaf whitefly but outstanding nematicidal action against *P. redivivus*. However, hexane and ethyl acetate extracts from juice were very effective insecticide and nematicide, killing 100% of the whiteflies and nematodes. *A. tequilana* juice contained alkaloids, flavonoids, and saponins that might cause the effect. It is important to identify and isolate molecules responsible for the activity, to establish analytical techniques for standardization of formulated organic products friendly to the environment and with potent bioinsecticidal and nematicidal action. *A. tequilana* leaves are a byproduct of the tequila industry that can be exploited economically for production of insecticide and nematicide products based on organic extracts.

References Cited

Abdelgaleil, S. A. M., M. A. Abbassy, A. S. H. Belal, and M. A. A. Abdel. 2008. Bioactivity of two major constituents isolated from the essential oil of *Artemisa judaica* L. Biores. Technol. 99: 5947-5950.

Botura, M. B., G. D. Silva, H. G. Lima, J. V. A. Oliveira, T. S. Souza, J. D. G. Santos, A. Branco, E. L. T. Moreirae, M. A. O. Almeida, and M. J. M. Batatinha. 2011. *In vivo* anthelmintic activity on aqueous extract from sisal waste (*Agave sisalana* Perr.) against gastrointestinal nematodes in goats. Veter. Parasit. 177: 104-110.

Botura, M. B., J. D. G. Dos Santos, G. D. Da Silva, H. G. De Lima, J. V. A. De Oliveira, M. A. O. De Almeida, M. J. M. Batatinha, and A. Branco. 2013. *In vitro* ovicidal and larvicidal activity of *Agave sisalana* Perr. (sisal) on gastrointestinal nematodes goats. Veter. Parasit. 192: 211-217.

Chaieb, I. 2005. Les saponines du Cestrum parqui nature chimique implications physiologiques et potential bio-pesticide. Thése de Doctorat en Sciences Agronomiques. Ecole Supérieure d´ Horticulture et d´Elevaga de Chott Mariem, Tunisia.

Cházaro-Basañez, M., J. A. Lomeli-Sención, and Y. L. Vargas-Rodriguez. 2006. *Agave vazquezgarciae* (Agavaceae), a new species from Jalisco, México. N. J. Bot. Nom. 16: 458-461.

Chen, P. Y., Y. C. Kuo, C. H. Chen, Y. H. Kuo, and C. K. Lee. 2009. Isolation and immunomodulatory effect of homoisoflavones and flavones from *Agave sisalana* Perrine ex Engelm. Molecules 14: 1789-1795.

Chigodi, M. O., D. K. Samoei, and M. Muthangya. 2013. Phytochemical screening of *Agave sisalana* perrine leaves (waste). Int. J. Appl. Biol. Pharma. Technol. 4: 200-204.

Chitwood, D. J. 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture – Agricultural Research Service. Pest Manag. Sci. 59: 748-753.

Collange, B., Navarrete, M., Peyre, G., and T. Mateille. 2011. Root-knot nematode (*Meloidogyne*) management in vegetable crop production: the challenge of an agronomic system analysis. Crop Prot. 30: 1251-1262.

CRT (Consejo Regulador del Tequila). 2014. crt.org.mx [Internet] México. [Citado: 13 Nov. 2014]. Disponible en: http://www.crt.org.mx/EstadisticasCRTweb/

Da Silva, B., and J. P. Parente. 2005. A new bioactive steroideal saponin from *Agave shrevei*. Zeits. F. Nat. 60: 57-61.

Da Silva, B., A. C. De Sousa, G. M. Silva, T. P. Mendes, and J. P. Parente. 2002. A new bioactive steroidal saponin from *Agave attenuata*. Zeits. F. Nat.57c: 423-428.

De Lara, R., T. Castro, G. Castro, J. Castro, and A. Malpica. 2003. La importancia de los nematodos de vida libre. ContactoS. 48: 43-46.

DEFRA. 2015. Department for Environment Food and Rural Affairs. Science search. Development of a robust assay to estimate the viability of potato cyst nematodes *Globodera* spp. - HP0143. http://randd.defra.gov.uk/. Accessed 13 July 2015.

EPA. 2015. US Environmental Protection Agency. Azadirachtin (121701) Clarified Hydrophobic Extract of Neem Oil (025007) Fact Sheet. http://www.epa.gov. Accessed 13 July 2015.

Ferreira-Domingues, L., M. Borges, A. C. Ferreira, C. C. Yuki, G. Dias da Silva, M. Silva, G. Murphy, E. L. T. Moreira, I. D. Santos, M. G. Ávila, R. De Almeida, A. Branco, M. A. Ornelas, and M. J. Moreira. 2010. Evaluation of anthelmintic activity of liquid waste of *Agave sisalana* (sisal) in goats. Rev. Bras. Parasitol. Vet. 19: 270-272.

Fuertes, C. M., B. Jurado, G. C. Gordillo, L. P. Negrón, E. Núñez, M. Esteban, and A. Távara. 2010. Estudio integral de plantas biocidas del algodonero. Cient. Invest. 13: 34-41.

Glazer, I., L. Salame, and D. Segal. 1997. Genetic enhancement of nematicide resistance in entomopathogenic nematodes. Biocontrol Sci. Techn. 7: 499-512.

González-Valdez, L. S., N. Almaraz-Abarca, J. B. Proal-Nájera, F. Robles-Martínez, G. Valencia-Del-Toro, and M. Quintos-Escalante. 2012. Surfactant properties of the saponins of *Agave durangensis*, application on arsenic removal. Int. J. Eng. Appl. Sci. 4: 87-94.

Good-Avila, S. V., V. Souza, B. S. Gaut, and L. E. Eguiarte. 2006. Timing and rate of speciation in Agave (Agavaceae). Proc. Nat. Acad. Sci. U.S.A. 103: 9124-9129.

Harborne, J. B. 1973. Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis. Chapman, London, GB.

Háuad-Marroquín, L. A., A. Pérez-Liñán, D. Resendez-Pérez, C. Moreno, A. Lara, D. Lázaro, and C. Luna. 2010. Determinación química y estudio terapéutico de *Agave tequilana* Weber. Rev.Sal. Pub. Nut. 5: 254-265.

Hernández, R., E. C. Lugo, L. Díaz, and S. Villanueva. 2005. Extracción y cuantificación indirecta de las saponinas *de Agave lechugilla* Torrey. Rev. Digit. Cient. Tecnol. 3: 1-9.

Iannacone, J., C. Cajachagua, B. Dueñas, L. Castillo, L. Alvariño, and G. Argota. 2013. Toxicidad de *Agave americana* y *Furcrea andina* (Asparagaceae) sobre *Culex quinquefasciatus* (Diptera) y *Heleobia cumingii* (Mollusca). Neotrop. Helmint. 7: 311-325.

Iñiguez-Covarrubias, G., R. Díaz-Teres, R. Sanjuan-Dueñas, J. Anzaldo-Hernández, and R. M. Rowell. 2001. Utilization of by-products from the tequila industry. Part 2: potential value of *Agave tequilana* Weber azul leaves. Biores. Technol. 77: 101-108.

Latournerie-Moreno, L., A. Ic-Caamal, E. Ruiz-Sánchez, H. Ballina-Gómez, I. Islas-Flores, W. Chan-Cupul, and D. González-Mendoza. 2014. Survival of *Bemisia tabaci* and activity of plant defense-related enzymes in genotypes of *Capsicum annuum* L. Chil. J. Agri. Res. 75: 71-77.

Lock, O. 1994. Investigación fitoquímica. Método en el estudio de productos naturales. Fondo editorial de la Pontifica Universidad Católica del Perú PUEP Lima, Lima (Perú).

Lowe, S., M. Browne, S. Boudjelas, and M. De Poorter. 2000. 100 of the world's worst invasive alien species a selection from the global invasive species database. Published by the Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), First published as special lift-out in Aliens 12, December 2000. Updated and reprinted version: November 2004, Auckland, New Zealand.

Marubayashi, J. M., V. A. Yuki, K. C. G. Rocha, T. Mituti, F. M. Pelegrinotti, F. Z. Ferreira, M. F. Moura, J. Navas-Castillo, E. Moriones, M. A. Pavan, and R. Krause-Sakate. 2013. At least two indigenous species of the *Bemisia tabaci* complex are present in Brazil. J. Appl. Entomol. 137: 113-121.

Meuser, F., I. Bauer, E. Hellwege, and J. Pilling. 2009. Inulin of very high chain length. Patent, US20090099129 A1.

Morales, F. J. 2007. Tropical whitefly IPM project. Advan.Virus Res. 69: 249-311.

Morales-Serna, J. A., A. Jiménez, R. Estrada-Reyes, C. Marquez, J. Cárdenas, and M. Salmón. 2010. Homoisoflavanones from *Agave tequilana* Weber. Molecules 15: 3295-3301.

Ogah, C. O., and H. B. Coker. 2012. Quantification of organophosphate and carbamate pesticide residues in maize. J. Appl. Pharmac. Sci. 2: 93-97.

Ongley, E. D., D. A. Birkholz, J. H. Carey, and M. R. Samoiloff. 1988. Is water a relevant sampling medium for toxic chemicals? An alternative environmental sensing strategy. J. Env. Qual. 17: 391-401.

Ortega, A. 2008. Bioecología de las moscas blancas. Moscas blancas temas selectos sobre su manejo, pp. 1-6. *En* S. Infante G. [ed.], 1ª. ed. Colegio de Postgraduados. Mundi prensa, México, DF.

Pérez, M. E., D. M. Ruiz, M. Schneider, J. C. Autino, and G. Romanelli. 2013. La química verde como fuente de nuevos compuestos para el control de plagas agrícolas. Revista Científica en Desarrollo 4: 83-91.

Perumalsamy, H., K. S. Chang, C. Park, and Y. J. Ahn. 2010. Larvicidal activity of *Asarum heterotropoides* root constituents against insecticide-susceptible and resistant *Culex pipiens* pallens and *Aedes aegypti* and *Ochlerotatus togoi*. J. Agri. Food Chem. 58: 10001-10006.

Pica, G. Y. 2008. Ensayo de toxicidad con el nematodo *Panagrellus redivivus*. SEMARNAT, México 1: 139-154.

Pineda, S., M. I. Schneider, and A. M. Martínez. 2007. El SPINOSAD, una alternativa para el control de insectos plaga. Cien. Nicol. 46: 29-42.

Ricci, M., A. P. Fifi, A. Ragni, C. Schlechtriem, and U. Focken. 2003. Development of a low-cost technology for mass production of the free-living nematode *Panagrellus redivivus* as an alternative live food for first feeding fish larvae. Appl. Microbiol. Biotech. 60: 556-559.

Roditakis, E., M. Grispou, E. Morou, J. B. Kristoffersen, N. Roditakis, R. Nauen, J. Vontas, and A. Tsagkarakou. 2009. Current status of insecticide resistance in Q biotype *Bemisia tabaci* populations from Crete. Pest Man. Sci. 65: 313-322.

Rodríguez-Garay, B. 2004. La materia prima *Agave tequilana* Weber var. Azul. Consejo Regulador del Tequila, México.

Romanelli, G. P., E. G. Virla, P. R. Duchowicz, A. L. Gaddi, D. M. Ruiz, D. O. Bennardi, E. Del Valle, and J. C. Autino. 2010. Sustainable synthesis of flavonoid derivates, QSAR study and insecticidal activity against the fall armyworm, *Spodoptera frugiperda* (Lep.: Noctuidae). J. Agric. Food Chem. 58: 6290-6295.

Ruvalcaba-Ruiz, D., and B. Rodríguez-Garay. 2002. Aberrant meiotic behavior in *Agave tequilana* Weber var. Azul. BMC Plan. Biol. 2: 1-14.

Samoiloff, M. R. 1987. Nematodes as indicators of toxic environmental contaminants. Vist. Nematol: 433-439.

Samoiloff, M. R., J. Bell, D. A. Birkholz, G. R. B. Webster, E. Arnott, R. Pulak, and A. Madrid. 1983. Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. Env. Sci. Technol. 17: 329-334.

SAS. 1979. SAS User´s Guide. Statistical analysis system Institute, Inc., Cary, NC.

Shinoda, T., T. Nagao, M. Nakayama, H. Serizawa, M. Koshioka, H. Okabe, and A. Kawai. 2002. Identication of a triterpenoid saponin for a crucifer, *Barbarea vulgaris*, as a feeding deterrent to the diamondback moth, *Plutella xylostella*. J. Chem. Ecol. 28: 587-599.

Silveira, R. X., A. C. S. Chagas, M. B. Botura, M. J. M. Batatinha, L. M. Katikic, C. O. Carvalho, C. M. L. Bevilaqua, A. Branco, E. A. A. Machado, S. L. Borges, and M. A. O. Almeida. 2012. Action of sisal (*Agave sisalana*, Perrine) extract in the in vitro development of sheep and goat gastrointestinal nematodes. Exp. Parasitol. 131: 162-168.

Silvestre, A., and J. Cabaret. 2014. Nematode parasites of animals are more prone to develop xenobiotic resistance than nematode parasites of plants. Parasite 11: 119-129.

Slomp, L., P. S. Pereira, S. De Castro, S. Zingaretti, and R. Oliveira. 2009. *In vitro* nematocidal affects of medicinal plants from Sao Paulo state, Brazil. Pharm. Biol. 47: 230-235.

Stahl, E. 1973. Drug analysis by chromatography and microscopy. Agris Records. FAO, USA.

Upasani, S. M., M. K. Hemlata, S. M. Prashant, and V. L. Mahashwari. 2003. Partial characterization and insecticidal propierties of *Ricinus communis* L foliage flavonoids. Pest Man. Sci. 59: 1349-1354.

Uribe, J. G., S .S. Saldivar, and A. V. Ozuna. 2009. Agave syrup extract having anticancer activity. Patent., US 20090124685 A1.

Wagner, H., and S. Bladt. 1996. Plant Drug Analysis. Springer, Heilderberg, Germany.
Yang, C. R., Y. Zhang, M. R. Jacob, S. I. Khan, Y. J. Zhang, and X. C. Li. 2006. Antifungal activity of C-27 steroideal saponins. Ant. Agent. Chem. 50: 1710-1714.
Zamora, P., F. Juárez, R. Aguirre, P. Ortiz, H. Godínez, and F. Álvarez. 2010. Variación de la concentración de azúcares y saponinas durante la cocción del maguey mezcalero potosino. R. Rev. Cient. Am. Lat. Car. Esp. Port. 8: 1-11.