Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol16-issue1-fulltext-6 *RESEARCH ARTICLE*

Insecticidal effects of plant extracts on immature whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyroideae)

Angel Cruz-Estrada[1]· Marcela Gamboa-Angulo[1]· Rocío Borges-Argáez[1]· Esaú Ruiz-Sánchez[2] ✉

[1]Centro de Investigación Científica de Yucatán, Unidad de Biotecnología, Colonia Chuburná de Hidalgo, Mérida, Yucatán, México
[2]Instituto Tecnológico de Conkal, División de Estudios de Posgrado e Investigación, Antigua carretera Mérida-Motul, Conkal, Yucatán, México

✉ Corresponding author: esau_ruiz@hotmail.com
Received October 14, 2012 / Accepted December 25, 2012
Published online: January 15, 2013

Abstract

Background: The whitefly (*Bemisia tabaci* Genn.) is a widely distributed and highly harmful plant pest species. The management of *B. tabaci* has been typically carried out by chemical pesticides. In the last decade however, there has been an increasing interest in natural products, particularly those of plant origin, to control this pest species. In the present work, aqueous and ethanolic extracts of native plants from the flora of the Yucatán peninsula (*Acalypha gaumeri*, *Annona squamosa*, *Carlowrightia myriantha*, *Petiveria alliaceae* and *Trichilia arborea*) and the introduced plant *Azadirachta indica* were collected and evaluated for insecticidal activity against eggs and nymphs *Bemisia tabaci*.
Results: Most of the aqueous and ethanolic extracts showed high insecticidal effects on *B. tabaci* eggs. The lowest LC_{50} values were recorded in the aqueous extracts of *A. gaumeri* (0.39% w/v), *A. squamosa* (0.36% w/v), *P. alliaceae* (0.42% w/v) and *A. indica* (0.30% /v), as well as in the ethanolic extracts of *P. alliaceae* (2.09 mg mL^{-1}) and *T. arborea* (2.14 mg mL^{-1}). On the other hand, *B. tabaci* nymphs were not affected by the aqueous extracts, but were highly sensitive to the ethanolic extracts of the tested plants. The lowest LC_{50} values were recorded in the ethanolic extracts of *P. alliaceae* (1.27 mg ml^{-1}) and *T. arborea* (1.61 mg mL^{-1}). The GC-MS analysis showed that phytol was the major component of the ethanolic extract of *P. alliaceae* and fatty acids were the major components of ethanolic extract of *T. arborea*.
Conclusions: Overall, results suggest that ethanolic extracts of *P. alliaceae* and *T. arborea* leaves showed the highest insecticidal effects on eggs and nymphs *B. tabaci*. The extracts from *P. alliaceae* and *T. arborea* are good candidates to be developed as sources of natural insecticides for the management of immature *B. tabaci* since their effects were comparable with that showed by the extracts of *A. indica*, a well-known plant species for its insecticidal activity.

Keywords: *Annona squamosa*, *Bemisia tabaci*, botanical insecticides, plant extracts, *Petiveria alliaceae*.

INTRODUCTION

The whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is a global plant pest that has caused enormous losses in crop production, mainly in Cucurbitaceae, Fabaceae and Solanaceae (Oliveira et al. 2001). This pest species is native to Southern Asia but currently distributed worldwide, particularly in tropical regions (Brown and Bird, 1992). High populations of *B. tabaci* induce losses in plant productivity by direct feeding, fungal growth associated with honey dew contamination, and plant physiological disorders. Losses also occur from *B. tabaci* due to the efficient transmission of *Begomovirus*, a genus of the taxonomic family *Geminiviridae* that causes leaf yellow mosaic and mottling, leaf distortion and stunting (Oliveira et al. 2001; Morales, 2007). The management of *B. tabaci*

has been typically carried out by chemical insecticides, which are associated with environmental contamination, high levels of resistance and damage to non-target organisms (Elberth and Nauen, 2000; Roditakis et al. 2009). Currently, natural products from plants have been considered one of the most promising sources of biorational products with new modes of actions to manage phytophagous insects (Dayan et al. 2009; Rattan, 2010).

Table 1. Botanic voucher specimens and ethno-botanical information of the plants tested.

Scientific name	Plant family	Common name	Voucher number
Acalypha gaumeri	Euphorbiaceae	Sak ch'ilib tux	PS 2584
Annona squamosa	Annonaceae	Saramullo	PS 2608
Azadirachta indica	Meliacea	Neem	PS 2607
Carlowrightia myriantha	Acanthaceae		PS 2589
Petiveria alliacea	Phytolaccaceae	Zorrillo	MMendez1417
Trichilia arborea	Meliacea	Ch'obenche	PS 2596

To protect crops in modern agriculture and an increasingly regulated world, natural plant-based insecticides can be a feasible plant pest management method and an attractive alternative to synthetic chemical insecticides because botanicals reputedly pose little threat to the environment, non-target organisms or to human health (Isman, 2006). A number of plant substances have been considered for use as insecticides, antifeedants or repellents, which include terpenes, flavonoids, alkaloids, phenols, and other related compounds (Adeyemi, 2010). Several factors, however, appear to limit the success of botanicals, most notably regulatory barriers. In this context, plant-derived products are best suited for use in organic food production and in the production and postharvest protection of food in developing countries (Isman, 2006; Dayan et al. 2009).

As part of our ongoing research on natural insecticides from the native flora of the Yucatan peninsula, five native species with chemotaxonomic precedents of insecticidal properties were collected and tested on immature *B. tabaci*. Plant species included *Acalypha gaumeri*, *Annona squamosa*, *Carlowrightia myriantha* (previously named *Justicia myriantha*) (Duran et al. 1998), *Petiveria alliaceae* and *Trichilia arborea*. The introduced and adapted species *Azadirachta indica* was also evaluated. The objective of the present work was to screen aqueous and ethanolic extracts from leaves of the selected plant species for insecticidal activity on eggs and nymphs *B. tabaci*. This approach will allow us to identify natural and safer agents for the development of biorational insecticides to manage *B. tabaci*.

MATERIALS AND METHODS

Plant Material

Leaves of the selected plants were collected from different localities of the Yucatan peninsula, Mexico and voucher samples (Table 1) were deposited in the herbarium at Unidad de Recursos Naturales of Centro de Investigación Científica de Yucatán. All plant material was dried under artificial light (50-60ºC) for three days and the leaves were subsequently ground.

Extract preparation

Ethanolic extracts. A sample (50 g) of dried and ground leaves of each plant was macerated three times (48 hrs each) with ethanol (300 ml) at room temperature. The macerated samples were filtered and the ethanol was removed under low pressure at 40ºC degree C, resulting in the corresponding organic crude extract (Cristóbal-Alejo et al. 2006). For the bioassays, extracts were diluted in a solution of ethanol:water (1:1 v/v) to the desirable concentrations.

Aqueous extracts. A sample (0.3 g) of dried and ground leaves of each plant was added to distilled water (10 ml) and boiled for 20 min. This procedure yielded a final extract concentration of 3%, and lower extract concentrations were obtained by diluting to 1.5 and 0.75% in distilled water. Extracts were filtered with filter paper (Whatman # 1), sterilized with a 0.22 µm Millipore membrane (Merk Millipore, MA, USA) and stored at -20ºC until required.

Analytical methods

GC-MS analyses of ethanolic extracts of *P. alliaceae* and *T. arborea* were performed on an Agilent Technologies 6890N chromatograph coupled to an Agilent Technologies 5975B mass selective detector, with an Ultra 1 column [methyl siloxane, 25 m long, 0.32 mm i.d., 0.52 µm film thickness, helium at flow rate = 1.0 ml/min]. Running conditions for the extracts are as follows: *P. alliaceae*, T_1 = 150ºC, gradient = 10ºC/min, T_2 = 280ºC (20 min); *T. arborea*, T_1 = 180ºC, gradient = 8ºC/min, T_2 = 300ºC (25 min). Each extract was analyzed using 0.4 µl of sample at 2% concentration. The components were identified by matching with the mass spectral library (NIST 05) of the corresponding compounds.

Table 2. Mortality of *Bemisia tabaci* eggs caused by aqueous (3% w/v) and ethanolic (10 mg mL^{-1}) plant extracts after 48 hrs exposure.

Plant species	% mortality of whitefly eggs	
	Ethanolic extracts (10 mg mL^{-1})	Aqueous extracts (3% w/v)
Imidacloprid	100 ± 0.00 a	100 ± 0.00 a
Acalypha gaumeri	95 ± 3.33 ab	98.0 ± 1.29 a
Annona squamosa	100 ± 0.00 a	99.3 ± 1.05 a
Azadirachta indica	99.3 ± 1.05 a	99.3 ± 1.05 a
Carlowrightia myriantha	95.3 ± 2.69 ab	99.3 ± 1.05 a
Petiveria alliacea	99.3 ± 1.05 a	100 ± 0.00 a
Trichilia arborea	100 ± 0.00 a	100 ± 0.00 a
Water	7.2 ± 4.03 c	9.8 ± 4.30b
EtOH/water	10.9 ± 4.00 c	

Mean mortalities (± standard error) followed by the same letter within the same column are not significantly different (Tukey, P < 0.05).

Bemisia tabaci colony

Adult whiteflies were collected from habanero pepper (*Capsicum chinense* Jacq.) at the Instituto Tecnologico de Conkal, in Conkal, Yucatan, Mexico. The stock colony of *B. tabaci* was maintained on habanero pepper plants in entomological cages (1.2 x 1.2 x 1.0 m) constructed of aluminum and anti-aphid mesh. Entomological cages were kept in a greenhouse at 25-35ºC, 55-75% relative humidity and natural light.

Bioassays on immature *Bemisia tabaci*

Bioassays were carried out on *B. tabaci* eggs and third instar nymphs. Groups of 15 *B. tabaci* adults were deposited into clipcages (2 cm diameter) set on habanero pepper leaves of 30-40 days-old plants as indicated by Muñiz and Nombela (2001). Adult whiteflies were removed at 48 hrs and plants were kept in cages as indicated above for 72 hrs for egg bioassays and 15 days for nymph bioassays. Leaves with eggs or nymphs were then detached and rounded sections (~2 cm in diameter) that had at least 30 individuals (eggs or nymphs accordingly) were marked. To keep moisturized, leaves were deposited individually on a bed of 2% agar in 6-cm-diameter Petri dishes. For bioassay of ethanolic extracts, samples of 10 µl containing 100, 50 and 25 µg crude extracts were taken with a Eppendorf micropipette and applied evenly on the individuals in the marked section of leaf (~3 cm^2), while for bioassays of aqueous extracts, 10 µl samples of 3, 1.5 and 0.75% concentrations were applied. Ethanol:water (1:1) and water were used as negative controls; imidacloprid (480 µg ml^{-1}) was used as positive control. Leaves in Petri dishes were incubated under laboratory conditions at 24 ± 3ºC, 75 ± 8% relative humidity and 12:12 hrs light:darkness. Evaluations of mortality were carried out 72 hrs after application of extracts. Unhatched and necrotic eggs as well as necrotic nymphs were considered dead individuals.

Data analyses

Results of the insecticide bioassays were reported as percentage of mortality. Prior to analysis, data were transformed to a square root function. Analysis of variance and Tukey mean comparison were carried out in Statistical Analysis System (SAS) version 8.1 for Windows. LC_{50} and LC_{90} were calculated for all plant extracts using Probit analysis.

RESULTS

Mortality of *B. tabaci* eggs

Ethanolic extracts of *A. gaumeri*, *A. squamosa*, *A. indica*, *C. myriantha*, *P. alliaceae* and *T. arborea* at concentration of 10 mg mL^{-1} caused high mortality (95 to 100%) on *B. tabaci* eggs (Table 2). Similarly, mortality caused by aqueous extracts of these plants ranged from 98 to 100% at concentration of 3% w/v. No significant differences on mortality within the same type of extract and the chemical insecticide imidacloprid were observed.

Table 3. Toxicity (LC_{50} and LC_{90}) of aqueous plant extracts on *Bemisia tabaci* eggs after 48 hrs exposure.

Plant species	n	Slope ± SE	% w/v		P
			LC_{50} (CI)	LC_{90} (CI)	
Acalypha gaumeri	5	2.28 ± 0.21	0.39 (0.30-0.46)a	1.41 (1.28-1.57)b	<0.0001
Annona squamosa	5	2.85 ± 0.30	0.36 (0.27-0.44)a	1.02 (0.93-1.11)a	<0.0001
Azadirachta indica	5	1.88 ± 0.21	0.30 (0.20-0.39)a	1.42 (1.28-1.62)b	<0.0001
Carlowrightia myriantha	5	4.00 ± 0.19	1.11 (1.06-1.16)b	2.33 (2.18-2.52)c	<0.0001
Petiveria alliacea	5	2.28 ± 0.21	0.42 (0.33-0.50)a	1.53 (1.39-1.71)b	<0.0001
Trichilia arborea	5	2.71 ± 0.20	0.60 (0.53-0.66)b	1.78 (1.63-1.98)b	<0.0001

n = number of groups tested containing 30 individuals each.
LC_{50} and LC_{90} values are in % w/v.
LC_{50} and LC_{90} values followed by the same letter within the same column are not significantly different.
CI = Confidence Interval.

Lethal concentrations (LC_{50} and LC_{90}) of all plant extracts were also estimated with serial dilutions of the plant extracts. Significant differences on LC_{50} and LC_{90} were observed based on non-overlapping confidence intervals. For aqueous extracts, those of *A. gaumeri*, *A. squamosa*, *A. indica* and *P. alliaceae* showed lower values for LC_{50} (0.30-0.42% w/v) compared to those of *C. myriantha* and *T. arborea*. For LC_{90}, however, the lowest value was only observed with the extract of *A. squamosa* (1.02% w/v) (Table 3). For ethanolic extracts, those of *P. alliaceae* and *T. arborea* showed the lowest values for LC_{50} (2.09 and 2.14 mg mL^{-1}, respectively) as well as for LC_{90} (3.83 and 3.32 mg mL^{-1}, respectively) (Table 4).

Mortality of *B. tabaci* nymphs

Ethanolic extracts of all plants tested at concentrations of 10 mg mL^{-1} caused high mortality (99-100%) on *B. tabaci* nymphs (Table 5). No significant differences on the effect of ethanolic extracts and the chemical insecticide imidacloprid were observed.

As for the aqueous extracts of the evaluated plants, with the exception of that of *C. myriantha*, no insecticidal effects on *B. tabaci* nymphs were observed. Mortality caused by all these extracts was significantly lower than that caused by the chemical insecticide imidacloprid (Table 5).

Therefore, lethal concentrations (LC_{50} and LC_{90}) on nymphs were only estimated for ethanolic extracts. In this regard, significant differences on LC_{50} and LC_{90} of ethanolic extracts were observed (Table 6). Ethanolic extracts of *P. alliaceae* and *T. arborea* had the lowest LC_{50} values, 1.27 and 1.61 mg mL^{-1}, respectively. The extract of *P. alliaceae*, however, had the lowest LC_{90} value (2.93 mg mL^{-1}) (Table 6).

GC-MS analyses of the most active extracts

GC-MS analyses of the ethanolic extracts of *P. alliaceae* and *T. arborea*, and identification of the components by matching with the mass spectral library of the corresponding compounds, showed that phytol (t_R = 12.09 min) was the major component of ethanolic extract of *P. alliaceae*, while hexadecanoic acid, ethyl ester (t_R = 7.32 min), octadecanoic acid, ethyl ester (t_R = 9.66 min) and eicosane (t_R = 16.60 min) were the major components of ethanolic extract of *T. arborea*.

DISCUSSION

Plant extracts are currently being studied as an ecologically friendly alternative to manage plant pests. Studies on botanical insecticides against *B. tabaci* have focused particularly on essential oils of different plants, such as *Thymus vulgaris*, *Allium cepa, Allium sativum, Satureja hortensis*, *Achillea biebersteinii, Cinnamomum verum, Syzygium aromaticum, Alkanna strigosa, Ballota undulate, Galium longifolium, Lepidium sativum, Peganum harmala, Pimpinella anisum, Ruta chalepensis, Retama raetam* and *Urtica pilulifera*, where 60-100% mortality has been reported (Aslan et al. 2004; Al-mazra'awi and Atteyyat, 2009; Ateyyat et al. 2009). Other authors have also documented the insecticidal effects of seed oil from *A. indica*, and their principle active ingredient azadirachtin on *B. tabaci* (Kumar et al. 2005; Pinheiro et al. 2009).

Table 4. Toxicity (LC_{50} and LC_{90}) of ethanolic plant extracts on *Bemisia tabaci* eggs after 48 hrs exposure.

Plant species	n	Slope	mg mL^{-1}		P
			LC_{50} (CI)	LC_{90} (CI)	
Acalypha gaumeri	5	2.84	3.54 (3.31-3.76)c	9.99 (9.05-11.24)e	<0.0001
Annona squamosa	5	4.77	2.71 (2.58-2.84)b	5.03 (4.75-5.39)c	<0.0001
Azadirachta indica	5	6.35	3.60 (2.50-2.70)c	4.14 (3.94-4.39)b	<0.0001
Carlowrightia myriantha	5	3.60	2.69 (2.52-2.85)b	6.11 (5.70-6.61)d	<0.0001
Petiveria alliacea	5	4.88	2.09 (1.94-2.23)a	3.83 (3.62-4.10)ab	<0.0001
Trichilia arborea	5	6.74	2.14 (2.01-2.25)a	3.32 (3.16-3.56)a	<0.0001

n = number of groups tested containing 30 individuals each.
LC_{50} and LC_{90} values are in mg mL^{-1}.
LC_{50} and LC_{90} values followed by the same letter within the same column are not significantly different.
CI = Confidence Interval.

In the present work aqueous and ethanolic extracts of plants from the Yucatan Peninsula, Mexico were evaluated on immature whitefly (*B. tabaci*), one of the most noxious pests in tropical agro-ecosystems. Plants were selected based on the precedent of insecticidal activity in the literature and for their chemotaxonomy. The extracts of a specimen of *A. indica*, growing in Yucatan was also tested in order to compare the insecticidal effects of the native species.

In general, the insecticidal properties of most of the native and adapted plants in Yucatan have been scarcely studied. In this work, all aqueous and ethanolic extracts of tested plants caused high mortality on *B. tabaci* eggs; however, on *B. tabaci* nymphs only ethanolic extracts were active. As for the aqueous extracts, that of *A. squamosa* had the lowest LC_{50} and LC_{90} values (0.36 and 1.02 mg mL^{-1}, respectively). Low LC_{50} and LC_{90} values were also observed on extracts of *A. gaumeri* and *P. alliaceae*. Extracts of these plant species showed similar insecticidal activity to that of *A. indica* leaves, herein tested.

Studies on insecticidal properties of *A. squamosa* have been focused mainly on the activity of its seed extracts, where activity has been attributed to the metabolites squamocin, annotemoyin and neoannonin, which target Diptera, Coleoptera and Lepidoptera, (Castillo-Sánchez et al. 2010). To the best of our knowledge, no data on insecticidal activity of *A. squamosa* leaves on *B. tabaci* was previously available. The only study on the use of low polarity extracts of *A. squamosa* leaves have been reported on the control of mosquitoes, an important group of human disease vectors (Kamaraj et al. 2011). Thus, this study is of importance in highlight the potential of *A. squamosa* leaves as a source of botanical insecticides.

As for the ethanolic extracts, the most effective on *B. tabaci* eggs and nymphs were those of *P. alliaceae* and *T. arborea*. Extracts of both plant species had the lowest LC_{50} values on eggs (2.09 and 2.14 mg mL^{-1}, respectively), and on nymphs (1.27 and 1.61 mg mL^{-1}, respectively). This is worth noting that among all plant species tested, only in *P. alliaceae* both types of extracts (aqueous and ethanolic) were active on immature *B. tabaci*. The insecticidal activity of extracts of leaves and other organs of *P. alliaceae* has been previously reported. For example, García-Mateos et al. (2007) reported that aqueous, methanolic and dicloromethane extracts of leaves caused 100% mortality on the whitefly *Trialeurodes vaporariorum*. Johnson et al. (1997) found that extracts of *P. alliaceae* roots had toxic effects on the tick *Boophilus microplus* and the phytophagous insects *Cylas formicarius elegantulus* and *Hypothenemus hampei*. Phytochemical analysis of the extracts showed that dibenzyltrisulfide compounds could be responsible for its effects. More recently, Rosado-Aguilar et al. (2010) showed that methanolic extracts of stem and leaves of *P. alliaceae* caused 100% mortality on the tick *B. microplus* larvae, and 91% inhibition of egg-laying on adults. Gas chromatography-mass spectrometry demonstrated that the chemical composition of the active fraction was mainly composed of dibenzyldisulfide and dibenzyltrisulfide, suggesting that these compounds might be responsible for the acaricidal activity of *P. alliaceae* stem extracts.

Table 5. Mortality of *Bemisia tabaci* nymphs caused by ethanolic (10 mg mL^{-1}) and aqueous (3% w/v) plant extracts after 48 hrs exposure.

Plant species	% Nymphal mortality	
	Ethanolic extracts	Aqueous extracts
Imidacloprid	100 ± 0.00 a	100 ± 0.00 a
Acalypha gaumeri	100 ± 0.00 a	3.3 ± 2.53 d
Annona squamosa	100 ± 0.00 a	6.6 ± 4.55 d
Azadirachta indica	99.3 ± 1.05 a	11.7 ± 8.13 cd
Carlowrightia myriantha	100 ± 0.00 a	42.3 ± 10.10 b
Petiveria alliacea	100 ± 0.00 a	27.6 ± 11.34 bc
Trichilia arborea	100 ± 0.00 a	9 ± 2.58 cd
Water	8.0 ± 5.43 b	2.7 ± 2.08 d
EtOH/water	7.8 ± 5.34 b	

Mean mortalities (± standard error) followed by the same letter within the same column are not significantly different (Tukey, $P < 0.05$).

As these sulfide compounds have been directly related to diverse biological effects of roots and stem extracts of *P. alliaceae*, we then analyzed the leaf extracts by GC-MS and compared these with the standard sulfide compounds (Sigma Aldrich). Standard compounds dibenzyldisulfide (t_R = 11.22 min) and dibenzyltrisulfide (t_R = 13.66 min) were not detected in three different extracts (ethanolic, ethyl acetate and hexanic) of *P. alliaceae* leaves. Instead, we observed phytol (t_R = 12.09 min) as the major component. This metabolite has been reported as an important component of the essential oil of *Salvia splendens*, such essential oil has also shown larvicidal activity against *Aedes albopictus* larvae with a LC_{50} value of 59.2 ppm (Mathew and Thoppil, 2011). Therefore, our findings suggest that phytol might be one of the responsible metabolites for the insecticidal activity of *P. alliaceae* extracts.

In the present work, we also observed that extracts *T. arborea* showed high insecticidal activity. To the best of our knowledge no reports on insecticidal activity or chemical constituents of this endemic species was previously available. Nevertheless, *Trichilia* genus belongs to the Meliacea family, as *A. indica*, for instance we might expect *T. arborea* to have insecticidal properties. Other species of *Trichilia*, like *T. pallida*, has shown insecticidal effects. For example, Baldin et al. (2007) documented that aqueous extracts of branches and leaves of *T. pallida* caused high mortality on *B. tabaci* nymphs in tomato plants under greenhouse conditions. Similarly, Da Cunha et al. (2005) and Roel and Vendramim (2006) found that the organic and aqueous extracts of *T. pallida* have insecticidal effects on larvae of some species of Lepidoptera, such as *Spodoptera frugiperda* and *Tuta absoluta*. Aqueous and acethonic extracts of *T. glauca* have also found to be toxic when ingested by *Acromyrmex lundi* (Caffarini et al. 2008). In previous work from our laboratory, we have found that *T. arborea* has activity against plant pathogens such as the knot nematode *Meloidogyne incognita* (Cristóbal-Alejo et al. 2006) and the fungal pathogen *Colletotrichum gloeosporioides* (Gamboa-Angulo et al. 2008). Phytochemical analyses of Trichilia species have shown that the insecticidal activity is due to the presence of tetranortriterpenoids, limmonoids, ω-phenylalkyl and fatty acids (Simmonds et al. 2001; Matos et al. 2009; Nebo et al. 2010). The GC-MS analysis that we performed for the ethanolic extract of *T. arborea* displayed the presence of fatty acids as major components, among them hexadecanoic acid, ethyl

ester (t_R = 7.32 min), octadecanoic acid, ethyl ester (t_R = 9.66 min) and eicosane (t_R = 16.60 min) were found. Eicosane is an alkane commonly found in essential oils of plants, which has been related to several activities as antimicrobial (Hsouna et al. 2011) and antitermitic (Manzoor et al. 2012).

Table 6. Toxicity (LC_{50} and LC_{90}) of ethanolic plant extracts on *Bemisia tabaci* nymphs after 48 hrs exposure.

Plant species	n	Slope	mg ml^{-1}		P
			LC_{50} (CI)	LC_{90} (CI)	
Acalypha gaumeri	5	7.39	3.15 (3.05-3.25)c	4.69 (4.48-4.94)cd	<0.0001
Annona squamosa	5	4.24	2.66 (2.55-2.77)b	5.33 (4.98-5.76)e	<0.0001
Azadirachta indica	5	6.06	2.57 (2.48-2.66)b	4.18 (3.98-4.43)b	<0.0001
Carlowrightia myriantha	5	5.67	3.10 (2.99-3.22)c	5.21 (4.93-5.55)d	<0.0001
Petiveria alliacea	5	3.53	1.27 (1.18-1.35)a	2.93 (2.73-3.18)a	<0.0001
Trichilia arborea	5	2.99	1.61 (1.50-1.71)a	4.30 (3.94-4.79)bc	<0.0001

n = number of groups tested containing 30 individuals each.
LC_{50} and LC_{90} values are in mg mL^{-1}.
LC_{50} and LC_{90} values followed by the same letter within the same column are not significantly different.
CI = Confidence Interval.

The plant extracts tested in this study, particularly those of *T. arborea* and *P. alliaceae* showed the potential to be developed into compounds for the management of immature whitefly. Further research will focus on evaluating these extracts through a bioassay-guided process, to determine the metabolites responsible for the insecticidal effect on inmatures of *B. tabaci*. In the long term, our goal is to develop safer alternatives to manage *B. tabaci*. These natural products might be considered an important component of the integrated pest management system.

ACKNOWLEDGMENTS

Authors thank Irma L. Medina-Baizabal, Paulino Simá-Polanco, Mirbella Cáceres-Farfán and Martha Mendez-Gonzales for their technical and taxonomical assistance. Authors also thank Dr. Andrew Donini for his assistance on the English translation of the manuscript.

Financial support: This work was supported by FOMIX Conacyt (Yuc-2011-C09-168624). A.E.C.E. thanks Conacyt for the Ph. D. scholarship (cvu 234514).

REFERENCES

ADEYEMI, M.M.H. (2010). The potential of secondary metabolites in plant material as deterrents against insect pests: A review. *African Journal of Pure and Applied Chemistry*, vol. 4, no. 11, p. 243-246.

AL-MAZRA'AWI, M.S. and ATEYYAT, M. (2009). Insecticidal and repellent activities of medicinal plant extracts against the sweet potato whitefly, *Bemisia tabaci* (Hom.: Aleyrodidae) and its parasitoid *Eretmocerus mundus* (Hym.: Aphelinidae). *Journal of Pest Science*, vol. 82, no. 2, p. 149-154. [CrossRef]

ASLAN, I.; ÖZBEK, H.; ÇALMAŞUR, Ö. and ŞAHİN, F. (2004). Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Industrial Crops and Products*, vol. 19, no. 2, p. 167-173. [CrossRef]

ATEYYAT, M.A.; AL-MAZRA'AWI, M.; ABU-RJAI, T. and SHATNAWI, M.A. (2009). Aqueous extracts of some medicinal plants are as toxic as imidacloprid to the sweet potato whitefly, *Bemisia tabaci. Journal of Insect Science*, vol. 9, no. 15.

BALDIN, E.L.L.; VENDRAMIM, J.D. and LOURENÇÃO, A.L. (2007). Interaction between resistant tomato genotypes and plant extracts on *Bemisia tabaci* (Genn.) biotype B. *Scientia Agricola*, vol. 64, no. 5, p. 476-481. [CrossRef]

BROWN, J.K. and BIRD, J. (1992). Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basin. *Plant Disease*, vol. 76, no. 3, p. 220-225.

CAFFARINI, P.; CARRIZO, P.; PELICANO, A.; ROGGERO, P. and PACHECO, J. (2008). Efectos de extractos acetónicos y acuosos de *Ricinus communis* (ricino), *Melia azederach* (paraíso) y *Trichillia galuca* (trichillia), sobre la hormiga negra común (*Acromyrmex lundi*). *IDESIA (Chile)*, vol. 26, no. 1, p. 59-64.

CASTILLO-SÁNCHEZ, L.E.; JIMÉNEZ-OSORNIO, J.J. and DELGADO-HERRERA, M.A. (2010). Secondary metabolites of the Annonaceae, Solanaceae and Meliaceae families used as biological control of insects. *Tropical and Subtropical Agroecosystems*, vol. 12, no. 3, p. 445-462.

CRISTÓBAL-ALEJO, J.; TUN-SUÁREZ, J.M.; MOGUEL-CATZÍN, S.; MARBÁN-MENDOZA, N.; MEDINA-BAIZABAL, L.; SIMÁ-POLANCO, P.; PERAZA-SÁNCHEZ, S.R. and GAMBOA-ANGULO, M.M. (2006). *In vitro* sensitivity of *Meloidogyne incognita* to extracts from native Yucatecan plants. *Nematropica*, vol. 36, no. 1, p. 89-97.

DA CUNHA, U.S.; VENDRAMIM, J.D.; ROCHA, W.C. and VIEIRA, P.C. (2005). Potencial de *Trichilia pallida* Swartz (Meliaceae) como fonte de substâncias com atividade inseticida sobre a traça-do-tomateiro, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology*, vol. 34, no. 4, p. 667-674. **[CrossRef]**

DAYAN, F.E.; CANTRELL, C.L. and DUKE, S.O. (2009). Natural products in crop protection. *Bioorganic & Medicinal Chemistry*, vol. 17, no. 12, p. 4022-4034. **[CrossRef]**

DURAN, R.; TREJO-TORRES, J.C. and IBARRA-MANRÍQUEZ, G. (1998). Endemic phytotaxa of the Peninsula of Yucatán. *Harvard Papers in Botany*, vol. 3, no. 2, p. 263-314.

ELBERT, A. and NAUEN, R. (2000). Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. *Pest Management Science*, vol. 56, no. 1, p. 60-64. **[CrossRef]**

GAMBOA-ANGULO, M.M.; CRISTÓBAL-ALEJO, J.; MEDINA-BAIZABAL, I.L.; CHÍ-ROMERO, F.; MÉNDEZ-GONZÁLEZ, R.; SIMÁ-POLANCO, P. and MAY-PAT, F. (2008). Antifungal properties of selected plants from the Yucatan peninsula, Mexico. *World Journal of Microbiology and Biotechnology*, vol. 24, no. 9, p. 1955-1959. **[CrossRef]**

GARCÍA-MATEOS, M.R.; ELIZALDE-SÁNCHEZ, E.; ESPINOSA-ROBLES, P. and ÁLVAREZ-SÁNCHEZ, M.E. (2007). Toxicity of *Petiveria alliacea* L. on greenhouse whitefly (*Trialeurodes vaporariorum* West.). *Interciencia*, vol. 32, no. 2, p. 121-124.

HSOUNA, A.B.; TRIGUI, M.; MANSOUR, R.B.; JARRAYA, R.M.; DAMAK, M. and JAOUA, S. (2011). Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratonia siliqua* essential oil with preservative effects against *Listeria* inoculated in minced beef meat. *International Journal of Food Microbiology*, vol. 148, no. 1, p. 66-72.**[CrossRef]**

ISMAN, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, vol. 51, p. 45-66. **[CrossRef]**

JOHNSON, L.; WILLIAMS, L.A.D. and ROBERTS, E.V. (1997). An insecticidal and acaricidal polysulfide metabolite from the roots of *Petiveria alliacea*. *Pest Management Science*, vol. 50, no. 3, p. 228-232. **[CrossRef]**

KAMARAJ, C.; BAGAVAN, A.; ELANGO, G.; ABDUZ-ZAHIR, A.; RAJAKUMAR, G.; MARIMUTHU, S.; SANTHOSHKUMAR, T. and ABDUL-RAHUMAN, A. (2011). Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. *Indian Journal of Medical Research*, vol. 134, no. 1, p. 101-106.

KUMAR, P.; POEHLING, H.M. and BORGEMEISTER, C. (2005). Effects of different application methods of azadirachtin against sweetpotato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants. *Journal of Applied Entomology*, vol. 129, no. 9-10, p. 489-497.**[CrossRef]**

MATHEW, J. and THOPPIL, J.E. (2011). Chemical composition and mosquito larvicidal activities of *Salvia* essential oils. *Pharmaceutical Biology*, vol. 49, no. 5, p. 456-463. **[CrossRef]**

MANZOOR, F.; NAZ, N.; MALIK, S.A.; SIDDIQUI, B.S.; SYED, A. and PERWAIZ, S. (2012). Chemical analysis and comparison of antitermitic activity of essential oils of neem (*Azadirachta indica*), vetiver (*Vetiveria zizanioides*) and mint (*Mentha arvensis)* against *Heterotermes indicola* (Wasmann) from Pakistan. *Asian Journal of Chemistry*, vol. 24, no. 5, p. 2069-2072.

MATOS, A.P.; NEBO, L.; VIEIRA, P.C.; FERNANDES, J.B.; DA SILVA, M.F.G.F. and RODRIGUES, R.R. (2009). Constituintes químicos e atividade inseticida dos extratos de frutos de *Trichilia elegans* e *T. catigua* (Meliaceae). *Química Nova*, vol. 32, no. 6, p. 1553-1556.

MORALES, F.J. (2007). Tropical Whitefly IPM Project. *Advances in Virus Research*, vol. 69, p. 249-311. **[CrossRef]**

MUÑIZ, M. and NOMBELA, G. (2001). Differential variation in development of the B- and Q-biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on sweet pepper at constant temperatures. *Environmental Entomology*, vol. 30, no. 4, p. 720-727.

NEBO, L.; MATOS, A.P.; VIERIA, P.C.; FERNANDES, J.B.; DA SILVA, M.F.G.F. and RODRÍGUEZ, R.R. (2010). Atividade inseticida dos frutos de *Trichilia claussenii* (Meliaceae) sobre *Spodoptera frugiperda*. *Química Nova*, vol. 33, no. 9, p. 1849-1852.

OLIVEIRA, M.R.V.; HENNEBERRY, T.J. and ANDERSON, P. (2001). History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection*, vol. 20, no. 9, p. 709-723.**[CrossRef]**

PINHEIRO, P.V.; QUINTELA, E.D.; DE OLIVEIRA, J.P. and SERAPHIN, J.C. (2009). Toxicity of neem oil to *Bemisia tabaci* biotype B nymphs reared on dry bean. *Pesquisa Agropécuaria Brasileira*, vol. 44, no. 4, p. 354-360. **[CrossRef]**

RATTAN, R.S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Protection*, vol. 29, no. 9, p. 913-920. **[CrossRef]**

RODITAKIS E.; GRISPOU, M.; MOROU, E.; KRISTOFFERSEN, J.B.; RODITAKIS, N.; NAUEN, R.; VONTAS, J. and TSAGKARAKOU, A. (2009). Current status of insecticide resistance in Q biotype *Bemisia tabaci* populations from Crete. *Pest Management Science*, vol. 65, no. 3, p. 313-322. **[CrossRef]**

ROEL, A.R. and VENDRAMIM, J.D. (2006). Efeito residual do extrato acetato de etila de *Trichilia pallida* Swartz (Meliaceae) para lagartas de diferentes idades de *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae). *Ciencia Rural*, vol. 36, no. 4, p. 1049-1054. **[CrossRef]**

ROSADO-AGUILAR, J.A.; AGUILAR-CABALLERO, A.; RODRIGUEZ-VIVAS, R.I.; BORGES-ARGAEZ, R.; GARCIA-VAZQUEZ, Z. and MENDEZ-GONZALEZ, M. (2010). Acaricidal activity of extracts from *Petiveria alliacea* (Phytolaccaceae) against the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). *Veterinary Parasitology*, vol. 168, no. 3-4, p. 299-303. **[CrossRef]**

SIMMONDS, M.S.J.; STEVENSON, P.C.; PORTER, E.A. and VEITCH, N.C. (2001). Insect antifeedant activity of three new tetranortritepenoids from *Trichilia pallida*. *Journal of Natural Products*, vol. 64, no. 8, p. 1117-1120. **[CrossRef]**

How to reference this article:

CRUZ-ESTRADA, A.; GAMBOA-ÂNGULO. M.; BORGES-ARGÁEZ, R. and RUIZ-SÁNCHEZ, E. (2013). Insecticidal effects of plant extracts on immature whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyroideae). *Electronic Journal of Biotechnology*, vol. 16, no. 1. http://dx.doi.org/10.2225/vol16-issue1-fulltext-6