

Aqueous Extracts of Some Medicinal Plants are as Toxic as Imidacloprid to the Sweet Potato Whitefly, *Bemisia tabaci*

Authors: Ateyyat, Mazen A., Al-Mazra'awi, Mohammad, Abu-Rjai, Talal, and Shatnawi, Mohamad A.

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Aqueous extracts of some medicinal plants are as toxic as Imidacloprid to the sweet potato whitefly, *Bemisia tabaci*

Mazen A. Ateyyat¹, Mohammad Al-Mazra'awi², Talal Abu-Rjai³ and Mohamad A. Shatnawi²

¹Ash-Shoubak University College, Al-Balqa' Applied University, 17119 Al Salt, Jordan

²Faculty of Agricultural Technology, Al-Balqa' Applied University, 17119 Al Salt, Jordan

³Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan

Abstract

Aqueous extracts of nine plants, known to have medicinal activity, were tested for their toxicity against the sweet potato whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleurodidae) compared to the toxicity of the insecticide, Imidacloprid. Extracts of *Lepidium sativum* L. (Brassicales: Brassicaceae) killed 71 % of early stage nymphs, which was not significantly different from mortality caused by Imidacloprid. Treatment of pupae with three plant extracts, *L. sativum*, *Achillea biebersteinii* L. (Asterales: Asteraceae), or *Retama raetam* (Forssk.) Webb and Berthel (Fabales: Fabaceae) prevented adult development, and treatment with *R. raetam* extract killed adults, at levels that were not significantly different from Imidacloprid. None of the other plants showed significant toxicity. However extracts of four plants, *Pimpinella anisum* L. (Apiales: Apiaceae), *Galium longifolium* (Sibth. and SM.) (Gentianales: Rubiaceae), *R. raetam* and *Ballota undulata* Benth (Lamiales: Lamiaceae) had a repellent effect.

Keywords: Biopesticides, botanicals, *Achillea biebersteinii*, *Artemisia inculta*, *Ballota undulata*, *Euphorbia hierosolymitana*, *Galium longifolium*, *Lepidium sativum*, *Pimpinella anisum*, *Phlomis syriaca* and *Retama raetam*

Correspondence: ateyyat@bau.edu.jo

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Introduction

Plants may provide an alternative to currently used pesticides for the control of plant pests, as they constitute a rich source of bioactive chemicals (Kim et al. 2005; Daoubi et al. 2005). Recent studies have demonstrated the insecticidal properties of chemicals derived from plants that are active against specific target species, biodegradable to non toxic products and potentially suitable for use in integrated management programs (Markouk et al. 2000; Tare et al. 2004).

The sweet potato whitefly, *Bemisia tabaci* Gen. (Homoptera: Aleurodidae), is a key pest of vegetables in Jordan (Al-Musa et al. 1987). It is also a serious economic pest of agronomic, horticultural, and ornamental crops throughout warm regions of the world (Byrne et al. 1990; Brown 1994).

In the present study, the toxicity and repellency of aqueous extracts of nine plants known to have medicinal activity, were investigated against the sweet potato whitefly, *B. tabaci*.

Materials and Methods

Plant material

Nine plants known to have medicinal activity, *Achillea biebersteinii* L. (Asterales: Asteraceae), *Artemisia inculta* Del. (Asterales: Asteraceae), *Ballota undulata* Benth. (Lamiales: Lamiaceae), *Euphorbia hierosolymitana* Boiss. (Malpighiales: Euphorbbiaceae), *Galium longifolium* (Sibth. and Sm.) (Gentianales: Rubiaceae), *Lepidium sativum* L. (Brassicales:

Brassicaceae), *Pimpinella anisum* L. (Apiales: Apiaceae), *Phlomis syriaca* Boiss. (Lamiales: Lamiaceae) and *Retama raetam* (Forssk.) Webb and Berthel (Fabales: Fabaceae), were collected from their natural habitats, from different localities of Jordan. The identity of each plant species mentioned was verified and confirmed by Prof. Dawud M. Al-Eisawi (Department of Biology, Faculty of Science, University of Jordan) using live specimens and photographs. The parts of the different plants used in the experiments are given in Table 1.

Preparation of the aqueous extracts

An aqueous extract was prepared by boiling 10 % wt/wt of the air-dried powdered plant part in sterile distilled water for 10 min and then cooled to room temperature overnight. The aqueous extracts were filtered using a Millipore filters (Millipore 0.2 mm, www.waters.com) to remove particulate matter. The final volume of each filtrate was completed to 100 ml with distilled water with 0.2% Tween 80 to account for the evaporated water during boiling. The aqueous extracts were prepared shortly before application. Negative controls represented by the distilled water contained the emulsifier Tween 80.

Insecticide

The insecticide Imidacloprid (Confidor® 200SC, Bayer Crop Science, www.bayercropscience.com) was used as positive control treatment at the recommended field application rate of 0.25 ml/l.

Insect culture

The colony of *B. tabaci* was maintained on tomato plants. To obtain immature whiteflies, 4-week-old greenhouse-grown tomato plants grown in a whitefly-free screened

Table 1. Voucher number, common name, family, time of collection, place of collection and partsused of the selected Jordanian medicinal plants.

Plant	Voucher #	Common name	Family	Time of collection	Place of collection	Parts used
<i>Pimpinella anisum</i> L.	Ateyyat 05-11	Anise	Umbelliferae	May 4, 2005	Mushagar	Fruits
<i>Achillea biebersteinii</i> Afan.	Ateyyat 05-25	Yarrow	Compositae	April 15, 2005	Mushagar	Aerial parts
<i>Galium longifolium</i> (Sibth & Sm.) Griseb.	Ateyyat 06-8		Rubiaceae	April 25, 2006	Mujib	Leaves
<i>Euphorbia hierosolymitana</i> Boiss.	Ateyyat 06-15	Spurge	Euphorbiaceae	May 15, 2006	Salt	Stem
<i>Artemisia inculta</i> Delile.	Ateyyat 05-5	Herba- alba wormwood	Compositae	May 4, 2005	Mushagar	Aerial parts
<i>Phlomis syriaca</i> Boiss.	Ateyyat 06-14		Lamiaceae	May 15, 2006	Salt	Leaves
<i>Retama raetam</i> (Forssk.)	Ateyyat 05-28	White broom, Juniper bush	Leguminosae	April 15, 2005	Dhana	Bark
<i>Ballota undulata</i> (Ghassa)	Ateyyat 06-13	Common black horehound	Labiatae	April 20, 2006	Dhana	Leaves
<i>Lepidium sativum</i> L.	Ateyyat 06-25	Garden cress	Cruciferae	April 15, 2006	Mushagar	Fruits

cage were trimmed to three fully expanded leaves and were transferred to the whitefly colony for 48 h. Adults were then aspirated from the plants, and the plants were placed in a separate cage. The synchronously-developing, uniformly-aged whitefly populations were then held until they developed to the appropriate stage.

Egg mortality

Immediately after the adult whiteflies were aspirated from the plants, the plants were sprayed with extracts. Water or Imidacloprid were the negative and positive controls Initial number of eggs prior application ranged from 140 to 300 per plant. There were five replicates (plants) for each treatment. Eight days after treatment, the unhatched eggs and newly emerged nymphs were counted and the percent hatch calculated.

Early stage nymphal mortality

Eight days after infestation, when first instar nymphs had emerged and attached to the leaf, the plants were sprayed as before. The number of first instar nymphs per plant ranged from 98 to 190. There were five replicates (plants) per treatment. Ten days after treatment, the number of dead nymphs were counted under a dissecting microscope. A nymph is considered dead if it was shrunken or its color changed. Normally developed nymphs to adult stage were also counted and the percentage of each was calculated.

Late stage pupal mortality

Fourteen days after infestation, when most nymphs were in the red-eye stage, the plants were sprayed as before. The number of nymphs per plant ranged from 75 to 150. There were five replicates (plants). Seven days after treatment, when most of the pupae had emerged from control plants, the number of empty pupal cases and pupae that failed to emerge were counted and the percent of emergence was calculated.

Adult mortality

A fully expanded leaf was placed in wet moss inside a Blackman box (Blackman 1971). The leaf was dipped into the solution of the required treatment and left overnight. About 30 adults were then introduced inside each box. Distilled water was used as negative treatment and imidacloprid was used as positive treatment. The number of dead whitefly adults was recorded after 48 hr. A whitefly adult was considered dead if it did not move after probing with a camel hair brush. Five replicates were made for each treatment.

Repellency tests

Two fully expanded leaves of tomato were placed individually in vials containing water. One leaf was dipped in the tested plant extract and the other one was dipped in distilled water. The vials were placed in a plastic jar (20 cm diameter, 30 cm high) covered with fine netting material. About 50 immobilized adults were placed between

the two vials. Numbers of adults attracted to each leaf was recorded after 3hr and 24hr. Five replicates were made for each treatment.

Statistical analysis

Arcsin-transformed data were analyzed by ANOVA and means were separated by Tukey’s Studentized Range test. Data of the repellency tests were analyzed using t-tests.

Results

Over 80% of the eggs hatched and nymphs were able to emerge, regardless of treatment and all plant extracts showed no differences with both negative and positive controls (F = 2.62, df = 10, 44; P = 0.0134) (Table 2). The highest percentage of dead early stage nymphs (71%) was caused by the extract of *L. sativum* that was not significantly different from the effect of Imidacloprid (F = 31.05, df = 10, 44; P = 0.0001) (Table 3). Extracts of three plants, *R. raelam*, *P. syriaca* and *A. inculata* were as toxic as the *L. sativum* extract, but not as toxic as Imidacloprid.

Table 2. % means of unhatched eggs of Bemisia tabaci exposed to a number of plant extracts.

Treatment	% mean of unhatched eggs(SE)
<i>Pimpinella anisum</i>	10.80 ^{ab} (1.74)
<i>Achillea biebersteinii</i>	14.20 ^{ab} (2.73)
<i>Galium longifolium</i>	14.40 ^{ab} (2.46)
<i>Euphorbia hierosolymitana</i>	20.80 ^a (3.57)
<i>Artemisia inculata</i>	15.20 ^{ab} (2.54)
<i>Phlomis syriaca</i>	19.00 ^{ab} (2.43)
<i>Retama raelam</i>	9.00 ^b (1.58)
<i>Ballota undulata</i>	17.00 ^{ab} (3.18)
<i>Lepidium sativum</i>	16.00 ^{ab} (0.84)
Water (–ve control)	9.80 ^{ab} (0.97)
Imidacloprid (+ve control)	18.20 ^{ab} (1.93)
(Fvalue, df)	(2.62, 44)
Pr > F	0.0134

* Arcsin-transformed data were analyzed by ANOVA and the means were separated by Tukey’s Studentized Range test. Means followed by the same letter are not significant (P = 0.05).

Treatment of pupae with three of the plant extracts, *L. sativum*, *A. biebersteinii*, or *R. raelam* prevented adult development as well as Imiacloprid (F = 5.51, df 10, 44; P = 0.0001) (Table 4). Treatment with *R. raelam* extract killed adults as as effectively as Imiacloprid (F = 6.68, df 1, 44; P = 0.0001) (Table 5).

Table 3. % means of dead early stage nymphs and % of early stage nymphs that developed to adult stage after exposure to a number of plant extracts.

Treatment	% mean of dead early stage nymphs (SE)	% mean of early stage nymphs that developed to adult stage (SE)
<i>Pimpinella anisum</i>	44.40 ^{cde} (3.79)	47.40 ^{bc} (2.94)
<i>Achillea biebersteinii</i>	42.80 ^{cde} (4.09)	45.00 ^{bcde} (6.38)
<i>Galium longifolium</i>	41.80 ^{cde} (2.15)	52.40 ^{bc} (1.94)
<i>Euphorbia hierosolymitana</i>	40.00 ^{de} (4.89)	55.20 ^b (4.26)
<i>Artemisia inculta</i>	55.40 ^{bcd} (6.01)	39.60 ^{bcde} (6.60)
<i>Phlomis syriaca</i>	59.60 ^{bc} (6.12)	34.00 ^{cde} (5.96)
<i>Retama raetam</i>	62.00 ^{bc} (6.24)	24.80 ^e (4.26)
<i>Ballota undulata</i>	29.60 ^e (2.11)	45.40 ^{bcd} (2.64)
<i>Lepidium sativum</i>	71.00 ^{ab} (1.79)	26.60 ^{de} (1.91)
Water (–ve control)	9.40 ^f (1.40)	81.80 ^a (2.85)
Imidacloprid (+ve control)	89.80 ^a (1.16)	3.00 ^f (0.32)
(Fvalue, df)	(31.05, 44)	(28.07, 44)
Pr > F	0.0001	0.0001

* Arcsin-transformed data were analyzed by ANOVA and the means were separated by Tukey's Studentized Range test. Means followed by the same letter are not significant (P = 0.05).

Table 4. % adult eclosion failure after pupal exposure to a number of plant extracts.

Treatment	% adult emergence failure (SE)
<i>Pimpinella anisum</i>	3.80 ^b (1.74)
<i>Achillea biebersteinii</i>	14.00 ^{ab} (1.10)
<i>Galium longifolium</i>	5.00 ^b (2.10)
<i>Euphorbia hierosolymitana</i>	6.40 ^b (2.62)
<i>Artemisia inculta</i>	5.00 ^b (1.67)
<i>Phlomis syriaca</i>	11.20 ^b (4.33)
<i>Retama raetam</i>	12.20 ^{ab} (1.10)
<i>Ballota undulata</i>	3.60 ^b (1.69)
<i>Lepidium sativum</i>	16.00 ^{ab} (3.33)
Water (–ve control)	5.60 ^b (2.18)
Imidacloprid (+ve control)	31.60 ^a (1.89)
(Fvalue, df)	(5.51, 44)
Pr > F	0.0001

* Arcsin-transformed data were analyzed by ANOVA and the means were separated by Tukey's Studentized Range test. Means followed by the same letter are not significant (P = 0.05).

Table 5. % mortality of adults exposed to a number of plant extracts.

Treatment	% adult mortality (SE)
<i>Pimpinella anisum</i>	8.80 ^c (1.93)
<i>Achillea biebersteinii</i>	13.60 ^c (6.33)
<i>Galium longifolium</i>	27.60 ^{bc} (5.36)
<i>Euphorbia hierosolymitana</i>	12.40 ^c (4.41)
<i>Artemisia inculta</i>	24.80 ^{bc} (8.15)
<i>Phlomis syriaca</i>	29.40 ^{bc} (6.65)
<i>Retama raetam</i>	42.00 ^{ab} (6.77)
<i>Ballota undulata</i>	25.40 ^{bc} (4.57)
<i>Lepidium sativum</i>	12.60 ^{bc} (2.11)
Water (–ve control)	13.60 ^{bc} (2.42)
Imidacloprid (+ve control)	65.40 ^a (3.88)
(Fvalue, df)	(6.68, 44)
Pr > F	0.0001

* Arcsin-transformed data were analyzed by ANOVA and the means were separated by Tukey's Studentized Range test. Means followed by the same letter are not significant (P = 0.05).

Table 6. Average number of adults attracted to treated and untreated plants

Plant extract	Whiteflies 3 hr. post application (SE)			Whiteflies 24 hr. post application (SE)		
	Treated	Untreated	t-value*	Treated	Untreated	t-value*
<i>Pimpinella anisum</i>		10.5 (2.8)	24.5 (3.7)	5.15	10.0 (3.0)	23.0 (4.4)
<i>Achillea biebersteinii</i>		9.4 (3.0)	7.4 (1.0)	-0.62	11.4 (0.8)	7.8 (0.7)
<i>Galium longifolium</i>		8.4 (1.5)	14.0 (2.8)	3.50	8.2 (2.5)	13.0 (4.1)
<i>Euphorbia hierosolymitana</i>		9.8 (1.7)	13.3 (4.9)	0.79	12.8 (2.1)	15.2 (2.4)
<i>Artemisia inculta</i>		10.3 (1.7)	11.2 (2.5)	0.53	8.7 (1.4)	10.8 (0.8)
<i>Phlomis syriaca</i>		9.0 (2.1)	10.0 (2.8)	1.07	10.3 (2.1)	9.5 (1.4)
<i>Retama raetam</i>		6.5 (1.1)	18.2 (5.31)	2.43	6.8 (1.0)	20.5 (5.3)
<i>Ballota undulata</i>		5.3 (1.4)	11.0 (2.5)	2.69	8.0 (1.5)	17.5 (2.6)
<i>Lepidium sativum</i>		8.8 (1.7)	12 (1.4)	1.15	9.0 (2.3)	13.5 (2.0)

t-value at 0.05 level = 1.94
*Plants with a positive t-value above 1.94 were significantly repellent to the insect. Plants with a negative t-value above 1.94 were significantly attractive to the insect .

The extracts of *P. anisum*, *G. longifolium*, *R. raetam* and *B. undulata* showed repellency effect to adults of whitefly compared with untreated plants (Table 6). However, leaves treated with extracts of *A. biebersteinii* were more attractive to the whitefly than untreated leaves by 24h after application.

Discussion

Whitefly management has traditionally depended on the use of synthetic insecticides. However, the increasing resistance of *Bemisia* species to insecticides provides an impetus to use integrated pest control measures, including biopesticides and biological control to combat this pest. Biopesticides are based on natural products and synthetic analogs of naturally occurring biochemicals and are more acceptable than conventional pesticides because of their reputation for being less hazardous to humans and other non-target organisms (McCloskey et al. 1993). Among the biopesticides are chemicals derived from a variety of plant families. The biological activity of plant extracts against bacteria, fungi, viruses and insects has been reported (Bozsik 1996; Macedo et al. 1997; Unicinì Manganelli et al. 2005).

In the present work, extract of *L. sativum* had toxicity that was not significantly different from the effect of Imidacloprid against early stage nymphs and pupae of *B. tabaci*. Treatment of pupae with three plant extracts, *L. sativum*, *A. biebersteinii*, or *R. raetam* prevented adult development, and treatment with *R. raetam* extract killed adults, at levels that were not significantly different from Imiacloprid. *P. anisum*, *G. longifolium*, *R. raetam* and *B. undulata* had repellent effects on adults of *B. tabaci*.

L. sativum belongs to Cruciferae family that contain glucosinolates (Burow et al. 2007). Glucosinolates are a class

of thioglycosides found predominantly in plants of the order Brassicales. An anti-herbivore defense has been attributed to the products formed by myrosinase-catalyzed hydrolysis upon plant tissue damage (Burow et al. 2007). The leaves of *L. sativum* are antiscorbutic, diuretic and stimulant (Uphof 1959; Chopra et al. 1986). The plant is administered in cases of asthma, cough with expectoration and bleeding piles (Chopra et al. 1986). The root is used in the treatment of secondary syphilis and tenesmus (Chopra et al. 1986). *A. biebersteinii* is rich in camphor, borneol and 1,8-cineole (Esmaeili et al. 2006). It is used as an antispasmodic, for abdominal pain and healing wounds. *R. raetam* contains flavinoids, has been used as an herbal remedy for diabetes and has been shown to have hypoglycemic activity in rats (Maghrani et al. 2005).

Repellent activity of some non-insecticidal agents could be attributed to the complex mixture of compounds that are detected by the susceptible insect (Schumutterer 1985). Anise is the common name of *P. anisum*, and is among the more ancient aromatic plants. The fruits of *P. anisum* are claimed to possess expectorant, stimulant, carminative, diuretic and diaphoretic properties. They are also used in flatulent colic and in some pharmaceutical preparations for asthma (Siddiqui et al. 2002). Phenylpropanoid derivatives from *Ballota nigra* exhibited a moderate antimicrobial activity against *Proteus mirabilis* and *Staphylococcus aureus* (Didry et al. 1999). Acetone extract of *Ballota hirsuta* leaves produced growth inhibition in larvae of the stored grain pest *Tribolium castaneum* (Passcual-Villalobos and Robledo 1999).

Considering toxic effects of *L. sativum*, *A. biebersteinii*, and *R. raetam*, and the repellent effects of *P. anisum*, *G. longifolium*, *R. raetam* and *B. undulata* against *B. tabaci*, it is possible that extracts of these plants can be used as natural control agents. Most of these plants are widely distributed and easy grown. Furthermore, the extraction method is

simple and cost-effective and the application techniques could be relatively easily designed for on-farm use. Since *B. tabaci* transmits tomato leaf curl virus, developing new methods of control is obviously important.

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