Fumigant and Contact Toxicity and Oviposition Deterrent Effects of Plant Essential Oils on *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Fumigant and contact toxicity and oviposition deterrent effects of plant essential oils on *Bemisia tabaci* (Hemiptera: Aleyrodidae)

*Tufail Ahmed Wagan, Yueping He, Wanlun Cai, Jing Zhao, and Hongxia Hua**

Abstract

Ethanol-extracted essential oils from sweet flag *Acorus tatarinowii* Schott (Acoraceae), cow parsnip *Heracleum hemsleyanum* Diels (Apiaceae), and wild asparagus *Stemona japonica* (Blume) Miq. (Stemonaceae) were examined for their contact and fumigant toxicity and oviposition deterrent potential against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) under laboratory and greenhouse conditions during Oct to Dec 2015. To determine fumigant toxicity, adult whiteflies were tested in airtight glass jars containing essential oil on filter paper. Mortality rates were recorded after 2, 4, 6, and 8 h of exposure. Essential oil from *A. tatarinowii* was the strongest toxicant, with mortality rates of 20.4, 37.1, 73.3, and 98.8%, respectively, followed by *S. japonica* and *H. hemsleyanum.* To test contact toxicity, females were released in a cage containing tomato leaves treated with essential oil in the laboratory, and females were released in a cage containing tomato plants sprayed with essential oil in the greenhouse. Mortality rates were examined after 6, 12, 18, and 24 h in the laboratory and after 24 and 48 h in the greenhouse. Leaves were examined for oviposition immediately after the last recording. Essential oils from *A. tatarinowii* showed the most insecticidal and anti-oviposition activity for all recording times in both the laboratory (41.3, 56.9, 85.6, and 95.6% mortality, respectively) and the greenhouse (58.3 and 80.8% mortality, respectively), followed by *H. hemsleyanum* and *S. japonica*. Based on our study, all 3 essential oils possess contact and fumigant toxicity and anti-oviposition properties against female whiteflies.

Key Words: tomato whitefly; anti-oviposition activity; laboratory; greenhouse

Resumen

Se examinaron los aceites esenciales extraeidos con etanol de acoro *Acorus tatarinowii* Schott (Acoraceae), vaca chirivía *Heracleum hemsleyanum* Diels (Apiaceae) y el espárrago silvestre *Stemona japonica* (Blume) Miq. (Stemonaceae) para su toxicidad de contacto, su efecto fumigante y su potencial de disuadir la oviposición de *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) en condiciones de laboratorio e invernadero desde octubre hasta diciembre del 2015. Para determinar la toxicidad como fumigante, adultos de la mosca blancas fueron probados en frascos de vidrio herméticos que contiene el aceite esencial sobre papel de filtro. Las tasas de mortalidad se registraron a 2, 4, 6, y 8 horas de la exposición. El aceite esencial de *A. tatarinowii* fue el tóxico más fuerte, con tasas de mortalidad del 20,4, 37,1, 73,3 y 98,8%, respectivamente, seguido de *S. japonica* y *H. hemsleyanum*. Para la prueba de toxicidad por contacto, las hembras fueron liberadas en una jaula que contiene las hojas de tomate tratadas con el aceite esencial en el laboratorio, y las hembras fueron liberadas en una jaula con plantas de tomate rociadas con el aceite esencial en el invernadero. Se examinaron las tasas de mortalidad después de 6, 12, 18, y 24 horas en el laboratorio y después de 24 y 48 horas en el invernadero. Las hojas fueron examinadas para la oviposición inmediatamente después del último registro. Los aceites esenciales de *A. tatarinowii* mostraron la mayor actividad insecticida y anti-oviposición para todos los tiempos de registro, tanto en el laboratorio (41.3, 56.9, 85.6, y 95.6% de mortalidad, respectivamente) y el efecto invernadero (el 58,3 y el 80,8% de mortalidad, respectivamente), seguido por *H. hemsleyanum* y *S. japonica*. Basado en nuestro estudio, los 3 aceites esenciales poseen propiedades de contacto y fumigante toxicidad y anti-oviposición contra las hembras de moscas blancas.

Palabras Clave: mosca blanca de tomate; actividad anti-oviposición; laboratorio; invernadero

The silverleaf whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), is a serious pest of agricultural crops and ornamental plants in fields and greenhouses throughout the World (Zhang et al. 2007). Both adults and nymphs feed on phloem, resulting in chlorosis in green plants (Cohen et al. 1998). They also excrete honeydew, which promotes the growth of sooty mould and disturbs normal photosynthesis. Indirectly, adults transmit viruses such as Melon yellows virus (MYV) and Tomato yellow leaf curl virus (TYLCV) (Nuez et al. 1999).

The presence of a waxy layer on the whitefly's body resists chemical insecticides penetration (James 2003), which makes it difficult to achieve effective control. Nevertheless, irrational applications of chemical insecticides are widely used in open fields and greenhouses while managing whitefly infestations. Excessive application of synthetic insecticides leads to several problems in the environment, besides causing resistance in the development stages of pests (Palumbo et al. 2001). Furthermore, natural enemies of *B. tabaci* suffer due to frequent pesticide applications (Gonzalez-Zamora et al. 2004), which limits the ability of these natural enemies to manage heavy whitefly infestations. Thus, there is an urgent need to develop effective control alternatives that are environmentally friendly and harmless to humans and other non-target organisms.

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Plant essential oils have been shown to have potential for development as eco-friendly alternative to chemical insecticides, and they may offer advantages over conventional insecticides in terms of low mammalian toxicity, rapid degradation, and local availability (Regnault-Roger et al. 2012). Several essential oils have been reported to have multiple modes of action like repellent, contact, fumigant, and fungicidal properties (Isman 2000). Essential oils are relatively non-toxic to mammals and fish, and they meet the criteria for "reduced risk" pesticides. Most of these oils are environmentally non-persistent and non-toxic to humans (Hjorther et al. 1997). However, there is not enough information on the contact and fumigant toxicity of the essential oils of *Acorus tatarinowii* Schott (Acoraceae), *Heracleum hemsleyanum* Diels (Apiaceae), and *Stemona japonica* (Blume) Miq. (Stemonaceae) to *B. tabaci*. Thus, we studied the contact and fumigant toxicity of these 3 aromatic plant essential oils to whiteflies under laboratory and greenhouse conditions using a screen cage method, and we quantified the repellent effect of these essential oils on oviposition of whiteflies.

Materials and Methods

CULTURE OF TEST INSECTS AND HOST PLANTS

A colony of *B. tabaci* was maintained on tomato plants for 1 yr without any application of chemical pesticides in a greenhouse. The toxicity of botanical oils to whiteflies and their anti-oviposition effect were studied during Oct to Dec 2015 in the Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory at Huazhong Agricultural University, China (30.59°N, 114.30°E; 23.3 m asl). Environmental conditions were 25 ± 2 °C, 50 ± 5% RH, and a 14:10 h L:D photoperiod in the laboratory and 25 ± 5 °C and 60 ± 10% RH in the greenhouse.

The susceptible tomato cultivar 'Xian Zao Hong' was used to culture the colony of *B. tabaci* and to conduct the bioassays. Plastic pots (15 cm diameter × 13 cm height) filled with 1.5 kg of soil were used to cultivate individual tomato seedlings. When the plants had 35 to 40 fully expanded leaves (7–8 branches, at 40 d after germination), they were ready for use in the greenhouse experiments. In addition, single leaves were used to test contact toxicity in the laboratory experiments.

ESSENTIAL OILS

Samples were extracted according to the methods described by Su et al. (2009) and Yao et al. (2011). The plant materials such as rhizomes of *A. tatarinowii* and roots of *H. hemsleyanum* and *S. japonica* for producing the botanical oils were purchased from a franchised outlet of Beijing Tongrentang Group, China. Before extraction, plant materials were dried in an oven at 45 °C up to 3 d. After being fully dried, the materials were crushed with an electric shredder and sieved through a size 40 mesh. Samples were then added to 5 times their weight of 95% ethanol (1 g powder with 5 mL ethanol), kept in the dark at 20 to 25 °C for 7 d, and shaken on a vortex twice a day. The solvent was subsequently filtered with a Buchner funnel, the filtrate was collected and preserved at room temperature, and the residue was re-extracted in 95% ethanol (1 g powder with 2.5 mL ethanol) and kept in the dark for 7 d. Filtrates from the 1st and 2nd extractions were combined and concentrated to dryness with a rotary evaporator, until no more droplets were coming off the samples.

The extracts were then weighed (29.22 g final essential oil from 414.68 g powder of *A. tatarinowii*, 22.29 g essential oil from 509.44 g powder of *H. hemsleyanum*, and 26.53 g essential oil from 400.91 g powder of *S. japonica*) and stored in brown collection bottles at 4 °C in a refrigerator. For the experiments, 0.05 g of crude oils were dissolved in 0.3 mL dimethyl sulfoxide (DMSO) + 1% Tween-20®, and the volume was brought to 5 mL by adding distilled water to get a final concentration of 10,000 ppm. A solution of 0.3 mL DMSO + 1% Tween-20® in a final volume of 5 mL distilled water was used as control.

LABORATORY EXPERIMENTS

Fumigant Toxicity

To determine the fumigant toxicity of essential oils, 0.1 mL of prepared oil was uniformly applied on 6 cm diameter discs of Whatman N° filter paper by using a micropipette. An equivalent volume of DMSO–Tween-20® solution was applied as a control. When the liquids had dried, the filter papers were attached to the bottoms of airtight glass jars (200 mL). Thirty 5-d-old adult whiteflies were aspirated into a collecting vial and released into the jars, and caps were kept tight on the jars. Jars were kept in the laboratory at 25 °C, 50% RH, and a 14:10 h L:D photoperiod. The insects had no contact with the filter paper throughout the experiments. Mortality rates were recorded after 2, 4, 6, and 8 h of exposure. The experiment was repeated twice, and 8 replications were performed for each essential oil and for the control.

Contact Toxicity

The prepared essential oils (0.1 mL of each) were applied uniformly on both sides of a tomato leaf with a Chinese brush, and DMSO–Tween-20® solution was applied as a control. When the liquids had dried, the leaves were placed in a cage (20 × 12 × 12 cm), so that the leaf petioles were submerged in water located under the cage. Twenty 5-d-old *B. tabaci* female adults were released in the cages. After 6, 12, 18, and 24 h, the numbers of live adults in each cage were counted, and after 24 h, the leaves were examined under a stereomicroscope (3075 Stereo Microscope Series) to locate the eggs. The number of eggs laid on each leaf surface was recorded. These experiments were conducted in an air-conditioned laboratory, with temperature set at 28 °C, 65 ± 5% RH, and a photoperiod of 14:10 h L:D. The experiment was repeated twice with a total of 8 replicates per treatment.

GREENHOUSE EXPERIMENT

Tomato plants with 35 to 40 fully expanded leaves (7–8 branches, at 40 d after germination) were sprayed with handheld sprayers. The essential oils (10 mL of each) were sprayed on treatment plants, and the same amount of DMSO–Tween-20® solution was sprayed on the control plants. When the liquids had dried, the potted plants were placed in an insect-proof cage (90 × 80 × 60 cm). Fifty 5-d-old female adults from the whitefly colony were collected and released into the cage. After 24 and 48 h of exposure, the plants were examined, and the number of live whiteflies was recorded. Fifteen leaves were removed randomly on each plant and examined using a stereomicroscope to record egg numbers. The experiment was repeated twice at different times, with 8 replicates per treatment.

STATISTICAL ANALYSES

One-way analysis of variance (ANOVA) was conducted using the SPSS 20.0 software package to analyze differences in toxicity. Differences among means were compared with Tukey's honest significant difference (HSD) test, and a $P \leq 0.05$ was considered significant. The percentage data were arcsine–square root transformed, and all count data were square root ($x + 1$) or log10 ($x + 1$) transformed before being subjected to data analysis. The untransformed means are presented in the results.

Results

LABORATORY EXPERIMENTS

Contact Toxicity

The insecticidal activity of the essential oils of *A. tatarinowii*, *H. hemsleyanum*, and *S. japonica* were tested against adult females of *B. tabaci*. The data indicate that all of the essential oils showed contact toxicity to *B. tabaci* during all experimental periods in the laboratory test. Among the tested oils, *A. tatarinowii* oil had the highest toxicity level at 12, 18, and 24 h of exposure, causing mortality rates of 56.9%, 85.6%, and 95.6%, respectively (Table 1). Compared with *A. tatarinowii* oil, essential oil of *H. hemsleyanum* showed similar contact toxicity at 6 h of exposure (causing 43.1% mortality), but its toxicity was reduced and maintained at the second highest toxicity level at 12, 18, and 24 h of exposure, causing 47.5, 63.8, and 69.4% mortality, respectively. Compared with the other 2 oils, essential oil of *S. japonica* had the third highest toxicity level at all observation times, with mortality rates ranging from 31.3% at 6 h of exposure to 51.9% at 24 h (Table 1). Mortality rates in the controls were low and ranged from 1.9% at 6 h to 13.1% at 24 h (Table 1).

Fumigant Toxicity

Essential oil of *A. tatarinowii* had the strongest fumigant action among the tested oils at 4, 6, and 8 h of exposure, at which mortality rates of 61.3, 94.6, and 98.8% were recorded, respectively (Table 2). In the first 2 h of exposure, essential oils of *S. japonica* and *H. hemsleyanum* showed fumigant toxicity similar to that of *A. tatarinowii* oil, causing 23.3 and 22.1% mortality, respectively (Table 2). The fumigant toxicity of *S. japonica* oil remained the second highest during 4, 6, and 8 h of exposure (causing 40.0, 80.4, and 86.7% mortality, respectively) (Table 2). Fumigant toxicity of *H. hemsleyanum* oil was similar to that of *S. japonica* oil at 4 and 8 h of exposure but less at 6 h of exposure (causing 73.3% mortality) (Table 2). Mortality rates in the controls were low and ranged from 2.9% at 2 h to 19.6% at 8 h (Table 2).

CONTACT TOXICITY IN THE GREENHOUSE EXPERIMENT

The results from the greenhouse experiment showed that all of the essential oils were highly toxic to adult whitefly females. The mortality of the exposed insects increased over time. ANOVA of essential oil comparisons at 24 and 48 h showed that *H. hemsleyanum* oil was the most toxic oil after 24 h of exposure, causing 60.5% mortality, and *S. japonica* oil was the least toxic oil (44.0% mortality), whereas toxicity of *A. tatarinowii* oil was intermediate (58.3% mortality), statistically not different from the other 2 oils (Table 3). After 48 h of exposure, *A. tatarinowii* oil was the most toxic oil, causing 80.8% mortality, and *S. japonica* oil was the least toxic oil (69.5% mortality), whereas toxicity of *H. hemsleyanum* oil was intermediate (73.3% mortality), statistically not different from the other 2 oils (Table 3). Mortality rates in the controls were low, with 12.5% at 24 h and 17.5% at 48 h (Table 3).

OVIPOSITION DETERRENT EFFECT

All essential oils were shown to have strong anti-oviposition properties compared with the control in both laboratory and greenhouse experiments. In the laboratory test, *A. tatarinowii* and *H. hemsleyanum* oils had the strongest oviposition deterrent effect, with mean numbers of 7.00 and 7.25 eggs per leaf, respectively, 24 h after exposure, representing a 4-fold reduction compared with the control (Table 4). Females in the treatment with *S. japonica* oil laid on average 12.62 eggs per leaf, representing a 2.3-fold reduction compared with the control (Table 4).

In the greenhouse experiment, all 3 essential oils showed statistically similar anti-oviposition activity, after both 24 and 48 h of exposure (Table 4). After 24 h, mean numbers of eggs per leaf in the treatments with essential oils ranged from 10.38 to 17.12, representing a 2.1-fold to 3.4-fold reduction compared with the control (Table 4). After 48 h, mean numbers of eggs per leaf in the treatments ranged from 14.25 to 19.50, representing a 2.4-fold to 3.2-fold reduction compared with the control (Table 4).

Discussion

This is the first study to show the fumigant and contact toxicity of the essential oils of *A. tatarinowii*, *H. hemsleyanum*, and *S. japonica* to

Table 1. Contact toxicity of plant essential oils determined as percentage of whitefly mortality after 6, 12, 18, and 24 h of exposure in the laboratory experiment.

	Mortality (%)			
Treatment	6 h	12 h	18 h	24 h
Acorus tatarinowii	41.25 ± 2.45a	56.87 ± 2.30a	85.62 ± 1.99a	95.62 ± 0.62a
Heracleum hemsleyanum	43.12 ± 2.82a	47.50 ± 2.50b	63.75 ± 2.63b	69.38 ± 2.20b
Stemona japonica	31.25 ± 2.45b	37.50 ± 2.67c	48.75 ± 2.79c	51.88 ± 2.66c
Control	1.88 ± 0.91c	5.00 ± 0.94d	6.88 ± 1.61d	13.12 ± 1.88d

Data are presented as mean ± SE of 8 replications. Means within a column followed by the same letter are not significantly different. The mean numbers of adults were analyzed by 1-way ANOVA, using Tukey's HSD post-hoc test ($P < 0.05$).

Table 2. Fumigant toxicity of plant essential oils determined as percentage of whitefly mortality after 2, 4, 6, and 8 h of exposure in the laboratory experiment.

	Mortality (%)			
Treatment	2 h	4 h	6 h	8 h
Acorus tatarinowii	20.42 ± 2.55a	61.25 ± 2.59a	94.58 ± 0.88a	98.75 ± 0.61a
Heracleum hemsleyanum	22.08 ± 1.88a	37.08 ± 1.83b	73.33 ± 2.36c	83.75 ± 2.04b
Stemona japonica	23.33 ± 2.67a	40.00 ± 2.09b	80.42 ± 1.94b	86.67 ± 1.54b
Control	2.92 ± 1.33b	9.58 ± 2.04c	14.58 ± 1.25d	19.58 ± 1.33c

Data are presented as mean ± SE of 8 replications. Means within a column followed by the same letter are not significantly different. The mean numbers of adults were analyzed by 1-way ANOVA, using Tukey's HSD post-hoc test ($P < 0.05$).

Table 3. Contact toxicity of plant essential oils determined as percentage of whitefly mortality after 24 and 48 h of exposure in the greenhouse experiment.

	Mortality (%)	
Treatment	24 h	48 h
Acorus tatarinowii	58.25 ± 3.47ab	80.75 ± 1.31a
Heracleum hemsleyanum	60.50 ± 2.32a	73.25 ± 2.03ab
Stemona japonica	44.00 ± 5.86b	69.50 ± 1.95b
Control	12.50 ± 2.26c	17.50 ± 2.58c

Data are presented as mean ± SE of 8 replications. Means within a column followed by the same letter are not significantly different. The mean numbers of adults were analyzed by 1-way ANOVA, using Tukey's HSD post-hoc test ($P < 0.05$).

B. tabaci under laboratory and greenhouse conditions. Essential oil of *A. tatarinowii* showed the most lethal effect on whiteflies and had anti-oviposition activity at all times during both fumigant and contact experiments under laboratory and greenhouse conditions. Our findings support results from previous studies on *Acorus* species. For instance, various plant parts of *Acorus calamus* L. (Acoraceae) have been used in treatments such as insecticides, anti-bacterial medications, anti-fungal medications, and toxicants (Mittal et al. 2009). Liu et al. (2013) examined the essential oil of *A. calamus*, which showed contact toxicity at 100.21 µg/cm^2 and fumigant toxicity at 92.21 µg/L to *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae). In our experiments, *A. tatarinowii* oil initially was similar in toxicity to the other 2 oils; however, its toxicity persisted over time and showed the maximum lethal effect in the end, eventually killing all the insects. This fluctuation may be caused by more chronic toxicity and less acute toxicity of the essential oil of *A. tatarinowii*.

The essential oil of *H. hemsleyanum* showed insecticidal and anti-oviposition activity in all experiments. The maximum lethal effects were recorded at 73.3 and 83.8% whitefly mortality in contact and fumigant tests, respectively. Previous studies clearly indicate the toxic effects of *Heracleum* plant parts. Most members of the genus *Heracleum* contain furanocoumarins and are known to be insect repellents that suppress the growth of several insect species (Moore & Debboun 2006). Essential oils from *Heracleum* species are ingredients of various commercially available insecticides, and they can be used as safe fumigants for controlling *P. interpunctella* (Ebadollahi & Ashouri 2011). Plants of the genus *Heracleum* can have toxic effects on humans and other organisms; if the sap comes in contact with skin, it can cause severe phytophotodermatitis (Wikipedia 2016). In our experiment, essential oil from *H. hemsleyanum* showed more acute and less chronic toxicity, and it was more effective upon contact than oil of *S. japonica*. Its insecticidal properties and anti-oviposition activity shown in this study prove its potential as an insecticide ingredient against whiteflies.

Table 4. Effect of plant essential oils on whitefly oviposition determined after 24 and 48 h of exposure in the laboratory and greenhouse experiments.

	Number of eggs deposited		
Treatment	24 h, laboratory	24 h, greenhouse	48 h, greenhouse
Acorus tatarinowii	7.00 ± 0.53c	10.38 ± 1.00b	14.25 ± 0.92b
Heracleum hemsleyanum	7.25 ± 0.45c	17.12 ± 0.95b	19.50 ± 1.43b
Stemona japonica	12.62 ± 0.46b	12.87 ± 1.01b	15.75 ± 1.25b
Control	28.37 ± 2.34a	35.75 ± 3.70a	46.13 ± 3.87a

Data are presented as mean ± SE of 8 replications. Means within a column followed by the same letter are not significantly different. The mean numbers of eggs were analyzed by 1-way ANOVA, using Tukey's HSD post-hoc test ($P < 0.05$).

The *S. japonica* essential oil maintained fumigant and contact toxicity and anti-oviposition activity at all times. Previous research has also revealed its insecticidal activity. *Stemona japonica* is one of the original plants in the Chinese Pharmacopoeia. As a traditional Chinese medicine, it has been used as an antitussive and an insecticidal agent (Greger 2006). Keys (1976) states that *S. japonica* tubers contain an alkaloid called stemonine, which is toxic and strongly effective against *Pediculus humanus capitis* (De Geer), *Pediculus corporis* (De Geer) (Phtiraptera: Pediculidae), and *Pthirus pubis* (L.) (Phtiraptera: Phtiridae) without causing irritation or toxicity to those handling the tubers. In our experiments, essential oil from *S. japonica* showed more acute and less chronic toxicity similar to essential oil from *H. hemsleyanum*. Regarding previous findings and results from our study, the lethal and anti-oviposition effects of *S. japonica* essential oil on whiteflies support its usefulness as an insecticide.

We here report for the first time that essential oils from *A. tatarinowii, H. hemsleyanum*, and *S. japonica* have contact and fumigant toxicity and oviposition deterrent properties against *B. tabaci* adults. We determined that essential oil from *A. tatarinowii* possessed the strongest toxicity—followed by oils of *H. hemsleyanum* and *S. japonica*—in laboratory and greenhouse experiments. Furthermore, all 3 essential oils significantly reduced oviposition in all experiments. These essential oils do not persist in nature, and no detrimental effects were found in the treated plants; therefore, it could be said that these essential oils are eco-friendly and could be used in integrated pest management programs. Further research is needed on the action of individual chemical constituents under laboratory and greenhouse conditions.

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