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Evaluation of fumigant toxicity of essential oils of Chinese medicinal herbs against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)

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ABSTRACT
Essential oils of sixteen Chinese medicinal plants were screened for fumigant toxicity against *Bemisia tabaci*. The essential oil of *Allium sativum* exhibited the strongest fumigant activity against *B. tabaci* with an LC_{50} value of 0.11 µg/l followed by the essential oils of *Agastache rugosa* (LC_{50} = 7.08 µg/l), *Illicium verum* (LC_{50} = 8.61 µg/l), *Chenopodium ambrosioides* (LC_{50} = 8.80 µg/l), and *Syzygium aromaticum* (LC_{50} = 13.54 µg/l). Three essential oils (*Curcuma aeruginosa, Schizonepeta tenuifolia* and *Valeriana officinalis*) did not show fumigant toxicity at a concentration of 50.00 µg/l. Chemical composition of garlic essential oil was determined using GC-MS. Two main constituents of the garlic oil, diallyl trisulfide and diallyl disulfide exhibited strong fumigant toxicity against the whitefly with LC_{50} values of 0.08 µg/l and 0.12 µg/l, respectively while diallyl sulfide has a LC_{50} value of 1.68 µg/l.

Keywords: *Bemisia tabaci*; *Allium sativum*; fumigation; essential oil composition, allyl trisulfide; diallyl disulfide.

1. Introduction
The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a major pest of economically important crops worldwide [1]. In China, it has been recorded since 1940's, but was not considered as a serious pest until recent years [2]. However, since 1996 it has been regarded as a serious pest of cotton, vegetables and ornamental crops in 22 provinces or cities in mainland China. Vegetable growers observed regular outbreaks and up to 70% losses in cucumber from *B. tabaci* infections in the Beijing districts [2]. The development of resistance of this pest to existing conventional pesticides and the increasing public concern over environmental pollution and health hazards created by synthetic pesticides generate a great need for new types of pest control agent's advantage with higher activity against the target pests, and lower impact on humans and environmental quality. The use of biologically based compounds in plant extracts or essential oils may be an alternative to currently used insecticides to control insects [3, 4]. Moreover, essential oils have a broad spectrum of insecticidal activity due to the presence of several modes of action, including repellent and antifeedant activities, inhibition of molting and respiration, reduction in growth and fecundity, cuticle disruption and effect on the invertebrate octopamine pathway [3, 5]. Essential oils derived from plants may also have minimal direct and/or indirect effects on natural enemies [6, 7]. Essential oils and aqueous extracts of some medicinal plants have been evaluated for insecticidal activity against the sweet potato whitefly (*B. tabaci*) and some of them show potential to be developed as natural insecticides in the control of the whitefly [8-22].
Several essential oils derived from Chinese medicinal herbs have been screened for repellency and insecticidal activities against grain storage insects and cockroaches as well as mosquitoes [23-28]. However, little information on fumigant activity of the essential oils of Chinese medicinal herbs against the sweet potato whitefly was available. In this study, we assessed fumigant activity of 16 essential oils derived from Chinese medicinal herbs/spices against the whitefly, *B. tabaci*. Moreover, the two main constituent compounds of garlic essential oil (the strongest fumigant toxicity among the essential oils tested) were also evaluated for fumigant activity against the sweet potato whitefly.

2. Materials and Methods
2.1 Insects
Colonies of the sweet potato whitefly, *B. tabaci* were established from individuals collected from sweet potato plants in a greenhouse (25-28 °C) of the Institute of Plant Protection, Fujian

Academy of Agricultural Sciences, Fuzhou, China and were maintained on sweet potato plants. To obtain uniform age in insects for bioassay studies, vigorous *B. tabaci* adults were selected from the population to produce eggs on potted, caged, insect-free plants for 36 h. The adults were removed to allow eggs of uniform age to develop in a growth chamber at 24.5-25.5 °C, 70-75 relative humidity (R.H.) and 16:8 h light: dark photoperiod for a certain period to obtain vigorous two-day-old adults.

2.2. Chinese medicinal herbs

Garlic (*Allium sativum*) was purchased fresh from a local supermarket in Beijing. The other medicinal herbs (Table 1) were obtained from Guangming Chinese Medicinal Herb Decoction Company (Hebei 071200, China). All the medicinal herbs were identified by Dr. Liu, QR (College of Life Sciences, Beijing Normal University, Beijing, China) and the voucher specimens were deposited in the museum of Department of Entomology, China Agricultural University, Beijing, China.

Table 1: Chinese medicinal herbs used for fumigant activity

Botanical name	Common name	Family	Plant material
Acorus tatarinowii Schott	Sweet flag	Araceae	Rhizomes
Agastache rugosa (Fisch et Mey) O. Ktze	Korean Mint	Labiatae	Aerial parts
Allium sativum L.	Garlic	Lilliaceae	Bulbs
Alpinia officinarum Hance	Lesser galangal	Zingiberaceae	Rhizomes
Cuminum cyminum L.	Cumin	Umbelliferae	Fruits
Curcuma aeruginosa Roxb.	Pink and blue ginger	Zingiberaceae	Rhizomes
Chenopodium ambrosioides L.	Mexican tea	Chenopodiaceae	Aerial parts
Cnidium monnieri (L.) Cusson	Monnier's snow parsley	Umbelliferae	Seeds
Elsholtzia ciliata (Thunb.) Hyland	Vietnamese balm	Labiatae	Aerial parts
Foeniculum vulgare Mill.	fennel	Umbelliferae	Fruits
Illicium verum Hook.f.	Star anise	Magnoliaceae	Fruits
Schizonepeta tenuifolia (Benth.) Briq.	Japanese mint	Labiatae	Flowers
Syzygium aromaticum (L.) Merr. et Perry	Cloves	Myrtaceae	Flower buds
Valeriana officinalis L.	Valerian	Valerianaceae	Rhizomes
Zanthoxylum bungeanum Maxim.	Pricky ash	Rutaceae	Fruits
Z. schinifolium Sieb. et Zucc	Green prickly ash	Rutaceae	Fruits

2.3. Essential oil extraction

Fresh garlic and lesser galangal and other dried spices (grinded to powdered form using a grinding mill) were subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulfate was used to remove water after extraction. Essential oils were stored in airtight containers in a refrigerator at 4 °C for subsequent experiments. Diallyl disulfide, diallyl trisulfide and diallyl sulfide were purchased from Aladdin-reagent Company (Shanghai, China).

2.4. Fumigant toxicity

Desiccators with a capacity of 4l were used as test chambers. Adults of *B. tabaci* on fresh leaves of sweet potato were exposed separately to the essential oils of Chinese medicinal herbs and constituent compounds of garlic oil, respectively. The leaves infested with *B. tabaci* were brought to desiccators. In order to maintain turgor of leaves, their petioles were dipped through a hole made in a rubber cap fitted to 15 ml capacity glass vials filled with tap water. The control consisted of a similar setup but without essential oils/compounds. Each replicate consisted of 20 *B. tabaci* adults placed on one leaf. For each dose, five replicates were used. The essential oils/compounds were applied with an automatic pipette on a blotting paper strip (6 cm×3 cm) attached to the bottom of the desiccators. Initial testing was establishing appropriate dose ranges and the six concentrations were used. No material was applied to the control desiccators. Mortality of insects was observed after 24 h.

2.5. GC-MS analysis

Gas chromatographic analysis was performed on an Agilent 6890N Gas Chromatograph (Agilent Technologies, Santa Clara, USA) while the essential oils were identified on an Agilent Technologies 5973N mass spectrometer. It was equipped with a flame ionization detector and HP-5MS (Agilent Technologies, Santa Clara, USA) capillary column (30 m × 0.25 mm × 0.25 μm). The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and increased at 10 °C/min to 180 °C, hold for 1 min, and then ramped at 20 °C/min to 280 °C, hold for 15 min. The injector temperature was maintained at 270 °C. The samples (1 µl, diluted 1:100 in acetone) were injected, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 ml/min. Spectra were scanned from *m/z* 20 to 550 at 2 scans/s. Most constituents were identified using gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [29]. Component relative percentages were calculated on GC peak areas.

2.6. Statistical analysis

The observed mortality data were corrected for control mortality using Abbott's formula. The results from all replicates were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LC_{50} values with their fiducial limits [30]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

3. Results and Discussion

3.1. Chemical composition of the essential oil

The yield of *A. sativum* essential oil was 1.83% (V/W) and the density of the essential oil was determined to be 1.05 g/ml. The chemical composition of the essential oil was summarized in Table 2. A total of 17 components of the essential oil were identified, accounting for 98.90% of the total oil. The principal compound in the essential oil was diallyl trisulfide (61.87%) followed by diallyl disulfide (19.47%), diallyl sulfide (4.41%) and diallyl tetrasulfide (3.67%).

Table 2: Chemical constituents of the essential oil derived from *Allium sativum* bulbs

Peak no.	Compounds	RI	Composition (%)
1	Dimethyl disulfide*	740	0.28
2	Diallyl sulfide*	848	4.41
3	Allyl isothiocyanate	890	0.49
4	Allyl methyl disulfide	915	1.46
5	Dimethyl trisulfide	975	1.38
6	1,3-Dithiane	1027	0.84
7	Diallyl disulfide*	1077	19.47
8	Linalool*	1097	0.11
9	Allyl methyl trisulfide	1134	0.69
10	Borneol*	1165	0.37
11	Methyl propyl trisulfide	1168	0.53
12	α-Terpineol*	1188	1.02
13	Dimethyl tetrasulfide	1224	0.24
14	Diallyl trisulfide*	1296	61.87
15	Diallyl thiosulfinate	1325	1.04
16	Allyl methyl tetrasulfide	1386	1.03
17	Diallyl tetrasulfide	1540	3.67
	Total		98.90

RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons. *Identification by co-injection of authentic compounds.

3.2. Fumigant toxicity of the essential oils

Among 16 essential oils tested (Table 3), the essential oil of *A. sativum* exhibited the strongest fumigant activity against *B. tabaci* adults with an LC_{50} value of 0.11 µg/l followed by the essential oils of *A. rugosa* (LC_{50} = 7.08 µg/l), *I. verum* (LC_{50} = 8.61 µg/l), *C. ambrosioides* (LC_{50} = 8.80 µg/l), *S. aromaticum* (LC_{50} = 13.54 µg/l) and *C. monnieri* (LC_{50} = 19.31 µg/l) (Table 3). The other essential oils of *A. tatarinowii*, *Z. bungeanum*, *Z. schinifolium*, *A. officinarum*, *E. ciliate*, and *C. cyminum* also possessed fumigant toxicity against *B. tabaci* adults with LC_{50} values ranged from 21.83 µg/l to 31.17 µg/l. However, the other three essential oils (*C. aeruginosa, S. tenuifolia* and *V. officinalis*) did not show fumigant toxicity at a concentration of 50.00 µg/l. The essential oil of *A. sativum* possessed almost 64 times stronger fumigant activity than the second active essential oil of *A. rugosa* (Table 3). In previous reports, the essential oil of *A. sativum* possessed fumigant activity against adult German cockroaches, *Blattella germanica* [31], larval sciarid fly, *Lycoriella ingénue* [32], the Japanese termite (*Reticulitermes speratus*) [33], pear pear psylla (*Cacopsylla chinensis*) [34], and several grain storage insects (*Ephestia kuehniella*, *Sitophilus oryzae*, *S. zeamais* and *Tribolium castaneum*) [35-37]. The essential oils of *A. rugosa*, *I. verum*, *C. ambrosioides*, and *S. aromaticum* have been also demonstrated to possess fumigant activity against several insect pests, e.g. head lice (*Pediculus humanus capitis*) [38], larval sciarid fly, *L. ingénue* [32], adult cigarette beetle (*Lasioderma serricorne*) [39], adults and larvae of the pulse beetle (*Callosobruchus chinensis*) [40], and adults of the maize weevil (*S. zeamais*) [41].

Table 3: Fumigant toxicity of the essential oils derived from Chinese medicinal herbs and constituent compounds of garlic oil against the adult whitefly

Treatment	LC_{50} (µg/l air)	95% fiducial limits	Slope ± SD	Chi square (χ^2)
A. tatarinowii	21.83	19.95-24.07	1.33 ± 0.18	17.48
A. rugosa	7.08	5.88-8.24	1.85 ± 0.11	18.61
A. sativum	0.11	0.09-0.14	1.23 ± 0.11	7.89
A. officinarum	25.77	22.89-28.27	2.78 ± 0.17	13.08
C. cyminum	30.17	26.98-33.98	9.58 ± 0.85	13.12
C. aeruginosa	>50.00	-	-	-
C. ambrosioides	8.85	8.16-9.87	2.70 ± 0.15	18.65
C. monnieri	19.31	18.04-21.26	2.13 ± 0.12	9.45
E. ciliata	26.64	24.23-28.54	2.56 ± 0.19	12.57
F. vulgare	10.86	9.50-12.35	2.87 ± 0.21	18.34
I. verum	8.61	7.34-9.41	2.68 ± 0.19	13.89
S. tenuifolia	>50.00	-	-	-
S. aromaticum	13.54	11.93-14.79	2.70 ± 0.19	13.57
V. officinalis	>50.00	-	-	-
Z. bungeanum	23.76	21.55-26.16	4.78 ± 0.27	12.36
Z. schinifolium	24.37	20.65-26.55	4.12 ± 0.29	9.12
diallyl disulfide	0.12	0.11-0.14	1.37 ± 0.09	9.37
diallyl trisulfide	0.08	0.07-0.09	1.19 ± 0.10	12.34
diallyl sulfide	1.68	1.55-1.81	1.78 ±0.19	13.45

LC_{50}, median lethal concentration; SD, standard deviation

The above findings suggest that the fumigant toxicities of these mentioned essential oils of Chinese medicinal herbs especially the essential oil of *A. sativum* are quite promising. They showed potential to be developed as possible natural fumigants for the control of the whitefly by considering the currently used synthetic fumigants (insecticides) because of being highly toxic to humans and other non-target organisms. Moreover, most of these Chinese medicinal herbs are widely cultivated and easily grown. However, for the practical application of the essential oils and their constituent compounds as novel fumigants, further studies on the safety of the essential oils/compounds to humans and plants are needed. A further study is also necessary to determine the toxicity of these essential oils and their constituent compounds on other economically important pests and their natural enemies in greenhouse conditions where pest management depends on chemical applications. Further studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce cost.

3.3. Fumigant toxicity of the constituents

The two main constituents of *A. sativum* oil, diallyl trisulfide and diallyl disulfide exhibited strong fumigant toxicity against the whitefly *B. tabaci* adults with LC_{50} values of 0.08 µg/l and 0.12 µg/l, respectively, while diallyl sulfide has a LC_{50} value of 1.68 µg/l. Diallyl trisulfide shows stronger fumigant activity than the crude essential oil and diallyl disulfide. Moreover, diallyl trisulfide exhibited 20 times stronger fumigant toxicity against the whitefly than another constituent compound, diallyl sulfide (Table 3). It suggested that the fumigant activity of the essential oil of *A. sativum* may be attributed to diallyl trisulfide. In the previous reports, diallyl trisulfide has been demonstrated to exhibit strong fumigant activity against the maize weevil (*S. zeamais*), rice weevil (*S. oryzae*) and red flour beetle (*T. castaneum*) [35, 42]. Diallyl trisulfide was also shown to have stronger fumigant toxicity than diallyl disulfide against the Japanese termite (*R. speratus*) [31]. Huang *et al.* [35] also demonstrated that diallyl trisulfide was strongest fumigant constituent in the essential oil of *A. sativum*. However, Tunaz *et al.* [31] found that allyl isothiocyanate derived from the essential oil of *A. sativum* exhibited fumigant toxicity against adult German cockroaches (*B. germanica*) with an LC_{50} value of 0.68 µl/l and allyl isothiocyanate also exhibited stronger fumigant activity than diallyl disulfide against larval sciarid fly (*L. ingénue*) [32]. However, allyl isothiocyanate was not detected from the essential oil of *A. sativum* in present study. Allyl isothiocyanate mainly comes from the cruciferous vegetables, e.g. seeds of black mustard (*Brassica nigra*) or brown Indian mustard (*Brassica juncea*) and only minor components in the essential oil of *A. sativum* [35, 43]. Moreover, diallyl disulfide derived from garlic essential oil exhibited strong larvicidal activity against *Culex pipiens* larvae [44].

4. Conclusions

Thirteen of sixteen essential oils of Chinese medicinal herbs and three main constituents of the essential oil of *A. sativum* demonstrated some fumigant toxicity against the whitefly. They showed potential to be developed as possible natural fumigants for control of the whitefly but needs to be further evaluated for safety in humans and to enhance its activity.

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