Effects of plant essential oils on immature and adult sweetpotato whitefly, *Bemisia tabaci* biotype B

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**Abstract**

Effects of essential oils derived from garden thyme, *Thymus vulgaris* L., patchouli, *Pogostemon cablin* (Blanco) Benth., and lemon-scent gum, *Corymbia citriodora* (Hook.) K. D. Hill & L. A. S. Johnson, on mortality of eggs, first-instar nymphs, and pupae, and on adult oviposition, of *Bemisia tabaci* (Gennadius) biotype B were determined under laboratory conditions. Three concentrations of essential oils, 0.125%, 0.25% and 0.5% (v/v), were applied in separate experiments, 0.5% essential oil treatment was applied for repellency. Greater mortality was observed with increasing dose of essential oils. No phytotoxicity was observed on plants treated with these essential oils. First-instar nymphs were more sensitive to essential oil treatments, compared with eggs and pupae. The greatest effect was found with essential oil extracted from *T. vulgaris*, which reduced the survival rate of *B. tabaci* by 73.4%, 79.0% and 58.2% after treatment of eggs, nymphs and pupae, respectively, as compared with controls. In no-choice tests, the cumulative survival rates of *B. tabaci* females treated with *T. vulgaris*, *P. cablin* and *C. citriodora* were 46.4%, 38.8% and 26.8% lower, respectively, as compared with controls. In choice tests, the mean numbers of eggs laid on *P. cablin*, *T. vulgaris* and *C. citriodora*-oil-treated plants were 74.5%, 59.0% and 48.0% fewer, respectively, than on control plants. Based on this study, essential oil derived from *T. vulgaris* possessed the greatest contact toxicity, while *P. cablin* oil exerted the strongest repellency to *B. tabaci*. Hence, these two oils could be used as effective and environmentally sustainable bio-insecticides for the control of *B. tabaci*.

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1. Introduction

The sweetpotato whitefly, *Bemisia tabaci* Gennadius biotype B (= *Bemisia argentifolii* Bellows & Perring) (Hemiptera: Aleyrodidae), is a serious pest of many ornamental and greenhouse crops throughout the world (Perring, 1996; Oliveira et al., 2001; Zhang et al., 2007). Since its invasion into China in the mid-1990s, *B. tabaci* has caused serious losses to cotton, muskmelon, various vegetables and ornamentals (Luo et al., 2002).

Adults and nymphs of *B. tabaci* feed on phloem, causing chlorosis on infested leaves (Cohen et al., 1998). Additionally, they excrete honeydew, which promotes growth of sooty mold and can transmit plant viruses such as tomato yellow leaf curl virus-Israel (TYLCV-Is) and melon yellow virus (MYV) (Schuster et al., 1996; Polston et al., 1999; Nuez et al., 1999).

Chemical control has been widely used for the management of *B. tabaci*. However, applications of chemicals have not been totally effective, partially due to the presence of waxy shelters formed by the pest. The waxy shelter resists penetration of chemicals and deters contact with the pest. The waxy shelter resists penetration of chemicals and deters contact with the pest (James, 2003). Since all *B. tabaci* feeding stages colonize the abaxial surface of leaves, it is difficult to achieve effective coverage by contact spraying. Additionally, frequent and often excessive chemical application has resulted in development of resistance against these chemicals, with subsequent population outbreaks (Palumbo et al., 2001). Moreover, all developmental stages of the pest are present on the host plants at the same time (Prabhaiker et al., 1989). Chemical applications alone are not sufficiently effective to suppress all developmental stages of the pest. Natural enemies of *B. tabaci* could be effective but populations of these...
natural enemies suffer after repeated chemical applications (Gonzalez-Zamora et al., 2004). Further, during heavy pest infestations, the activities of natural enemies alone are insufficient to prevent economic losses of the host crops.

The present situation has led to a search for efficient alternatives, such as essential oils of plants, or complementary pest control strategies. Essential oils are valuable secondary metabolites obtained through steam distillation of herbs and medicinal plants (Yatagai, 1997). These oils have been traditionally used as medicines in many countries, and as odorants in fragrances and flavor enhancers in many food products. Ancient peoples were aware of their pesticidal properties; however, it is only in recent years that these oils have been commercialized as pest control products (Isman, 2000). Most of these oils are environmentally non-persistent and non-toxic to humans (Cockayne and Gawkrodger, 1997; Hjorther et al., 1997), while being effective against several pest species (Calderone and Spivak, 1995; Obeng-Ofori et al., 1997; Kim et al., 2003; Choi et al., 2004). In China, public pressure against chemical pesticide impacts on the food supply, water and other environmental components has been strong in recent years.

Fig. 1. Mean percentages (±SE) of survival of *Bemisia tabaci* eggs, nymphs and pupae on plants treated with various concentrations of oil and Tween-20 (control) when eggs were 6 days old.

Fig. 2. Mean percentages (±SE) of survival of *Bemisia tabaci* nymphs and pupae on plants treated with various concentrations of oil and Tween-20 (control) when first-instar nymphs were 2 days old.

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Extensive prospects exist for the application of essential oils in a controlled environment, e.g., glasshouse, for production of vegetables, ornamentals, and certified organic production of high-value crops. In addition, essential oils could be used in field crops in rotation with other insecticides, lessening the total quantities of more persistent products applied over a growing season.

Essential oils derived from summer savory, *Satureja hortensis* L. (Lamiaceae), oregano, *Origanum vulgare* L. (Lamiaceae), race me catnip, *Nepeta racemosa* Lam. (Lamiaceae) and white leaved savory, *Micromeria fruticosa* L. (Lamiaceae) are effective against *B. tabaci* (Aslan et al., 2004; Çalmasur et al., 2006). Essential oil vapors caused more than 90% mortality of *B. tabaci* adults (Aslan et al., 2004; Çalmasur et al., 2006), while ginger oil had faint repellent activity to *B. tabaci* biotype B adults (Zhang et al., 2004). However, these oils are only effective against one life stage (adults) of *B. tabaci*, which is inadequate to suppress reproducing populations of this pest.

Previously, we had screened a number of essential oils for their possible efficacy against *B. tabaci* biotype B. Based on initial results, we selected garden thyme, *Thymus vulgaris* L. (Lamiaceae), patchouli, *Pogostemon cablin* (Blanco) Benth. (Lamiaceae), and lemon-scent gum, *Corymbia citriodora* (Hook.) K. D. Hill & L. A. S. Johnson (Myrtaceae) essential oils for further research. The present study aimed to further test the efficacy of these three essential oils against *B. tabaci* biotype B, and specifically aimed to determine the degree of contact toxicity of these essential oils to egg, nymphal and pupal stages of *B. tabaci* and quantify the repellent effect on female settlement and oviposition.

2. Materials and methods

2.1. Stock culture of test insects and host plants

A stock culture of *B. tabaci* biotype B was established using three hundred individuals from a colony which had been maintained on tomato plants for the last 5 years without any exposure to pesticides, and obtained from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (CAAS). The stock culture was mass-reared on tomato plants, *Solanum lycopersicum* L. var. *lycopersicum* (Solanaceae), variety Zhong-Za No. 9, in an air conditioning-equipped glasshouse, under the condition of 26 ± 2 ºC, and natural light regime (39°57′ N, 116°19′ E), at the Institute of Plant Protection, CAAS, Beijing, China. A small culture, which was transferred weekly from the greenhouse, was kept in an air-conditioned laboratory, under the condition of 26 ± 1 ºC, 65 ± 5% RH and light regime 14:10 (hours L:D). The tomato plants were maintained in insect-proof cages (60 × 60 × 60 cm).

For the experiments, tomato seeds were first grown in large plastic trays. When seedlings reached 4–5 cm height, the seedlings were transplanted into plastic pots (9 cm in diameter) and allowed to reach approximately 15 cm height with 5–7 fully expanded leaves, for use in the experiments. Two days before the initiation of experiments, older leaves were removed from the plants, while retaining the top two fully expanded leaves.

![Fig. 3. Mean percentages (±SE) of survival of *Bemisia tabaci* pupae on plants treated with various concentrations of oil and Tween-20 (control) when pupae were 2 days old.](image)

Table 1

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<td>Total</td>
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**P < 0.001 (Tukey’s HSD test).**

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2.2. Essential oils

Essential oils extracted from *T. vulgaris*, *P. cablin* and *C. citriodora* were tested. *T. vulgaris* and *P. cablin* plants and *C. citriodora* leaves were collected from various localities of Yunnan province in China. Voucher specimens were deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, China. Essential oils were extracted from the plant materials by steam distillation, and the oils yielded were kept at 4°C. Essential oils were diluted in distilled water plus Tween-20 (Amersco) (0.5%) to 0.125%, 0.25% and 0.5% (v/v) concentration before tests. These three concentrations of essential oils were applied in contact toxicity experiments. In separate experiments, 0.5% essential oil treatments were tested for repellency.

2.3. Bioassay for contact toxicity experiments

The developmental stages that were treated in toxicity experiments consisted of 6 d-old eggs, 2 d-old first-instar nymphs and 2 d-old pupae. All developmental stages of *B. tabaci* were obtained by introducing adults (100–120 unsexed per plant) on fresh tomato plants in cages. After 24 h, the adults were removed, and the eggs on the plants were allowed to develop to appropriate stages for experiments. The experiments were conducted in an air-conditioned laboratory, with temperature set at 26 ± 1°C, 65 ± 5% RH and light regime 14:10 (hours L:D). The number of *B. tabaci* individuals was counted on each test leaf before treatment. Each experiment consisted of 6–8 replications, with 100 insects per replication (a total of 600–800 insects per treatment).

2.3.1. Effect of oils on *B. tabaci* eggs

Six days after removal of the adults, the leaves with eggs (6 d old) were dipped in essential oils (0.125%, 0.25% and 0.5%) for 5 s (Zhang et al., 2004; Wang et al., 2003). For the controls, leaves with eggs were dipped in Tween-20 (0.5%). The number of nymphs was counted on 3 d after eggs were treated with oils. Subsequently, the number of pupae and adults (based on empty pupal cases) were also recorded 10 and 15 d after eggs were treated with oils, respectively.

2.3.2. Effect of oils on *B. tabaci* nymphs

Nine days after removal of the *B. tabaci* adults, the leaves with first-instar nymphs (2 d old) were treated with oils as 2.3.1. The number of pupae and adults were recorded 7 and 12 d after nymphs were treated with oils, respectively.

2.3.3. Effect of oils on *B. tabaci* pupae

Sixteen days after removal of the *B. tabaci* adults, the pupae (2 d old) were treated with oils as described in 2.3.1. The adult eclosion was determined 5 d after pupae were treated with oils.

2.4. Bioassay for repellency effect

2.4.1. No-choice test

Pairs (one adult male and one adult female) of *B. tabaci* (<2 h old) were placed on a fresh tomato plant and allowed to feed and mate for 2 d. The treated and control tomato leaves were dipped in different essential oils (0.5%), and in Tween-20 (0.5%) for 5 s. The leaves were allowed to dry for 1 h at room temperature. The
females were identified with the help of a binocular microscope and subsequently individually placed in clip cages (2.5 cm diameter × 2.5 cm height) on the abaxial surface of the top two leaves of treated or control tomato plant. One day after release, and for 4 consecutive days, females were transferred to fresh leaflets of the same plant every 24 h. Thus the observation of any one replication lasted for a total of 5 days. The numbers of eggs laid per female per 24 h, and survival of females, were recorded. The experiment consisted of 5 replications, and each replicate consisted of 10 clip cages per treatment (a total of 50 females per treatment).

2.4.2. Choice test

Experimental plants were treated as per the no-choice tests. Pairs of tomato plants (one 0.5% Tween-20—treated control and the other 0.5% oil-treated) were randomly assigned to cages (60 × 60 × 60 cm). Sixty randomly chosen B. tabaci adult females were released into each cage by placing the open vial in the center of the cage. The cumulative number of individuals settled per plant was counted 2, 4, 6, 8 and 24 h after release without interference. The adults were removed 24 h after release, leaves were removed from the plant, and the eggs were counted on both sides of the leaf surfaces under a dissecting microscope (20× magnification). The experiment consisted of 7 replications, and each replicate consisted of three cages per treatment (a total of 21 cages per treatment).

2.5. Data analysis

One-way ANOVA (SPSS 13.0 software package) was used to analyze the toxicity differences on B. tabaci survival indices (i.e., eggs, nymphs and pupae) among different dosages, for each essential oil. Percentages (p) were transformed to arcsine (p/100)0.5 before analysis. Differences among means were compared with Tukey's Honestly Significant Difference (HSD) test at α = 0.05. Two-way ANOVA was used to analyze the interactions between essential oils dosage and whitefly stage on B. tabaci. For no-choice repellency experiments, one-way ANOVA was used to analyze the difference in the numbers of eggs per female per 24 h among different essential oil treatments and control treatment, and among days after particular treatment. Tukey's HSD was used to examine the significance of the differences. For the analysis of the data on settlement or oviposition preference in repellent choice experiments, number of females or eggs of all replicates per oil or control treatment were pooled and analysed by performing a χ² test with a null hypothesis of equal distribution.

3. Results

3.1. Contact toxicity of essential oils to B. tabaci immature

3.1.1. Effect of oils on B. tabaci eggs

Different dosages of T. vulgaris (F = 164.26; df = 3, 24; P < 0.001), P. cablin (F = 93.08; df = 3, 24; P < 0.001) and C. citriodora (F = 50.13; df = 3, 24; P < 0.001) resulted in significant reductions of eggs hatchability when compared with the controls (Fig. 1). Unhatched eggs or individuals unable to come out of the egg shells were considered to be dead. As for mortalities of B. tabaci nymphs which survived from the egg treatment, all the essential oil treatments, T. vulgaris (F = 24.56; df = 3, 24; P < 0.001), P. cablin (F = 19.22; df = 3, 24; P < 0.001) and C. citriodora (F = 10.39; df = 3, 24; P < 0.001), resulted in higher mortalities of B. tabaci nymphs than the controls. Overall, when eggs were treated with essential oil from T. vulgaris, P. cablin and C. citriodora, the survival rate of B. tabaci during the entire immature period was 61–74%, 41–65% and 54–55% lower than the controls, respectively.

3.1.2. Effect of oils on B. tabaci nymphs

Essential oils from all three plants, T. vulgaris (F = 121.60; df = 3, 21; P < 0.001), P. cablin (F = 133.11; df = 3, 21; P < 0.001) and C. citriodora (F = 39.22; df = 3, 20; P < 0.001), significantly reduced development of B. tabaci nymphs into pupae (Fig. 2), whereas the proportion of emerged adults was not affected by the essential oil treatments. The survival rate of B. tabaci immatures treated with different dosages of essential oil from T. vulgaris, P. cablin and C. citriodora on nymphs was reduced by 47–79%, 23–81% and 38–54%, respectively, as compared with control.

3.1.3. Effect of oils on B. tabaci pupae

Different dosages of T. vulgaris (F = 1179.63; df = 3, 20; P < 0.001), P. cablin (F = 588.04; df = 3, 20; P < 0.001) and C. citriodora (F = 1062.01; df = 3, 20; P < 0.001) resulted in significantly higher mortalities of B. tabaci pupae, when compared with controls (Fig. 3). Some oil-treated individuals remained in the early...
pupal stage and dried out; while those that survived until the late pupal stage exuded tawny body fluid, which in some cases caused a speckled appearance on the leaf where the insects were adhered. The survival rates of \textit{B. tabaci} treated with essential oil from \textit{T. vulgaris}, \textit{P. cablin} and \textit{C. citriodora} on pupae was 55–59\%, 49–60\% and 47–66\% lower respectively, than control groups.

The efficacy of three essential oils increased with increasing concentration for all \textit{B. tabaci} stages (Table 1). This increase was most significant for \textit{T. vulgaris}-derived essential oil, followed by essential oil extracted from \textit{P. cablin} and \textit{C. citriodora}. The concentration of 0.5\% was most effective, and at this concentration, nymphs were more sensitive to essential oil treatments compared with eggs and pupae. Among the three tested oils, essential oil extracted from \textit{T. vulgaris} was the most effective, reducing the survival rate of eggs, nymphs and pupae of \textit{B. tabaci} 73.4\%, 79.0\% and 58.2\%, respectively, as compared with the control.

3.2. Repellency effect of the essential oils against \textit{B. tabaci}

3.2.1. No-choice test

As compared to controls, the average cumulative numbers of eggs per female of \textit{B. tabaci} in five days was significantly reduced by 0.5\% concentrations of \textit{T. vulgaris} (63.1\%), \textit{P. cablin} (58.5\%) and \textit{E. citriodora} (52.9\%) (\(F = 32.59\); \(d.f. = 3, 213\); \(P < 0.001\)) (Fig. 4A). In 0.5\% essential oil treatments the number of eggs laid daily increased, control (\(F = 15.70\); \(d.f. = 4, 270\); \(P < 0.001\)), \textit{T. vulgaris} (\(F = 10.50\); \(d.f. = 4, 195\); \(P < 0.001\)), \textit{P. cablin} (\(F = 13.90\); \(d.f. = 4, 209\); \(P < 0.001\)) and \textit{C. citriodora} (\(F = 9.32\); \(d.f. = 4, 214\); \(P < 0.001\)). Survival rates also decreased in all the oil treatments (Fig. 4B). The cumulative survival rates of \textit{B. tabaci} females treated with \textit{T. vulgaris}, \textit{P. cablin} and \textit{C. citriodora} were 46.4\%, 38.8\% and 26.8\% lower than the control group, respectively.

3.2.2. Choice test

There were fewer instances of \textit{B. tabaci} adults settling on essential oil-treated plants, than control plants during all observation times (Fig. 5). Fewer numbers of \textit{B. tabaci} were observed on plants treated with \textit{T. vulgaris}, \textit{P. cablin} and \textit{C. citriodora} (57.5\%, 69.3\% and 39.6\%, respectively) than on control plants at 24 h after release.

The mean numbers of eggs laid on 0.5\% \textit{T. vulgaris}, \textit{P. cablin} and \textit{C. citriodora} oil-treated plants were 59.0\%, 74.5\% and 48.0\% fewer than on control plants, respectively (Fig. 6). Also, the numbers of laid eggs/female/24 h on \textit{T. vulgaris}, \textit{P. cablin} and \textit{C. citriodora} -derived essential oil-treated plants were 0.2, 0.5 and 1.0 lower than that on control plants respectively.

4. Discussion

The results of the present study revealed that essential oils obtained from \textit{T. vulgaris}, \textit{P. cablin} and \textit{C. citriodora} can efficiently reduce egg hatchability, nymph and pupae survival and oviposition of \textit{B. tabaci} biotype B. No phytotoxicity was observed on oil-treated plants.

\textit{B. tabaci} egg hatchability was reduced \(>50\%\) with essential oils from the three plant species. Survival rates of nymph and pupae development were also significantly reduced as compared to the control. The oils caused some eggs to die without any morphological changes, while others turned into half-emerged dead crawlers. The former phenomenon was possibly due to disruption or inhibition of embryogenesis, and the latter phenomenon was attributed to the effect of essential oils on crawlers after eclosion from viable eggs, presumably when they came into contact with the residues on the egg chorion. Essential oil treatments of 6 d-old eggs caused higher mortalities of nymphs entering subsequent developmental stages compared with the control group. This was probably due to contact of emerging white nymphs with the remaining residues from essential oil treatments (Elling et al., 2002). The residual effect of essential oil treatments indicated that the non-volatile ingredients might be responsible for the insecticidal activity of these oils.

\textit{T. vulgaris} and \textit{P. cablin} oils at concentration of 0.5\% had the strongest effects on young \textit{B. tabaci} nymphs. Proportions of pupae developed and adults emerged were both reduced after oil treatments. Similar observations in reduction were seen in \textit{B. tabaci} nymphal with 0.3\% water extract of neem kernels treatment (Price and Schuster, 1991). However, the proportion of emerged adults was not affected by the neem treatment, reflecting successful pupation.

The results of choice experiments demonstrated repellent effects of the oils in terms of fewer adults alighting on the oil-treated tomato plants as compared with control. The number of \textit{B. tabaci} on leaves did not change over the course of the 24-h experiment. The adult females probably settled at the suitable feeding and oviposition sites and seldom moved afterwards (Zhang et al., 2004).

The females deposited significantly lower number of eggs on all oil-treated tomato plants. Reduced oviposition is a normal consequence if adults avoid settling on a host plant. In the present experiments, the number of eggs laid per female in 24 h on oil-treated plants was lower than in the control, in both choice and no-choice conditions. The number of eggs laid in the control in the choice experiment was comparable with that on oil-treated plants in the no-choice experiment. It is clear from the results that in choice tests, egg laying by females in the controls may have been affected by the essential oil treatments, which might be due to the fact that volatile components of the oils function as oviposition deterrents (Ngoh et al., 1998; Koschier and Sedy, 2003; Koschier et al., 2002; Pascal-Villalobos and Ballesta-Acosta, 2003).

In the no-choice experiment, the number of eggs laid on oil-treated plants was consistently lower than in the control. This might be due to the mildly persistent effect of essential oils, which caused reduced phloem sap uptake by adults with frequent changing of feeding sites, resulting in reduced numbers of mature eggs ready for deposition (Kumar et al., 2005). It is also possible that essential oil has an irreversible affect on the physical condition of females, thus reducing reproductive capacity. Rao et al. (1999) reported that \textit{Artemisia annua} oil could inhibit ovarioc development and reduce oocyte number in the treated insects, which suggests interference of the oil in egg production.

It has been reported in previous research that all essential oils tested in this study are chemically characterized as natural mixtures.
of terpenoids. The essential oil extracted from *T. vulgaris* contains thymol, p-Cymene, carvacrol, α-Pinen, linalool, myrcene, α-terpineol and 1,8-cineole; *P. cablin* derived essential oil contains patchouli alcohol, β-patchoulen and α-guaiene; and *C. citriodora* oil mainly consists of 1,8-cineole. Most of these organic compounds have been found to be poisonous to insect and mite pests (Traboulsi et al., 2002, 2005; Miresmailli et al., 2006), or repellent. Lemon-scent gum is strongly repellent to ticks (Jaenson et al., 2006) and mosquitoes (Schreck and Leonhardt, 1991), while monoterpenes derived from thyme are repellent to mosquitoes as well (Park et al., 2005).

In order to reduce the possibility of target site resistance developed by *B. tabaci* after chemical treatments, a mixture of different active compounds is suggested for *B. tabaci* control. It is probable that the pest will develop resistance more slowly to an insecticide composed of a mixture of different active compounds than to a single active ingredient (Isman, 2000). *Myzus persicae* Sulzer developed resistance to pure azadirachtin (the major ingredient of neem insecticide), but not to a refined neem seed extract containing the same absolute amount of azadirachtin together with many other constituents present (Feng and Isman, 1995).

In conclusion, essential oils from *T. vulgaris* exerted the strongest contact toxicity, while *P. cablin* oil exerted the strongest repellency to *B. tabaci*. The oils are environmentally non-toxic and safe for humans as well as other mammals. Hence, the oils can be used as bio-insecticides for the control of *B. tabaci*. However, future research needs to be conducted on compatibility of the essential oils combined with a biocontrol agent in an IPM program against *B. tabaci*. No detrimental effects of botanical insecticide UDA-245, extracted from *Chenopodium* (*Chenopodium ambrosioides* variety near ambrosioides) essential oil, was found on *Encarsia formosa* Gahan (Chiasson et al., 2004). Another important research area is to investigate ecological natural control functions of aroma plants when intercropped in main host plants on the pest. Aroma plants beefsteakplant, *Perilla frutescens* (L.) Britton and wild mint, *Mentha arvensis* L., when intercropped with tomato plants, reduced the population of *Triaurodes vaporariorum* Westwood by 39.1% and 41.5% as compared to the control, respectively (Wang et al., 2006). If *P. cablin* were intercropped with the tomato host, this might reduce the attraction of the major host to the pest, hence suppressing pest population.

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