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ANTI-OVIPOSITION AND REPELLENCE ACTIVITIES OF ESSENTIAL OILS AND AQUEOUS EXTRACTS FROM FIVE AROMATIC PLANTS AGAINST GREENHOUSE WHITEFLY *TRIALEURODES VAPORARIORUM* WESTWOOD (HOMOPTERA: ALEYRODIDAE)

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Abstract

DEHGHANI, M. and K. AHMADI, 2013. Anti-oviposition and repellence activities of essential oils and aqueous extracts from five aromatic plants against greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). *Bulg. J. Agric. Sci.,* 19: 691-696

The present study assessed the repellence and anti-oviposition activities of essential oils and aqueous extracts isolated from five aromatic plants against *T. vaporariorum*. Cucumber plants were treated with essential oils or aqueous extracts (40 µL/mL), with control plants sprayed with distilled water. Approximately 250 greenhouse whitefly adults were released in to cage. To evaluate repellent and anti-oviposition effect, 3 and 6 days after infestation, the number of eggs and adults per leaf were recorded. The highest anti-oviposition effect at 3 days after infestation occurred with essential oil of *Achillea millefolium* L. treatment. The greatest repellence effect 3 and 6 days after infestation occurred with water extracts of *Cuminum cyminum* L. and *Thymus vulgaris* L. treatment, respectively. The essential oil and aqueous extract from orange fruit peelings (*Citrus sinensis* (L.) Osbeck)) treatments produced the least repellence and antioviposition effects. These results indicate a potential use of essential oils and aqueous plant extracts in pest management of greenhouse whiteflies.

Key words: Botanical insecticides; navel orange; cumin; fennel; plant extracts; thyme; yarrow; *Trialeurodes vaporariorum*

Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), is one of most serious pests of vegetables in the world, particularly in greenhouses (Lindquist, 1972). Whiteflies damage plants through direct sap feeding (Russell, 1977), transmission of plant pathogenic viruses (Nuez et al., 1999), and honeydew excretion, a substrate for sooty mould that reduces photosynthetic and economically very harmful (Van Lenteren et al., 1995). Traditionally, whitefly management has used mainly on the synthetic insecticides, which are toxic to the environment, and caused outbreaks of whiteflies (Menn, 1996). Moreover, chemical control of whitefly is difficult because of widespread resistance against different insecticides (Gerling, 1990). Plants provide an alternative to currently used pesticides for pest control, as they are a rich source of bioactive chemicals (Kim et al., 2005) that are active against specific target species, are not toxic products, and are potentially suitable for use in integrated pest management (Tare et al., 2004). Essential oils are valuable secondary metabolites obtained through steam distillation of herbs and medicinal plants (Yatagai, 1997). Most of them have no persistence and are nontoxic to humans and environment (Hjorther et al., 1997). Moreover, those of many common spices have also been evaluated for their repellence and insecticidal effects against insect pests (Isman et al., 2000). Chaubey (2008) studied the fumigant activity of from dill (*Anethum graveolens* L.) and cumin (*Cuminum cyminum* L.) essential oils on cowpea weevil (*Callosobruchus chinensis* L.) (Bruchidae). In another experiment,

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the insecticidal property of cumin oil vapors against carmine spider mite (*Tetranychus cinnabarinus* Boisduval) and cotton aphid (*Aphis gossypii* Glover) has been determined and major components have been reported (Tuni and Sahinkaya, 1998). The insecticidal activities extracts of Korean mint (*Agastache rugosa* Fisch & C.A. Mey.) whole plant, star anise (*Illicium verum* Hook. f.) fruit and fennel (*Foeniculum vulgare* Mill.) fruit, horseradish (*Armoracia rusticana* G. Gaertn., B. Mey. & Scherb) and mustard (*Brassica juncea* L.) oils have been proved against the adults of tobacco beetle (*Lasioderma serricornae* F.) (Kim et al., 2003).

The insecticidal and repellent activities of thyme (*Thymus vulgaris* L.) have been reported against red flour beetle (*Tribolium castaneum* Herbst) (Clemente et al., 2003), rice weevil (*Sitophilus oryzae* L.) (Lee et al., 2001), Indian meal moth (*Plodia interpunctella* Hübner) (Passino et al., 2004), cotton leafworm (*Spodoptera litura* F.) (Hummelbrunner and Isman, 2001). The essential oils of thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.), fennel (*F. vulgare*), caraway (*Carum carvi* L.) and rosemary (*Rosemarinus officinalis* L.) have been reported for its insecticidal activities against greenhouse whitefly (*T. vaporariorum*) (Aroiee et al., 2005). The repellent effects of the garden cress (*Lepidium sativum* L.), yellow milfoil (*Achillea biebersteinii* L.), have been proved against sweet potato whitefly (*Bemisia tabaci* Gennadius) (Ateyyat et al., 2009). In addition, Pavela and Herda (2007) investigated the properties of pongam oil, as an antifeedant, repellent, and anti-oviposition agent against greenhouse whitefly. The current study was initiated to investigate the repellent activity and anti-oviposition effect of some plants on *T. vaporariorum*, obtained in greenhouses in Iran.

Materials and Methods

Insect cultures

T. vaporariorum was collected from experimental greenhouses of Shahid Bahonar University, Kerman, Iran and used to infest cucumber (*Cucumis sativus* L.) plants in 80 × 60 × 60 cm fine cloth cages in greenhouse condition (6×4 m) (related to local condition such as temperature (15-20°C) and humidity (60±10%) in greenhouse). To perform each test, whiteflies were collected from this stock culture with a hand aspirator.

Extraction of essential oils and aqueous extracts

The aromatic plants such as *Thymus vulgaris* (leaves) and *Achillea millefolium* (leaves and flowers) (areas: Baft, Kerman, Iran: 29°14’00.18”N 56°36’07.70”E, 2271 m (altitude)], *Foeniculum vulgare* (seed) and *Cuminum cyminum* (seed) (areas: Mahan, Kerman, Iran: 30°03’41.12”N 57°17’29.29”E, 1903 m (altitude)], *Citrus sinensis* (peel) (area: Jiroft, Kerman, Iran: [(28°40’43.64”N 57°44’25.00”E, 688 m (altitude)], were collected from different areas of Kerman province, Iran. The dried parts of each plant (100 g) to sterile distilled water were boiled by Clevenger instrument for 4 h. The extract was provided in different plant derived which the aqueous portion (aqueous phase) was isolated from the essential oil (oily phase). For the short time, these plant products were kept at 4°C in refrigerator (Traboulsi et al., 2005; Tawatsin et al., 2006).

Chemical components

Plant components were determined using GC–MS analyses on a Finnigan GCQ instrument and equipped with a 30m×0.25mm×0.25mm on with a Zebron ZB-5 column (Phenomenex, USA), using the following temperature program: initial temperature 60°C, hold for 1 min, then gradient 4°C min^{-1} to 180°C, then gradient 10°C min^{-1} to 275°C and keep 5 min at this temperature. Temperature of the transfer line was 275°C; ion source was 200°C. linear velocity of the carrier gas (helium) was 40 cm/s. Full scan spectra in the range of relative mass *m/z* 50–450 Da were obtained (Adams, 2007). The quantity of each major component was determined by comparison with a standard solution i njected under identical GC/MS conditions. Analyses of these essential oils revealed that the substances for *T. vulgaris* were α-Thujene, α –Pinene, 1-Octen-3-ol, Myrcene, α-Terpinene, p-Cymene, γ-terpinene, 1,8-Cineol (eucalyptol), cis-Sabinene hydrate, Terpinolene, Linalool, Camphore, Borneol, Terpinen-4-ol, α –Terpineol, Thymol, methyl ether, Carvacrol, methyl ether, Thymol, Carvacrol. Major components of essential oils in *C. sinensis* were Limonene, Linalyl acetate, Linalool, Bergaptene. The major substances such as Limonene, Anethole, Fenchyl acetate, *α*-Pinene, Myrcene, Estragole was obtained by *F. vulgare.* The constituents for *A. millefolium* were sabinene, 1,8-cineole, borneol, bornyl acetate, *α* –pinene, terpinine-4-ol and chamazulene. Also, β-pinene, p-cymene, 1,8-cineole, γ-terpinene, borneol, terpinen-4-ol, pulegone, anethole, carvacrole was determined by analyses of *C. cyminum* (Traboulsi, 2005; Pavela, 2009).

Repellent experiment

The repellent and anti-oviposition effects of plant products were determined using a spraying test bioassay in greenhouse condition. The experiment was carried out from 11 January to 24 February 2011 in the Experimental Agriculture Research and Natural Resource Center of Kerman, Kerman, Iran. The cucumber plants ‘Negin’ with five fully expanded leaves were used as host plant and oviposition substrate of the greenhouse whitefly. The plants were treated with extract at 40 µL/mL and control plants were sprayed with distilled water. The essential oils of plant derived above mentioned at same volume (5ml) (for avoid the phytotoxicity effect on the cucumber plant) (except *C. cyminum* and *T. vulgaris*) were sprayed on filter paper (Whatman, 9 cm diameter) (The size of filter paper was sprayed with essential oils: 2 × 4 cm). One treated filter paper was attached at the each petiole cucumber plants, via plastic string. Moreover, extract was sprayed on the plants under the same condition described above with the same volume (40 mL). Randomly, four treated plants with the plant products and four control plants were placed simultaneously in a double row of plants with 18 cm spacing between and within rows in each cabin of the experiment (80 × 60 × 60 cm). Into each cage, 250 greenhouse whitefly adults were released. From each plant, 3 and 6 d after infesting with *T. vaporariorum*, one leaf, at the same areas, were detached to evaluate the repellent and anti-oviposition effects of plant products. The numbers of the greenhouse whitefly adults and eggs on each detached cucumber leaf were recorded. At least two cages were used for each plant product. The repellency index (RI) and anti-oviposition index were calculated from the formula (Pascual-Villalobos and Robledo, 1998):

$$R = \frac{(C-T)}{(C+T)} \times 100$$

according to the amount of the adults or eggs in the control (C) and treatment (T).

Statistical analysis of data

Differences among the RI and anti-oviposition index of plant products were subjected to one-way ANOVA. Mean were compared using the Tukey test (P < 0.05). These analyses were conducted using StatPlus (version 4.9, 2007, Croydon, UK).

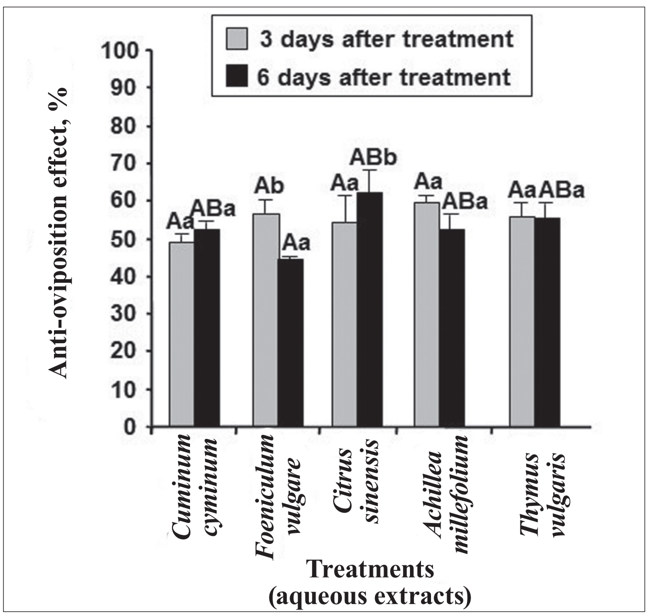
Results and Discussion

Anti-oviposition activities

All the tested plant products that affected oviposition behavior of greenhouse whitefly are shown in Figure 1. Among different plant products there were no significant differences in the anti-oviposition index 3 d after infesting (P < 0.05). The highest anti-oviposition effect after 3 d was caused by essential oil of *A. millefolium* (67.77%), while the lowest anti-oviposition index was for aqueous extract of *C. cyminum* (48.78%). Anti-oviposition effect caused by all plant products after 6 d ranged from 44.40% to 70.98% in aqueous extract and essential oil of *F. vulgare* treatments, respectively. There were significant differences (P < 0.005) among aqueous extract and essential oil of *F. vulgare* as well as *C. sinensis* treatments.

Repellence effects

The RI of different plants products is shown in Figure 2. Based on repellent effect of the plant products, the aqueous



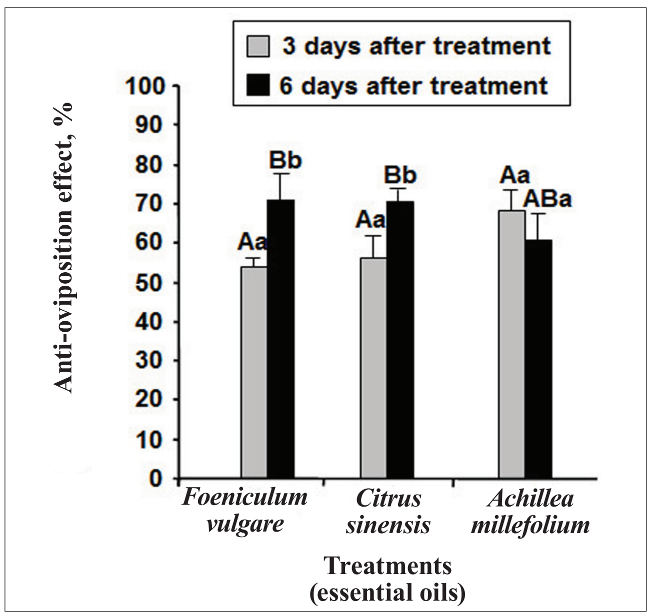


Fig. 1. Anti-oviposition activity of various plant essential oils and aqueous extracts against greenhouse whitefly *Trialeurodes vaporariorum*

Note: Bars with different small letters indicate significant differences days within the same plant derived chemical. Bars with different upper case letters indicate significant differences between the plant derived chemical within the same time periods at (P ≤ 0.05) (one-way ANOVA) extract of *C. cyminum* showed the highest repellency effect on greenhouse whitefly after 3 d (66.11%), while the RI of the essential oil of *C. sinensis* was the lowest (29.48%). The results revealed no significant different between the repellent effect of different plant products on the greenhouse whitefly after 6 d ($P < 0.05$). The aqueous extract of *T. vulgaris* had the highest repellent effect on *T. vaporariorum* after 6 d (62.46%) and the essential oil of *F. vulgare* had the lowest (40.22%).

To compare anti-oviposition effect of each plant product on greenhouse whitefly between 3 and 6 d, aqueous extract of *F. vulgare* ($P < 0.005$), essential oil of *F. vulgare* ($P < 0.05$) and essential oil of *C. sinensis* ($P < 0.05$) treatments were significantly different. In addition, the compare repellent effect of different plant products on greenhouse whitefly adults, between 3 and 6 d that indicated the aqueous extract of *C. cyminum* ($P < 0.005$), essential oil of *C. sinensis* ($P < 0.005$), aqueous extract of *A. millefolium* ($P < 0.05$) and aqueous extract of *T. vulgaris* ($P < 0.05$) had significant differences.

The search for deterrent or repellent compounds is imperative for developing less toxic pest management tools (Isman, 2006). It has been well recognized that insect repellent agents are selective to pests, have no or little harmful effects on non target organisms and the environment (Curtis et al., 1990). Many plant products and essential oils with high volatility, such as alkanes, terpenoids, alcohols, aldehydes, and etc., act on greenhouse whiteflies in the vapor phase. Some volatile compounds were effective against greenhouse whiteflies for a relatively short period. Pavela and Herda (2007) reported repellency of Pongam oil on adult greenhouse whitefly under greenhouse conditions. Zhang et al. (2004) informed the repellent effects of ginger oil against adult silver leaf whitefly *Bemisia argentifolii* Bellows & Perring on tomato under greenhouse conditions. Al-Mazra'awi and Ateyyat (2009) showed that the plant extracts roman nettle (*Urtica pilulifera* L.) and headed savory (*Thymus capitatus* L.) were repellent to *B. tabaci* adults. Repellency of *T. vulgaris* is attributed to monoterpenes such as carvacrol, alpha-terpinene, and thymol derived from the essential oil of thyme (Park et al., 2005). In the presented studies, essential oils and aqueous plant extracts specially, essential oil of *A. millefolium*, *F. vulgare* and aqueous extract of *C. cyminum* showed a high anti-oviposition and repellent effect for greenhouse whitefly. Similar result on these plant products obtained from other pests studies. For example, Kordan et al. (2003) reported that wheat weevil (*Sitophilus granarius* L.) was most effectively deterred (90%) by the powders obtained from *A. millefolium*. The results of Chaubey (2006) showed that the essential oils of *C. cyminum*, *Piper nigrum* L. and *F. vulgare* reduced the oviposition potential of the red flour beetle *Tribolium castaneum* (Herbst). In addition, Pavela (2009) has been reported that the oil of

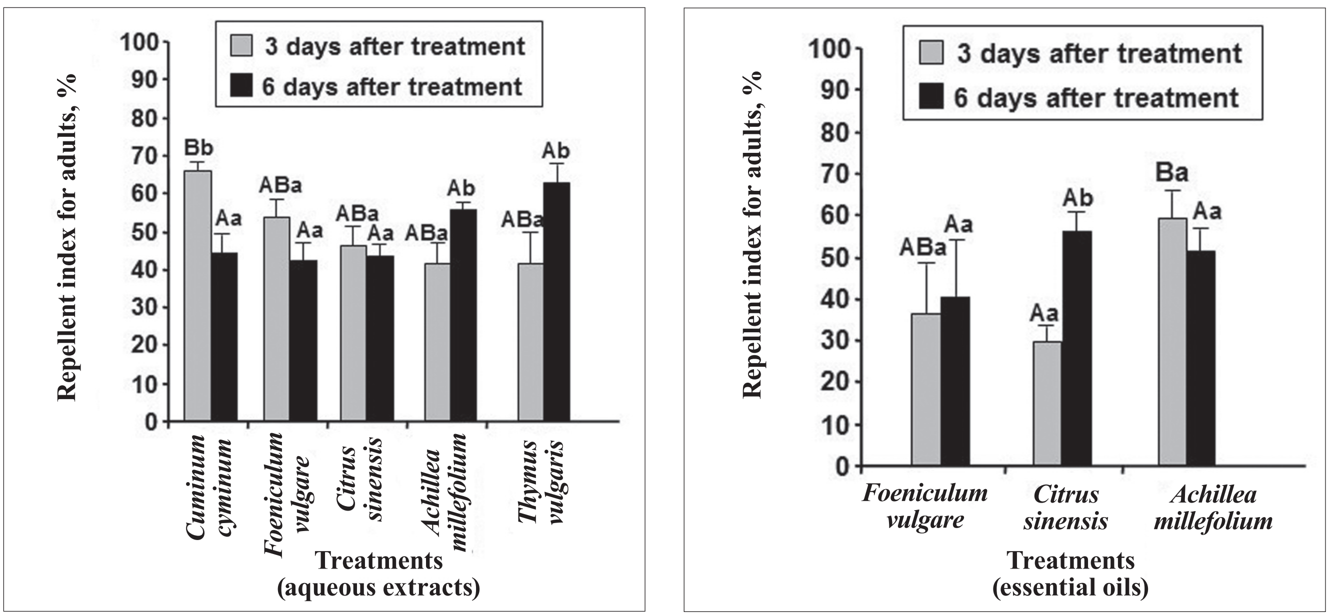


Fig. 2. Repellent activity of various plant essential oils and aqueous extracts against greenhouse whitefly *Trialeurodes vaporariorum*

Note: Bars with different small letters indicate significant differences days within the same plant derived chemical. Bars with different upper case letters indicate significant differences between the plant derived chemical within the same time periods at ($P \leq 0.05$) (one-way ANOVA)

T. vulgaris had most repellent and high anti-oviposition effect against brown house mosquito (*Culex quinquefasciatus* Say). In this experiment, the plants treated with essential oils, aqueous extracts showed relatively long lasting repellent, and anti-oviposition effect on the adults of greenhouse whitefly. The effectiveness and duration of repellency of chemicals depend on the type of repellent (active ingredient and formulation), the mode of application, local condition (temperature, humidity and wind) (Barnard, 2000). Repellent and oviposition deterrence effect is often connected with pest reduction.

Conclusions

Therefore, these results show a prospective utilization of essential oils and aqueous plant extract in plant protection against greenhouse whiteflies. The results provided that repeated treatment would have a decremented influence on greenhouse whitefly populations. In cases of large whitefly attacks, it could be useful to mix preparations with natural enemies. In addition, this provides an important example of the potential for synergy when using an integrated pest management approach. However, the plant products influence on beneficial organism is not known and it is necessary to clarify this issue.

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