J. Crop Prot. 2019, 8 (1): 57-67

Research Article

Insecticidal effects of essential oils from two medicinal plants against *Aleuroclava jasmini* (Hemiptera: Aleyrodidae)

Saeid Javadi Khederi[1], Mehdi Khoobdel[1*], Mohammad Khanjani[2], Asghar Hosseininia[2, 3], Behzad Sadeghi Sorkhe Dizaji[2], Sayed Masoud Hosseini[4] and Hossein Sobati[1]

1. Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Department of Plant protection, Faculty of Agriculture, University of Bu-Ali Sina, Hamedan, Iran.
3. Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI) Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran.
4. Department of Pharmacodynamy and Toxicology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract: The whitefly, *Aleuroclava jasmini* Takahashi (Hemiptera: Aleyrodidae) is an important pest on paper mulberry *Brousson etiapapyrifera* (L.) Vent. (Moraceae) plants in the green space of Tehran, Iran. Considering the importance of essential oils in the integrated management program of pests, the present survey were carried out to assess the insecticidal effects of essential oils extracted from garden thyme, *Thymus vulgaris* L. (Lamiaceae), and neem seed, *Azadirachta indica* A. Juss (Meliaceae) on mortality of immature stages of *A. jasmini* including eggs, first-instar nymphs, and pupae, and adult repellency. Three doses of the above mentioned essential oils consisting of 0.125%, 0.25% and 0.5% (v/v), were tested in contact toxicity assays. Separately, various essential oils (0.5%), as well as Tween-20 (0.5%) were applied as treated and control for adult repellency. Responses differed according to the type and concentration of oils, as well as growth stage of *A. jasmini*. The highest mortality was detected with increasing concentration of assayed essential oils. The results showed that younger nymphs were more sensitive to treatments than other growth stages. Essential oil derived from *T. vulgaris* was the most effective, decreasing the survival rate of eggs, nymphs and pupae 64%, 76% and 50%, respectively, as compared with controls. In free-choice assays, the mean numbers of eggs laid on 0.5% *A. indica* and *T. vulgaris* oil-treated plants were 80.98% and 58.57% lower than on control plants, respectively. In conclusion, extracted oils from *T. vulgaris* and *A. indica* represented the highest contact toxicity, and repellency to *A. jasmini* respectively. Finally, the assayed essential oils show promise for inclusion in integrated *A. jasmini* management programs in green space of Tehran, Iran.

Keywords: insecticide, repellency, essential oil, paper mulberry, whitefly

Introduction

Paper mulberry *Brousson etiapapyrifera* (L.) Vent. (Moraceae) is cultivated in nearly all green spaces of Tehran, Iran. The intensive cultivation of plants often leads to injuries by pathogens or insect pest and may require the implementation of pest control measures (Javadi Khederi *et al.*, 2018a). Since 2008, paper mulberry production has been threatened by a major insect pest, *Aleuroclava jasmini* Takahashi (Hemiptera: Aleyrodidae) (Bagheri *et al.*, 2012). *Aleuroclava jasmini* is a

Handling Editor: Saeid Moharramipour

* **Corresponding author**, e-mail: khoobdel@yahoo.com
Received: 22 May 2018, Accepted: 8 December 2018
Published online: 2 February 2019

polyphagous insect that is responsible for great economic damage in many agricultural crops such as paper mulberry (Bagheri *et al.*, 2012). In addition, this pest excretes honeydew, which promotes the growth of sooty mold fungi, affecting the process of photosynthesis (Bi *et al.*, 2001).

Aleuroclava jasmini control in Iran depends heavily on insecticides due to their ease of application, quick action, and high performance (Hosseininia *et al.*, 2017). Since all feeding stages of *A. jasmini* colonize the underside of leaves, it is difficult to obtain efficient coverage by pesticide spraying. In addition, repeated application of these compounds can lead to overuse of insecticides and induce different issues such as environmental pollution, food contamination, affect biological agents (Desneux *et al.*, 2007; Guedes *et al.*, 2016) and the selection of resistant pest populations (Horowitz *et al*. 2007; Liang *et al*. 2012).

In general, the adverse effects of pesticide use and the increased restrictions on the application of pesticides have stimulated the exploration of eco-friendly pest control methods (Javadi Khederi *et al.*, 2014), such as plant essential oil application with the lowest risk and the highest compatibility with the environment. Essential oils are valuable secondary metabolites of plants which are obtained by steam distillation or other methods (von Elling, 2002; Hosseininia *et al.*, 2017). Most of them are potentially effective in control of whiteflies because many of them are selective to herbivores, and have little adverse effects on the environment as well as biological control agents (Isman, 2000; Kim *et al.*, 2003; Choi *et al.*, 2004). Plant essential oils can be applied against herbivores in the same way as the other pesticides and act in many ways on different types of insect pests (Isman, 2000). Many of them are known to have repellent and pesticide activities against different pest species (Desmarchelier, 1994; Isman, 2000). Essential oils have been extensively studied because they are deemed as potential alternative to replacing synthetic pesticides and because of also being easier to apply (von Elling, 2002; Park *et al.*, 2005; Aroiee *et al.*, 2005; Yang *et al.*, 2010; Hosseininia *et al.*, 2017). Aroiee *et al.*, (2005) determined the repellent effectiveness of the essential oil of *T. vulgaris* on greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). Yang *et al.*, (2010) investigated repellent and anti-oviposition effects of essential oils obtained from *Thymus vulgaris* L. (Meliaceae), *Pogostemon cablin* (Blanco) Benth. (Lamiaceae), and *Corymbia citriodora* (Hook.) K. D. Hill and L. A. S. Johnson (Myrtaceae) on *Bemisia tabaci* Gennadius biotype B (Hemiptera: Aleyrodidae). In other studies, the activities of the essential oil of *Azadirachta indica* A. Juss (Meliaeceae) on different developmental stages of *T. vaporariorum* were evaluated (von Elling, 2002; Hosseininia *et al.*, 2017).

The present survey conducted to investigate the toxicity of essential oils extracted from garden thyme, *T. vulgaris* and neem seed, *A. indica* against *A. jasmini*, and specifically designed to detect the degree of contact toxicity of these oils to developmental stages and specify their repellent effect on *A. jasmini* adult females.

Materials and Methods

Rearing of insect and host plant

The initial population of *A. jasmini* was established using nymph-infested foliage collected from paper mulberry fields located in the area of Garm Dare (Tehran, Iran; 35˚ 45' 28.4" N, 51˚ 4' 1.06" E 1,287 m asl). Briefly, the leaves bearing nymphs and pupae were brought to the laboratory and were placed with paper mulberry plants in insect rearing cages at 24 ± 1 °C, 60 ± 5% RH and 16L: 8D h photoperiod. The stock culture was maintained on paper mulberry plants for at least five generations in a rearing chamber prior to the study. The paper mulberry plants were raised free of insects under greenhouse conditions. This culture was used as the source throughout this study.

Paper mulberry bare-root seedlings were planted in 2.6-L pots filled with a 1.3: 1 sand:

[DOR: 20.1001.1.22519041.2019.8.1.1.1]

peat moss mixture (v/v) in a greenhouse and allowed to reach approximately 15cm height with at least five fully expanded leaves. At the time of planting, 5g of osmocote fertilizer (14N-14P-14K) was applied to each pot. Two days before beginning the experiments, the plants were severely pruned in order to induce the production of new leaves, keeping only the top three fully expanded leaves which are much more suitable to sucking pest colonization at their early stage (Javadi Khederi *et al*., 2016; Javadi Khederi *et al*., 2018b).

Preparation of essential oils

Essential oils derived from garden thyme plant, *T. vulgaris* and neem seed, *A. indica* were obtained from National Ornamental Plant Institute, Mahallat, Iran. Essential oils were extracted by steam distillation using a Clevenger-apparatus, and the produced oils were stored at 5 °C until required. The extracted oils were diluted in de-ionized water plus Tween-20 (Amersco, 0.5%) to 0.125%, 0.25% and 0.5% (v/v) doses before the experiments. These concentrations were used in contact toxicity assays whereas 0.5% essential oils of *T. vulgaris* and *A. indica* were applied for adult repellency. The applied concentrations were selected according to data presented in the related literature of *Aleuroclava jasmini* (Hemiptera: Aleyrodidae) (Khoobdel *et al*., data unpublished).

Contact toxicity of oils on *A. jasmini* immature stages

Six days-old eggs, two days-old first-instar nymphs and two days-old pupae were evaluated in contact toxicity bioassays. The above mentioned growth stages of *A. jasmini* were obtained by introducing 100-150 unsexed adults on paper mulberry plants in insect rearing cages. The adults were removed after 24h, and plants were kept under controlled conditions (24 ± 1 °C, 60 ± 5% RH, and photoperiod 16:8 (L: D) h) until the eggs developed to proper stages for bioassays. Six days after removal of the adults, the recently fully expanded leaves with eggs (six days old) were dipped for 10 s in three serial dilutions of essential oils (0.125%, 0.25% and 0.5%) and Tween-20 (0.5%) as control (Cahill *et al.*, 1996; Nauen and Konanz, 2005). The number of nymphs, pupae and adults (based on the number of empty pupae) was recorded three, ten and fifteen days after treatment with the oils (Yang *et al.*, 2010). Unhatched eggs or those unable to leave from the egg shells were considered as dead. Also, nine days after removal of the *A. jasmini* adults, the leaves with first-instar nymphs (two days old) were treated with oils as mentioned above. The number of pupae and adults were determined seven and twelve days after essential oils treatment. In addition, sixteen days after removal of the *A. jasmini* adults the leaves with, pupae (two days old) were dipped in oils as described above and the adult eclosion was counted five days after treatment. There were six replicates in all contact toxicity bioassays and each replicate consisted of 100 insects (a total of 600 insects per treatment).

Repellent effect of oils on adults of *A. jasmini*

In the no-choice assay, the couple of adult male and female of *A. jasmini* (< five h old) were placed on a fresh paper mulberry plant and allowed to feed and mate for two days. The paper mulberry treated and control leaves were dipped in various essential oils (0.5%), and in Tween-20 (0.5%) for 10 s respectively, and subsequently, the leaves were dried at room temperature (about 25 °C). The females were individually placed in clip cages (2cm diameter, 1.5cm high) on the underside of the treated or control leaves. One day after beginning the experiment, and for four consecutive days. Females were then transferred to new leave of the same plant every 24h (Yang *et al.*, 2010). Therefore, the observation of each replication lasted for a total of five days. The number of eggs laid per female as well as their survival rate were recorded per each 24h. There were five replicates in no-choice assay and each replicate included twelve cages per treatment (a total of 60 females per treatment).

In the free-choice assay, potted plants were treated as described in the no-choice experiment. The couple of paper mulberry plants (i.e. 0.5% Tween-20-treated as well as 0.5% essential oil-treated) were randomly arranged in fine cloth cages (80 × 60 × 60cm) in greenhouse condition. Then, one hundred randomly selected *A. jasmini* adult females were released into each cage using an open vial in the center of cage. The cumulative number of females settled per plant was recorded at 2, 4, 6, 8 and 24h after beginning the experiment. In addition, 24h after *A. jasmini* release, the adults were removed and number of eggs were counted on both sides of the leaves. There were five replicates in free-choice assay, and each replicate included three cages per treatment (a total of 15 cages per treatment).

Statistical analysis

The data obtained from no-choice repellency experiments and contact toxicity of essential oils on survival rate of *A. jasmini* immature stages were analyzed by one-way ANOVA (SAS, 2004). Mean values were separated using Tukey's test for post hoc comparison ($p \leq 0.05$). Two-way ANOVA was used to analyze the interactions between essential oils concentration and whitefly stage of *A. jasmini* (SAS, 2004). The related data of *A. jasmini* preference in repellent choice bioassays were analyzed by performing an X^2 test with a null hypothesis of equal distribution (SPSS, 2004). All graphs were produced by Sigma Plot version 11.0 (Systat Software, 2008).

Results

Contact toxicity of essential oils on immature stages of *A. jasmini*

Various concentrations of *T. vulgaris* ($F_{3,\ 22}$ = 175.33, $P < 0.0001$) and *A. indica* ($F_{3,\ 22}$ = 72.63, $P < 0.0001$) significantly decreased egg hatch compared to the controls. The essential oils extracted from *T. vulgaris* ($F_{3,\ 22}$ = 16.48, $P < 0.0001$) and *A. indica* ($F_{3,\ 22}$ = 22.43, $P < 0.0001$), showed higher mortalities than control on *A. jasmini* nymphs which survived through egg treatment. The survival rate of *A. jasmini* eggs, during entire immature period was 52-64%, 34-61% lower than the control groups, respectively when they were treated with the oils derived from *T. vulgaris* and *A. indica* (Fig. 1a, b).

Development of *A. jasmini* nymphs into pupae significantly decreased on the plants treated with essential oils of *T. vulgaris* ($F_{3,\ 20}$ = 153.22, $P < 0.0001$) and *A. indica* ($F_{3,\ 20}$ = 142.53, $P < 0.0001$) while the proportion of emerged adults was not affected. As compared to control, various concentrations of *T. vulgaris* and *A. indica* essential oils reduced the survival rate of *A. jasmini* immature nymphs by 44-76% and 15-74%, respectively (Fig. 1c, d).

Essential oils from two plants, *T. vulgaris* ($F_{3,\ 18}$ = 724.23, $P < 0.0001$) and *A. indica* ($F_{3,\ 18}$ = 433.62, $P < 0.0001$), significantly reduced development of pupae into adult when compared with controls. Treatments with essential oil from *T. vulgaris* and *A. indica* on pupae stage reduced the survival rates of *A. jasmini pupae* by 45-50% and 38-50% compared to controls (Fig. 1e, f). Moreover, the toxicity of the treated oils increased with increasing dose for all developmental stages. This increase was most pronounced for *T. vulgaris* extracted oil. Also, *A. jasmini* first-instar nymphs were more sensitive to essential oils compared with eggs and pupae at the most effective concentration 0.5%. Besides, the greatest effect was observed with *T. vulgaris* extracted oil, which decreased the survival rate of eggs, nymphs and pupae by 64%, 76% and 50% respectively, as compared to controls (Fig. 1a-f).

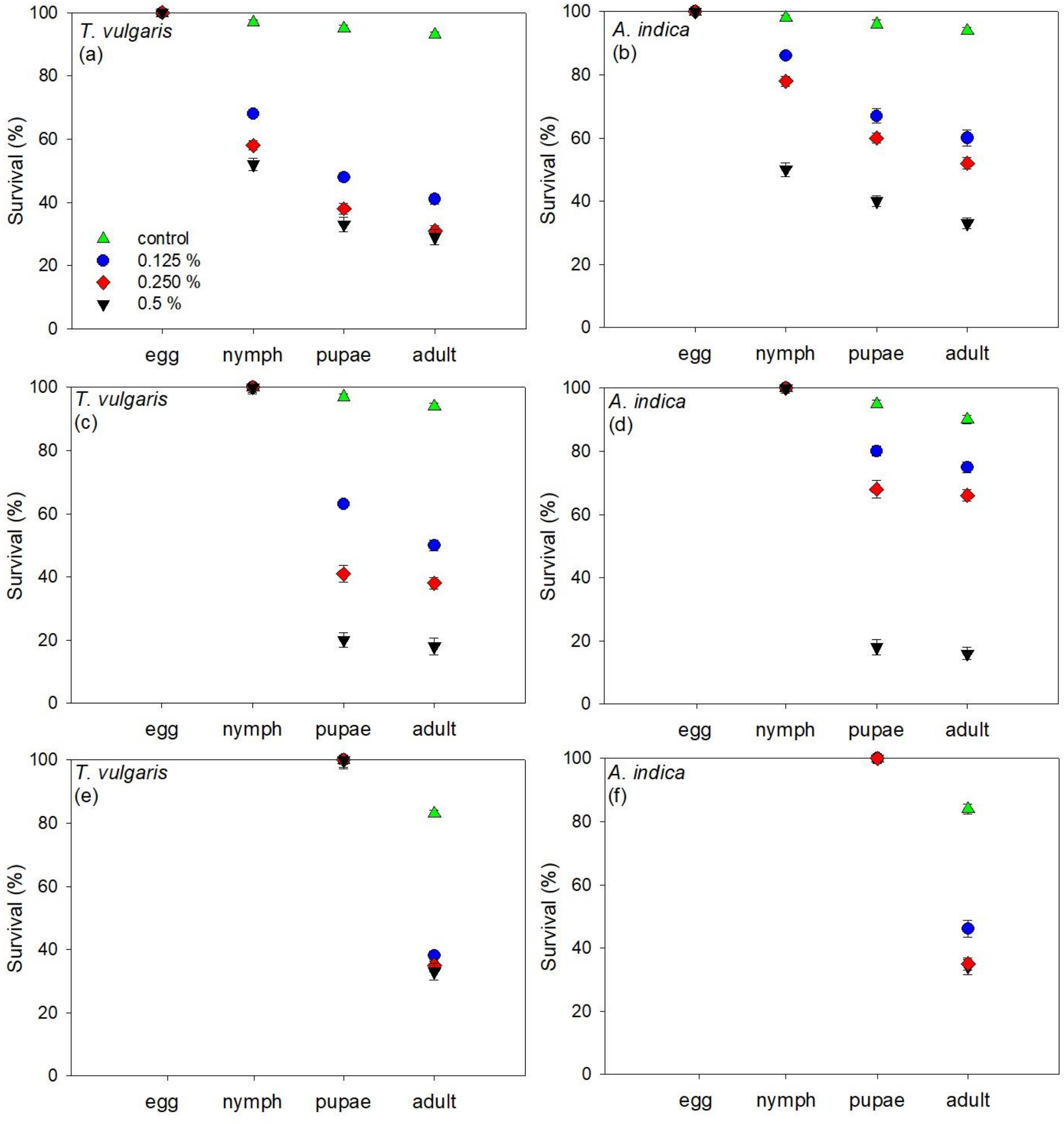

Figure 1 Mean percentages of survival (± SE; n = 6) in *Aleuroclava jasmini* immature stages reared on *Brousson etiapapyrifera*. Insects treated at 0.125%, 0.25% and 0.5% of *Thymus vulgaris* and *Azadirachta indica* oils compared with Tween-20 (control). (a, b) treated on eggs (six days old), (c, d) treated on nymphs (nymphs 1^{st} = two days old) and, (e, f) treated on pupae (two days old).

Repellent activities of the essential oils

In a no-choice experiment, the cumulative survival rates of *A. jasmini* females treated with *T. vulgaris* and *A. indica* were 40% and 33% lower than the control, respectively (Fig. 2a). Also the mean cumulative numbers of eggs per female during five days was significantly decreased by 0.5% doses of essential oil treatments of *T. vulgaris* and *A. indica* as compared to controls ($F_{3,\ 312}$ = 64.73, P < 0.0001) (Fig. 2b).

During the free-choice assay, a lower density of *A. jasmini* adults settling on essential oil-treated plants was recorded than in control plants. 24h after beginning the experiments, smaller numbers of *A. jasmini* female were counted on the plants treated with *A. indica* and *T. vulgaris* (65.71% and 48.89% respectively) than on control plants (Fig. 3).

Also, the average numbers of eggs laid per females on *A. indica* and *T. vulgaris* treated plants were 80.98% and 58.57% less than on non-treated plants, respectively, under free-choice condition (Fig. 4).

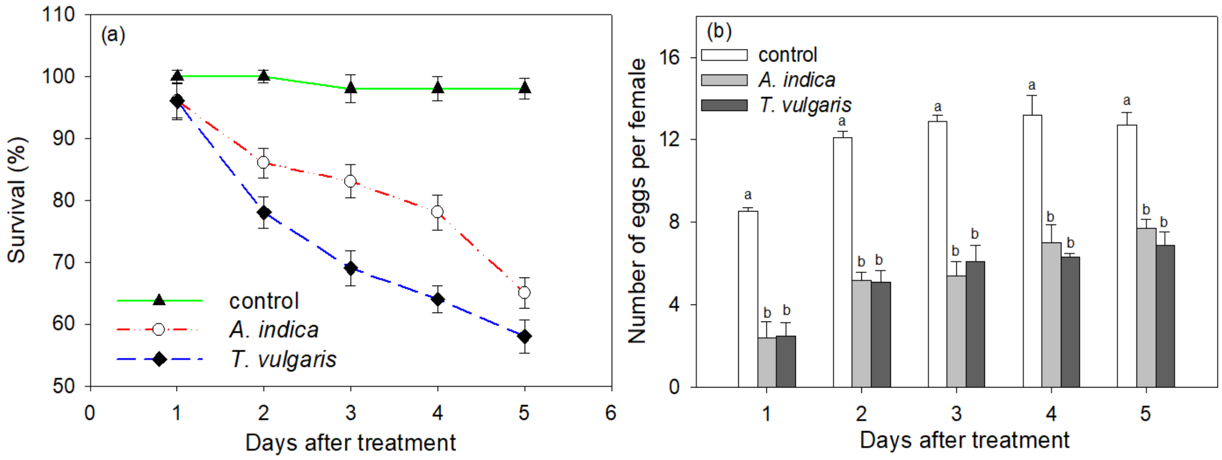

Figure 2 No-choice assay: mean (± SE; n = 5) percentage of female survival rate (a), and number of eggs per *Aleuroclava jasmini* female (b) five days after feeding on the *Brousson etiapapyrifera* plants treated by 0.5% *Thymus vulgaris* and *Azadirachta indica* oils. For each cluster, means with the same letter are not significant (Tukey's test; P ≤ 0.05).

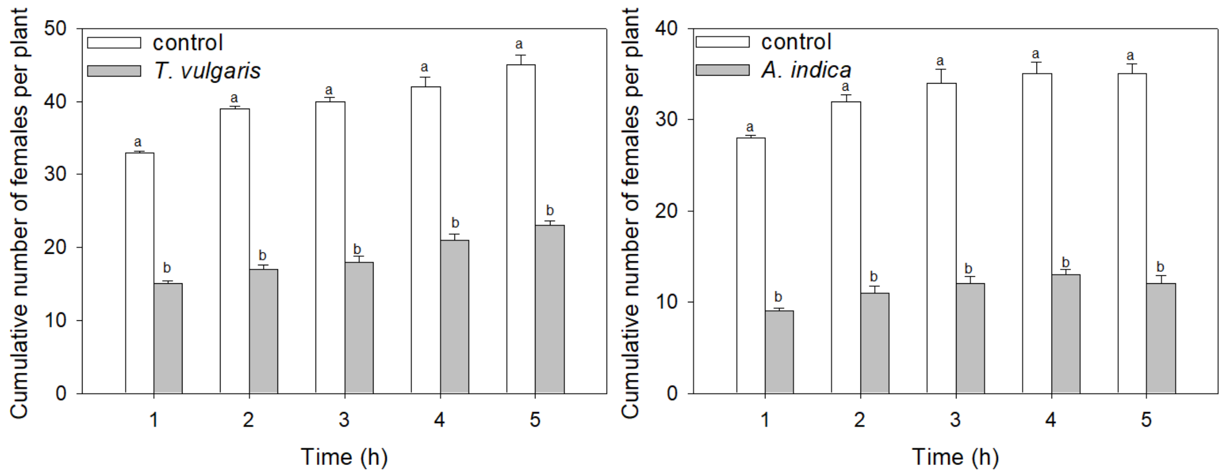

Figure 3 Free-choice assay: mean (± SE; n = 5) cumulative number of *Aleuroclava jasmini* female counted on control and treated *Brousson etiapapyrifera* plants with 0.5% *Thymus vulgaris* and *Azadirachta indica* oils at 2, 4, 6, 8 and 24h after beginning the experiments. For each cluster, means with the different letter are significantly different (χ^2 test; P < 0.01).

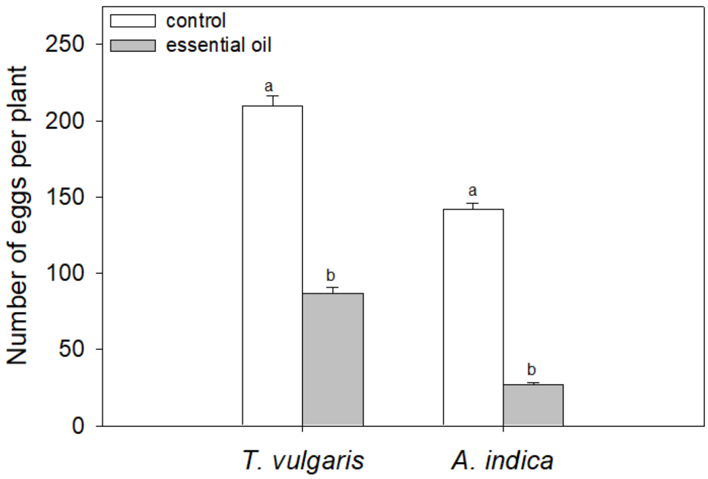

Figure 4 Free-choice assay: mean (± SE; n = 5) number of *Aleuroclava jasmini* eggs counted on control and treated *Brousson etiapapyrifera* plants with 0.5% *Thymus vulgaris* and *Azadirachta indica* oils 24h after exposure to adults. For each cluster, means with the different letter are significant (χ^2 test; P < 0.01).

Discussion

The present survey showed that essential oils derived from *T. vulgaris* and *A. indica* can effectively decrease egg hatchability, nymph and pupae survival rate and oviposition of *A. jasmini*. The essential oils caused death of some eggs without any morphological changes, whereas some others became semi-emerged dead crawlers. The first event was probably attributed to infraction or inhibition of embryogenesis, and the second one was presumably due to the effect of essential oils residues on crawlers after emergence from viable eggs (Yang *et al.*, 2010). *T. vulgaris* and *A. indica* treatments on the six days-old eggs caused higher mortalities of nymphs entering next immature stages compared to the control. Presumably due to emerging nymphs coming in contact with still active residues of essential oils (von Elling *et al.*, 2002; Yang *et al.*, 2010; Hosseininia *et al.*, 2017). The derived oils from *T. vulgaris* and *A. indica* plants at dose of 0.5% were more effective on the first-instar nymphs of *A. jasmini*. Essential oil treatments decreased both pupa development and adult emergence. Similarly, von Elling *et al.*, (2002) observed that treated *T. vaporariorum* eggs with NeenAzal-T/S, five and seven days after female's oviposition, substantially increased mortality of larvae, resulting in a significant reduction in the number of pupae formed.

Our study has shown that essential oils tested may successfully control this pest because of their high volatility and repellent efficacy. Fewer adult females alighting on the *T. vulgaris* and in particular on *A. indica* treated plants in comparison to the control plant in free-choice assays evidenced repellent effects of the treated oils. The female oviposition was significantly reduced on *A. indica* -treated paper mulberry plants. Reducing the number of eggs laid is a normal reaction if adults avoid host plant (Smith, 2005). von Elling *et al.*, (2002) observed that exposing *T. vaporariorum* adult to fresh residues of NeemAzal-T/S significantly reduced the number of laid eggs. Similarly, Coudri *et al.*, (1985) obtained 80% less oviposition of *B. tabaci* on cotton plants *Gossypium hirsutum* L. (Malvaceae) treated with neem kernels. They believed that repellent effects are the main cause for this reduction. In the current survey, the number of eggs laid per female on oil-treated plants was less than control, in both free-choice and no-choice bioassays. Free choice assays indicated that number of eggs 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 act as oviposition

[DOR: 20.1001.1.22519041.2019.8.1.1.1]

deterrents (Koschier *et al.*, 2002; Koschier and Sedy, 2003; Yang *et al.*, 2010; Hosseininia *et al.*, 2017).

In the no-choice bioassay, the females laid significantly less number of eggs on oil-treated plants. It might be due to the persistent impact of essential oils on the reduction of phloem sap uptake by adults with frequent changing of feeding areas, which may decrease numbers of developed eggs for deposition (Kumar *et al.*, 2005). Previous studies have confirmed that essential oils tested in the current survey are chemically characterized as natural mixtures most of which are poisonous or repellent to insects (von Elling *et al.*, 2002; Yang *et al.*, 2010; Dehghani and Ahmadi, 2013). The results of our study are mostly in the agreement with the results of other investigators. von Elling *et al.*, (2002) have reported that plant extracts of *A. indica* had repellent effects on *T. vaporariorum* females. Some researchers have evidenced that concentrations of 0.5% *T. vulgaris* were most effective at repelling *B. tabaci* and *T. vaporariorum* adults. Moreover, the essential oils derived from *T. vulgaris* offer a high potential for the control of *B. tabaci* and *T. vaporariorum* whiteflies (Yang *et al.,* 2010; Dehghani and Ahmadi, 2013).

Finally, essential oils extracted from *T. vulgaris* and *A. indica* showed the good contact toxicity, and repellency to *A. jasmini* whiteflies respectively. Therefore, they may be used in integrated pest management programs of this pest, especially where the emphasis is placed on food and environmental safety and replacing the more dangerous and toxic pesticides. Hence, it may lead to new and more effective strategies to prevent and control of *A. jasmini* whitefly in paper mulberry landscapes of Tehran, Iran.

Acknowledgements

The authors are grateful to the anonymous referee for comments on the manuscript. This work is part of a military dissertation of the first author that was funded by financial assistance from National Ornamental Plant Institute, Mahallat, Iran and Bu-Ali Sina University of Hamedan, Iran.

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اثرات حشره‌کشی اسانس‌های دو گیاه دارویی روی سفیدبالک *Aleuroclava jasmini* (Hemiptera: Aleyrodidae)

سعید جوادی خدری[1]، مهدی خوبدل[1*]، محمد خانجانی[2]، اصغر حسینی‌نیا[2، 3]، بهزاد صادقی سرخه‌دیزجی[2]، سیدمسعود حسینی[4] و حسین ثباتی[1]

۱- مرکز تحقیقات بهداشت، دانشگاه علوم پزشکی بقیه‌الله، تهران، ایران.
۲- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه بوعلی‌سینا، همدان، ایران.
۳- گروه مدیریت و فناوری تولید، پژوهشکده گل و گیاهان زینتی محلات، مؤسسه علوم تحقیقات باغبانی، محلات، ایران.
۴- گروه داروشناسی و سم‌شناسی، دانشکده داروسازی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: khoobdel@yahoo.com
دریافت: ۱ خرداد ۱۳۹۷؛ پذیرش: ۱۷ آذر ۱۳۹۷

چکیده: سفیدبالک *Aleuroclava jasmini* Takahashi (Hemiptera: Aleyrodidae) یکی از آفات مهم گیاه توت کاغذی *Brousson etiapapyrifera* (L.) Vent. (Moraceae) در فضای سبز شهر تهران محسوب می‌شود. با توجه به اهمیت اسانس‌های گیاهی در امر کنترل تلفیقی آفات، مطالعه کنونی به‌منظور بررسی اثرات حشره‌کشی اسانس آویشن باغی *Thymus vulgaris* L. (Lamiaceae)، و عصاره بذر چریش *Azadirachta indica* A. *Juss* (Meliaceae) روی مرگ‌ومیر مراحل نابالغ سفیدبالک *A. Jasmini* شامل تخم، پوره سن اول و شفیره و هم‌چنین اثرات دورکنندگی آن‌ها روی حشرات بالغ انجام شد. به‌طور خلاصه سه غلظت ۰/۱۲۵، ۰/۲۵ و ۰/۵ درصد (حجم/حجم) از ترکیبات مذکور در ارتباط با اثر تماسی آن‌ها مورد استفاده قرار گرفت. به‌علاوه در یک مطالعه جداگانه، اثر دز ۰/۵ درصد از اسانس‌های گیاهی روی دورکنندگی حشرات بالغ بررسی شد. واکنش‌ها با توجه به نوع، غلظت ترکیبات و هم‌چنین مرحله رشدی سفیدبالک *A. jasmini* متفاوت بود. به‌طور کلی میزان مرگ‌ومیر بیش‌تری از این آفت با افزایش غلظت ترکیبات مورد مطالعه، مشاهده گردید. هم‌چنین، نتایج این مطالعه نشان داد که پوره‌های سن اول به‌مراتب از حساسیت بیش‌تری نسبت به مراحل تخم و شفیرگی برخوردار هستند. در نتیجه، به‌ترتیب اسانس گیاه آویش باغی و عصاره بذر گیاه چریش بیش‌ترین اثر تماسی را روی مراحل نابالغ و اثر دورکنندگی را روی حشرات بالغ این سفیدبالک نشان دادند. ترکیبات مورد بررسی نتایج امیدوارکننده‌ای در ارتباط با کنترل این آفت نشان دادند که می‌توان آن‌ها را به‌عنوان ترکیبات سازگار با محیط‌زیست، در برنامه‌های مدیریت تلفیقی این آفت در فضای سبز شهر تهران مورد توجه قرار داد.

واژگان کلیدی: حشره‌کش، دورکنندگی، اسانس‌های گیاهی، توت کاغذی، سفیدبالک