

Research Article

Effect of ethanolic plant extracts along with releasing eggs of *Orius horvathi* (Heteroptera: Anthocoridae) against the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae)

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Abstract: The present research aimed to study the insecticidal activity of four ethanolic extracts of *Cercis siliquastrum* L., *Calendula officinalis* L., *Peganum harmala* L., *Melia azedarach* L. against *Frankliniella occidentalis* (Pergande) as well as hatching time and hatching rate of *Orius horvathi* (Reuter). The results revealed that the extracts had no significant effect on the hatching time of the predatory bug. The result showed concomitant use of the extracts with releasing eggs of the *O. horvathi* could control thrips effectively. The lowest ($73.66 \pm 7.01\%$) hatching rate belonged to *P. harmala*, indicating concomitant use of *P. harmala* extract with releasing eggs of *O. horvathi* can cause effective control of adult thrips. Moreover, concomitant use of *C. siliquastrum*, *C. officinalis* extracts with releasing eggs of *O. horvathi* can control immature thrips effectively.

Keywords: Integrated Pest Management, natural enemy, *Orius horvathi*, Western Flower Thrips, plant-derived chemicals.

Introduction

The Western Flower Thrips (WFT), *Frankliniella occidentalis* (Pergande), was first described in California in 1895. Since the 1970s, it has invaded much of the world and become dominant in most areas where it was introduced (Kirk and Terry, 2003; Morse and Hoddle, 2006). It has become a significant pest of semi-protected strawberry crops (*Fragaria × ananassa* Duchesne) in the U.K. (Sampson *et al.*, 2021). It was detected in cucumber greenhouses in Varamin, Iran, and has dispersed to many agricultural areas and crops, including cucumbers, tomatoes, strawberries, and eggplants (Gholami and Sadeghi, 2016). The

main host plants identified for the western flower thrips belong to the Asteraceae family, followed by the Rosaceae family (Afsharizadeh Bami *et al.*, 2018). The thrips cause direct feeding damage to rose flowers and transmit tospoviruses (Khavanad *et al.*, 2019). The main strategies of the Integrated Pest Management program for *F. occidentalis* are biological, chemical, cultural, and mechanical controls (Gholami and Sadeghi, 2016). The application of insecticides is the most commonly used tactic to control WFT. The current insecticides have mainly caused insect resistance and have resulted in thrips outbreaks. One alternative for controlling pests is using natural insecticides, i.e., active ingredients isolated primarily from plants (Schmutterer, 1990; Valizadeh *et al.*, 2013a, b, 2020). Compounds containing active insecticidal phytochemicals seem promising for solving some of these problems (Rattan, 2010). Some compounds have no toxic effect or low toxicity on non-target

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organisms and mammals and are less harmful to the environment (Wei *et al.*, 2011; Valizadeh *et al.*, 2014; 2021). Among the most important natural enemies of the thrips are minute pirate bugs, *Orius* spp. Predation by *O. insidiosus* (Say) (Heteroptera: Anthoridae) on life stages of western flower thrips in pepper flowers was checked. *Orius insidiosus* to prey successfully on different life stages and species of thrips in complex environments indicating that this insect is an efficient predator of thrips and an important biological control agent (Baez *et al.*, 2004). Razavi and Ahmadi (2016) investigated compatibility between four ethanolic plant extracts and a predatory bug, *Orius horvathi* (Reuter), against *F. occidentalis*. The side effect of two compounds derived from neem, vijayneem, and neembicidine, was evaluated on the predatory bug *Rhynocoris marginatus* (Fab.) (Heteroptera: Reduviidae). No significant impact was observed on the incubation period. However, neem-based biopesticides significantly reduced egg hatching, prolonged nymphal development, and reduced the nymphal survival rate (Sahayaraj *et al.*, 2003). Effects of extract of *M. azedarach*, on a braconid wasp, *Cotesia ayerza* (Brethes) revealed this extract could represent an exciting tool for integrated pest management of the wasp population (Defago *et al.*, 2011). Insecticidal activities of five plant-derived chemicals on *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) showed that the mortality of the 1-2 days old thrips was more than 64% (Najmizadeh *et al.*, 2013). Additionally, the efficacy of spinosad against the *F. occidentalis* and its impact on associated biological control agents on greenhouse cucumbers in south Ontario was studied (Jones *et al.*, 2004). The influence of botanical pesticides and biological agents on *O. laevigatus* (Fieber) (Heteroptera: Anthoridae) and *F. occidentalis* dynamics under greenhouse conditions were assessed. As a result, botanical insecticide rotenone and neem were found to reduce *O. laevigatus* numbers, and the effects were evident in the adult stage of *O. laevigatus* (Bonsignore *et al.*, 2012).

Due to the lethal and sub-lethal effects of plant extracts on natural enemies, their

application is sometimes limited. Finding compounds that cause fewer mal effects on natural enemies is significant in IPM programs. In the present study, four ethanolic plant extracts of *Cercis siliquastrum* L., *Calendula officinalis* L., *P. harmala* L., *M. azedarach* L. were evaluated on controlling of *F. occidentalis*, also, hatching time, and hatching rate of *O. horvathi*.

Materials and Methods

Rearing the western flower thrips

The initial population of WFT was collected from the experimental teaching greenhouse of Shahid Bahonar University of Kerman, Kerman, Iran. After identification, the *F. occidentalis* was reared on pots of Borlotti bean (*Phaseolus vulgaris* L.). The pots were kept in 80 × 60 × 60 cm wood-framed cages that were covered with cloths at 25 ± 5 °C, 60 ± 10% RH, and 14:10 h (L: D) in the greenhouse. No insecticides were applied.

Rearing the natural enemy

The predatory bug, *O. horvathi*, was collected from the alfalfa research farm of Shahid Bahonar University of Kerman, Kerman, Iran. No insecticides were used at the farm, so the insects were not exposed to insecticides previously. The predatory bug was reared on fresh bean leaf discs (2.5 cm in diameter) infested simultaneously with *Aphis fabae* (Scopoli) nymphs and eggs of *Ephesia kuehniella* (Zeller) as well as on 10% diluted honey. These discs were placed upside down in plastic Petri dishes. The dishes were 4 cm in diameter and had a meshed hole on the lid. The Petri dishes were partially filled with a 0.5-cm-thick layer of 0.7% agar gel. The Petri dishes were held in a growth chamber at 25 ± 1 °C, 60 ± 10% relative humidity, and a photoperiod of 16:8 h (L: D). Twenty adult females were taken from the stock culture for oviposition and then confined on bean leaf discs in the round plastic Petri dishes described above for 48 h. The bean leaves with the eggs were incubated until the eggs hatched. As mentioned above, the first

nymphal instars were transferred to plastic Petri dishes with the mesh-covered hole in the lid. A camel hair brush was used to transfer the instars. The paper shelters were used to prevent cannibalism. The Petri dishes were kept in a growth chamber under the conditions mentioned above until the bugs reached the desired stage for the experiments.

Preparation of ethanolic plant extracts

The fruits of *M. azedarach* and seeds of *P. harmala* and *C. siliquastrum* were collected from their natural habitat in Kerman, Iran (30°18'4.6"N, 57°1'57.6"E, 1761 m). The seeds of *C. officinalis* were collected from Safa Blvd., Kerman, Iran (30°15'6" N, 57°6'16" E, 1761 m). The fruits and seeds were dried in the shade at room temperature and then completely powdered by an electrical steel grinder. The powder (50 g) was poured into an Erlenmeyer flask, and 100 ml of ethanol (95%) was added. The flasks were sealed with Parafilm® and shaken for one hour, and placed in the refrigerator for one day. Then, the extract was filtered using filter paper and poured into a vial (10 ml). The vial was covered with aluminum foil to prevent light incidents on the extract. The concentration of the stock extract was measured, and then 10 mg.ml⁻¹ was obtained by dilution with ethanol. The resultant compound was kept in the freezer at (18 °C) until the day of the experiment.

Greenhouse bioassay

For the experiment, each pot of Borlotti beans *P. vulgaris* cv. Borlotti with two expanded leaves was placed inside a transparent plexiglass cylinder. The cylinders were 70 cm in height and 25 cm in diameter, and had a mesh-covered hole in the lid to prevent the escape of insects. The cylinders were kept under greenhouse conditions at 25 ± 5 °C, 60 ± 10% RH, and 14:10 h (L: D). The immature thrips (larval stages (1-2 days) were taken from the stock culture and transferred to bean leaves (5 thrips/plant). After two days, each bean plant, with thrips on its leaves (with seven replications), was sprayed with the ethanolic plant extract 10 mg.ml⁻¹. The volume of each

extract was 5 ml. While each control treatment with seven replications was sprayed with 95% ethanol. Ten days after spray, the number of immature and adult thrips was recorded.

Bioassay procedure

In this experiment, freshly prepared bean leaf discs (2.5 cm in diameter) were placed upside down inside the round plastic Petri dishes (4 cm in diameter). A meshed hole in the lid was prepared to prevent insect escape. The Petri dishes were partially filled with a 0.5-cm-thick layer of 0.7% Agar gel. The bean leaves were infested with nymphs of *A. fabae* as prey, eggs of *E. kuehniella*, and honey diluted to 10% for feeding the predatory bug. The round plastic Petri dishes were held in a growth chamber at 25 ± 1 °C, 60 ± 10% RH, and a photoperiod of 16: 8 h (L: D). For oviposition, adult females of *O. horvathi* were taken from the stock culture and then confined on bean leaf discs in the round plastic Petri dishes as described above for 24 hours. Then the bean leaves that contained *O. horvathi* eggs were used for experiments. Each unit (bean leaves with *O. horvathi* eggs) was sprayed individually with the ethanolic plant extracts 10 mg.ml⁻¹, while each control treatment was sprayed with ethanol 95%. The units were held in a growth chamber as described above. The number of hatched-egg was recorded daily.

Data analysis

To affirm the basic assumptions of the data to be analyzed, they were first tested for the normal distribution and the homogeneity of variance using the Bartlett- test (Köhler *et al.*, 2002). The data that had not conformed to the assumptions of normal distribution were transformed to conform to the assumptions using the Box-Cox formula:

$$Y = (X^2 - 1)/\lambda \text{ if } \lambda \neq 0,$$

$$Y = \ln X \text{ if } \lambda = 0,$$

where: Y is the transformed value, X is the untransformed value, and $0 < \lambda < 1$ (Anonymous, 1996).

The data were subjected to a one-way analysis of variance followed by a Fisher LSD method ($p \leq 0.05$). Statistical analyses were run in Statplus version 4.9, 2007.

Results

The four plant extracts had no significant effect on the hatching time of *O. horvathi* (Fig. 1). The highest mean belonged to *P. harmala* treatment (5.42 ± 0.12 days), and the lowest mean was observed in the control treatment (5.28 ± 0.11 days).

The plant extracts significantly affected the hatching rate of the predatory bug (Fig. 2). The lowest mean ($73.66 \pm 7.01\%$) belonged to *P. harmala* treatments. The highest mean ($99.99 \pm 0.002\%$) was related to control treatments.

Also, immature thrips population was significantly different among plant extracts (Fig. 3). The highest mean of immature thrips per leaf was related to the control treatment (1.86 ± 0.26), and the lowest mean of immature thrips belonged to the *C. siliquastrum* treatment.

Results showed that the number of female thrips was significantly different among treatments (Fig. 4). The highest mean of female thrips population was observed in control (2.29 ± 0.36), and the lowest mean in *P. harmala* treatment.

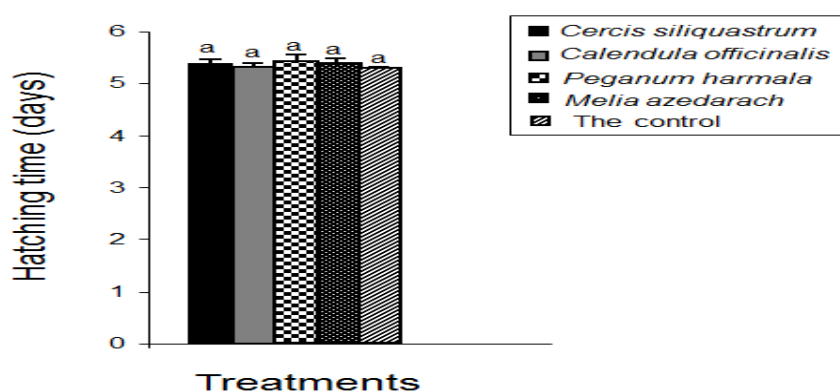


Figure 1 Hatching time of *Orius horvathi*. Each leaf is treated individually with one of the four plant extracts: *Cercis siliquastrum*, *Calendula officinalis*, *Peganum harmala*, *Melia azedarach*.

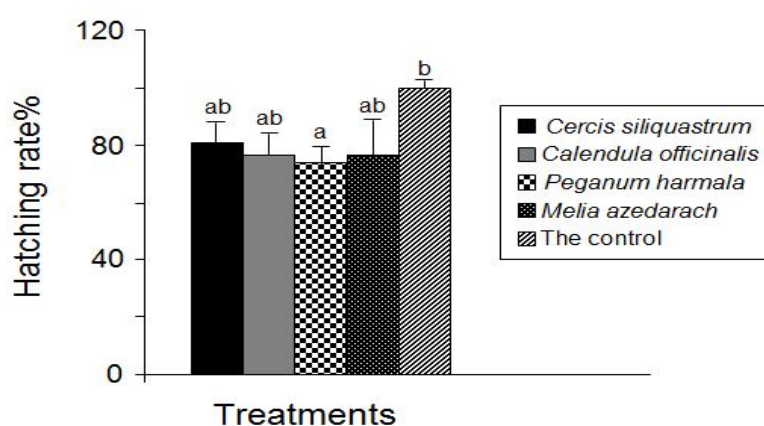


Figure 2 Hatching rate of *Orius horvathi* 6 days post-treatment. Each leaf is treated individually with the four plant extracts: *Cercis siliquastrum*, *Calendula officinalis*, *Peganum harmala*, *Melia azedarach*.

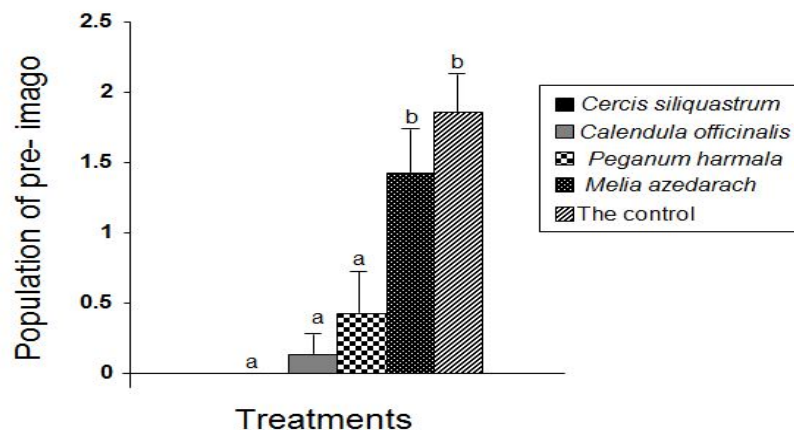


Figure 3 The mean number of pre-imago thrips *Frankliniella occidentalis* per leaf, 10 days after spraying the extracts. Each leaf is treated individually with the four plant extracts: *Cercis siliquastrum*, *Calendula officinalis*, *Peganum harmala*, *Melia azedarach*.

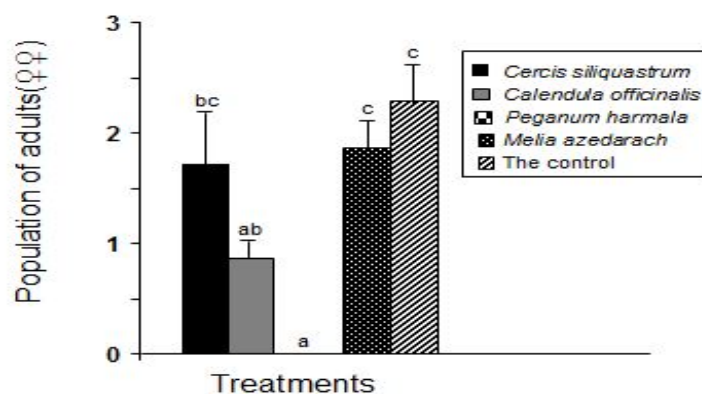


Figure 4 The mean number of female thrips *Frankliniella occidentalis* per leaf, 10 days after spraying the extracts. Each leaf is treated individually with the four plant extracts: *Cercis siliquastrum*, *Calendula officinalis*, *Peganum harmala*, *Melia azedarach*.

Discussion

The growing demand for natural products has intensified in the past decades as they are extensively used as biologically active compounds. Pesticides of plant origin are gaining increased attention and interest among those concerned with environment-friendly, safe and integrated crop management approaches (Oftadeh *et al.*, 2020). The results indicated

concomitant use of *P. harmala* extract with releasing eggs of *O. horvathi* could cause effective control of adult thrips. Also, the application of *C. officinalis* extract can control thrips effectively. These results agree with the study on the insecticidal activity of the ethanolic plant extract of *C. siliquastrum*, *C. officinalis*, *P. harmala*, and *M. azedarach* against the *T. tabaci* at 30 mg.ml⁻¹. After 72 h of treatment, mortality was more than 64% (Najmizadeh *et al.*, 2013).

The effects of ethanolic extracts of *P. harmala* seeds on olive fruit fly *B. olea*, i. e., adult repellency, reproductive activity, larval growth, and parasitism levels by *P. concolor* were investigated. Larval parasitism was not affected by infested fruit treatment with 2% *P. harmala* extract. *P. harmala* extracts as a potential control for insect pest species were discussed (Rehman *et al.*, 2009). A side effect of two compounds derived from neem, vijayneem, and neembicidine, on predatory bug *Rhynocoris marginatus* (Fab.) was evaluated. No significant impact was observed on the incubation period; however, neem-based biopesticides significantly reduced the egg hatching prolonged the nymphal development and reduced the nymphal survival rate (Sahayaraj *et al.*, 2003). The effect of four ethanolic plant extracts (*C. siliquastrum* L., *C. officinalis* L., *P. harmala* L., *M. azedarach* L.) on the hatching time of *O. horvathi* was investigated. The four plant extracts had no significant effect on the hatching time of *O. horvathi*. The result suggested that application of ethanolic plant extracts in combination with releasing the eggs of the predator can cause effective control of pests (Razavi and Ahmadi, 2018). Predation by *O. insidiosus* (Heteroptera: Anthocoridae) on life stages and species of flower thrips in pepper flowers was checked. The ability of *O. insidiosus* to prey successfully on different life stages, and species of thrips in complex environments indicate its efficiency as predator of thrips and an important biological control agent (Baez *et al.*, 2004). The investigated biological control of western flower thrips with Orius species in an eggplant greenhouse in Turkey showed that *O. laevigatus* is a more efficient predator in controlling *F. occidentalis* than *O. niger* in the greenhouse (Kececi and Gurkan, 2013). An assessment of four ethanolic plant extracts against *O. horvathi* indicated that all extracts had insecticidal activity in different proportions (Razavi and Ahmadi, 2016). While the plant extract plays an important role in the thrips control, it is required to consider a specified time interval between the application of plant extract and the release of the natural enemy (Razavi and Ahmadi, 2016).

Effect of five Medicinal plant extracts on *Aphis craccivora* (Koch) (Hemiptera: Aphididae) and its predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) was evaluated. Accordingly, *Ochradenus baccatus* extract strongly affected aphids and was safest for the predator (Sayed *et al.*, 2020). Effects of *M. azedarach* extract on *C. ayerza*, a parasitoid of the alfalfa defoliator *C. lesbia* have been studied. The result suggested that *M. azedarach* extracts could represent an exciting tool for integrated pest management of *C. lesbia* population (Defago *et al.*, 2011). Controlling the western flower thrips by releasing the predatory phytoseiid mites and pesticide application on pepper in a greenhouse was evaluated. Results showed that releasing the predatory mites in the blue sticky traps reduced the WFT population by 45 to 56%. The best control management of the WFT was by the following insecticide: chlorfenapyr, imidacloprid, azadirachtin, and agricultural potassium soap (Sanad and Hassan, 2019). Field evaluation of water extracts of plants on sucking insect pests and their associated predator in transgenic Bt cotton showed that extract of *Azadirachta indica* A. Juss to control sucking insect pests of cotton can be as effective as a synthetic insecticide in crop yield. Besides, they are safer for natural enemies in the field (Arshad *et al.*, 2019).

Conclusion

Since the extracts have no significant effect on the hatching time of *O. horvathi*, the natural enemy can continue its activity. Applying *P. harmala* extract in combination with releasing eggs *O. horvathi* can cause effective control of adult thrips. Moreover, concomitant use of *C. siliquastrum*, *C. officinalis*, with releasing eggs of *O. horvathi* can control immature thrips effectively.

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Statement of conflicting interests

The authors state that there is no conflict of interest.

Author contribution

Kamal Ahmadi designed the experiments and gave protocol. Nooshin Razavi did all experiments and wrote manuscript. Kamal Ahmadi revised the manuscript. Haji Mohammad Takolozadeh was advisor in written the manuscript. All authors approved the final version of the manuscript.

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تأثیر استفاده هم‌زمان از عصاره‌های گیاهی همراه با رهاسازی تخم سن شکارگر *Orius horvathi*
(Heteroptera: Anthocoridae) در کنترل تریپس غربی گل *Frankliniella occidentalis*
(Thysanoptera: Thripidae)

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چکیده: هدف از پژوهش حاضر بررسی فعالیت حشره‌کشی چهار عصاره اتانولی گیاهان ارغوان، گل همیشه‌بهار، زیتون تلخ و اسپند روی تریپس غربی گل *Frankliniella occidentalis* و هم‌چنین طول مدت زمان تفریخ تخم و میزان تفریخ تخم سن شکارگر اورپوس *Orius horvathi* می‌باشد. نتایج به‌دست آمده حاکی از آن بود که عصاره‌های گیاهی روی تفریخ تخم سن اورپوس با هم اختلاف معنی‌داری نداشتند. پایین‌ترین میانگین تفریخ تخم مربوط به عصاره گیاهی اسپند ($7/01 \pm 73/66$ عدد) بود و این نتیجه نشان داد که استفاده هم‌زمان از عصاره اتانولی اسپند و رهاسازی تخم سن شکارگر اورپوس در کنترل تریپس‌های بالغ بسیار مؤثر است. علاوه بر آن، تلفیق عصاره‌های گیاهی گل همیشه‌بهار و ارغوان به‌همراه رهاسازی تخم سن شکارگر برای کنترل تریپس‌های نابالغ مؤثر بود.

واژگان کلیدی: تریپس غربی گل، ترکیبات گیاهی، دشمنان طبیعی، سن اورپوس، مدیریت تلفیقی کنترل آفات