

Research Article

Resistance of different almond cultivars/genotypes to almond fruit wasp, *Eurytoma amygdali* (Hymenoptera: Eurytomidae)

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Abstract: Resistance of eight almond genotypes/cultivars, including Sefid, Mamaei, Rabie, Shahrood₇, Ferragnes, Shahrood₁₃, Nonpareil, and Hooreh, to almond fruit wasp (AFW) *Eurytoma amygdali* Enderlein, was investigated using choice and no-choice tests. The infested fruits ranged from $4.60 \pm 1.45\%$ in the Hooreh genotype to $86.87 \pm 2.01\%$ in the Ferragnes cultivar in the choice test. The highest and lowest premature dropped fruits were recorded in Ferragnes ($80.70 \pm 3.21\%$) and Hooreh genotype ($2.43 \pm 1.12\%$), respectively. The highest and lowest numbers of alive larvae were observed in Nonpareil (1.27 ± 0.70 larvae/fruit) and Ferragnes cultivar (0.04 ± 0.02 larvae/fruit). The No-choice test indicated the highest premature dropped fruits ($79.21 \pm 3.76\%$) and the lowest number of alive larvae (0.09 ± 0.03 larvae/fruit) in the Ferragnes cultivar. The olfactory response revealed that *E. amygdali* females were strongly attracted to fruits and fruit extracts of Mamaei and Ferragnes cultivars compared to the Hooreh genotype. Our finding demonstrated that certain chemical stimuli emitted from the unripe fruits of almond might influence the host finding behavior of AFW females.

Keywords: Almond fruit wasp, Host susceptibility, Choice test, No-choice, Olfactory response

Introduction

The almond fruit wasp (AFW), *Eurytoma amygdali* Enderlein (Hymenoptera: Eurytomidae), is a severe pest of almonds *Prunus amygdalus* Batch, in southeastern Europe, the Middle East, and Central Asian countries (Talhouk, 1977; Kouloussis and Katsoyannos, 1995; Doganlar *et al.*, 2006), which seriously reduces the yield and its quality (Nourbakhsh, 1998). It is a univoltine pest that primarily damages almond fruits but is also

observed on apricot and plum (Baspinar *et al.*, 2018). The damage to almond fruits caused by this pest is reported as 60 to 95% in unsprayed orchards (Kouloussis and Katsoyannos, 1995; Nourbakhsh, 1998; Faraj, 2018). Different methods such as collecting and destroying mummified fruits (Doganlar *et al.*, 2006; Faraj, 2018), using sex pheromones, and protecting natural enemies (Doganlar *et al.*, 2006) have been reported to control the pest, but the primary tactic for management of the pest in many countries is based on the chemical control (Kouloussis and Katsoyannos, 1995; Nourbakhsh, 1998; Faraj, 2018). However, continual reliance on pesticides may eventually result in several potential ecological problems, including insect resistance, secondary pest outbreaks, killing non-target organisms, and

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contamination of the environment (Mahmood *et al.*, 2016).

As an inseparable element of the integrated pest management (IPM) program, host plant resistance is compatible with other methods of control (Smith, 2005) and, in many cases, useful to enhance the efficiency of biological control agents (Bong *et al.*, 1991; Saeidi and Raeesi, 2020). Using insect-resistant host plants is an efficient, economical, ecologically, and environmentally advantageous control method within any IPM program (Stenberg, 2017). The use of resistant cultivars has been suggested in previous studies to control this pest (Talebi Chaichi, 1987; Arambourg *et al.*, 1985; Katsoyannos *et al.*, 1992). According to Talhouk (1977), female wasps select fruits based on certain physical factors, and larger fruits are usually preferred for oviposition to smaller ones. According to Kouloussis and Katsoyannos (1995), pericarp thickness is another factor that may stimulate or deter wasp oviposition. In another study, Nourbakhsh (1998) reported that the pest preferred soft-shell cultivars for oviposition. The rate of fruit infestation to almond fruit wasp was 21.21% in Sefid cultivar (a soft-shell), whereas it was 7.31 and 6.35% in hard-shell cultivars Mamaei and Sangi, respectively (Nourbakhsh, 1998). An investigation of Tzanakakis *et al.* (1997) on the effect of *E. amygdali* oviposition on fruit drop of three almond cultivars showed that infested fruits suffered a heavy premature drop in the “Texas” (Mission) and “Ferragnes”, but not in “Truoto” cultivar. Mohammadi-Khoramabadi and Arzani (2010) studied five almond cultivars and showed no significant relationship between morphological characteristics of fruits and their infestation rate.

Fruit volatile compounds are other factors that attract almond fruit wasps and play a key role in host selection and female oviposition. In this regard, Kouloussis and Katsoyannos (1994) examined the olfactory response of adult insects to the fruit chemical compounds and found that females responded significantly to fruit odor for oviposition. A review of the related literature showed that limited research had been

conducted on the host plant resistance to *E. amygdali*. Therefore the current research was undertaken to study the resistance of eight almond genotypes to the almond fruit wasp and understand the olfactory mechanism and its role in the host plant selection by *E. amygdali*. This information could provide essential knowledge for future behavioral, physiological, and chemical studies to understand the olfaction mechanism in *E. amygdali*.

Materials and Methods

Plant materials and cultural practices

Eight almond genotypes/cultivars were evaluated in this study, including one early flowering (Sefid), three medium flowerings (Mamaei, Rabie, and Nonpareil), three late flowerings (Shahrood₇, Ferragnes, and Shahrood₁₃) commercial cultivars, and one local landrace (Hooreh). These genotypes/cultivars have been planted in the Emamieh collection, Saman, by Dept. of Horticulture, Agricultural, and Natural Resources Research and Education Center, Chaharmahal and Bakhtiari province, Iran. Trees were approximately 10 years old, 3–4 m in height, and planted at 5 × 4 m distances between and along the rows. According to the soil analysis, fertilizers (micro and macro elements) were used (Dept. of Soil Sciences, Agricultural and Natural Resources Research and Education Center, Chaharmahal and Bakhtiari province, Iran). Trees were irrigated once a week, and weeds were controlled mechanically. The insecticide Confidor 35% SC (Bayer Crop Science, a.i. imidacloprid, 350 g/l) was applied at the recommended dosage of 0.5 ml. L⁻¹ to control almond aphids after falling of the petals. To control almond spider mites, *Schizotetranychus smirnovi* Wainstein, spraying was carried out using Neuron 25% EC (Golsam Gorgan Company, a. i. bromopropylate, 250 g/l) at a rate of 2 ml. L⁻¹, when the population exceeded three mites (nymphs and adults) on the abaxial surface of each leaf (Saeidi *et al.*, 2014).

During the study period (2011–2012), no insecticides were applied against almond fruit

wasp. Fruits were collected from selected trees at the appropriate stage for oviposition (immature, fresh, green fruits with an approximate diameter of 1.5-2.5 cm). The fruits on the branches were covered with an insect-proof net on May 5 (a few days before the adult emergence) under natural conditions to prevent the wasp oviposition. On the day of the experiment (May 15), immature, fresh, green fruits were removed from the trees and transferred to the laboratory for the olfactory experiment.

Insects' materials

In the first week of May, the mummified almond fruits (the infested fruits of the previous year that remained on the trees) from the Saman orchards were collected and kept in plastic boxes (60 cm long $\times$ 20 cm wide $\times$ 15 cm deep) under the natural conditions. In total 10 boxes were used. Each box contained 100 mummified fruits covered by insect-proof nets (0.5 mm mesh) to prevent adult escape. The newly emerged wasps (< 24 h old) were collected using an aspirator and kept in separate plastic boxes (20 cm long $\times$ 10 cm wide $\times$ 7 cm deep) for three days under the laboratory conditions (25 ± 1 °C, $50 \pm 10\%$ RH and a photoperiod of 14 light:10 darkness) and fed with 10% sugar – water solution. In each box, 20 adult wasps (10 males and 10 females) were released and allowed to mate. Male and female wasps were identified based on the morphology of genitalia (Kouloussis, 2004). Mating usually takes place within 1-2 days after emergence (Talebi-Chaichi, 1987). Therefore, three days old wasps were used in the experiments.

Choice test under the field conditions

The experiment was conducted under heavy natural infestation to *E. amygdali*. A completely randomized block design with eight treatments (almond cultivars/genotypes) and five replicates was used. Each replicate consisted of five trees (same-age) from each genotype. Before the adult emergence, four branches (1 m in length) from different tree directions were selected randomly, and a polyester mesh cloth (0.5 m in

width $\times$ 1.5 m in length) was hung 20 cm below the selected branch to collect the dropped fruits. The observation was done weekly from May 15 to Jun 15 (from the first to fourth weeks after the maximum emergence of the adults), and the dropped fruits were collected and inspected for the female drilling and oviposition. Four weeks after the maximum emergence of the adults, fruits of the selected branches (dropped and remaining on the branch) were collected and transferred to the laboratory. The fruits were cut into two pieces using a sharp knife and inspected for wasp oviposition under a binocular microscope at $10 \times$ magnification.

No-choice test

No choice experiment was arranged in a completely randomized blocks design with eight treatments (almond genotypes/cultivars) and five replications under natural conditions. Before the emergence of the adults, five same-aged trees of each genotype were randomly selected, and four branches (1 m in length) were marked in different directions of each tree. Insect-proof nets covered marked branches to avoid any contamination. Infestation of the genotypes was done by introducing one female (3 days old). After two weeks, the number of dropped fruits due to the pest oviposition was recorded. Indeed, after four weeks, all fruits of each branch were collected separately and transferred to the laboratory. Fruits were cut into two pieces using a sharp knife and observed under a binocular microscope at $10 \times$ magnification, and the number of active larvae in each fruit was recorded. The percent loss in fruits due to wasp oviposition activity was determined as (Number of infested fruits / total number of fruits) $\times 100$.

Olfactory response of *E. amygdali* to fruit volatiles

Two genotypes that showed resistance at the previous stages (Ferragnes and Hooreh) and Mamaei cultivar (as control) were studied in the olfactory test. A Y-tube glass olfactometer was used to test the attraction of the adult AFW to almond fruit volatile. All bioassays were

conducted during the photo phase, between 09:00 and 12:00 h. The bioassay room was maintained at 25 ± 1 °C, with $50 \pm 10\%$ RH. The olfactometer consisted of a central tube (15 cm in length, 1 cm in diameter) and two lateral arms (10 cm in length, 1 cm in diameter), which were separately connected to an extending glass box (10 cm in length $\times$ 10 cm in width $\times$ 5 cm in height). At 150 ml min^{-1} , purified air was passed into the extending glass box through activated charcoal to filter the room air and prevent other odors from entering. Illumination was provided by hanging an office lamp (20 W) vertically, 50 cm above the olfactometer.

In each experiment, two genotypes/cultivars were compared. Each experiment consisted of 10 replicates, and 10 adults were used per replicate. Five almond fruits were placed in the extending glass box for the bioassay, and the purified air was passed through the fruits. A single mated female wasp (3 days old) was introduced individually into the central arm of the Y-tube. Response of each wasp to the examined cultivars was recorded as positive when the wasp walked into one of the arms (choice chambers) and remained there for at least 30 seconds. If a wasp did not choose within five minutes after release into the olfactometer, it was considered a no-responder and excluded from the analysis. After five wasps had been tested, the olfactometer arms were rotated 180°, to randomize any positional effects. When ten wasps were bioassayed, the olfactometer was replaced with a clean one, and the fruits were also replaced. After each replicate, the olfactometer was washed with odor-free dishwashing detergent and 70% ethanol and then dried in the oven at 110 °C for one hour. Similar experiments were used to compare the olfactory response of male wasps.

Olfactory response of *E. amygdali* to fruit extract

Fruits of selected genotypes/cultivars (Ferragnes, Mamaei, and Hooreh) were collected appropriately from the plants covered by insect-proof nets (as mentioned above). The pericarp of the fruits was separated and placed

in liquid Nitrogen for grinding. The cold extraction method by ethanol was used to avoid damaging the compounds (Ghabbari *et al.*, 2018). From each sample, 10 g was weighed, ground, and solved in 100 ml pure Ethanol. The mixture was shaken at 300 rpm for 5 min. The supernatant was collected and filtered by Whatman grade 1 filter paper. The solvent was evaporated using a rotary vacuum evaporator to reduce the volume to 20 ml and stored at 5 °C. For the bioassay, 2 ml of each sample was injected on cotton wool and let the solvent evaporate at room temperature (25 °C), then it was used in olfactory bioassays. In each experiment choice, two genotypes/cultivars in 10 replicates were compared.

Statistical analysis

Statistical analysis was performed using SAS (version 9.1) and SPSS (version 22) software. Analysis of variance (Proc ANOVA) was performed to identify significant differences among the treatments, and means were compared using LSD test at 5% level. Olfactometer data were compared using Student's t-test at 1% and 5% probability levels (SAS Institute, 2001).

Results

Choice test

Results indicated significant differences in the percentage of infested fruits ($F = 49.42$, $df = 7$, $p = 0.0001$) and dropped fruits ($F = 34.71$, $df = 7$, $p = 0.0001$) among the studied genotypes/cultivars. The infested fruits ranged from $4.60 \pm 1.45\%$ in the Hooreh genotype to $86.87 \pm 2.01\%$ in the Ferragnes cultivar. The highest dropped fruits were recorded on Ferragnes ($80.70 \pm 3.21\%$) followed by Shahrood₁₃ and Shahrood₇, whereas the lowest recorded on the Hooreh genotype ($2.43 \pm 1.12\%$) (Table 1). Studied genotypes/cultivars significantly influenced the number of larvae/infested fruits ($F = 35.26$, $df = 7$, $p = 0.0001$). The highest number of alive larvae was observed in Nonpareil (1.27 ± 0.7 larvae/fruit) followed by Shahrood₁₃ ($0.84 \pm$

0.25), whereas the lowest was supported by Ferragnes cultivar (0.04 ± 0.02 larvae/fruit) (Table 1).

No-choice test

Results showed no significant differences among the studied genotypes/cultivars in the percentage of infested fruits ($F = 0.22$, $df = 7$, $p = 0.98$), whereas they significantly differed in percentage of dropped fruits ($F = 9.19$, $df = 7$, $p = 0.0001$) and the number of alive larvae/fruit ($F = 41.02$, $df = 7$, $p = 0.0001$). In the no-choice test, the damage caused by *E. amygdali* ranged from 55.83 to 81.23% on the studied genotypes/cultivars. The highest and lowest premature dropped fruits were recorded in Shahrood₁₃ ($81.23 \pm 4.57\%$) and Mamaei ($55.83 \pm 4.66\%$) cultivars. The highest number of alive larvae were observed in Nonpareil (1.32 ± 0.18 larvae/fruit) followed by

Shahrood₁₃ (0.95 ± 0.15), whereas the lowest was supported by Ferragnes cultivar (0.09 ± 0.03 larvae/fruit) (Table 2).

Olfactory response of the wasp to fruit volatiles

Almond wasp females were strongly attracted to the Mamaei fruits in Hooreh genotype and Mamaei cultivar. Among the 100 assayed females, $66.43 \pm 8.90\%$ were attracted to Mamaei, whereas $27.14 \pm 8.30\%$ preferred the odors of the Hooreh genotype, and $6.43 \pm 3.60\%$ did not respond to the examined genotypes/cultivars (Table 3). When the Hooreh genotype was compared to the Ferragnes cultivar, $64.16 \pm 7.30\%$ of females were attracted to Ferragnes, $25.84 \pm 4.90\%$ attracted to the Hooreh genotype, and $10 \pm 7.90\%$ remained not responding (Table 3).

Table 1 Mean comparison ($\pm$ SE) of infested fruits, dropped fruits, and density of larvae among different almond genotypes/cultivars under natural infestation to *Eurytoma amygdali* in the choice test.

Variety	Infested fruits (%)	Dropped fruits (%)	No. larvae/infested fruits
Sefid	65.91 ± 5.56 c	51.75 ± 4.50 c	0.57 ± 0.08 c
Ferragnes	86.87 ± 2.01 a	80.70 ± 3.21 a	0.04 ± 0.02 d
Mamaei	40.93 ± 4.67 d	35.85 ± 3.60 d	0.61 ± 0.15 c
Rabie	47.62 ± 6.36 d	41.25 ± 4.35 d	0.65 ± 0.12 c
Shahrood ₁₃	77.23 ± 4.07 b	61.25 ± 4.50 b	0.84 ± 0.25 b
Nonpareil	38.70 ± 3.89 d	34.31 ± 4.70 d	1.27 ± 0.70 a
Shahrood ₇	72.69 ± 5.95 bc	62.49 ± 5.45 b	0.69 ± 0.15 bc
Hooreh	4.60 ± 1.45 e	2.43 ± 1.12 e	0.62 ± 0.16 c

Means with the same letter(s) in each column are not significantly different at $P = 0.05$ using LSD test.

Table 2 Mean comparison ($\pm$ SE) of infested fruits, dropped fruits, and density of larvae among different almond genotypes/cultivars under artificial infestation to *Eurytoma amygdali* in the no-choice test.

Variety	Infested fruits (%)	Dropped fruits (%)	No. larvae/infested fruits
Sefid	84.58 ± 3.68 a	66.92 ± 5.16 bc	0.67 ± 0.08 c
Ferragnes	93.07 ± 1.34 a	79.21 ± 3.76 b	0.09 ± 0.03 d
Mamaei	80.31 ± 4.33 a	55.83 ± 4.66 c	0.71 ± 0.12 c
Rabie	83.52 ± 4.73 a	61.42 ± 6.36 bc	0.72 ± 0.07 c
Shahrood ₁₃	79.87 ± 5.38 a	81.23 ± 4.57 a	0.95 ± 0.15 b
Nonpareil	79.72 ± 4.31 a	62.94 ± 5.85 bc	1.32 ± 0.18 a
Shahrood ₇	81.87 ± 4.02 a	81.10 ± 5.33 a	0.80 ± 0.15 bc
Hooreh	79.93 ± 3.45 a	57.72 ± 3.10 c	0.71 ± 0.07 c

Means with the same letter(s) in each column are not significantly different at $P = 0.05$ using LSD test.

In the choice experiment between Mamaei and Ferragnes cultivars, females showed no significant difference in their choice. The attracted females to Mamaei, and Ferragnes cultivars were $49 \pm 6.30\%$ and $47 \pm 6.70\%$, respectively (Table 3). When the adult males were used in the olfactory test, they showed no significant choice difference between the odors of examined genotypes/cultivars. In the choice between Hooreh and Mamaei, $46.87 \pm 8.80\%$ and in choice experiment between Hooreh and Ferragnes, $44.50 \pm 4.90\%$ were attracted to Hooreh genotype, respectively (Table 4).

Olfactory response of the wasp to fruit extracts

The same results were obtained when the fruit extracts were used in olfactory bioassays. Females of *E. amygdali* were strongly attracted to Mamaei fruit extracts ($50.83 \pm 4.80\%$) in a choice experiment between Hooreh genotype and Mamaei cultivar. Interestingly and astonishingly, they have strongly attracted the fruit extracts of the Ferragnes cultivar ($55.83 \pm 5.30\%$) in the choice between Hooreh genotype and Ferragnes cultivar (Table 5).

Table 3 Response of *Eurytoma amygdali* females to fruit volatiles of different almond genotypes/ cultivars in an olfactory test.

Experiment	No. of replicates	Total no. of released wasp	No. of non-responding wasps	Choices (variety)	Responding wasps	
					No. of wasps	% of responding wasps (Mean $\pm$ SE)
Experiment 1	14	140	9	Mamaei	93	$66.43 \pm 8.90^{**}$
				Hooreh	38	27.14 ± 8.30
Experiment 2	12	120	12	Ferragnes	77	$64.16 \pm 7.30^{**}$
				Hooreh	31	25.84 ± 4.90
Experiment 3	10	100	4	Mamaei	49	49.00 ± 6.30^{ns}
				Ferragnes	47	47.00 ± 6.70^{ns}

** and ns: significant at 1% probability level and not significant, using Student's t-test, respectively.

Table 4 Response of *Eurytoma amygdali* males to fruit volatiles of different almond genotypes/cultivars in an olfactory test.

Experiment	No. of replicates	Total no. of released wasps	No. of non-responding wasps	Choices (variety)	Responding wasps	
					No. of wasps	% of responding wasps (Mean $\pm$ SE)
Experiment 1	10	100	2	Mamaei	45	46.87 ± 8.80^{ns}
				Hooreh	53	53.00 ± 7.30
Experiment 2	10	100	7	Ferragnes	49	48.50 ± 6.00^{ns}
				Hooreh	44	44.50 ± 4.90

ns: none significant using Student's t-test.

Table 5 Response of *Eurytoma amygdali* females to fruit extract volatiles of different almond genotypes/cultivars in the olfactory test.

Experiment	No. of replicates	Total no. of released wasps	No. of non-responding wasps	Choices (variety)	Responding wasps	
					No. of wasps	% of responding wasps (Mean $\pm$ SE)
Experiment 1	12	120	13	Mamaei	61	$50.83 \pm 4.80^{**}$
				Hooreh	46	38.33 ± 2.00
Experiment 2	12	120	12	Ferragnes	67	$55.83 \pm 5.30^{**}$
				Hooreh	41	34.17 ± 3.90
Experiment 3	10	100	5	Mamaei	46	46.0 ± 6.10^{ns}
				Ferragnes	49	49.0 ± 6.90

** and ns: significant at 1% probability level and not significant using Student's t-test, respectively.

Discussion

The current study indicated significant differences in the amount of damage caused by *E. amygdali* to different almond genotypes/cultivars. Under the field conditions, most of the damaged fruits dropped, and only a few remained on the trees (Table 1). According to Nourbakhsh (1998), young almond fruits are sensitive to wasp drilling and usually drop in the early stages of growth. In contrast, the larger ones remain on the tree, and *E. amygdali* larvae feed in their kernel. In our study, the percentage of dropped fruits in the early flowering cultivars such as Sefid was significantly lower than late-flowering cultivars (Ferragnes). Talhouk (1977) reported that female wasps choose the fruits based on some physical factors, and for oviposition, the larger fruits were preferred to smaller fruits.

Like the choice test, the most infested fruits dropped significantly in late flowering cultivars in the no-choice test. Results also showed no significant differences in the percentage of infested fruits among the studied genotypes/cultivars. It might be explained that there were no morphological barriers in the studied genotypes/cultivars against the wasp drilling. In contrast to Kouloussis and Katsoyannos (1995), the pericarp thickness was one factor that stimulated or prevented AFW oviposition. Both choice and no-choice tests revealed significant differences in the density of larvae among the studied genotypes/cultivars. Many studies have shown that only one larva was able to survive in each fruit due to cannibalism behavior (Kouloussis and Katsoyannos, 1991; Faraj, 2018; Tolga and Yoldas, 2018), but in some cases, owing to sufficient food, two larvae may survive in each kernel (Nourbakhsh, 1998). In another study, Kouloussis and Katsoyannos (1991) showed that the females of *E. amygdali* used a host-marking pheromone immediately after oviposition. Therefore other females were able to distinguish the infested fruits from non-infested fruits for depositing eggs.

According to the results of both choice and no-choice tests, the highest and the lowest number of alive larvae were observed in Nonpareil and Ferragnes cultivars, respectively. Our observations indicated a hypersensitivity reaction against AFW in the Ferragnes cultivar. In this cultivar, in addition to the secretion of sticky gum, the infested kernels wrinkled and could not develop after oviposition by the wasp. Therefore the larvae died due to starvation. As shown in tables 1 and 2, only a few larvae (4 and 9% in choice and no-choice tests, respectively) could survive in the Ferragnes cultivar. Hypersensitive reaction is a rapid localized cell death that occurs at the point of pathogen or invader penetration. This host response includes morphological and histological changes that cause the death of attacked tissue and, finally, the death of aggressive agents (Fernandes, 1990; Singh and Singh, 2005). Despite many hypersensitive reactions against pathogens, there are few examples against insect herbivores. Most reports are related to galling insects, bark beetles, adelgids, and siricids (Fernandes, 1990). Hypersensitivity mechanism was reported as the basis of resistance in wheat to Hessian fly, *Mayetiola destructor* (Say) (Grover, 1995), in rice against the Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Bentur and Kalode, 1996), in potato to egg masses of *Leptinotarsa decemlineata* (Say) (Balbyshev and Lorenzen, 1997) and *Bauhinia hrevipes* Vogel to a leaf galling *Contarinia* sp. (Fernandes, 1998).

Results of the choice test indicated the lowest attraction of *E. amygdali* females (Table 1) to fruits of Hooreh genotype under natural conditions. On the other hand, results of olfactory response indicated strong attraction of AFW females to fruits (Table 3) and fruit extracts of Mamaei and Ferragnes cultivars (Table 5). These findings demonstrated that olfactory cues were required for females to approach their host plant for oviposition. Locating a host plant is crucial for a herbivorous insect to fulfill its nutritional requirements and find suitable oviposition sites

(Bruce *et al.*, 2005). According to Kouloussis and Katsoyannos (1994), the extract of undamaged unripe almond fruits stimulated female aggregation on the glass surface treated with these extracts. The olfactory response of male AFW was not similar to the females. They did not respond to the host plant odors (Table 4). Similar results were reported by Kouloussis and Katsoyannos (1994).

In phytophagous insects, olfaction is crucial to execute innate behaviors crucial for survival and reproduction, such as recognizing mates, location of food sources, and selecting the suitable host for oviposition (Bernays and Chapman, 1994). Chemical cues released by the host plant could modulate the behavior of host selection for oviposition. This study revealed that certain chemical stimuli (kairomones) emitted from the unripe fruits of almond (Mamaei and Ferragnes) cultivars might influence the host finding behavior of AFW females. These compounds may not be present at adequate or specific ratios in Hooreh genotype. According to Nayasembe and Torto (2014), the volatile compounds emitted by plants depend on their quality, quantity, and recipient insect species. Bruce *et al.* (2005) demonstrated that recognizing a host plant by olfactory signals could occur using either species-specific compounds or specific ratios of ubiquitous compounds. Isolation, identification, and synthesis of these compounds may be helpful to develop an environmentally safe method for sustainable control of the pest on almonds and reduce the application of pesticides.

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Declaration of conflicting interests

The author declares that he has no conflict of interests.

References

- Arambourg, Y., Fauvel, G. and Chevin, H. 1985. *Eurytoma amygdali* End. and its presence in France, a new chalcidoid pest of almond. Review of Agricultural Entomology, 73: 691-698.
- Balbyshev, N. F. and Lorenzen, J. H. 1997. Hypersensitivity and egg drop: a novel mechanism of host plant resistance to Colorado potato beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology, 90(2): 652-657.
- Baspinar, H., Doll, D. and Rijal, J. 2018. Pest management in organic almond, In: Vacante, V. and Kreiter, S. (Eds.), Handbook of Pest Management in Organic Farming. CAB International, pp. 328-347.
- Bentur, J. S. and Kalode, M. B. 1996. Hypersensitive reaction and induced resistance in rice against the Asian rice gall midge *Orseolia oryzae*. Entomologia Experimentalis et Applicata, 78: 77-81.
- Bong, C. F. J., Sikorowski, P. P. and Davis, F. M. 1991. Effects of a resistant maize genotype and cytoplasmic polyhedrosis virus on growth and development of the corn earworm (Lepidoptera: Noctuidae). Environmental Entomology, 20: 1200-1206.
- Bruce, T. J. A., Wadhams, L. J. and Woodcock, C. M. 2005. Insect host location: a volatile situation. Trends in Plant Science, 10(6): 269-274.
- Bernays, E. A. and Chapman, R. F. 1994. Host-plant Selection by Phytophagous Insects. Chapman & Hall, London, 312 pp.
- Doganlar, O., Yildirim, A. E. and Doganlar, M. 2006. Natural enemy complex of *Eurytoma amygdali* Enderlein, (Hymenoptera, Eurytomidae) in eastern Mediterranean region of Turkey. Research Journal of Agriculture and Biological Sciences, 2(6): 282-286.
- Faraj, F. M. H. 2018. Effect of some plant extracts on the population density of

- Eurytoma amygdali* and their impact on almond production in Sulaimani province. Euphrates Journal of Agriculture Science, 10 (1): 1-9.
- Fernandes, G. W. 1990. Hypersensitivity: A neglected plant resistance mechanism against insect herbivores. Environmental Entomology, 19(5): 1173-1182.
- Fernandes, G. W. 1998. Hypersensitivity as a phenotypic basis of plant induced resistance against a galling insect (Diptera: Cecidomyiidae). Environmental Entomology, 27(2): 260-267.
- Ghabbari, M., Guarino, S., Caleca, V., Saiano, F., Sinacori, M., Baser, N., Ben-Jemaa, J. M. and Verde, G. 2018. Behavior-modifying and insecticidal effects of plant extracts on adults of *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae). Journal of Pest Science, 91: 907-917.
- Grover, J. P. B. 1995. Hypersensitive response of wheat to the Hessian fly. Entomologia Experimentalis et Applicata, 74(3): 283-294.
- Katsoyannos, B. I., Kouloussis, N. A. and Vasiliou, A. 1992. Monitoring populations of the almond seed wasp populations (*Eurytoma amygdali*), with sex pheromone traps and other means, and optimal timing of chemical control. Entomologia Experimentalis et Applicata, 62(1): 9-16.
- Kouloussis, N. A. and Katsoyannos, B. I. 1991. Host discrimination and evidence for a host marking pheromone in the almond seed wasp, *Eurytoma amygdali*. Entomologia Experimentalis et Applicata, 58: 165-174.
- Kouloussis, N. A. and Katsoyannos, B. I. 1994. Adult response of almond seed wasp, *Eurytoma amygdali*, to chemicals from its host and certain non-host. Entomologia Experimentalis et Applicata, 73(3): 211-220.
- Kouloussis, N. A. and Katsoyannos, B. I. 1995. Distribution and activities of *Eurytoma amygdali* (Hym.; Eurytomidae) wasps on almond trees. Annals of the Entomology Society of America, 88(4): 547-553.
- Kouloussis, N. A. 2004. Almond seed wasp, *Eurytoma Amygdali* Enderlein (Hymenoptera: Eurytomidae). In: Encyclopedia of Entomology. Springer, Dordrecht. p. 137.
- Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A. and Hakeem K. R. 2016. Effects of pesticides on environment. In: Hakeem, K., Akhtar, M. and Abdullah, S. (Eds), Plant, Soil and Microbes. Springer International Publishing, Switzerland. pp. 253-269.
- Mohammadi-Khramabadi, A. and Arzani, A. 2010. Evaluation of some morphological characteristics in five almond cultivars and their relationships with seed infestation and resistance to the almond seed wasp, *Eurytoma amygdali* Enderlein (Hym.: Eurytomidae), in Yazd province, Iran. Journal of Agricultural Sciences and Natural Resources, 16 (2): 197-205.
- Nayasembe, V. O. and Torto, B. 2014. Volatile phytochemicals as mosquito semiochemicals. Phytochemistry Letters, 8: 196-201.
- Nourbakhsh, S. H. 1998. Determination of the infested fruits percentage in almond cultivars by almond seed wasp *Eurytoma amygdali* (Hym.: Eurytomidae) and evaluation of mechanical control and natural enemies. Project Report, Agricultural and Natural resources Research Center, Shahrekord, Iran. 15 pp.
- Tzanakakis, M. E., Papadopoulos, N. T., Katsoyannos, B. I., Drakos, G. N. and Manolakis, E. 1997. Premature fruit drop caused by *Eurytoma amygdali* (Hymenoptera: Eurytomidae) on three almond varieties. Journal of Economic Entomology, 90(1): 1635-1640.
- Saeidi, Z. and Raeesi, M. 2021. Integration of resistant variety and biological agent to control tomato leaf miner, *Tuta absoluta* (Meyrick), under greenhouse conditions. Bulletin of Entomological Research, 111: 357-363.
- Saeidi, Z., Shabani, F., Nourbakhsh, S. H. and Nemati A. 2014. Effectiveness of some acaricides on almond spider mite (*Schizotetranychus smirnovi* Wainst.). Journal of Plant Protection, 28(1): 11-17.
- SAS Institute Inc. 2001. SAS/ Stat users Guide, version 9.1 SAS Institute Inc, Cary, NC.

- Singh, D. P. and Singh, A. 2005. Disease and Insect Resistance in Plants. Science Publisher, Enfield, New Hampshire, USA. 396 pp.
- Smith, C. M. 2005. Plant Resistance to Arthropods, Molecular and Conventional Approaches. Springer Netherlands. 426 pp.
- Stenberg, J.A. 2017. A conceptual frame work for integrated pest management. Trends in Plant Sciences, 22: 759-769.
- Talebi-Chaichi, P. 1987. Study on the Bioecology of the *Eurytoma amygdali* End. (Hym.: Eurytomidae). Amidi Publication, Tabriz, Iran, 40 pp
- Talhouk, A. S. 1977. Contribution to the knowledge of almond pests in east Mediterranean countries. V. Fruit-feeding insects, *Eurytoma amygdali* and *Anarsia lineatella*. Zeitschrift für Angewandte Entomologie, 83: 145-154.
- Tolga, M. F. and Yoldas, Z. 2018. Investigation on the damage rate of *E. amygdali*, with first record of its parasitoid, *Exeristes roborator* (Fabricius) in almond orchards of Mugala and Manisa. VII Turkish Plant Protection Congress. 14-17 November, Dslaman, Turkey.

مقاومت ارقام مختلف بادام نسبت به زنبور مغزخوار بادام (*Eurytoma amygdali* (Hymenoptera: Eurytomidae)

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چکیده: مقاومت هشت ژنوتیپ بادام (شامل سفید، مامایی، ربیع، شاهرود ۷، فرانسیس، شاهرود ۱۳، نان پاریل و هوره) نسبت به زنبور مغزخوار بادام *Eurytoma amygdali* Enderlein به کمک آزمون‌ها انتخاب و عدم انتخاب مورد مطالعه قرار گرفت. در روش انتخاب آزاد، درصد میوه‌های آلوده از $1/45 \pm 4/60$ در ژنوتیپ هوره تا $2/01 \pm 86/87$ در رقم فرانسیس متغیر بود. بیش‌ترین و کم‌ترین میزان ریزش میوه‌های نارس به ترتیب در رقم فرانسیس ($3/21 \pm 80/70$ درصد) و ژنوتیپ هوره ($1/12 \pm 2/43$ درصد) ثبت شد. بیش‌ترین و کم‌ترین تعداد لارو زنده در میوه به ترتیب در رقم نان پاریل ($0/70 \pm 1/27$) و فرانسیس ($0/04 \pm 0/02$) مشاهده شد. نتایج آزمون عدم انتخاب، بیش‌ترین میزان ریزش میوه نارس ($3/76 \pm 79/21$ درصد) و کم‌ترین تعداد لارو زنده در میوه ($0/03 \pm 0/09$ درصد) را در رقم فرانسیس نشان داد. نتایج آزمایش پاسخ بویایی نشان داد که افراد ماده زنبور مغزخوار بادام به شدت جذب میوه و عصاره میوه ارقام مامایی و فرانسیس در مقایسه با ژنوتیپ هوره شدند. براساس نتایج به دست آمده، برخی از محرک‌های شیمیایی که از میوه‌های نارس بادام منتشر می‌شوند ممکن است بر رفتار میزبان‌یابی حشرات ماده زنبور مغزخوار بادام تأثیر بگذارند.

واژگان کلیدی: زنبور مغزخوار بادام، حساسیت میزبان، آزمون انتخاب، عدم انتخاب، پاسخ بویایی