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Research Article

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Determination of the preferred two different coccinellid species on different aphid species feeding on broad bean plants

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Abstract

In this study, the orientation of two different predator species [(*Coccinella septempunctata* L., *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)) to two different aphids [*Aphis fabae* Scopoli and *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)] was investigated. Y-type olfactometer which was made of glass material with one entrance and two exit openings was used in the study. Different applications were applied to the exit ends of the olfactometer and the orientation of the predators dropped from the entrance end was examined. Individuals passing the marked area (10 cm) on the olfactometer were considered to have turned to that plant. All of the trials were conducted separately for each predator and prey with 10 replications. The counts of the insects advancing on the arms of the olfactometer were made 1, 4 and 8 hours after the release. When looking at the data obtained in the first stage of the study, it was determined that the predator insects mostly gravitate towards the leaves infected with aphids; In the second stage, it was observed that *C. septempunctata* individuals mostly turned towards the side that was contaminated with *A. pisum*, and *H. variegata* individuals mostly turned towards the side that was contaminated with *A.fabae*. When looking at the data obtained from the study, it was determined that the plants damaged by herbivorous insects show an attractive feature for predators. Accordingly, it was concluded that chemicals obtained from broad bean plants damaged by aphids should be analyzed.

Keywords: *Coccinella*, *Hippodamia*, *Aphis fabae*, *Acyrthosiphon pisum,* Olfactometer

Introduction

Living organisms are in constant interaction with each other and with the inanimate environment in which they live (Odum and Barrett, 2005). In this context, arthropods, like all living things, are under the influence of chemicals secreted by the plants in their environment. Accordingly, both herbivores and their natural enemies benefit from these chemicals in finding their food (Bell and Cardé, 1984; Visser, 1986; Roitberg and Isman, 1992; Vet and Dicke, 1992; Cardé and Bell, 1995; Schoonhoven et al., 1998). It is also known that these chemicals are used for defense purposes in plants (Dicke and Vet, 1999; Vet, 1999). When the researches in recent years are examined, it is observed that the reactions of herbivores after the damage on the plant are focused on, and how these reactions affect the herbivores and natural enemies (Price et al., 1980; Turlings et al., 1991; Vet and Dicke, 1992; Thaler, 1999; Kessler and Baldwin, 2001; Becker et al., 2016; Giunti et al., 2016; Lin et al., 2016; Gençer et al., 2017; Silva et al., 2017). In order to achieve positive results in the control of harmful organisms in agriculture, the host and foraging behaviors of the factors used in biological control should be known (Tunca et al., 2011).

Aphids are harmful organisms that cause economic losses around the world. As a result of feeding on the plant, they stop growing and if the population reaches high numbers, they kill the plant on which it is fed. This pest group causes indirect losses in plants due to their secretion of toxic substances and

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carrying virus diseases (Lodos, 1982; Catherall et al., 1987; Kovalev et al., 1991; Elmalı and Toros, 1997; Blackman and Eastop, 2000). Among the hosts of *Aphis fabae* are more than 200 wild plants as well as vegetables, sugar beet, broad beans, beans, potatoes (Völkl and Stechmann, 1998; Barnea et al., 2005; Fericean et al., 2012). Although Acyrthosiphon pisum is a pest of weeds, it also causes damage to beans, lentils, alfalfa, sainfoin and some legumes (Stary, 1970; Ali and Habtewold, 1994).

Coccinellidae is one of the families that are effective in biological control (Khan et al., 2007). *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) belonging to this family, is a species living in the Palearctic region (Central and North Africa, Europe, Arabia, India and China) (Korchefsky, 1932; Horion, 1961). The subject of our study is polyphagous and has been observed especially on aphids that feed on weeds. In addition, species belonging to Aleyrodidae (Hemiptera) and Chaitophoridae (Hemiptera) families are among the groups they feed on (Horion, 1961; Klausnitzer, 1966; Elmalı and Toros, 1994; Aslan and Uygun, 2005; Elekçioğlu and Şenal, 2007). *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), an important aphid predator, is an oval-shaped and 6-8 mm long (Uygun, 1981) and very common species in the palearctic region (Korcschefsky, 1932; Horion, 1961). This predator mostly feeds on aphids; in addition, it has been reported that they are effective on soft bodied insects that cause damage to plants (Ali and Rizvi, 2009).

Beneficial insects are sensitive to chemical aspects of the multitrophic environment, particularly host location (Poppy, 1997) and can learn to associate plant volatiles in the presence of prey (Drukker et al., 2000). Leaves of plants normally secrete small amounts of volatile compounds, but when a plant is damaged by an insect, an increase is observed in the amount of compounds secreted (Reddy, 2012). Beneficial organisms respond significantly to volatile substances released from plants after damage caused by herbivores (Turlings et al., 1990). Predators use numerous clues released by plants alone or when damaged to locate their prey in their natural habitats (Vet and Dicke, 1992). Looking at the studies conducted, it is observed that predator insects use semiochemicals to find their prey (Ninkovic et al., 2001). These volatile chemicals released by plants may differ in different plant and herbivore combinations (Boom et al., 2004). Looking at the studies conducted, it is seen that mass production can be made to use coccinellids against aphids and other harmful organisms. Coccinellids' ability to distinguish odors is effective here. Due to the presence of volatile compounds released by plants due to the feeding of herbivores, coccinellids can distinguish the attacked plant (Dicke, 2009; Heil, 2008).

Considering the researches carried out in the world, the reproductive potential of aphids and their damages on the products are quite high. Farmers prefer intensive chemical control against these pests. This event negatively affects the environment and human health. In order to prevent these negative effects, there are different methods. Accordingly, in this study, predator behaviors of *H. variegata* and *C. septempunctata* (*A. fabae* and *A. pisum*) were investigated.

Materials and Methods

The main materials in the study are two different predators [*Coccinella septempunctata* L., *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae)], Broad bean (*Vicia faba* L.) and two aphids [*Aphis fabae* Scopoli and *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)]. In the study, all plants, hunts, hunters production and experiments were carried out in Isparta Applied Sciences University, Faculty of Agriculture, Plant Protection Department, Biological Control Research and Application Laboratory.

Production of Plants

The broad bean seeds used in the experiments were planted in 2-liter pots with a soil: peat: perlite mixture at a ratio of 1: 1: 1. After this process, the pod seeds were expected to germinate, after the germination process, the daily maintenance of the plant was made and no chemicals were used in pest controls on the plants. Plant production was carried out in climate cabinets with 27±1 °C temperature and 65±5% proportional humidity and long day lighting (16: 8) conditions.

Culture of Aphids

Aphis fabae and *Acythosiphon pisum* individuals used as food in the study were obtained from mass production in the laboratory. Aphids were transferred to clean plants in separate net cages with the help of a sable brush and their reproduction was achieved. Then, clean plants were left next to the infected plants with the increased aphids and aphids were passed on to the clean plants. This process was carried out as long as the trials continued. All of the aphid production was carried out in climate chambers with 27±1 °C temperature and 65±5 % relative humidity and long day lighting (16: 8).

Providing Predators from Nature

Polyphagous hunters *H. variegata* and *C. septempunctata* were collected from agricultural production areas with aphid damage with the help of a pad, taken to a container with their food, brought to the laboratory and mass production started.

Culture of Predators

H. variegata and *C. septempunctata* individuals who were used as hunters and collected from field conditions in the study were brought to the laboratory and put into mass production. In this process, cages made of plexiglass material covered with tulle on the sides and top are used. In order to make the production easy and fast, hunter individuals were left separately in the cages with aphids used in the experiments and the individuals used in the experiments were obtained from these productions. All of the hunter productions were carried out in climate chambers with 27 ± 1 ° C temperature and 65 ± 5% proportional humidity and long day lighting (16: 8).

Establishment of Trials

In the study, a Y-type olfactometer made of glass material with an entrance and two exit openings was used. In addition, an air pump has been used to keep the air flow in the arms of the olfactometer moving regularly. In the first stage of the trials, a clean plant leaf was placed at one of the exit ends of the olfactometer and a leaf infected with aphids was placed at the other end. Then, 10 hunter individuals were placed at the entrance end of the olfactometer and they were expected to move. In the second stage of the experiments, the plant

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contaminated with *A. fabae* was placed on one end of the olfactometer and the plant infected with *A. pisum* on the other end, and the movements of the predator individuals released from the entrance end were observed as in the first stage. Individuals passing the marked area (10 cm) on the olfactometer were considered to have turned to that plant. All of the trials were conducted separately for each hunter and food with 10 replications. The counts of the insects moving on the arms of the olfactometer were made 1, 4 and 8 hours after the release. These experiments were carried out in climate chambers with 27 ± 1 ° C temperature, 65 ± 5% relative humidity and long day lighting (16: 8).

Results and Discussion

In this study, two different predator insects (*C. septempunctata*, *H. variegata*) and two aphids (*A. fabae* and *A. pisum*) were investigated with the help of an olfactometer. In the first stage of the experiment in which the orientation of *Coccinella septempunctata* was examined (1 arm of clean plant and the other plant infected with aphids), it was determined that the predator insect mostly turned towards the arm where the infected plants were found (p<0.05). According to the counts made at the end of the 8th hour in this part of the study, the hunter insect preferred the arm with *A. pisum* rather than the arm with *A. fabae* (Table 1). At this stage, two different aphids and a broad bean plant were placed at the two exit ends of the olfactometer and the predator's orientation was investigated. *Coccinella septempunctata* individuals showed more orientation towards the arm with *A. pisum* than the counts made at the end of the 4th hour (p <0.05) (Figure 1, Table 2).

Table 1. Amounts of *Coccinella septampunctata* at the different hours to be directed to clean broad bean plant with two different aphids

Coccinella septempunctata	Infested	Cleaned	P	F
Application of *Acyrthosiphon pisum* (1 h)	1.8±0.249 a*	0.7±0.153 b	0.001	14.14
Application of *Acyrthosiphon pisum* (4 h)	4.1±0.379 a*	0.7±0.213 b	0.001	61.2
Application of *Acyrthosiphon pisum* (8 h)	8.0±0.258 a*	1.3±0.153 b	0.001	498.78
Application of *Aphis fabae* (1 h)	1.7±0.260 a*	0.6±0.221 b	0.005	10.37
Application of *Aphis fabae* (4 h)	4.4±0.306 a*	0.4±0.221 b	0.001	112.5
Application of *Aphis fabae* (8 h)	7.5±0.307 a*	1.1±0.233 b	0.001	275.5

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

Table 2. Amounts of *Coccinella septampunctata* turning to broad bean plant with two different aphids at different times

Coccinella septempunctata	*Aphis fabae*	*Acyrthosiphon pisum*	P	F
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (1 h)	1.4±0.163 a	1.3±0.213 a	0.714	0.14
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (4 h)	3.5±0.167 a*	4.5±0.224 b	0.002	12.86
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (8 h)	4.4±0.267 a	5.0±0.333 a	0.177	1.98

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

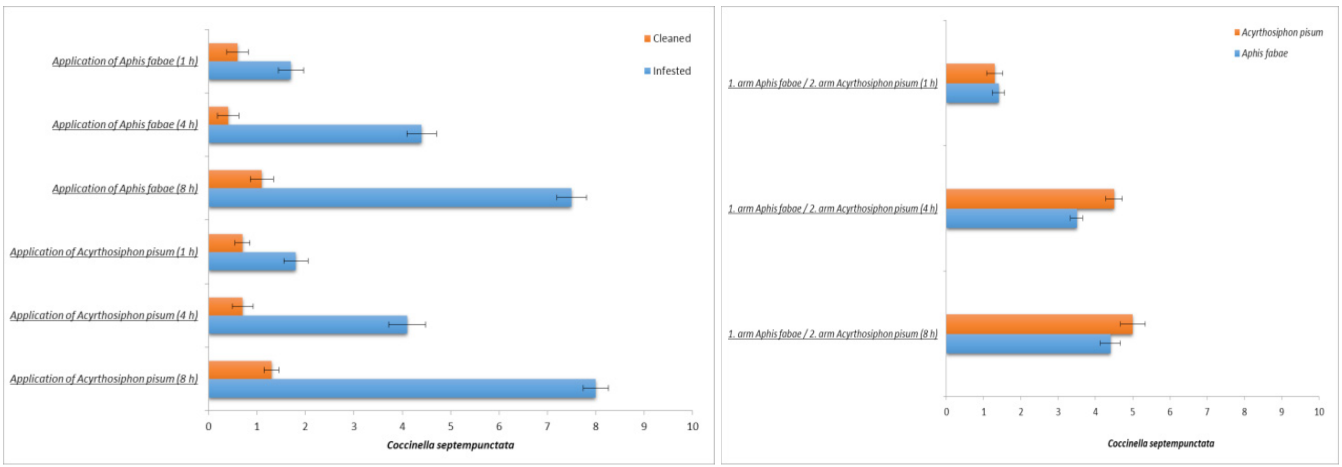

Figure 1. The amount of *Coccinella septempunctata* moving to aphids at different times

In the second stage of the experiment in which the orientation of *Hippodamia variegata* was examined (1 arm of clean plant and the plant infected with the other arm aphid), it was determined that the hunter insect mostly directed towards the arm where the infected plants were ($p < 0.05$). According to the counts made at the end of the 8th hour, the hunter insect preferred the arm with *A. fabae* rather than the arm with *A. pisum* (Table 3). At this stage, two different aphids and a broad bean plant were placed at the two exit ends of the olfactometer and the predator's orientation was investigated. *Hippodamia variegata* individuals showed more orientation towards the arm with *A. fabae* than the counts made at the end of the 4th and 8th ($p < 0.05$) (Figure 2, Table 4).

Table 3. Amounts of *Hippodamia variegata* at different hours to be directed to clean broad bean plant with two different aphids

Hippodamia variegata	**Infested**	**Cleaned**	**P**	**F**
Application of *Acyrthosiphon pisum* (1 h)	1.6±0.306 a*	0.7±0.213 b	0,027	5,83
Application of *Acyrthosiphon pisum* (4 h)	4.1±0.379 a*	0.7±0.213 b	0,001	61,2
Application of *Acyrthosiphon pisum* (8 h)	7.6±0.267 a*	1.1±0.233 b	0,001	336,5
Application of *Aphis fabae* (1 h)	1.6±0.221 a*	0.5±0.167 b	0,001	15,78
Application of *Aphis fabae* (4 h)	4.8±0.249 a*	0.5±0.269 b	0,001	137,53
Application of *Aphis fabae* (8 h)	8.0±0.258 a*	0.7±0.213 b	0,001	474,86

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

Table 4. Amounts of *Hippodamia variegata* turning to broad bean plant with two different aphids at different times

Hippodamia variegata	***Aphis fabae***	***Acyrthosiphon pisum***	**P**	**F**
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (1 h)	1.5±0.224 a	1.0±0.255 a	0,16	2,14
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (4 h)	4.5±0.167 a*	3.7±0.213 b	0,008	8,79
1. arm *Aphis fabae* 2. arm *Acyrthosiphon pisum* (8 h)	5.4±0.221 a*	4.3±0.260 b	0,005	10,37

*Different letters on the same line indicate that there is a statistical difference between the averages according to the Tukey multiple comparison test

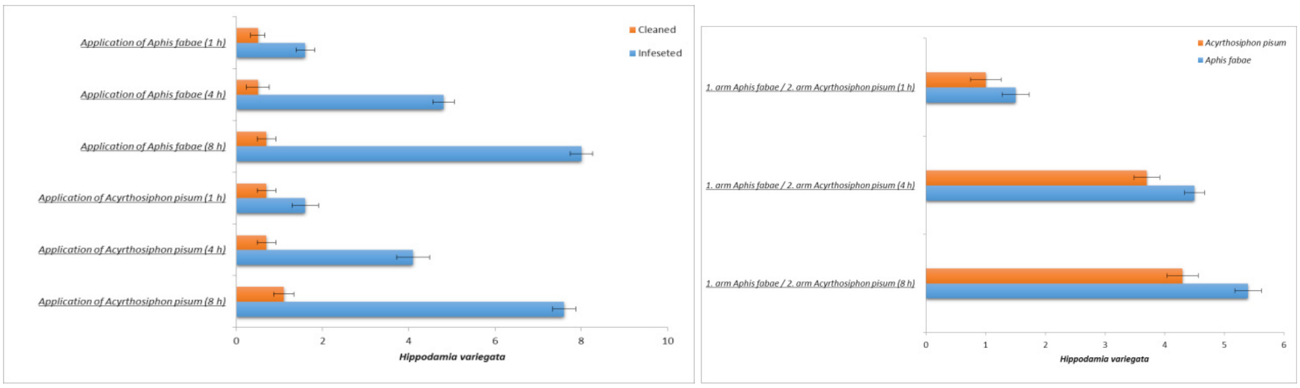

Figure 2. The amount of *Hippodamia variegata* moving to aphids at different times

De Moares et al. (1998) stated that volatile compounds secreted from the plant vary according to the pest and that these compounds are detected by parasitoids. According to the data obtained, when they were damaged by *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) and *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae), which are harmful on some products (cotton, corn and tobacco), the mentioned products secreted different compounds. De Moares and Lewis (1999) determined that the compounds released from cotton and tobacco plants damaged by some herbivores have attractive effects on *Cardiochiles nigriceps* (Vier.) (Hymenoptera: Braconidae) and *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). Llusià and Peñuelas (2001) conducted a study with two apple varieties (Golden Delicious and Starking) damaged by *Pananychus ulmi* Koch (Acarina: Tetranychidae) whether the substances secreted due to the effects of the pests attract predatory mites (*Amblyseius veersoni* Chant and *A. californicus* McGregor). They investigated. In their experiments with the olfactometer, they observed that the damaged plants attracted 85% of the predatory mites. Lin et al. (2016) examined the orientation of *Propylaea japonica* with an olfactometer. As a result, *P. japonica* has been observed to react to volatile organic compounds from citrus plants damaged by *Diaphorina citri* and *Candidatus liobacter*.

When looking at the studies conducted in recent years, different studies on the compounds released into the environment after herbivores damage plants and how these affect natural enemies stand out (Price et al., 1980; Turlings et al., 1991; Vet and Dicke, 1992; Bernays and Chapman, 1994; De Moares et al., 1998; Dicke et al., 1990; Dicke et al., 1993; Dicke et al., 1994; Geervliet et al., 1994; Mattiacci and Dicke, 1995; Rosenthal and Berenbaum, 1992; Schoonhoven et al., 1998; Thaler, 1999; Kessler and Baldwin, 2001; Becker et al., 2016; Giunti et al., 2016; Lin et al., 2016; Gençer et al., 2017; Silva et al., 2017). When plants are damaged by herbivores, some volatile compounds and secondary metabolites are released as a defense behavior. It has been determined in researches that these compounds secreted have attractive effects on many parasitoids and predators (Kester and Barbosa, 1991; Lecomte and Thibout, 1984; Mattiacci et al., 1994; McAuslane et al., 1991; Paré and Tumlinson, 1999; Steinberg et al., 1993; Udayagiri and Jones, 1992; 1993).

Conclusion

When the data obtained as a result of the study were evaluated, it was determined that both predator insects were directed towards plants damaged by aphids. While *C. septempuntata* individuals, among the predatory insects used in the experiments, mostly turned to the side where *A. pisum* was; It has been determined that *H. variegata* individuals mostly turn to the side where *A.fabae* is located.

Considering the data obtained from the study, it was determined that plants damaged by herbivorous insects show an attractive feature for predatory insects. Accordingly, it was concluded that by analyzing the chemicals obtained from the broad bean plant damaged by aphids, it was concluded that experiments should be conducted to determine to what extent which compound would affect the predator insects.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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