

Research Article

Interactions between *Orius albidipennis* and *Aphidius colemani* (Hymenoptera: Braconidae) for the control of *Aphis gossypii* on greenhouse cucumber

Mehran Attarzadeh¹, Ali Rajabpour^{1*}, Mohammad Farkhari² and Arash Rasekh³

1. Department of Plant Protection, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Ahvaz, Iran.

2. Department of Genetics and Plant Productions, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Ahvaz, Iran.

3. Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Abstract: *Aphis gossypii* Glover (Hemiptera: Aphididae) is an important cucumber pest especially in greenhouse. The efficacy of simultaneous release of generalist predator, *Orius albidipennis* Reuter (Hemiptera: Anthracoridae) and a specialist parasitic wasp, *Aphidius colemani* Viereck (Hymenoptera: Braconidae), was evaluated in laboratory conditions against the pest. For this purpose, investigations were carried out on preference of the predator between parasitized and non-parasitized aphids. In addition, production of volatile infochemicals between the natural enemies (NEs) was studied by olfactometry trials. In another part of this research, systemic production of volatile synomone by the infested cucumber plants for attraction of each NE was examined by the olfactometry tests. Results revealed that *O. albidipennis* had no obvious preference to either the parasitized or non-parasitized aphids, while both NEs were significantly attracted to volatiles emitted from infested host plants. Our findings revealed that each of the NEs avoided odors which indicated the presence of another intraguild competitor. The documented facts from the entire study reveal that the NEs are good biocontrol agents against *A. gossypii* on cucumber, but their avoidance from each other makes simultaneous release of the predator and parasitic wasps unsuitable for biological control of this aphid.

Keywords: predatory bug, parasitoid wasp, simultaneous release, infochemicals, aphid biocontrol

Introduction

Problems associated with chemical pesticides, such as pest resistance, side effect on non-target organisms, secondary pest outbreaks, environmental contaminations etc. (Pedigo, 2002)

resulted in the development of integrated pest management (IPM) theory in 1970 (Knipling, 1972). Biological control has been a valuable method in IPM programs around the world for many years (Orr, 2009). Two natural enemy species are frequently released simultaneously to control one pest species in greenhouse biological control programs (Orr, 2009). In some cases, releasing of two or more biocontrol agents increased mortality by 12.97% and reduced pest abundance by 27.17% compared to single release

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*Corresponding author, e-mail: rajabpour@ramin.ac.ir

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against aphid pests (Stiling and Cornelissen, 2005). In simultaneous release of two natural enemies against a pest, intraguild predation (IGP) and interspecific competition (IC) between the natural enemies might affect the biological control and these phenomena can directly or indirectly disrupt biological control programs (Orr, 2009). The effect of IGP and IC on biocontrol efficacy of some anthocorid bugs and some parasitoid wasps were investigated by some authors. For example, occurrence of IGP between *Anthocoris nemorum* L. and *Aphidius colemani* Viereck on *Myzus persicae* Sulzer (Hom., Anthocoridae) (Meyling *et al.*, 2004), *Orius majusculus* Reuter and *Encarsia formosa* Gahan (Hym., Aphelinidae) on *Bemisia tabaci* Gennadius (Hom., Aleurodidae) pupa (Sohrabi *et al.*, 2012) were studied in laboratory conditions. Moreover, IGP between other generalist predators like *Hippodamia convergens* GuérinMénéville (Col., Coccinellidae) and *Lysiphlebus testaceipes* Cresson (Hym., Braconidae) on *Aphis gossypii* Glover (Colfer and Rosenheim, 2001), *Harmonia axyridis* Pallas (Col., Coccinellidae) and *Aphelinus asychis* Walker (Hym., Aphelinidae) on *Macrosiphum euphorbiae* Thomas (Hom. Aphididae) (Synder *et al.*, 2004), *Macrolopus pygmeus* Wagner (Het., Miridae) and *Trichogramma macro* Nagaraja and Nagarkatti (Hym., Trichogrammatidae) on *Tuta absoluta* Meyreck eggs (Chailleux *et al.*, 2013), *Coccinella septempunctata* L. (Col. Coccinellidae) and *Lysiphlebus fabarum* Marshall (Hym., Braconidae) on *Aphis fabae* Scopoli (Meyhofer and Klug, 2002), *H. axyridis* and *A. asychis* on *Myzus persicae* Sulzer (Fu *et al.*, 2017) were previously evaluated.

Chemical information plays a critical role in foraging behavior of natural enemies. The chemical information can originate from herbivore, its food, or other natural enemies (Takabayashi *et al.*, 1994). The importance of infochemicals, in foraging behavior of parasitoids and predators has been previously documented. For instance, olfactory tests revealed that *Anthocoris* spp. respond to pear trees infested by *Psylla pyrii* L. (Hem., Psyllidae) (Drukker *et al.*, 1995). In addition,

positive behavioral response of *O. tristicolor* (White) to bean plants damaged by *Frankliniella occidentalis* Pergande (Thys., Thripidae) *Tetranychus urticae* Koch (Acari, Tetranychidae), *O. sauteri* Poppius to eggplant and infestation by *Thrips palmi* Karny (Thys., Thripidae) (Mochizuki and Yano, 2007) were reported. Effect of plant infochemicals on behavior and preference of some parasitoid wasps like *Cotesia glomerata* L. (a parasitoid of *Pieris rapae* L. on cabbage) (Steinberg *et al.*, 1992), *C. sesamiae* Cameron (a parasitoid of *Chilo* spp. on maize and sorghum) (Ngi-Song *et al.*, 1996), *Trichogramma chilonis* Ishii (a parasitoid of *Helicoverpa armigera* Hubner in sorghum) (Romeis *et al.*, 1997) and *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) (a parasitoid of *Listronotus oregonensis* LeConte on gramineae) (Cournoyer and Boivin, 2004) were studied in a tritrophic system.

Our ability to develop successful biocontrol programs will be enhanced by experimental or field studies which address the probable trophic interactions occurring in an agricultural system (Rosenheim *et al.*, 1995).

Predatory bugs which belong to the genus *Orius* are generalist predators of various soft bodied arthropods like aphids (Reitz *et al.*, 2006). *O. albidipennis* Reuter is a common predator in several regions of Iran and its ability as a potential biocontrol agent has been reported especially in greenhouse conditions (Rajabpour *et al.*, 2011, Salehi *et al.*, 2016, Banihashemi *et al.*, 2017). Another important biological control agent of aphids is *A. colemani* which is released commercially against the pest in greenhouses (Enkegaard, 2005). The wasp larvae develop entirely inside aphid body and do not kill their host until the larvae are ready to pupate (Enkegaard, 2005). Among the many species of greenhouse aphids, *A. gossypii* is an economically important aphid which attack many greenhouse plants including cucumber (Blackman and Eastop 2000).

Generalist predators, including *Orius* spp., and parasitoids are considered as important natural enemies of aphids. The generalist predators not

only feed on the aphids, but might also consume aphidophagous parasitoids at all stages of development (Traugott *et al.*, 2012). The aim of the present study is to investigate interactions between two important biocontrol agents (*O. albidipennis* and *A. colemani*) for simultaneous release against *A. gossypii* in greenhouse cucumber. In addition, production of volatile infochemicals by the infested host plants or interspecific competitors in response to each natural enemy will be evaluated in laboratory conditions.

Materials and Methods

Host plant

Cucumber (*Cucumis sativus* cv. superdaminos) seeds were sown in pots filled with a perlite-cocopeat mix (1: 1, v: v) moistened regularly with half-strength Hoagland nutrient solution. The plants were grown in cages, 0.6 × 0.6 × 2m, in growth chamber with photoperiod of 14:10h (light: dark), at 20 ± 5 °C, and maximum photon flux density of 1000 μmolm⁻²s⁻¹.

Insect cultures

Wingless females of *A. gossypii* were provided from an experimental colony at Insect Ecological Laboratory of Shahid Chamran University of Ahwaz, Khuzestan province, south west Iran. The aphids were released in the rearing cages, 1 × 1 × 0.6m, with the cucumber plants. The cages were kept inside an air conditioned room at a temperature of 25 ± 3 °C, relative humidity of 65 ± 5%, with a photoperiod of 14: 10h (light: dark). After the colony establishment, the wingless female adults or 2nd instar nymphs from the colony were used in the trials or for *A. colemani* rearing, respectively.

Mummies of *M. persicae* Sulzer and *A. gossypii* (parasitized by *A. colemani*) were provided from Koppert BV, Netherlands. After emergence of the parasitoid adults, 100 adults were introduced to the insect rearing cages with cucumber plants infested by *A. gossypii* at ambient conditions earlier mentioned for aphids rearing.

Adults of the predatory bugs, *O. albidipennis*, were collected from unsprayed

sunflower fields in Mollasani region, Khuzestan province, south west Iran. Female bugs were isolated in a Plexiglas cylinder (18cm high, 7.5cm diameter) covered with a fine gauze lid on the top and margin for ventilation. At least one male was selected from the offspring of each female bug and was identified using keys of Pericart (1972). The bugs were reared on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) while date palm pollen was used as daily diet and bean pod as oviposition substrate at 25 ± 1 °C, 60 ± 5%RH, and 16: 8h (light: dark) in an incubator. The Plexiglas cylinders were lined with crumpled wipe papers to provide a hiding place to rest and reduce cannibalism.

Experimental design

Prey preference trials

Experiments were performed at 25 ± 1 °C, 60 ± 5% RH within a photoperiod of 16: 8h (light: dark) in an incubator. Parasitized and non-parasitized female adults of *A. gossypii* were used as prey of *O. albidipennis*. For this purpose, five females of *A. colemani* (3-4 days-old) were introduced into a standard 10cm plastic Petri dish that contained 20 wingless aphids which located upside a wet filter paper. To facilitate ventilation, a hole was made on top of the dishes (2.5cm diameter) and covered with fine net. After 8h, the parasitic wasps were removed from the Petri dishes. The parasitized aphids were used in the prey preference experiments 3 days post removal of parasitic wasp.

The experimental arena included the Petri dishes (with characteristics mentioned above) that comprised a cucumber leaf disk placed upside down on a 20ml layer of agar (5%) (Montserrat *et al.*, 2000). Based on preliminary tests, densities of 5 or 10 parasitized aphids and 5 or 10 non-parasitized aphids per arena were used in the trials. To identify parasitized from non-parasitized aphids, one antenna of parasitized aphid was separated by fine pincet. Adult of the predatory bug (2-4 days old) was introduced to the experimental arena. Experiments were performed in a completely randomized design with 10 replications. After 2h, the predator was removed

and the numbers of killed preys were recorded separately.

Olfactory trials

A Y-shaped olfactometer device was applied in the trials. Various parts of the device are shown in Figure 1. A leaf of cucumber plants was infested by adults of *A. gossypii* in a clips cage (5cm diameter). After 24h, another leaf from the plant was picked and placed in chamber 2. The leaf was washed by distilled water before use. Another cucumber leaf from plant without previous infestation was placed in chamber 3. Air flow (3.5lit/sec.) was passed across chambers 2 and 3 and reached location 1. A female wasp (2-4 days old and starved for 8h) was introduced to location 1. In another trial, a female predatory bug (3-4 days old and starved for 8h) was released in location 1.

After 10 min, location of the parasitic wasp or predatory bug in chamber 2 was recorded. If

the wasp or bug did not move, and location 2 or 3 were not selected by the natural enemies, the trial was canceled and repeated again.

To investigate the production of volatile infochemicals by the intraguild competitors, a cucumber leaf with five wingless female aphids + one female predatory bug were placed in chamber 1 and five wingless female aphids were placed alone in chamber 2. Then a female parasitic wasp (2-4 days old) was introduced to location 1. In another scenario, five female aphids + a female parasitoid and five female aphids were placed alone in chambers 2 and 3, respectively. The location of the wasp or bug was determined after 10min.

All olfactory experiments were conducted in an incubator at 25 ± 2 °C, $60 \pm 5\%$ RH and a photoperiod of 16:8h (light: dark). Each trial was repeated for 20 times based on a completely randomized design.

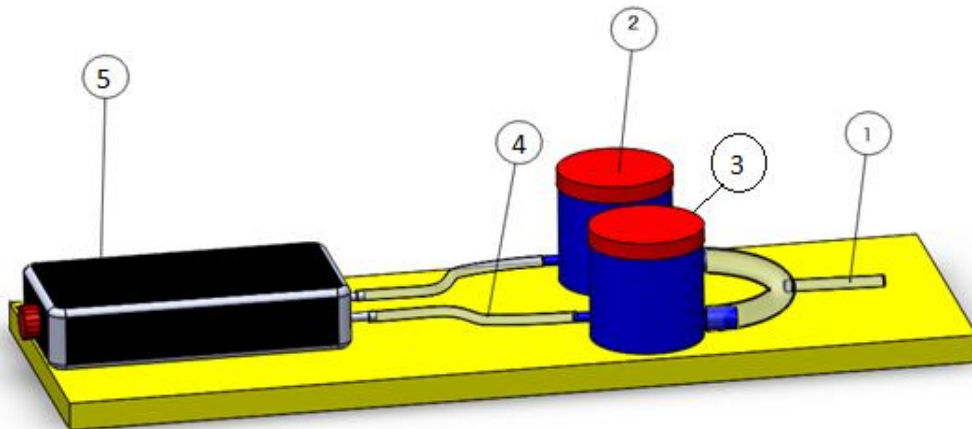


Figure 1 Schematic figure of Y-shaped olfactometry device (1. Common entrance tube, 2 and 3. Sample chamber, 4. Connective tube, 5. Electric engine to create airflow).

Data analyses

Manly’s α index was used to evaluate prey preference (Manly, 1974; Chesson, 1984):

$$\alpha = \frac{\ln\left(\frac{n_{i0} - r_i}{n_{i0}}\right)}{\sum_{j=1}^m \ln\left(\frac{n_{j0} - r_j}{n_{j0}}\right)}, i = 1, \dots, m$$

Where α = Manly’s α index for prey type, n_{i0} is initial number of prey items of type i , r_i is the number of prey items of type i consumed by the predator, n_{j0} is initial number of prey items of type j , r_j is the number of prey items of type j consumed by the predator and m is the number of prey types in the experiment. The α index give values between zero and one, and the number of the different prey types is always summed to be one and in several

experiments, all individuals of both prey species were consumed. To calculate Manly's α index in these cases, the formula was modified by the addition of one individual prey of the completely depleted prey type to corresponding n_{i0} and n_{j0} in the above equation. This correction is based on the assumption that if another individual of the prey in question is present, it would survive. The corresponding estimate of α_i is slightly conservative (Klecka, 2010).

One sample t-test was used to compare Manly's α index mean of each sample with 0.5 in prey preference trials. In addition, chi-square test was used to analyze olfactory trials. All statistical analyses were performed using SPSS statistical analysis package (SPSS, 1993).

Results

Prey preference experiments

Manly's α index of *O. albidipennis* preference was calculated between parasitized and non-parasitized aphids at 5 and 10 densities for each prey (Table 1).

Results show that Manly's α index of the predatory bug was not significantly different when fed on parasitized and non-parasitized aphids at different densities. Therefore, *O. albidipennis* has no obvious preference for either of the two preys.

Olfactory experiments

Synomone production studies

Responses of *O. albidipennis* and *A. colemani* female adults to volatile cues which originated from leaves of plants previously infested (IP) and not infested (NIP) by *A. gossypii* are presented in Table 2.

Results indicated that adults of *O. albidipennis* were significantly attracted to leaf of IP in comparison with NIP. Same behavior was observed for female adults of *A. colemani* in the olfactometry tests.

Inter-specific competition

Results of olfactometry trials for indicating IC between *O. albidipennis* and *A. colemani* are shown in Table 3.

Table 1 Manly's α indices of *Orius albidipennis* for the parasitized and unparasitized *Aphis gossypii*.

Number of Parasitized: Unparasitized	Unparasitized aphid			Parasitized aphid		
	Manly's α index	t (df=8)	P-value	Manly's α index	t (df=8)	P-value
5:5	0.533 ± 0.088	0.379	0.715	0.466 ± 0.088	-0.379	0.715
10:10	0.504 ± 0.054	0.075	0.942	0.490 ± 0.054	-0.075	0.942

Table 2 Responding of *Aphidius colemani* and *Orius albidipennis* adults to cucumber plant with previous infestation (IP) and no infestation (NIP) by *Aphis gossypii*.

Species	Volatile cue sources	Total no.	Number of response		Number of no response	χ^2	P-value
			IP	NIP			
<i>A. colemani</i>	IP/NIP	100	63	31	6	10.8	0.001
<i>O. albidipennis</i>	IP/NIP	100	67	29	4	15.0	< 0.001

Table 3 Response and non-response of *Aphidius colemani* and *Orius albidipennis* adults to volatile cues from the aphid + intraguild competitor (IC) and the *Aphis gossypii* only.

Species	Volatile cue sources	Total no.	Number of response		Number of no response	χ^2	P-value
			Aphid + IC	Aphid			
<i>A. colemani</i>	Aphids + IC/aphids	100	38	58	4	4.16	0.041
<i>O. albidipennis</i>	Aphids + IC/aphids	100	37	60	3	5.45	0.020

Results indicated that both the wasps and bugs significantly avoided the chamber where the competitors were present which is associated with aphid patch. It seems that the *O. albidipennis* and *A. colemani* can recognize volatile odor of their intraguild competitors and avoid the IC when they had another choice.

Discussion

The predatory bug, *O. albidipennis*, did not exhibit any preference between parasitized and unparasitized adults of *A. gossypii*. Insect pest predators may prey unparasitized pests or pests which are parasitized and contain the immature stage of endoparasitoids, a form of IGP. Therefore, the biological control of the pest can either be enhanced or disrupted by introducing a predator species to an existing host-parasitoid system. When the predator exhibits a relative preference to unparasitized pest, it can be expected to improve control of the pest even if it produces high levels of IGP. In contrast, if the predator shows significant preference for parasitized pest, the biological control by the parasitoid can be disrupted (Colfer and Rosenheim 2001). Therefore, predator preference for hosts (unparasitized vs. parasitized) may be a key factor in determining the net effect of predation in simultaneous release of a generalist predator and a specialist parasitoid to control a pest (Erbilgin *et al.*, 2004). Various findings were obtained by researchers when the preference of a generalist predator was investigated between parasitized and unparasitized preys by specialist parasitoids. For instance, three predators of *B. tabaci* nymphs, *Geocoris punctipes* Say, *Orius insidiosus* Say, and *Hippodamia convergens* Gue'rin-Me'nevil, exhibited a significant preference for parasitized nymphs by *Eretmocerus* sp. nr. *emiratus* (Hym., Aphelinidae) compared with unparasitized nymphs (Naranjo, 2007). Moreover, adults and 5th instar nymphs of *O. majusculus* exhibited significant preference for parasitized over unparasitized nymphs of *B. tabaci* by *E. formosa* (Sohrabi *et al.*, 2012).

In contrast, adults of *C. septempunctat* and larvae of *Episyrphys balteatus* De Geer (Dip., Syrphidae) showed significant preference to unparasitized nymphs of *A. fabae* in comparison to parasitized nymphs by *L. fabarum* (Meyhofer and Klug 2002). Also, Takizawa *et al.* (2000) demonstrated that the larvae of aphidphagous ladybirds (*C. septempunctat*, *Harmonia axyridis* Pallas and *Propylea japonica* Thunberg) had significant tendency to feed on unparasitized aphids compared with mummies of *Aphis craccivora* Koch which contained larvae of *A. colemani*. *H. axyridis* showed significant preference for unparasitized nymphs of *B. tabaci* in comparison with whitefly nymphs parasitized by *E. formosa* and *E. sophia* Girault and Dodd (Tan *et al.*, 2016). The tendency of generalist predator to feed on the pest's mummies or unparasitized preys may be attributed to the effect of the preys on performance of the predators. For instance, Takizawa *et al.*, (2000) documented that the coccinellid larvae exhibits different survival, developmental time and body weight when reared with parasitized or unparasitized aphids by *A. colemani*. In addition, the aphids paralyzed by the parasitic wasp result in decrease of its defensive behavior, therefore, it can be a crucial reason of more attack preference of the predators.

Similar to our findings, no significant preference was observed between parasitized and unparasitized eggs of *T. absoluta* (parasitized by *T. achaeae*) by *M. pygmeus* (Chailleux *et al.*, 2013).

Ikegawa *et al.* (2015) stated that types and combinations of behaviour of prey and predators may greatly affect qualitative outcomes of biological control by multiple natural enemies. Therefore, it is expected that different results were observed for different pest-parasitoid-predator complexes.

It has been proved that adults of both natural enemies: *O. albidipennis* and *A. colemani*, could recognize volatile cues of infested cucumber plant by the aphids. The cues are probably produced systemically. Many host plants release volatile compound when infested

by herbivorous insects. The compounds are used as cues by predators or parasitoids foraging for their preys or hosts, respectively (Neveu *et al.*, 2002). The attractive volatiles may be emitted only by infested parts of the host plant or systemically released by uninfested parts of the infested host plant, which can probably enhance the detectability of the signal (Dicke *et al.*, 1990; Neveu 2002). The host-derived cues have been shown to guide female parasitoids to locate and evaluate host patches before oviposition. For instance, Neveu *et al.* (2002) demonstrated that *Brassica campestris* L. whose roots are attacked by *Delia radicum* L. larvae, emits volatile cues attracting *Trybliographa rapae* Westwood (Hymenoptera: Figitidae). In addition, the infochemicals were detected in cereals that were attacked by *Sitobion avenae* Fabricious. The infochemicals guided *Aphidius rhopalosiphi* De Stepani-Perez (Hym., Braconidae) to the infested plants in olfactometry tests (Micha *et al.* 2000). Sasso *et al.* (2007) showed that Aphid-infested tomatoes (by *Macrosiphum euphorbia* Thomas) were significantly more attractive towards *Aphidius ervi* Holiday (Hym., Braconidae) than undamaged plants and aphids themselves. The authors distinguished 8 compounds, α -pinene, (*Z*)-3-hexen-1-ol, α -phellandrene, limonene, (*E*)- β -ocimene, p-cymene, methyl salicylate, (*E*)- β -caryophyllene, which were released from the infested plants. The synthetic standard of the compound significantly affected the behavior of the parasitic wasp.

The volatiles released from several aphid and host plant species, alone or in association, were studied for their infochemical role in prey location by the generalist predators. For example, it is documented that *Vicia fabae* L., *Brassica napus* L. and *Sinapis alba* L. are infested by some aphid species, viz *M. persicae*, *Acyrtosiphon pisum* Harris and *Brevicoryne brassicae* L., emit volatile synomone, (*E*) - β -Farnesene, which attract *Adalia bipunctata* L. (Col., Coccinellidae) larvae and adults (Francis *et al.*, 2004). Soybean plants infested by *A. glycines* released volatile cues composed of methyl salicylate, (D)-limonene and (E, E)-a-

farnesene which affected the behavior of *C. septempunctat*, *Chrysopa carnea* Stephens (Neu., Chrysopidae) and syrphid flies. However, the behavioral effect was not observed in *H. axyridis* (Zhu and Park, 2005). Tan and Liu (2014) demonstrated that tomatoes infested by *M. persicae* distributed attractive volatile cues which stimulate three predator species; *C. septempunctata*, *P. japonica* (Col., Coccinellidae), and *O. sauteri* Poppius (Het., Anthocoridae), two whitefly parasitoid species (*E. formosa* and *E. sophia* Girault and Dodd) (Hym., Aphelinidae), and one aphid parasitoid species *Aphidius gifuensis* Ashmead (Hym., Aphidiidae).

Glinwood *et al.* (2011) demonstrated that predatory coccinellids can learn to associate the odor of aphid-infested plants with the presence of prey, and this olfactory learning ability is sensitive enough to discriminate variability between different genotypes of the same plant.

Our findings showed that both *A. colemani* and *O. albidipennis* avoided odors which indicated the presence of another intraguild competitor. It seemed that the natural enemies use the cues for reduction of intraguild competition between each other. Cotes *et al.* (2015) demonstrated that parasitoids of herbivorous insects utilize volatiles to evaluate predator-derived infochemicals to reduce predation risks. Our findings are in line with the results of Gnanvossou *et al.* (2003) who demonstrated that phytoseiid mite, *Typhlodromalus manihoti* Moraes, *T. aripo* DeLeon and *Euseius fustis* Pritchard and Baker, avoided patches of *Mononychellus tanajoa* Bondar inhabited by the other heterospecifics or by conspecifics when tested against a patch without predators. Same results were reported for *Neoseiulus californicus* McGregor and *Phytoseiulus cucumeris* Athias-Henriot (Cakmak *et al.* 2006).

In conclusion, in this study, it was proved that *O. albidipennis* and *A. colemani* have some valuable characteristics for control of *A. gossypii* in greenhouse cucumber e.g. there is no preference of the predatory bugs to the aphid mummies and systemic production of

synomone by the host plant for attraction of the natural enemies to infested plants. Although these characteristics make the natural enemies good biocontrol agents against *A. gossypii* on cucumber, especially in greenhouses; avoidance of the natural enemies from each other causes simultaneous release of the predator and parasitic wasp unsuitable for biological control of aphids in greenhouse or field. Results of the study can be applied in biocontrol program of *A. gossypii* on cucumber.

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برهم کنش بین گونه‌ای *Aphis gossypii* و *Orius albidipennis* (Hymenoptera: Braconidae) برای کنترل *Aphis gossypii* روی خیار گلخانه‌ای

مهران عطارزاده^۱، علی رجب‌پور^{۱*}، محمد فرخاری^۲ و آرش راسخ^۳

- ۱- گروه گیاه‌پزشکی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، ایران.
 ۲- گروه زراعت و اصلاح نباتات، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، ایران.
 ۳- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، اهواز، ایران.
 پست الکترونیکی نویسنده مسئول مکاتبه: rajabpour@ramin.ac.ir
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چکیده: شته جالیز *Aphis gossypii* Glover (Hemiptera: Aphididae) یک آفت مهم خیار به‌خصوص در شرایط گلخانه‌ای به‌حساب می‌آید. کارایی رهاسازی هم‌زمان شکارگر عمومی‌خوار *Orius albidipennis* Reuter (Hemiptera: Anthocoridae) و زنبور پارازیتوئید تخصصی *Aphidius colemani* Veireck علیه این آفت در شرایط آزمایشگاهی مورد ارزیابی قرار گرفت. برای این هدف، بررسی روی ترجیح این شکارگر بین شته‌های پارازیته شده و پارازیته نشده انجام شد. علاوه بر این، تولید پیام‌رسان‌های شیمیایی بین این دشمنان طبیعی توسط آزمایشات بویایی‌سنجی صورت گرفت. در قسمت دیگری از این تحقیق، تولید سیستمیک سینومون‌های تدخینی توسط گیاهان خیار برای جلب این دشمنان طبیعی توسط آزمایش‌های بویایی‌سنجی مطالعه شد. نتایج نشان داد که شکارگر *O. albidipennis* بین شته پارازیته شده و پارازیته نشده ترجیح میزبانی مشخصی نداشت درحالی‌که هر دو دشمن طبیعی به صورت معنی‌داری به بوهای متضاد شده توسط گیاهان میزبان آلوده جلب می‌شدند. یافته‌های ما نشان داد که هر دشمن طبیعی از بویی که بیانگر حضور دشمن رقیب درون رسته‌ای دیگرش است، اجتناب می‌کند. در کل این آزمایش‌ها نشان داد که این دشمنان طبیعی عوامل کنترل بیولوژیکی خوبی علیه *A. gossypii* در خیار هستند ولی اجتناب این دشمنان طبیعی از یک‌دیگر موجب می‌شود که رهاسازی هم‌زمان این شکارگر و زنبور پارازیتوئید گزینه مناسبی برای کنترل بیولوژیک این شته نباشد.

واژگان کلیدی: سن‌های شکارگر، زنبورهای پارازیتوئید، رهاسازی هم‌زمان، پیام‌رسان‌های شیمیایی، بیوکنترل شته‌ها