

## Intraguild Predation on the Parasitoid Wasp *Aphidius colemani* by the Predator *Aphidoletes aphidimyza*: Effect of Host Plant Cultivars

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### ABSTRACT

In a tri-trophic system, guild members may engage in IntraGuild Predation (IGP) and their interactions may be affected by the host plants. We used a system composed of the predatory gall midge, *Aphidoletes aphidimyza* Rondani, the parasitoid wasp, *Aphidius colemani* Viereck, and the melon aphid, *Aphis gossypii* Glover, to test how the outputs of IGP were affected by two cultivars of cucumber (Khasib and Karim). IGP between natural enemies were examined on a single cucumber plant of each cultivar infested with mummified, parasitized or healthy individuals of melon aphid in a controlled environment room at  $25\pm 2^\circ\text{C}$ ,  $65\pm 5\%$  RH, and a photoperiod of 16L: 8D hours. According to the results, *A. aphidimyza* was the effective predator of parasitized aphids, but not on mummified ones. In treatments with either healthy or parasitized aphids alone, *A. aphidimyza* consumed significantly less numbers of healthy aphids or parasitized ones on Karim cultivar. When predators were provided with healthy aphids and parasitized aphids together on plants, the risk for parasitized aphids of being predated upon by *A. aphidimyza* larvae was significantly reduced on Karim cultivar. Manly's Preference Index for healthy aphids on Karim cultivar was significantly the highest. The results revealed that the strength of IGP on IG-prey on Karim cultivar was less than Khasib cultivar. Therefore, better control of melon aphid population can be expected on this cultivar.

**Keywords:** *Aphis gossypii*, Cucumber cultivars, Interspecific interactions, Predatory gall midge.

### INTRODUCTION

One of the possible tactics for pest control is the introduction of multiple natural enemies (Cakmak *et al.*, 2009). The introduced natural enemies, sharing a pest species as a host, may be involved in IntraGuild Predation (IGP), which has been recognized as an important factor disrupting effective pest control. IGP is a kind of predation that a member of a guild kills and eats another member of the same guild

(Polis *et al.*, 1989). In this system, the predator is defined as IG-predator, the prey as IG-prey, and their shared resource as the extraguild prey (Lucas, 2005). According to Polis *et al.* (1989), guild members may attack each other symmetrically or asymmetrically. Symmetric IGP happens when two species have mutual predation. Asymmetric IGP occurs when one species is always the predator of the other species.

Several factors may affect IGP (Polis *et al.*, 1989; Lucas, 2005), one of which is

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bottom-up factor. Host plants are considered as bottom-up factors and may have the ability to affect not only the herbivores, but also their natural enemies (Tahriri Adabi et al., 2010; Fallahpour et al., 2015; Doryanizadeh et al., 2016). For example, differences in morphological traits of host plants such as the presence of trichomes potentially interferes in biological control by hindering predation and mobility of natural enemies (Styrsky et al., 2006). Similarly, leaf surface wax of plants can reduce the searching efficiency of natural enemies (White and Eigenbrode, 2000). It has been also reported that different genotypes (Schädler et al., 2010) and cultivars (Tahriri Adabi et al., 2010) of a plant or different host plants (Madadi et al., 2008) can affect tri-trophic interactions by influencing the performance of natural enemies or the intensity of IGP. Changing nutrient contents of host plants via application of fertilizers, as reported by Hosseini et al. (2010), can also influence IGP among aphidophagous predators. Furthermore, plant metabolites ingested by pests can negatively affect the suitability and quality of herbivores as resources for natural enemies (Turlings and Benrey, 1998).

The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a sap-sucking pest of worldwide importance that attacks several agricultural crops such as cotton, cucurbits, citrus, coffee, cocoa, eggplant, peppers, potato, and okra (Deguince et al., 1994; Blackman and Eastop, 2000). The aphid frequently builds up large population on the crops and causes both direct and indirect damages on them (Blackman and Eastop, 2000). Two types of natural enemies associating with *A. gossypii* are the predatory gall midge, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), and the parasitoid wasp, *Aphidius colemani* Viereck (Hymenoptera: Braconidae) (van Lenteren, 2003; Hosseini et al., 2010).

*A. aphidimyza* is a specialist predator of aphids, which has the ability to attack more than 80 aphid species (Yukawa et al., 1998).

The larva is the predacious stage of *A. aphidimyza*, feeding on the host aphids, while adult midge only feed on aphid honeydew or plant nectar (Helyer et al., 2003). The larva can usually kill more aphids than it consumes, which makes it a very successful biocontrol agent. It sucks out the fluids from the body of an aphid, but before that, the aphid is paralyzed by injection of a toxin in the leg (Helyer et al., 2003).

*A. colemani*, another specialist natural enemy of aphids, is considered as an effective endoparasitoid wasp of *A. gossypii*, and *Myzus persicae* Sulzer (van Lenteren, 2003). The larvae feed and develop within the body of aphids. The host aphid is killed when wasp's pupation occurs and its body becomes a rigid, leathery, golden-brown mummy. Later, a mature wasp emerges from the mummified aphid by cutting a circular hole in the back of the mummy (Helyer et al., 2003).

Both *A. aphidimyza* and *A. colemani* are often found in fields or used simultaneously in protected cropping systems, and may temporally overlap in their phenology that could result in IGP. Between predator and parasitoid guilds, the IGP interaction is always asymmetrical, the parasitoid being the inferior antagonist (Meyhöfer and Hindayana, 2000). In this situation, using beneficial insects together may reduce the output of the biological control programs. For example, Colfer and Rosenheim (2001) found that *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) reduced survival of immature parasitoid *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) by predation on mummified aphids. However, it may be possible that the combination of a predator and a parasitoid could improve biocontrol success and reduce pest outbreaks on the crop. In this condition, as reported by Snyder et al. (2004) the successful control of the pest would be expected.

In the present study, in order to determine whether two biological control agents, i.e. a predator and a parasitoid, could be better

than either alone for biological control of *A. gossypii*, no-choice and choice experiments were conducted in the clear plastic containers housing each a single cucumber plant to assess whether 1) the predator *A. aphidimyza* preferentially consumes parasitized versus healthy aphids, and 2) different cultivars affect the IGP between the predator *A. aphidimyza* and the parasitoid *A. colemani*. Characteristics of the IGP interactions would highly influence the ability of these naturally co-occurring enemies to suppress *A. gossypii* populations.

## MATERIALS AND METHODS

The experiments were performed using the tri-trophic system of cucumber (*Cucumis sativus* L.) as a host plant, the melon aphid (*A. gossypii*) as a pest, the parasitoid wasp *A. colemani* as an IG-prey, and the predatory gall midge, *A. aphidimyza* as the IG-predator.

### Host Plants

Two greenhouse cucumber (*C. sativus*) cultivars including Karim and Khasib were used in the experiments. The cultivars were selected based on their different morphological structure (i.e., trichome) (Basij *et al.*, 2011), and for being among the most cultivated cultivars. In order to supply plants for insects rearing, the seeds were sown and grown in plastic pots (20 cm diameter×18 cm height), filled with a mixture of 30% vermicompost and 70% soil, in a greenhouse at 24±4°C, 60±10% of RH and a natural photoperiod. To provide plants for the main experiments, the seeds were grown in small size plastic pots (7.5 cm diameter×8 cm height) with the same growing medium and conditions as described above. When seedlings in the small pots reached the 2-3 leaf stages, they were transferred to the laboratory for the experiments outlined below.

### Insect Rearing

*A. gossypii* colony was established from the individuals collected from cucumber plants cv. Zohal grown in a greenhouse in Ardabil, Iran. The aphids were transferred on tested cucumber cultivars, and separately reared on them for more than three generations.

*A. colemani* was obtained from a commercial supplier (Koppert Biological Systems, The Netherlands) as mummified aphids. The parasitoid wasps were emerged by placing the mummies in a growth chamber, under a net-covered cage (80×40×50 cm) containing aphid-infested cucumber plants. It was separately reared on *A. gossypii* on both cultivars for three generations.

*A. aphidimyza* was obtained as pupae from a laboratory culture maintaining in the Ferdowsi University of Mashhad, Mashhad, Iran. The predatory gall midge was separately reared on each cucumber cultivar for three generations, in a net-covered cage (80×40×50 cm) containing the melon aphid-infested cucumber plants. All insect cultures were maintained in a controlled environment room at 25±2°C, 65±5% of RH, and 16L:8D photoperiod.

### Experimental Procedure

Each experimental unit (microcosm) consisted of a clear plastic container (24×14×7 cm) with a fine-mesh net opening (14×6 cm) on the lid for ventilation. Inside each microcosm, a single pot of cucumber plant (at 2-3 leaf stages as described above) was placed. In order to synchronize individuals of similar ages of aphids on plants, 15-20 apterous female of *A. gossypii* were randomly collected from the stock culture and placed on each experimental plant by a fine paintbrush. After 24 h, the adult aphids were removed, leaving a total of 40-50 aphid nymphs per plant. The nymphs were left on the plants to develop to the desired ages.



In order to study the IGP between the 24 hour-old-larvae of *A. aphidimyza* and *A. colemani* in a whole plant study, no-choice and choice experiments were performed. For this reason, four combinations (with 10 replicates for each) were established on both cultivars inside the microcosms as described below:

### No-Choice Experiments

(1) 30 healthy aphids, one larvae of *A. aphidimyza*,

(2) 30 parasitized aphids, one larvae of *A. aphidimyza*,

(3) 30 mummified aphids, one larvae of *A. aphidimyza*,

#### Choice Experiment

(4) 30 healthy aphids, 30 parasitized aphids, one larvae of *A. aphidimyza*

Furthermore, mortalities of the aphids or the wasps were studied on separate plants without the predator. To prepare plants with parasitized aphids, 20 adult parasitoids per plant were released and allowed to parasitize 40-50 of the 2<sup>nd</sup> instar nymphs (preferred age) of *A. gossypii* for 24 h. Then, the adult parasitoids were removed from the microcosms by using an aspirator. By observing the parasitized aphids with their swollen body on plants (4-5 days later), the number of parasitized aphids was reduced to 30 parasitized aphids per plant by removing the extra parasitized or non-parasitized aphids. Plants with mummified aphids were prepared in the same way, except the time needed to detect mummified aphids on plants. In these treatments, mummified aphids were detected on plants 6-8 days after the introduction of the adult parasitoids. Plants with healthy aphids were prepared in a similar way as used for treatments with parasitized or mummified aphids, but without releasing of any parasitoids. Furthermore, the 4<sup>th</sup> instar nymphs of *A. gossypii* were used as healthy aphids.

After 24 hours, the larvae of the predator were excluded and the number of consumed

healthy aphids was immediately counted. In treatments with parasitized or mummified aphids, counting was delayed until the mummification or the emergence of the adult parasitoids, respectively. All experiments were performed in a controlled environment room at 25±2°C, 65±5% RH, and a 16L: 8D photoperiod.

### Statistical Analysis

In this study, since the mortality of insects without the predator was negligible, the main data was not corrected. Data on the predation of *A. aphidimyza* when encountered with healthy or parasitized aphids on each cultivar were analyzed using the independent-sample *t*-test and those on both cultivars were subjected to a two-way Analysis Of Variance (ANOVA), followed by a Tukey's test at 5% significance level (SPSS, 2007).

The preference index for *A. aphidimyza* preying on healthy aphids and parasitized aphids was calculated on the basis of the proportional predation of the two prey items (Manly, 1974). It was calculated as:

$$\beta_1 = \frac{\log\left(\frac{e_1}{A_1}\right)}{\log\left(\frac{e_1}{A_1}\right) + \log\left(\frac{e_2}{A_2}\right)}$$

Where,  $\beta_1$  (Beta= Manly's preference index ) is the preference for healthy aphids,  $e_1$  is the number of healthy aphids remaining after the experiment,  $A_1$  is the number of healthy aphids offered,  $e_2$  is the number of parasitized aphids remaining, and  $A_2$  is the number of parasitized aphids offered. The value of the index falls between 0 and 1. An index equal to 0.5 indicates that the predator selects prey randomly. A value of the index larger than 0.5, indicates the preference for healthy aphids. The index lower than 0.5 shows the preference for parasitized aphids. Independent-samples *t*-test was used to test the significance of Manly's index (SPSS, 2007). Figures were created in Excel 2010.

## RESULTS

In this study, the predation did not occur on mummified aphids, so the related treatment was eliminated from the analysis. In no-choice experiment, there were significant differences in the consumption number of healthy aphids and parasitized aphids when the predator larvae encountered them on each cultivar ( $t= 5.035$ ,  $df= 18$ ,  $P< 0.0001$  for Khasib cultivar and  $t= 3.394$ ,  $df= 18$ ,  $P= 0.003$  for Karim cultivar). On Khasib cultivar, the predator consumed  $8.20 \pm 0.42$  healthy aphids and  $5.60 \pm 0.30$  parasitized aphids, which the predation rate on healthy aphids was significantly more than parasitized ones (Figure 1). Similar trend was observed on Karim cultivar; with the mean number of  $6.20 \pm 0.41$  healthy aphids and  $4.60 \pm 0.22$  parasitized aphids were consumed by the predator (Figure 1). Generally, by comparing both cultivars, mean numbers of consumed healthy aphids ( $t= 3.397$ ,  $df= 18$ ,  $P= 0.003$ ) or parasitized

aphids ( $t= 2.652$ ,  $df= 18$ ,  $P= 0.016$ ) by *A. aphidimyza* on Khasib cultivar were significantly higher than the other cultivar (Figure 1).

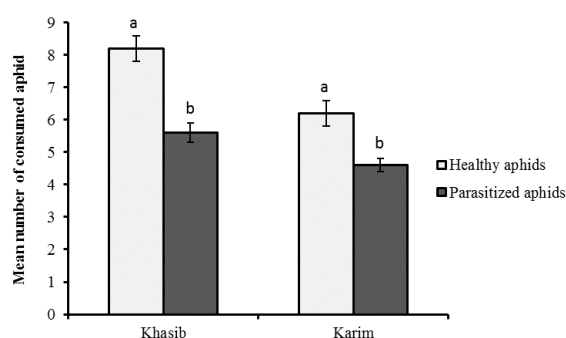
In choice experiment, the main effect of cultivar ( $F= 2.690$ ,  $df= 1, 36$ ,  $P= 0.110$ ) and prey items ( $F= 0.299$ ,  $df= 1, 36$ ,  $P= 0.588$ ) were not significant. However, significant difference was found for their interactions ( $F= 11.989$ ,  $df= 1, 36$ ,  $P= 0.001$ ). In this test, the numbers of consumed parasitized aphids on Khasib cultivar and the healthy ones on Karim cultivar were highest. The lowest numbers of consumed preys belonged to the parasitized aphids on Karim cultivar (Figure 2).

Manly's  $\beta$  index for healthy aphids was  $0.45 \pm 0.03$  and  $0.58 \pm 0.02$  on Khasib and Karim cultivars, respectively. Manly's  $\beta$  index for parasitized aphids was  $0.55 \pm 0.03$  and  $0.42 \pm 0.02$  on Khasib and Karim cultivars, respectively (Table 1). Comparing the preference indices using *t*-tests indicate a significant preference of *A. aphidimyza* for parasitized aphids on Khasib cultivar ( $t= -$

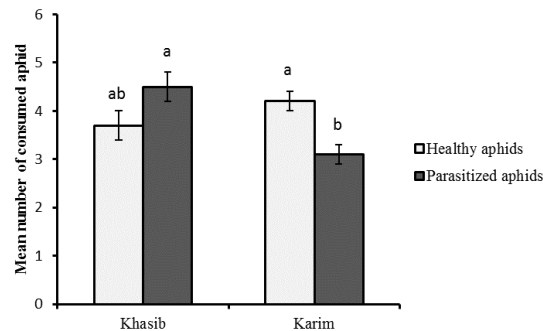
**Table 1.** Manly's preference index ( $\pm$ SE) of *Aphidoletes aphidimyza* when provided with healthy and parasitized aphids containing *Aphidius colemani* immatures on each cucumber cultivar.

Cultivar	Prey items	Manly's preference index
Khasib	Healthy aphids	$0.45 \pm 0.03^*$
	Parasitized aphids	$0.55 \pm 0.03^*$
Karim	Healthy aphids	$0.58 \pm 0.02^{**}$
	Parasitized aphids	$0.42 \pm 0.02^{**}$

\*, \*\* Significant at  $P \leq 0.5$  or  $P \leq 0.01$ , respectively.



**Figure 1.** Mean number of prey ( $\pm$ SE) consumed by *Aphidoletes aphidimyza* larvae in a no-choice experiment. Columns with different letters represent significant differences within a cultivar (Independent-samples *t*-test).



**Figure 2.** Mean number of prey ( $\pm$ SE) consumed by *Aphidoletes aphidimyza* larvae when offered a choice between healthy aphids and parasitized aphids by *Aphidius colemani*. Columns with different letters represent significant differences among treatments (Tukey's test).

2.350,  $df= 18$ ,  $P= 0.030$ ) and for healthy aphids on Karim cultivar ( $t= 4.808$ ,  $df= 18$ ,  $P< 0.0001$ ).

## DISCUSSION

In the present study, the predation on the parasitized aphids happened by the IGP predator which indicates the asymmetrical IGP between *A. aphidimyza* and *A. colemani*. Among predators and parasitoids, the most common form of IGP is asymmetric as reported by Colfer and Rosenheim (2001), Snyder *et al.* (2004), Naranjo (2007), Chacon and Heimpel (2010), and Velasco-Hernandez *et al.* (2013).

In no-choice experiment, healthy aphids on each cultivar were more preferred by *A. aphidimyza* larvae and consumed more than parasitized individuals, because the swollen body of parasitized aphids is abnormally larger than healthy aphids, as described by Enkegaard *et al.* (2005), and normally less number of larger preys is expected to be consumed. Furthermore, host plant cultivars significantly influenced the predation of *A. aphidimyza*, since different numbers of preys were consumed by the predator larvae. Similar results were reported by Gholami Moghaddam *et al.* (2013) who found that different wheat cultivars can affect the predation rate of *Orius albidipennis* Reuter

(Hemiptera: Anthocoridae) on barely aphid, *Sipha maydis* (Passerini).

When given a choice, *A. aphidimyza* larvae predated high numbers of parasitized and healthy prey individuals on Khasib and Karim cultivars, respectively. According to literatures, preference of aphidophagous predators to healthy aphids or parasitized aphids might be different. For example, Bilu and Coll (2009) reported that *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) larvae had no preference for either parasitized or healthy aphids. Colfer and Rosenheim (2001) showed that *H. convergens* had a preference to non-parasitized aphids. Furthermore, in most of the cases, discrimination between healthy and parasitized aphids did not happen by the predators (Almohamad *et al.*, 2008), which is consistent with the feeding behavior of *A. aphidimyza* larvae in our study, since preying upon both of the prey types (healthy and parasitized aphids) happened.

Different levels of intraguild predation of *A. aphidimyza* might be associated with the different host plant cultivars. The lowest IGP on immature parasitoids when healthy aphids and parasitized aphids were used in combination on Karim cultivar may be related to the reduced predation on larger prey (i.e. parasitized aphids). Furthermore, the predation of *A. aphidimyza* on both prey items when they were offered alone was significantly lower on Karim cultivar

compared to Khasib cultivar. The results on Karim cultivar confirmed the results obtained in our early investigation (Mottaghinia *et al.*, 2015), which demonstrated that the predation rate of the predatory gall midge on Karim cultivar was lower. A reason for the lower predation of the predator larvae on Karim cultivar may be associated with the presence of trichomes on the leaf surface. The density of trichomes on Karim cultivar was significantly more than Khasib cultivar (Mottaghinia *et al.*, 2015), which may impede the movements of *A. aphidimyza* larvae more than the other cultivar and cause its decreased attack rate. Trichome density has been reported to influence IGP. For example, Madadi *et al.* (2008) showed that the intraguild predation on the predatory mite, *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) by the minute pirate bug, *O. albidipennis* could be different on three host plants (pepper, eggplant and cucumber) due to their various trichome densities. Less mobility of the predator larvae on the cultivar with more dense trichomes is in accordance with findings of Fordyce and Agrawal (2001), who showed that trichomes on the pipe vine (*Aristolochia californica* Torrey) slowed the walking speed of the green lacewings, *Chrysopa carnea* (Stephens) (Neuroptera: Chrysopidae) and reduced the capture rate of prey. However, our results on less preference of *A. aphidimyza* larvae for the high pubescent cultivar is in contrast to the results reported by Lucas and Brodeur (1999) for the adult midge. In this study, they showed that *A. aphidimyza* females preferred to oviposit more eggs on leaves with high trichomes density compared to the ones with low trichomes density. They concluded that the preference of adult midge to oviposit in pubescent leaves is a strategy to lessen being predated by coccinellids. Although the leaf characteristics of Karim cultivar may negatively affect the *A. aphidimyza* larvae mobility, it should not be neglected that the larvae showed less IGP on the parasitoid immatures on this cultivar. So, it seems that disruption of the

biological control programs may not occasionally happen.

In this study, we found no sign of predation on mummified aphids, because all mummies were undamaged and almost all the parasitic wasps safely emerged from them. This result is in line with the findings of Enkegaard *et al.* (2005) who reported that the aphid mummies were not predated by the predatory gall midge. It was perhaps not so surprising because the mandibles of some predator larvae were not sharp enough to break the hard exoskeleton of mummies. According to the literature, *Episyrphus balteatus* De Geer (Diptera: Syrphidae) larvae (Meyhöfer and Klug, 2002; Pineda *et al.*, 2007) and the fourth instar of *C. undecimpunctata* (Bilu and Coll, 2009) were not able to feed on mummies. Mummification is a strategy for the parasitoids to get protection from some of their natural enemies. However, some other researchers reported that the aphid mummies were broken and consumed by other aphidophagous predators (Synder and Ives, 2001; Royer *et al.*, 2008). Colfer and Rosenheim (2001), for example, observed that *H. convergens* readily consumed mummies of *A. gossypii*, harboring *L. testaceipes*.

In conclusion, the results of our study demonstrated that plant cultivars may have significant effects on higher trophic levels. We showed that the intensity of IGP on IG-prey on Karim cultivar was less than Khasib cultivar. So, we can expect that the partial preference for healthy aphids on this cultivar may weaken the predation of IG-prey and strengthen the suppression of the melon aphid. Although leaf surface structure was described as one of the possible reasons for low predation of the IG-predator on this cultivar, other possibilities such as chemical marks left by the parasitoid females and secondary metabolites of the host plant may be involved in these interactions. Our experiment in microcosm conditions relatively mimicked the natural situation where both parasitized and healthy aphids co-occur. However, the occurrence of IGP in



small scaled experiments does not mean that biocontrol was disrupted in natural conditions. According to Synder *et al.* (2004), despite the occurrence of IGP between *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) and *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) in microcosm feeding trials, no signs of IGP were observed in greenhouse release of both natural enemies. So, further investigations need to focus on the mechanisms of the interactions and the consequences of these interactions on the population dynamics of the third trophic level in field and greenhouse conditions.

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### شکارگری درون رسته‌ای روی زنبور پارازیتوئید *Aphidius colemani* توسط پشه شکارگر *Aphidoletes aphidimyza*: تاثیر ارقام گیاه میزبان

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#### چکیده

در یک سامانه غذایی سه سطحی، اعضای یک رسته ممکن است درگیر شکارگری درون رسته‌ای (IGP) شوند و برهمکنش‌های آن‌ها تحت تاثیر گیاهان میزبان قرار گیرد. در این تحقیق سیستمی متشکل از پشه شکارگر *Aphidoletes aphidimyza* Rondani، زنبور پارازیتوئید *Aphidius colemani* Viereck و شته جالیز *Aphis gossypii* Glover استفاده شد تا مشخص گردد که چگونه نتیجه نهایی IGP تحت تاثیر دو رقم خیار (خسیب و کریم) قرار می‌گیرد. IGP بین دشمنان طبیعی مورد استفاده روی گیاه کامل هر رقم خیار که با شته‌های مومیایی، پارازیته یا سالم شته جالیز آلوده شده بود در دمای  $25 \pm 2$  درجه سلسیوس، رطوبت نسبی  $65 \pm 5$  درصد و دوره نوری تاریکی: روشنایی (8: 16) در اتاقک رشد مورد بررسی قرار گرفت. بر اساس نتایج به دست آمده، *A. aphidimyza* شکارگر موثر شته‌های پارازیته بود اما روی شته‌های مومیایی شکارگری نداشت. در تیمارهایی که به تنهایی به هر یک از شته‌های سالم یا پارازیته آلوده بودند *A. aphidimyza* به طور معنی‌داری تعداد کم‌تری از شته‌های سالم یا پارازیته را در رقم کریم تغذیه کرد. زمانی که شته‌های سالم و پارازیته به طور همزمان در اختیار شکارگر قرار گرفت احتمال شکار شدن شته‌های پارازیته توسط لارو *A. aphidimyza* روی رقم کریم به طور معنی‌داری کاهش یافت. شاخص ترجیح منلی برای شته‌های سالم در رقم کریم به طور معنی‌داری بیش‌ترین بود. نتایج حاکی از آن بود که شدت IGP روی شکار درون‌رسته در رقم کریم کم‌تر از رقم خسیب بود. بنابراین، کنترل بهتر جمعیت شته جالیز را در این رقم می‌توانیم انتظار داشته باشیم.