

Research Article

Natural enemies of the currant lettuce aphid, *Nasonovia ribisnigri* (Mosely) (Hemiptera: Aphididae) and their population fluctuations in Ahvaz, Iran

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Abstract: *Nasonovia ribisnigri* (Mosely) is one of the most important pests of the lettuce plant and it was reported for the first time in Ahvaz in 2008. In order to investigate the dominant species of its natural enemies and their population fluctuations, sample were taken arbitrarily from fifty plants twice a week during the growing season in 2010-2012. In this study, ten species of predators, three species of parasitoids and two species of hyperparasitoids were collected and identified. Hoverflies with a relative frequency of 55% were the dominant predators. Peaks of lacewings and subsequently ladybird beetles were more coincident with peaks of aphid population in mid-March in the first year of studies. But their densities in the second year were very low. Also, hoverflies and parasitoids were mainly observed in the high densities in late March-early April, in both years. Regression analysis indicated that populations of aphids were mainly affected by ladybird beetles and lacewings in the first year of study, as well as by ladybird beetles, hoverflies and parasitoids in the second year. Therefore, additional studies are required for further evaluation on the potential abilities of these natural enemies being a good candidates for the future biological control programs.

Keyword: *Nasonovia ribisnigri*, natural enemies, population fluctuation, multiple regressions, biological control

Introduction

One of the world's most important vegetables is *Lactuca sativa* (L.) (Asteraceae). Many aphid species are known to attack this outdoor lettuce. They cause a reduced and abnormal growth of the plant and are vectors of numerous viral diseases (Blackman and Eastop, 2000). Among them, the currant lettuce aphid, *Nasonovia ribisnigri* (Mosely) (Aphididae) is a primary pest of the

lettuce that has spread throughout Europe, Canada, Asia, the Middle East, North and South America (Blackman and Eastop, 2000) and recently invaded the New Zealand (Stufkens and Teulon, 2003) and Australia (Diaz and Fereres, 2005). This pest was reported on *Crepis* sp. (Asteraceae) in the Alborz Mountains of Iran in 1994 (Rezvani, 2001) and for the first time in Ahvaz, south of Khuzestan province, in romaine lettuce fields (Bagheri *et al.*, 2008).

The overcrowded population *N. ribisnigri* and its covertly feeding habits on the central part of lettuce makes product non-marketable for consumption due to the severe damage by this infestation. Mackenzie (1986) has estimated that the economic threshold of the lettuce aphid in the

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fields was 0.5 aphids per plant. *N. ribisnigri* is particularly difficult to control as it is characterized by colonization of the innermost leaves of lettuce that protects them from contact insecticides. Therefore, the widespread use of insecticides to control this pest has created a serious resistance problem (Kift *et al.*, 2004). For this reason, there is a growing interest in a more effective management of the aphid, with the potential utilization of the natural enemies. In California, a number of indigenous natural enemies have found this invasive aphid as a suitable host. This led to further attention for their potential role in aphid biological control programs (Bugg *et al.*, 2008).

Nearly all previous studies on this pest have focused on the ecological aspects and biological control of *N. ribisnigri* on the lettuce infestation (Griffiths, 1960; Mackenzie, 1986; Palumbo *et al.*, 2000; Liu, 2004; Poole *et al.* 2004; Nebreda *et al.*, 2005; Smith and Chaney, 2007; Walker *et al.*, 2007). The common beneficial insects that were reported included the brown lacewing, 11-spotted ladybird, transverse ladybird, hoverflies, several spider species, damsel bugs and many parasitoids (Griffiths, 1960; Nebreda *et al.*, 2005; Smith *et al.*, 2008). Smith and Chaney (2007) indicated that the hoverflies are the only predator group consistently found in all infested romaine fields, with average of up to nine hoverfly larvae per lettuce head. Studies in Iran on the ecology and population dynamics of this pest and its natural enemies are limited (Bagheri, *et al.*, 2008; Mossadegh *et al.*, 2011; Nazari *et al.*, 2012). Our objective in this study was to determine the dominant species of the natural enemies of the lettuce aphid, and their population fluctuations in the lettuce fields in the Ahvaz region. In addition, we compared the significance of the natural enemies relative to suppressing the aphids' density.

Material and Methods

All samplings were conducted on a 20 × 25m farmland of lettuce of Romaine variety, at Research Center of Agriculture of Ahvaz, Iran. No pesticides were used during growth of the plants.

For the purpose of identification and defining population dynamics of the natural enemies, numerous samples were taken arbitrarily and diametrically from fifty plants, twice a week from early December to late May during 2010-2012. On each plant, three leaves from frame (outer), wrapper (middle) and head (inner) parts were selected randomly (Palumbo, 1999). Samples were transferred to the laboratory in separate labeled plastic bags. Number of winged and wingless aphids as well as the nymphs was counted separately. Immature stages of the predator insects including eggs and larvae and the parasitized aphids were kept in a separate ventilated container and were checked daily for the appearance of adults. Emerged insects were counted according to their species and transferred to test tube containing 75% ethanol. Specimens were identified to possible level, according to available keys and resources (Butler and Ritchie, 1970; Stary, 1976; Gordon, 1985; Dousti, 2000). Parasitoids were sent to Dr. Petr Stary, Czech academy of science for final identification.

The dominant species of the natural enemies was estimated according to equation 1 (Seraj, 2009):

$$D = \sum \left(\frac{n_i}{n} \right)^2 \quad (1)$$

Where, D is the Dominance parameter, n_i is the number of individuals of the species i and n is the number of all collected individuals of each natural enemy. Also, dominance parameter was estimated for predators.

To evaluate the parameters of the relationships among number of *N. ribisnigri* and densities of its predators, a multiple linear regression model (SAS Institute, 2009) was calculated as:

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 \quad (2)$$

Where x_1 , x_2 and x_3 are average density of ladybirds, hoverflies and lacewings, respectively and y is the average number of lettuce aphids per plant for each of the stages;

included winged and wingless adults, nymphs and total populations.

Parasitism was obtained as number of mummies per total number of aphids at each sampling date (Russell, 1987). Additionally, linear regressions (SAS Institute, 2009) were used to evaluate the relationship between the average number of stages of the lettuce aphid per plant and an average percent of parasitism.

Results

During two years of sampling, the major aphid species was *Nasonovia ribisnigri* based on population density. However, *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) were observed in a lower population density.

Also, ten species belonging to three orders and four families of predators, three species of wasp parasitoids and two species of hyperparasitoids were collected and identified. Among predators, hoverflies with relative frequency of 55% were dominant. Lacewings and ladybird beetles were ranked second and third with relative frequencies of 23% and 22%, respectively.

In the first year, following the appearance of aphids in early July, the activity of predators began in mid February. Lacewings seemed to become increasingly abundant with the increased population of the aphids (Fig. 1. a). Peaks of lacewings were coincident with peaks of aphid population on the 9th of March. It was followed by a peak of ladybird beetles on the 13th of March. Conversely, the population of hoverflies increased slowly in March and reached its peak, on the 27th of March. At this time, it was observed that the aphid population decreased. In the second year, the development of the lettuce aphid population as well as predators began comparatively later than noticed in the previous year (Fig. 1. b). However, the density of the ladybird beetles and lacewings were lower in the second year, although, hoverflies had two peaks and their population mainly increased in April. Activities

of the aphid parasitoids were mainly observed in early March to late April (Fig. 2. a-b). The highest percentage of the parasitism occurred on 3rd of April (51.02%) and on 6th of April (81%) and the average percent of parasitism was 11% and 15%, in 2010/11 and 2011/12, respectively. In addition, it was observed that with increased abundance of parasitoids, the densities of aphid were decreased.

Predators

Among hoverflies, *Episyrphus balteatus* (De Geer) (Syrphidae) was the most common syrphid predator with relative frequency of 45%, throughout the two years of sampling (Table 1). This species was mainly observed in high density during late March and early April in 2010/11 and 2011/12, respectively (Fig. 3. a-b). Its population was lower in the first year of sampling than the following year (Fig. 3. a). *Eupeodes corollae* (Fabricius) (Syrphidae) composed 36% of the species observed from lettuce. Its population increased dramatically in mid March but peaks mainly occurred on 27th of March 2010/11 (Fig. 3. a). This species was observed in a much lower density following year (Fig. 3. b). Other syrphids including *Sphaerophoria bengalensis* (Maquart.) (Syrphidae) and *Eupeodes nuba* (Wiedemann) (Syrphidae) were less common and their populations never exceeded an average of 0.34 and 0.28 larvae per plants, the two consecutive years, respectively (Table 1).

Scymnus levaillanti (Mulsant.) (Coccinellidae) with relative frequency of 68% was the most common species of ladybird beetles (Table 1). High density of this predator was observed from late March to mid April, but occurrence of their maximum number was on 13th and 26th of March, in the first and second years of sampling, respectively (Fig. 3. c-d). *Coccinella septempunctata* (L.) (Coccinellidae) appeared earlier than other Coccinellid predators in February. However, its population increased very slowly but peaks mainly occurred in March (Fig. 3. c-d). A relative frequency of *C. septempunctata* in lettuce field was 23%.

Moreover, *C. novemnotata* (Herbst) (Coccinellidae) and *C. undecimpunctata* (L.) (Coccinellidae) were recorded in this research. Their densities were much lower than those of the other two species, respectively (Table 1).

Hemerobius sp. (Hemerobiidae) with a relative frequency of 52% was the most abundant lacewing in the field during the two years (Table 1). In 2010/11, higher populations of this predator were coincident with occurrence of maximum number of aphid on the 9th of March (Fig. 3. e). Alternatively, this species was found in very low density, in next year of sampling (Fig. 3. f). Also, *Chrysoperla carnea* (Stephens) (Chrysopidae) was recorded in this research. Population dynamics of this species were similar to those for *Hemerobius* sp.

Parasitoids

Aphidius matricariae (Haliday) (Braconidae); *Praon volucre* (Haliday) (Braconidae) and *Diaeretiella rapae* (McIntosh) (Braconidae) were recorded in association with *N. ribisnigri* on lettuce. Among them, *A. matricariae* composed 94% of species of parasitoids (Table 2).

Hyperparasitoids

Additionally, in this research two hyperparasitoid species *Alloxysta* sp. (Figitidae, Charipinae) and *Dendrocerus* sp. (Megaspilidae) were also reared and identified.

Aphid density relationships with predators and parasitoids:

In 2010/11, regression analysis indicated significant, positive relationship between average abundance of ladybird beetles and lacewings versus total number of aphid as well as nymphs in the lettuce field (Table. 3). In contrast, the ladybird beetle populations were in high correlations with wingless aphids. Winged aphids were affected by all of the predators and the hoverflies had a negative effect on them. No significant relationship was observed between density of parasitoids and each stage of aphid’s population (Table 5).

In following year of sampling, hoverflies with negative and ladybird beetles with positive relationships affected significantly nymphs and total aphid populations (Table 4). Regression analyses for wingless and winged aphids were similar to those of previous year. Significant correlation was found between the population stages of the aphid and parasitoids (Table 5).

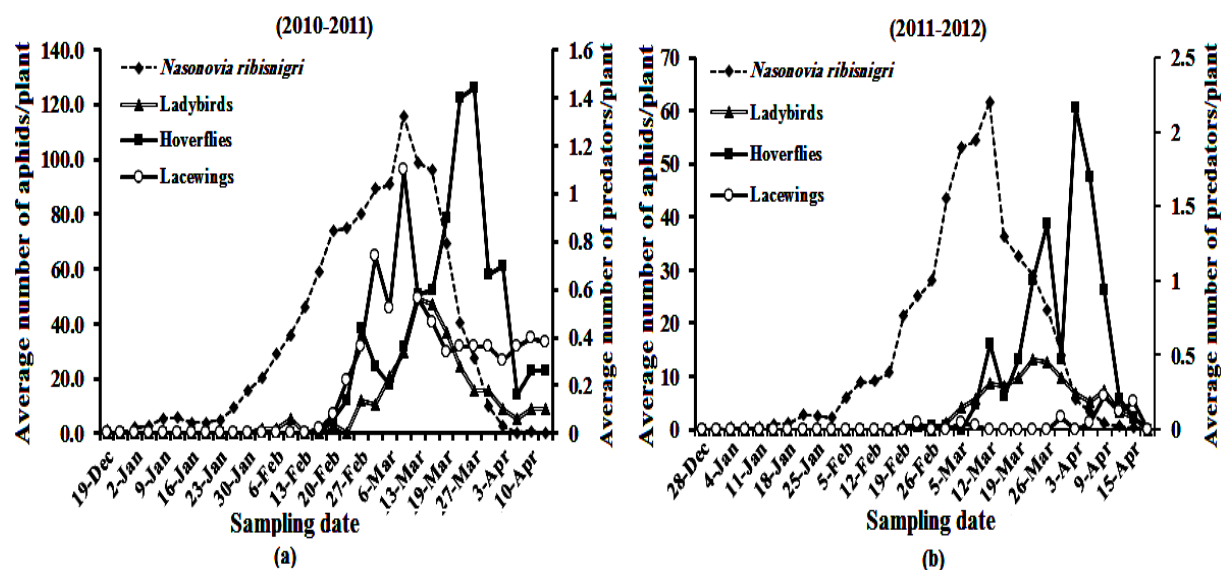


Figure 1 Abundance of lettuce aphids and their predators during two years of sampling in Ahvaz (a-b).

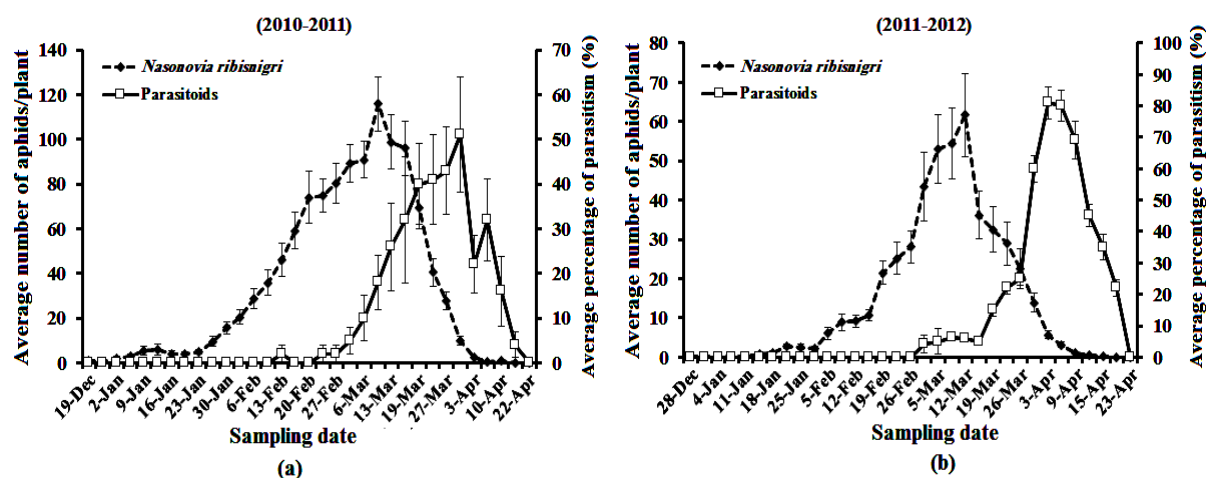


Figure 2 Abundance of lettuce aphids and percentage of parasitism during two years of sampling in Ahvaz (a-b).

Table 1 Average density of predators of lettuce aphid with their relative frequencies of the population during two years of sampling.

Predators	Species	No. of predators (Mean \pm SE)	Relative Frequency (%)	Total
Hoverflies	<i>Episyrphus balteatus</i>	8.04898 \pm 1.270	45	17.86857 (55%)
	<i>Eupeodes corolla</i>	6.42653 \pm 0.820	36	
	<i>Sphaerophoria bengalensis</i>	1.79571 \pm 0.034	10	
	<i>Eupeodes nuba</i>	1.59734 \pm 0.011	9	
Ladybird beetles	<i>Scymnus levaillanti</i>	4.74653 \pm 0.050	68	7.030612 (22%)
	<i>Coccinella novemnotata</i>	0.38449 \pm 0.013	5	
	<i>C. septempunctata</i>	1.63591 \pm 0.020	23	
	<i>C. undecimpunctata</i>	0.26367 \pm 0.013	4	
Lacewings	<i>Hemerobius</i> sp.	4.067347 \pm 0.011	52	7.675102 (23%)
	<i>Chrysoperla carnea</i>	3.607755 \pm 0.015	48	

Discussion

Nasonovia ribisnigri was attacked by several species of predators and parasitoids as observed in this study. Hoverflies composed 55% of predators collected from infested lettuce; other predators were less abundant. *Episyrphus balteatus*, *Scymnus levaillanti* and *Hemerobius* sp. were important species of predators. These results are consistent with those obtained in California's central coastal area, that show the larvae of syrphids are the most

abundant predators often making up > 85% of these predators. However, some other syrphid species viz, *Toxomerus marginatus* (Say), *Toxomerus occidentalis* (Curran), *Platycheirus stegnus* (Say) and *S. sulfuripes* (Thomson) were also commonly found in those fields (Smith and Chaney, 2007; Smith et al., 2008). Whereas, other studies in New Zealand and Australia showed that *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) was the most important predator of *N. ribisnigri* (Walker et al., 2007).

In this study, *Aphidius matricaria* was the important parasitoid of aphid, which occurred in the greatest numbers during the two-year sampling. Comparable to our findings, other studies in Spain showed that *Aphidius hieraciorum* (Stary) (Braconidae) was the most abundant parasitoid and it was the first report of its acting as a parasitoid of *N. ribisnigri* (Nebreda *et al.*, 2005). A survey for the identification of the aphid parasitoids in Khuzestan, Iran showed that *A. hieraciorum* and *Praon pubescens* (Stary) (Braconidae) were newly recorded for the fauna of Iran in association with *N. ribisnigri* on *L. sativa*. (Mossadegh *et al.*, 2011; Nazari *et al.*, 2012).

Additionally, other studies conducted in the United Kingdom indicated that *Monoctonus paludum* (Marshall) (Braconidae) was the primary and common parasitoid species found to suppress this pest (Griffiths, 1960). The reason for the dissimilar results in this research was not clear and may be due to other factors including seasonal occurrence, limited area of studies, rarity, and misidentification.

Our data indicated that the activity of predators began when population levels of lettuce aphid were increased in February. In the first year, high population of lacewings and subsequently ladybird beetles were coincident with peak of aphids. Accordingly, regression analysis showed a significant and positive relationship between average densities of these predators and aphids. In the following year, in contrast to the clear decline that was observed in the population of lacewings, hoverflies had two peaks of high populations, and very well adapted to survive the environment. There was a significant relationship between populations of ladybird beetles, hoverflies as well as parasitoids versus total density of lettuce aphids. These result also showed that hoverflies and parasitoids had negative effects on aphids. Winged aphids were the only stage that was attacked by all predators. It seems that due to the aphids' activity on the wrapper and frame leaves, predators can easily detect the aphid. The reason for the dissimilar results in the two subsequent years was not clear and may be due

to other factors that affected the natural enemies' abundance. Smith and Chaney (2007) have demonstrated a direct relationship between syrphid egg and larvae densities and reductions of aphid densities in the lettuce fields. Also there was no relationship between non-syrphid predators and aphid densities. They concluded that the hoverflies were primarily responsible for the suppression of this pest in the organic lettuce infestation in the California central coastal area. In addition, according to Smith *et al.*, (2008), aphid density was significantly higher in plots where syrphids were suppressed with Entrust, whereas untreated romaine was marketable.

Our data and other studies demonstrated that the species diversity and the relative importance of different natural enemies vary in different regions and different weather conditions. In the Ahvaz region, lacewings could disperse rapidly and colonize shortly after the aphids became established. Consequently, they increased coincident with aphid's peaks. During the growing season, if farmers use insecticides, these generalist predators will be able to leave the field temporarily and prey on the mites, thrips and egg of moths (Bugg, *et al.*, 2008). As a consequence, specialist predators including ladybird beetles and hoverflies that only prey on lettuce aphids, seems to keep them in low levels of population. Moreover, hoverflies were the only predator group consistently found with high relative frequency during two year observations. The factors mentioned above and abilities of larvae's predators to forage efficiently inside the lettuce heads can play an important role in integrated pest management. Therefore, additional studies are required for further evaluation on the potential abilities of these predators being good candidates for the future biological control programs.

It should be noted that the *A. matricaria* is an oligophagous species of parasitoids but our result suggested that this species with a relative frequency of 94% among parasitoids, could play a collaborative role with predators for controlling aphids in greenhouse lettuce.

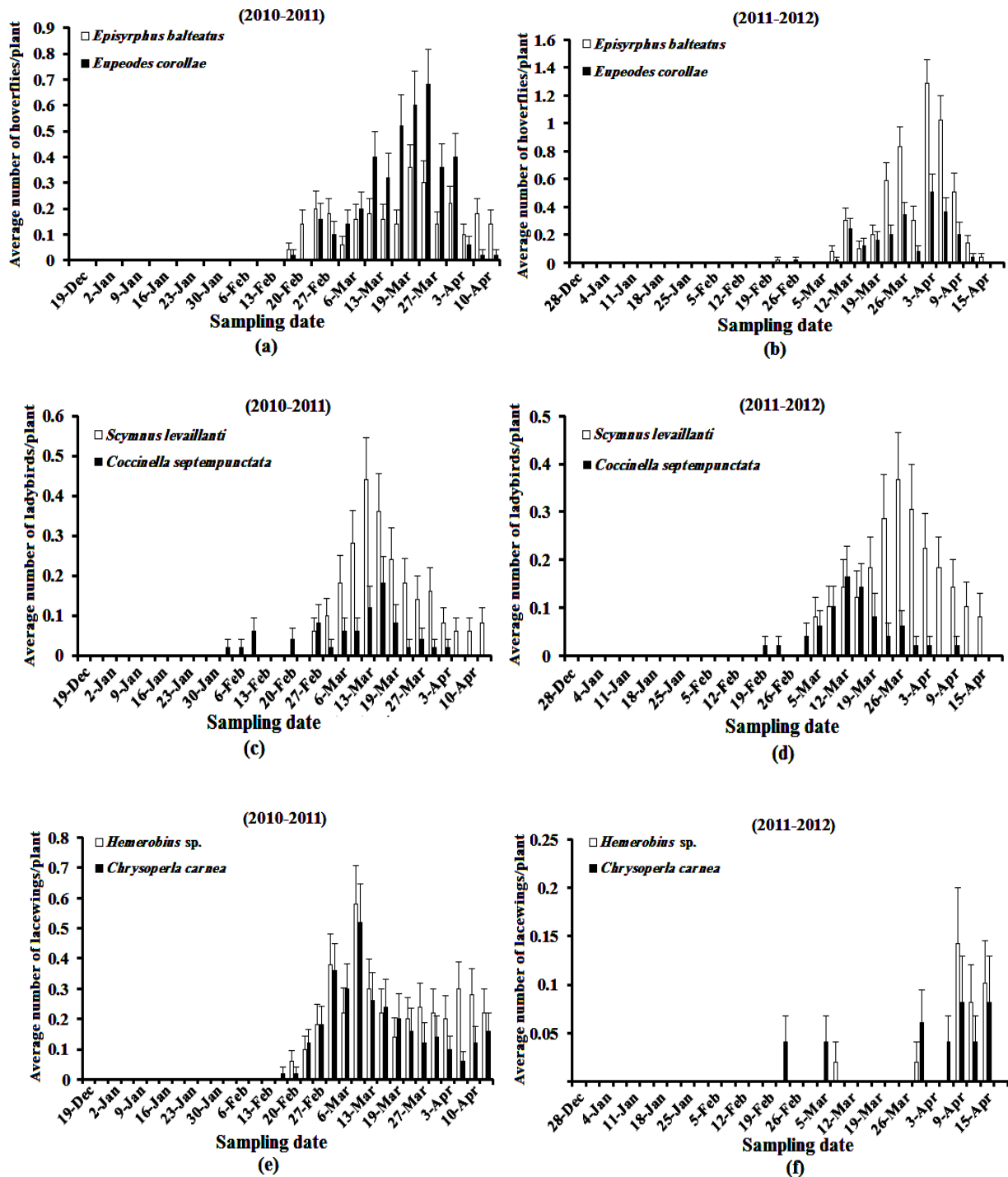


Figure 3 Population fluctuations of *Episyrphus balteatus*, *Eupeodes corollae* (a-b) *Scymnus levaillanti*, *Coccinella septempunctata* (c-d) *Hemerobius sp.* and *Chrysoperla carnea* (e-f) during two years of sampling in Ahvaz.

Table 2 Average density of parasitoids of lettuce aphid with their relative frequencies of the population during two years of sampling.

Parasitoids species	No. of parasitoids (Mean \pm SE)	Relative frequency (%)	Total
<i>Aphidius matricariae</i>	0.229462 \pm 0.063	94.0	0.24431
<i>Praon volucre</i>	0.014242 \pm 0.008	5.8	
<i>Diaeretiella rapae</i>	0.000606 \pm 0.00063	0.2	

Table 3 Multiple linear regression analysis between mean number of developmental stages of lettuce aphids (y) and average density of ladybird beetles (x_1), hover flies (x_2) and lacewings (x_3) in 2010/11.

Stage	Equation	R^2	P	C_p
Nymph	$y = 11.96 \pm 80.32x_1 \pm 48.40x_3$	0.490	<0.0001*	5.51
Wingless	$y = 1.305 \pm 3.36x_1$	0.162	0.02*	2.92
Winged	$y = 0.616 \pm 12.35x_1 - 1.96x_2 \pm 2.73x_3$	0.746	<0.0001*	4.00
Total	$y = 13.7 \pm 93.14x_1 \pm 50.95x_3$	0.504	<0.0001*	5.68

* Significant correlation at $P < 0.05$.**Table 4** Multiple linear regression analysis between mean number of developmental stages of lettuce aphids (y) and average density of ladybird beetles (x_1), hover flies (x_2) and lacewings (x_3) in 2011/12.

Stage	Equation	R^2	P	C_p
Nymph	$y = 7.81 + 90.5x_1 - 15.71x_2$	0.353	0.001*	5.36
Wingless	$y = 0.615 + 2.09x_1$	0.156	0.025*	8.18
Winged	$y = 0.531 + 8.33x_1 - 1.258x_2 - 6.53x_3$	0.568	<0.0001*	4.00
Total	$y = 8.86 + 102x_1 - 17.59x_2$	0.365	0.001*	5.63

* Significant correlation at $P < 0.05$.**Table 5** Linear regression analysis between mean number of developmental stages of lettuce aphids and average percentage of parasitism during two years of sampling.

Development stage	2010-2011			2011-2012		
	Equation	R^2	P	Equation	R^2	P
Nymph	$y = 62.8 - 65.4x$	0.078	0.31	$y = 38.6 - 54.9x$	0.604	0.001*
Wingless	$y = 2.54 - 2.81x$	0.092	0.27	$y = 1.74 - 2.00x$	0.450	0.006*
Winged	$y = 4.09 - 2.86x$	0.025	0.57	$y = 2.83 - 3.95x$	0.555	0.001*
Total	$y = 69.4 - 71.1x$	0.075	0.33	$y = 43.2 - 60.9x$	0.600	0.001*

* Significant correlation at $P < 0.05$.

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دشمنان طبیعی شته رایج کاهو، (*Nasonovia ribisnigri* (Mosely) (Hemiptera: Aphididae) و تغییرات جمعیت آنها در اهواز، ایران

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چکیده: شته *Nasonovia ribisnigri* Mosely مهم‌ترین آفت کاهو محسوب می‌شود که از اهواز برای اولین بار در سال ۱۳۸۷ گزارش شده است. به منظور بررسی دشمنان طبیعی مهم شته کاهو و تغییرات جمعیت آنها، در طول سال‌های ۱۳۸۹-۱۳۹۱ به طور تصادفی هفته‌ای دو بار از ۵۰ بوته کاهو نمونه برداری صورت گرفت. در این بررسی، ده گونه شکارگر، سه گونه پارازیتوئید و دو گونه هایپرپارازیتوئید جمع‌آوری شناسایی گردید. مگس‌های گل با فراوانی نسبی ۵۵٪ گونه غالب شکارگرها بودند. در سال اول بررسی، اوج جمعیت بالتوری‌ها و بعد از آن کفشدوزک‌ها هم‌زمانی بیشتری با اوج جمعیت شته‌ها در اواخر اسفند ماه داشت. ولی در سال دوم جمعیت آنها بسیار پایین بود. همچنین در طول دو سال، حداکثر جمعیت مگس‌های گل و پارازیتوئیدها اساساً در اول فروردین ماه مشاهده گردید. نتایج آنالیز رگرسیون نشان داد که جمعیت شته‌ها اساساً تحت تأثیر کفشدوزک‌ها و بالتوری‌ها در سال اول و کفشدوزک‌ها، مگس‌های گل و پارازیتوئیدها در سال دوم نمونه برداری قرار گرفتند. بنابراین مطالعات بیشتری جهت ارزیابی توانایی بالقوه این دشمنان طبیعی به‌عنوان یک کاندیدای خوب برای برنامه‌های آبی کنترل بیولوژی این آفت مورد نیاز است.

واژگان کلیدی: *Nasonovia ribisnigri* دشمنان طبیعی، تغییرات جمعیت، رگرسیون چندگانه، کنترل بیولوژیک