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Population fluctuations of grape leafhopper, *Arboridia kermanshah* (Hemiptera: Cicadellidae), under natural conditions of Kermanshah grapes

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Abstract. The grape leafhopper, *Arboridia kermanshah*, is an important pest species in Iranian vineyards. Adults and nymphs cause damage by piercing the leaf tissues and sucking the intracellular contents. This study was focused on the biology of grape leafhopper in Kermanshah Province. Grape leaves were used as sampling units to estimate egg and nymph populations and sticky yellow traps were used to estimate the adult populations. A vineyard was sampled weekly, during growing seasons in 2017 and 2018. Our findings indicated that the leafhopper has three generations per year in Kermanshah. Overwintering of adults happened in the remnants of sheds and under grape barks. Population dynamics of *A. kermanshah* were studied in relation to temperature and relative humidity. Population dynamics of grape leafhopper were significantly and positively related to temperature in all biological instars of the pest in 2017 except the second and the third nymphal instars. Population fluctuation was significantly related to relative humidity only for the fifth nymphal instar in 2017. There were significant and positive relationships between temperature and population dynamics of eggs, and the fifth nymphal instars and total immature stages in 2018. Relative humidity changes also had significant relation with all stages except the fourth and the fifth instar nymphs. The density of parasitized eggs by *Oligosita pallida* Kryger was 93.24±1.1% and 89.74±4.9% in the first and second sampling years, respectively. Hence, the egg parasitoids were very effective in the last generation, therefore, chemical control for this pest is not recommended.

Keywords: Cicadellidae, Life cycle, natural conditions, parasitism, population density

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Introduction

Grape, *Vitis vinifera* L, is an economically valuable crop in Iran and its cultivation area is growing increasingly, as a result of its high economic and nutritional value (Faramarziani, 2012). A large area of fruit orchards all over the world is devoted to this valuable product, and their product constitutes a major part of the fruit production in the world. Iran has suitable ecological conditions for growing grapes. The area under cultivation and the amount of grape production are much higher than other horticultural products. It can be processed in various domestic and foreign markets, and it is an interesting export product (Afshari *et al.,* 2014). Several agents affect the yield of grapes. Pests, diseases, and weeds cause significant damage in some years (Javadi khedari, 2011). Insects of the family Cicadellidae are amongst the most harmful groups of sucking insects that reduce plant's tolerance to undesirable conditions. These insects feed on a wide range of plants. In addition to the direct damage, plants suffer some indirect damage by transmission of viruses, bacteria, and phytoplasmas (Haji Alilou Banab *et al.,* 2016). The grape's leafhopper, *Arboridia kermanshah* Dlabola (Hemipetra: Cicadellidae) is an economically important pest in Iranian vineyards (Mostaan & Akbarzadeh Showkat, 1995). Adults and nymphs

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suck intracellular contents out of mesophyll tissues and leave the cells empty and withered, under the leaves. Because of the symptoms, inflicted damages manifest themselves as whitening and eventual defoliation (Latifian *et al.,* 2005). Based on performed investigations, the immature instars of this pest exhibit five nymphal instars as the most damages are caused by adults and nymphs, especially by the fifth instar nymphs. Leaf margins and the periphery of secondary veins were determined as preferred places for laying eggs by females, followed by periphery of midribs and the inner region of the veins. Mostly, different stages were observed in the middle parts of vine shrubs, after which lower and upper parts are the next preferences, respectively (Latifian *et al.,* 2004). Investigations have shown that *A. kermanshah* exhibits three or four generations per year in Isfahan province, of which the first period of adult activity occurred in May and June, the second period occurred in July, the third period occurred in August, and the fourth period occurred in September and October (Latifian *et al.,* 2004). Another grape leafhopper, *Empoasca vitis* Gothe, is a polyphagous pest distributed in Europe. The adults of this pest hibernate on softwoods outside the vineyards. In early spring, as the temperature rises, the insects migrate to the vineyard and may have 2-4 generations per year based on the region's climate (Cerutti *et al.,* 1991؛ Boll & Herman, 2004). The grape leafhopper, *Scaphoideus titanus* Ball, is a monophagous pest and hibernates in the form of eggs beneath the barks of grape shrubs, and in mid-May, eggs begin to hatch, awaiting five nymph stages (Vidano, 1964). Usually, insects appear in early June, the population of which reaches its peak at the end of July until mid-August, and are active in the vineyards until the end of October (Lessio & Alma, 2004). Numerous factors can affect the activity and population dynamics of grape leafhopper, which are temperature, relative humidity, light, competition between species, and natural enemies (Martinson & Dennhy, 1995). Temperature and relative humidity are the most important regulating factors in the life cycle of grape leafhopper. Although active species in desert areas are adapted to arid conditions, high humidity is essential for the survival of many species of grape leafhoppers. Temperature is also one of the most important promoters for migrants at the host level and their area of activity (Delong, 1971). Lessio & Alma (2004) showed that daily capture increases were correlated with daily minimum and maximum temperatures and were negatively correlated with maximum values of relative humidity.

Most researchers who have studied the bio-ecology of grape leafhoppers have designated that parasitoids play an important role in the population changes of these insects (Chantrasa *et al*., 1984). For example, the use of egg parasitoids has been shown as an effective factor in controlling the population by demolishing the biomass of eggs before initiating the damaging stage (Settle & Wilson, 1990). In pest management programs, it is necessary to acquire deep knowledge of pest biology, its position in the agricultural ecosystems, its life cycle and seasonal period, its habits and behaviors, estimated population density of the pest, and types and levels of these pest damages. The more awareness and knowledge of the pest and ecological factors, the more successful and reliable the control methods for this pest will be (Sami', 2004). The aim of the present study was to determine the grape leafhopper bio-ecology, *A. kermanshah*, in a natural condition. We also evaluated the effect of temperature, relative humidity, and natural enemies potential on the population dynamics of the grape leafhopper. It can be used to find proper methods for the management of this pest and to obtain accurate information for use in integrated management programs.

Materials and methods

This research was done in Deh-Pahn village, located east of Kermanshah city (34°20'18" N, 47°8'3" E, Altitude: 1302 m.) in 2017 and 2018. Since the eggs and nymphs were located on the lower surfaces of the grape leaves, one leaf was taken as a sampling unit. At the first sampling date, 30 leaves were collected (on 7 Jul 2017 and 8 Jun 2018) to determine the required sample size. Based on the results, RV (Relative variation) was calculated from formula 1:

$$RV = \frac{SE}{\bar{x}} \qquad (1)$$

Where:

$\bar{\chi}$: The mean of primary sampling data. SE: the standard error of the mean (Pedigo & Buntin, 1994).

Relative variation value from primary sampling data less than 25 percent and the number of samples needed for the next sampling was determined from equation 2:

$$N = \left(\frac{t * SD}{D * \bar{x}}\right)^2 \qquad (2)$$

Where:

N: required sample size, t: t-student, SD: Standard deviation, D: desired fixed proportion of mean and $\bar{X}$: mean of primary sampling data. If the value is RV>D, the number of samples is increased in the primary sampling (Pedigo & Buntin, 1994).

The main samples were taken weekly, from grape shrubs (Number and age of trees: 110 shrubs and ten years old, Variety type of grape: Askari, Type of pruning: Scott- Henry training, Irrigation interval: weekly, Size of the garden: 4000 m^2). For studying the population dynamics of *A. kermanshah* in relation to nymph and egg stages, samples were taken randomly in four different geographical directions. After being removed from the branches, the leaves were placed in plastic bags, and labeled. Then, the samples were transferred (with zip-kip nylon) to the laboratory and kept in the refrigerator (Temperature: 5 °C). To observe the different nymphal instars, the collected grape leaves were segregated carefully and one-by-one using a stereo-microscope, then were counted and recorded.

In addition, sticky yellow traps (20×15×0.3 cm) were used to estimate the population density of grape leafhopper adults. During the sampling process, on each sampling date, 10 grape shrubs were randomly selected and a sticky yellow trap (at height: 1.6m) was installed on each one, replaced by a new one every week.

The effect of temperature and relative humidity on the population fluctuation

The temperature and relative humidity of the sampling dates were obtained from the nearest weather station (In Havanirouz). Correlation between the mean daily temperature and the population variability of the pest was used to observe the response of the population fluctuation to temperature changes (Minitab version 16 software). To investigate the effect of relative humidity on the population fluctuation of this insect, the mean relative humidity on each sampling date was considered. Like temperature, the relationship between relative humidity and population changes was determined using a correlation coefficient.

Measuring body length in different nymphal stages

In order to obtain the body length of different nymphal instars of *A. kermanshah*, 30 specimens of each nymphal instar were collected, and microscopic slides were prepared in the laboratory for the identification process. All measurements of specimens were done under a stereomicroscope using a graticule.

Collection of natural enemies

Samples of infested leaves were collected weekly from the vineyards. The insects were studied using a stereomicroscope. The collected mites were clarified and mounted in Hoyer's medium before identification with a microscope. The leafhopper eggs were marked in leaf tissues. Then each parasitized egg on the leaf tissue was transferred to a petri dish with wet cotton and incubated at 25±2°C and 60±2% RH, (14L: 10D) until parasitoids emerged. Petri dishes were monitored daily. All specimens were identified by Dr. Maryam Darbemamieh (the third author) for the mite species and Dr. Hossein Lotfalizadeh from East-Azarbaijan Agricultural and Natural Resources Research & Education Center, for parasitoid wasps. Samples were deposited in the insect collection of the Department of Plant Protection, Razi University.

Determining rate of parasitism of grape leafhopper eggs

Equation 3 was used to determine the parasitism percentage of grape leafhopper eggs

Equation 3:

$$\text{Egg parasitism (\%)} = \frac{\text{number of parasitized eggs}}{\text{number of unparasitized eggs} + \text{number of parasitized eggs}} \times 100$$

Results

Measuring body lengths in different nymphal instars

The body lengths of the first to fifth instars were 0.74±0.02, 0.86±0.01, 1.43±0.01, 1.85±0.07 and 2.23±0.06 mm, respectively. The nymphal body is elongated and somewhat wide, in the dorsal part of the abdomen. There are some delicate hairs placed in two symmetrical rows. The antenna of all instars is long and setaceous, two distal segments are globous, and the rest are uncountable or barely countable (Fig. 1). Adults are yellowish and the ends of their heads are almost rounded. On the vertex, there are two distinct black spots. The abdominal sternites and legs are cream to yellow. Yellow pronotum contains dark black spots on its surface. The front wings are yellow and the hind wings are light yellow in both males and females. The males' antennae are longer than in females (11 segments in males and 9 in females). In males, the stylet has extended to the front of the sheet. The aedeagus is long, almost straight, and has two angles at a slightly angle to the axis of the aedeagus. The female leafhopper has a yellow abdomen and genitals (Fig. 2 and 3). Only the tip of the reproductive area is distinctly blackish-brown. Different stages of nymphs are distinguished by pronotum width and the wing pad development. Therefore, wing pad was indeterminate at the first instar. In the second instar, it extended to the end of the first abdominal segment, and in the third instar elongates to the end of the second abdominal segment. For the fourth instar, it reaches the end of the third abdominal segment, and in the fifth instar, it continues to the end of the fourth abdominal segment. After the emergence of the third instar, two black spots appear on their pronotum. The first and second nymphs are without these spots, and for the fourth and the fifth nymphal instars, these spots are more clearly distinguished (Fig. 1).

Population dynamics and effect temperature and Relative humidity

Population variability of grape leafhopper adults, *A. kermanshah*, in 2017 and 2018 are shown in Fig. 4 and 5. In 2017, sampling was done from the 7th of July. Three generations are shown in Fig. 4 and 5 and the first was in its peak on the first sampling date. The second generation started its activity in early August, and its peak occurred in mid-August, then ended in mid-September with a duration of 35 days. The third generation appeared in September and reached its peak simultaneously at the end of September, then ended in mid-November with a complete period of 63 days. The shortest period was related to the second generation (35 days) in 2017. Based on the field studies, it was determined that this pest began its activity in the second decade of June 2018. As it is shown in Fig. 5, this pest had three generations per year in the region. The first generation appeared in mid-June, reached its peak in mid-July, and ended in mid-August, with the duration of 56 days. The second generation appeared in mid-August, reached its peak at the end of August, and ended in mid-September, with the duration of 35 days. The third generation appeared at the end of September, reached its peak in early October, and ended in mid-November with the duration of 49 days. The longest and the shortest period of activity were 56 days (the first generation) and 35 days (the second generation) in 2018, respectively. Investigations revealed that this pest hibernated in the form of an adult, inside the remains of the shed as well as under the barks of other trees in the orchards. In 2018, adult insects entered the overwintering phase earlier in mid-November (Fig. 5). The population density trend of nymphs in 2017 is shown in Fig. 6. For the first to third generations, peaks of eggs and the first instar occurred on July 14th, August 25th, and September 15th, respectively. The peaks of the second instar occurred on July 14th, September 1st, and September 29th, and for 3rd instar on July 14th, September the first, and October 6th. In 2018 (Fig. 8), a peak of eggs was observed on July 20th, August 24th and, September 21st for first to third generations, respectively. For the first to third generations, peaks of the first and the second instars were recorded in July 20th, September 7th, and September 21st, respectively. As it is shown in Fig. 8, nymphal density in the second and third generations showed lower peaks. This can be attributed to the activities of egg parasitoid wasps, which were highly active in the second and third generations. The results of the effect of temperature and relative humidity on the pest population dynamics in 2017 and 2018 are shown in Table 1. According to the obtained results in 2017, there was a significant and positive correlation between the stages of eggs, the first nymphs, the fourth nymphs, the fifth instars, and the adult population and daily temperature. There was no such significant relationship between the second and third nymphs. Regarding the effect of relative humidity, there was only a significant relationship and positive correlation between the fifth nymph's population and relative humidity, and there was not such a significant positive relationship between other stages (Fig. 6).

Fig. 1. The different immature stages of*, Arboridia kermanshah*, A) Egg, B) the first instar , C) the second instar , D) the third instar , E) the fourth instar , F) the fifth instar

Also, the results of the temperature and relative humidity effects on the density of the pest population in 2018 showed that there was a positive and significant correlation between the populations of eggs, the fifth nymphs, and adults, and such relationship was not observed for the first to the fourth nymphs. Regarding the effect of relative humidity, there was no significant relationship between the fourth and the fifth nymphal instars and adults, but there was a significant and negative correlation between the different stages of this pest and relative humidity, which means that the population of the pest increased with the decrease in relative humidity (Fig. 8).

Also, the relationship between the population dynamics of grape leafhoppers and temperature for total immature stages was positive and significant in 2017. Otherwise, this relation with relative humidity was insignificant for total immature stages (Fig. 7). There was a positive and significant relationship between temperature and population dynamics of total immature stages in 2018. Relative humidity changes had also this significant relation with total immature stages (Fig. 9).

Natural enemies

During the third generation, when the nymphs showed lower activity, the parasitoids were perfectly active so a few eggs hatched. Preliminary results showed that predatory mite, *Anystis baccarum* (L.) (Trombidiformes: Anystidae), and lacewing larvae, *Chrysoperla* sp. (Neuroptera: Chrysopidae), fed on different nymphs. Moreover, the parasitoids reared from leafhopper eggs were identified as *Anagrus atomus* (L.) (Hymenoptera: Mymaridae), *Gonatocerus thyrides* Debauche, (Hymenoptera: Mymaridae), *Oligosita pallida* Kryger (Hymenoptera: Trichogrammatidae) (Deh-pahni *et al*., 2019).

Discussion

Study of the biology and population dynamics of pests can help understand their behavior and determine the factors affecting their population variability in field conditions. Kohansal *et al.* (2019) evaluated seasonal fluctuations of *A. kermanshah* on nine grape cultivars in Isfahan province. They observed adult emergence from late April in each sampling year. The maximum population density of adults was recorded in late June. Based on their results, maximum and minimum population density in each sampling year was observed on White Yaghoti and Black Yaghoti, respectively.

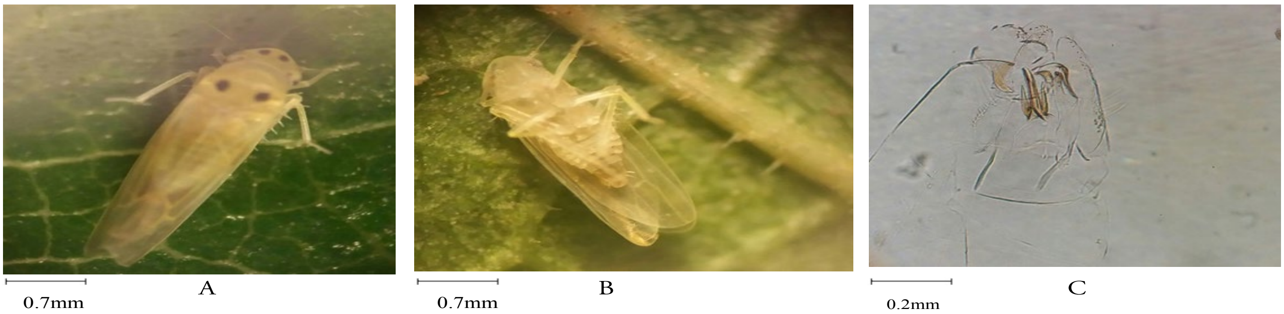

Fig. 2. The male adult stage of*, Arboridia kermanshah*, A) Dorsal surface, B) Abdominal surface, C) Aedeagous

In this study, the activity of nymphs in the second generation began in early July in the first studied year and from mid-June in the second year, then the density of nymphs gradually increased. The oviposition activity in both years began in early and mid-May and peaked in early July. Then from mid-July to mid-September, the number of healthy grape leafhopper eggs decreased, which was due to the activity of their egg parasitoids. At the end of November in the first year and mid-November in the second year, the oviposition activity of the grape leafhopper stopped. The total population density of this pest was higher in Isfahan (350 in 2005 and 2006) (Kohansal *et al.,* 2019), but as a result of the different sampling methods, the population density of this pest was calculated lower (30 in 2017 and 40 in 2018) in Kermanshah. Although in the Kermanshah region, the activity of adults in the vineyards started later, three generations of the pest were observed per year as in Isfahan province (Kohansal *et al.,* 2019).

The egg parasitoid, *O. pallida*, was identified as the most abundant parasitoid in this province in both sampling seasons. Eggs parasitized by other parasitoids were very few because the number of other parasitoid species was low and did not play a significant role in parasitizing eggs in this area. In Urmia region, the parasitism percentage of *A. kermanshah* eggs has been reported to be more than 90% in favorable conditions (Mostaan & Akbarzade Showkat, 1995).

Hesami *et al.* (2002) showed that the percentage of parasitism in *A. kermanshah* eggs by *A. atomus* reached 90% at the end of the season. In both sampling seasons, the highest percentage of parasitism was observed at the end of the season, which is similar to the study of Hesami *et al.* (2002). It seems that at the end of the season, the parasitoid uses more leafhoppers to recover its population and maintain it until the next year. Therefore, at that time the percentage of parasitism was very high. The activity of predators and parasitoids can be considered also as an alternative factor for the reduction of nymphs' peaks. According to the results in both sampling seasons, temperature and relative humidity affected the population dynamics of *A. kermanshah* in all three generations. In 2017, increasing in temperature at every sampling date affected some various life stages of the pest and caused population dynamics in all three generations, but relative humidity had less effect. Otherwise, population dynamics were more dependent on the relative humidity for all 3 generations in 2018, which is similar to the findings of Latifian *et al.* (2005) in Isfahan.

Table 1. Correlation relationship between the population changes of total immature stages of the grape leafhopper, *Arboridia kermanshah*, and temperature and relative humidity in 2017-2018.

	Temperature		Relative humidity	
Biological stage	**correlation**	**P-value**	**correlation**	**P-value**
	2017		2017	
Total immature stages	0.671	0.004^{**}	-0.157	0.56^{ns}
	2018		2018	
Total immature stages	0.512	0.018^{*}	-0.626	0.002^{**}

^{ns}: No significant difference, *: significant difference at the level of 0.05, **: significant difference at the level of 0.01

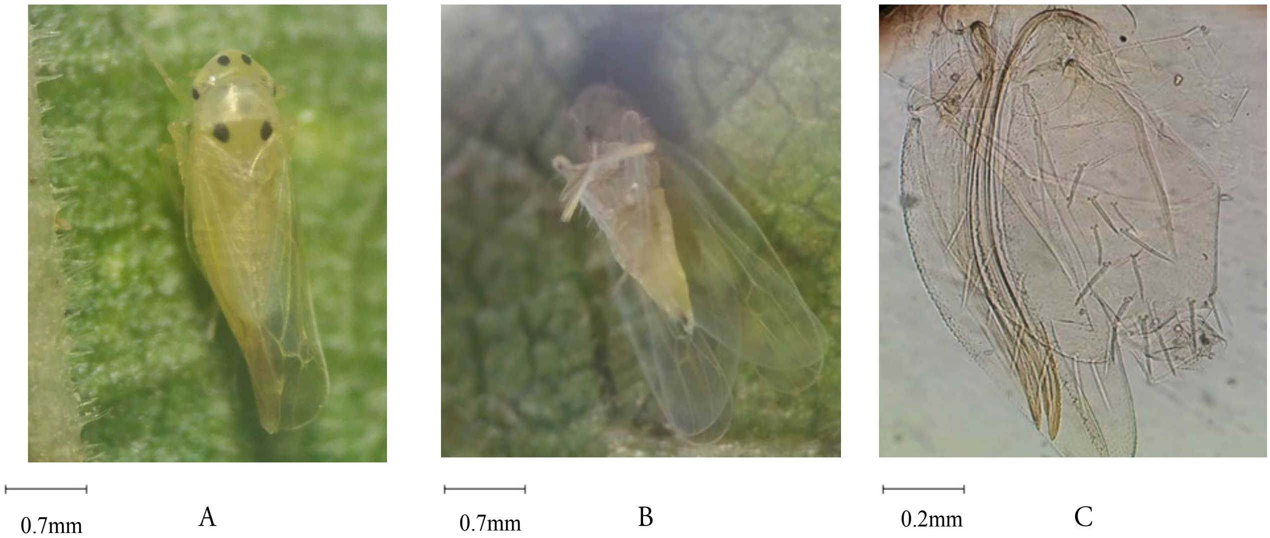

Fig. 3. The female adult stage of, *Arboridia kermanshah*, A) Dorsal surface, B) Abdominal surface , C) Ovipositor

Since the climatic conditions were different in the two sampling seasons, the results were not comparable. The findings of Lessio & Alma, (2004) suggest that high relative humidity could result in a decrease in *S. titanus* flight activity. However, an increase in relative humidity is the consequence of a decrease in atmospheric motion. Zhang & Shipp (1998) showed how an increase in vapor pressure deficit, which is negatively related to relative humidity, causes an increase in flight activity of the hemipteran *Orius insidiosus* (Say) (Hem., Anthocoridae), and they suggest how an increase in take-off because of a decrease in relative humidity could be a means of avoiding desiccation by seeking a moister environment.

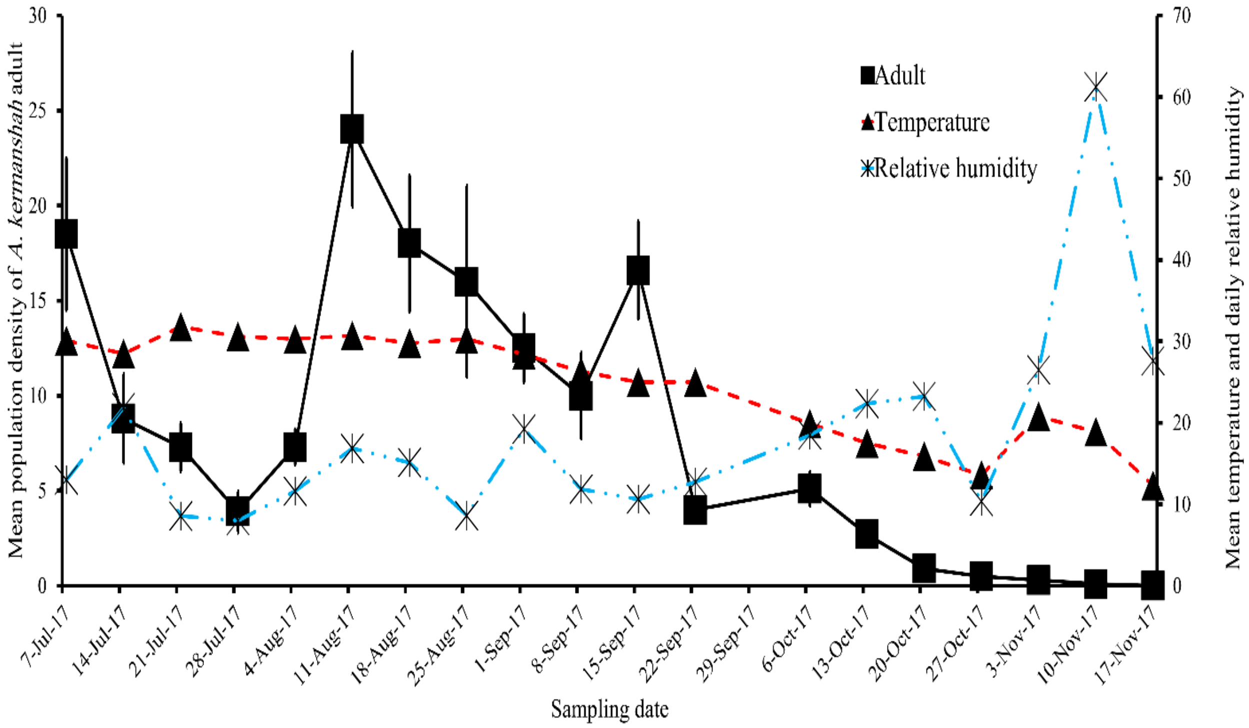

Fig. 4. Population fluctuation of the adult of *Arboridia kermanshah*, temperature and relative humidity records of Kermanshah Meteorological Station during the sampling period in 2017.

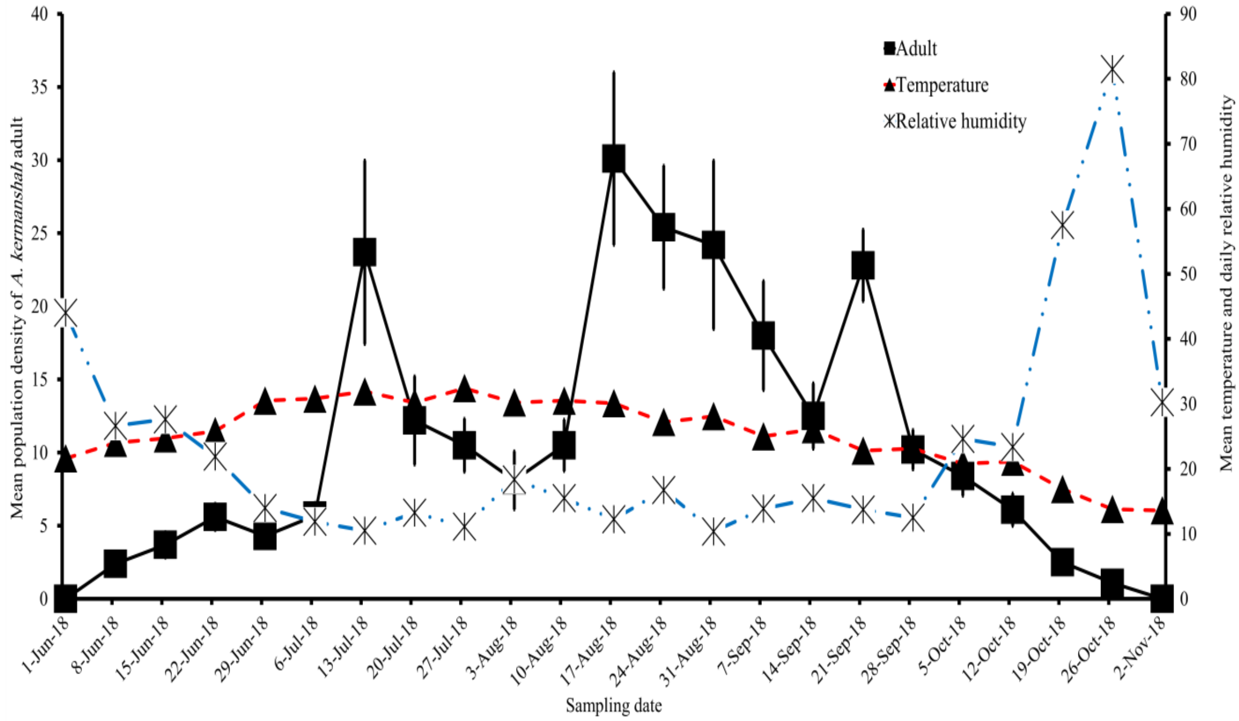

Fig.5. Population fluctuation of the adult stage of *Arboridia kermanshah*, temperature and relative humidity records of Kermanshah Meteorological Station during the sampling period in 2018.

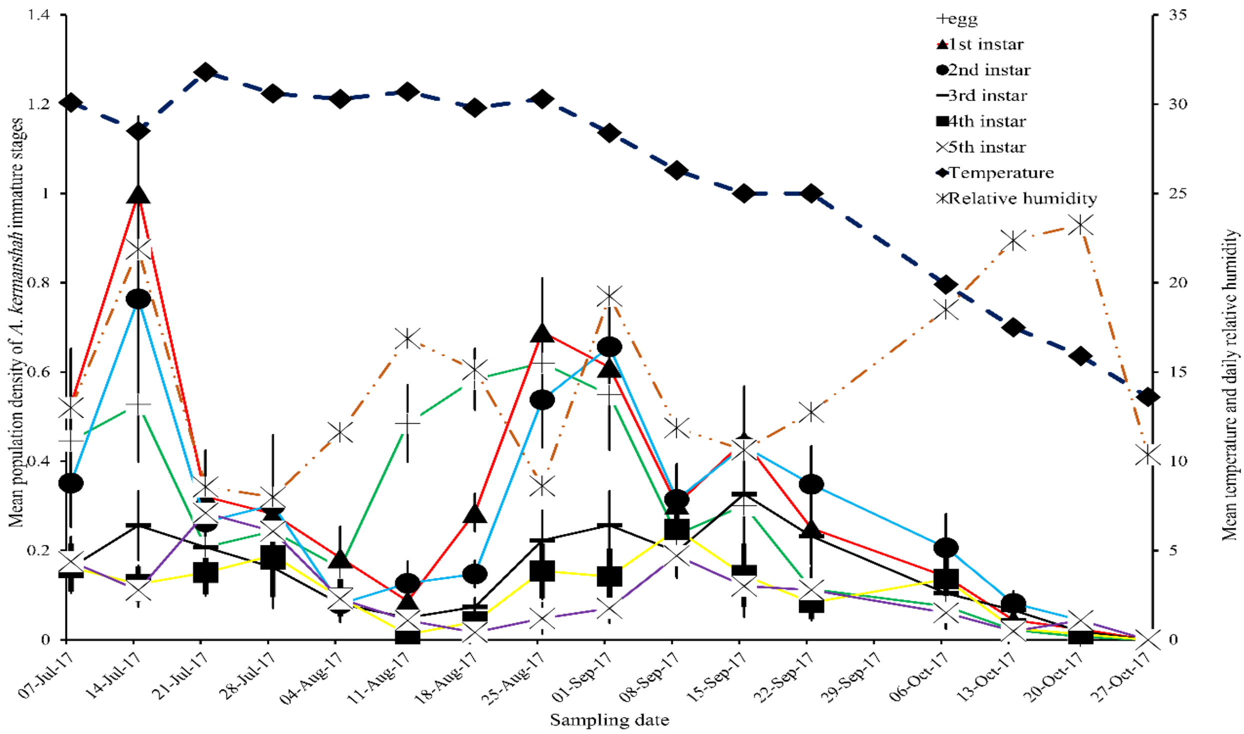

Fig. 6. Population fluctuation of the different immature stages of *Arboridia kermanshah*, temperature and relative humidity records of Kermanshah Meteorological Station during the sampling period in 2017.

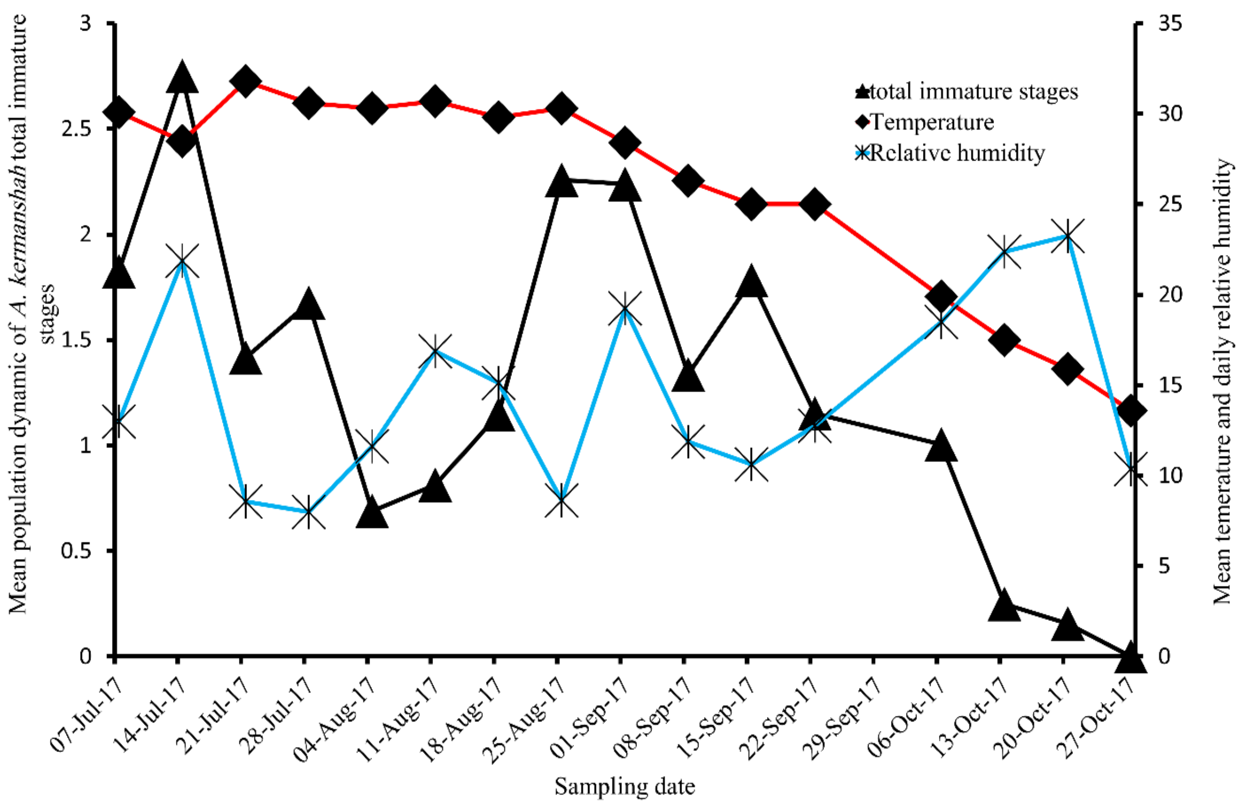

Fig. 7. Population fluctuation of the total immature stages of *Arboridia kermanshah*, temperature and relative humidity records of Kermanshah Meteorological Station during the sampling period in 2017.

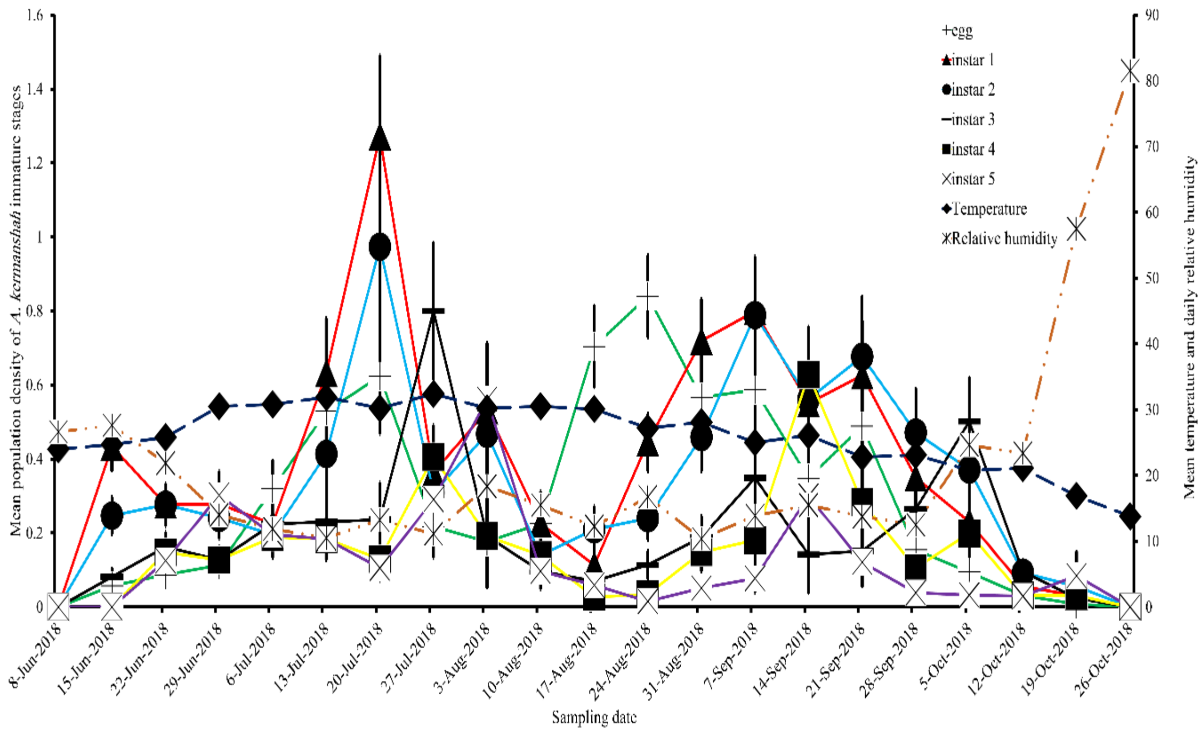

Fig. 8. Population fluctuation of the different immature stages of *Arboridia kermanshah*, temperature and relative humidity records of Kermanshah Meteorological Station during the sampling period in 2018.

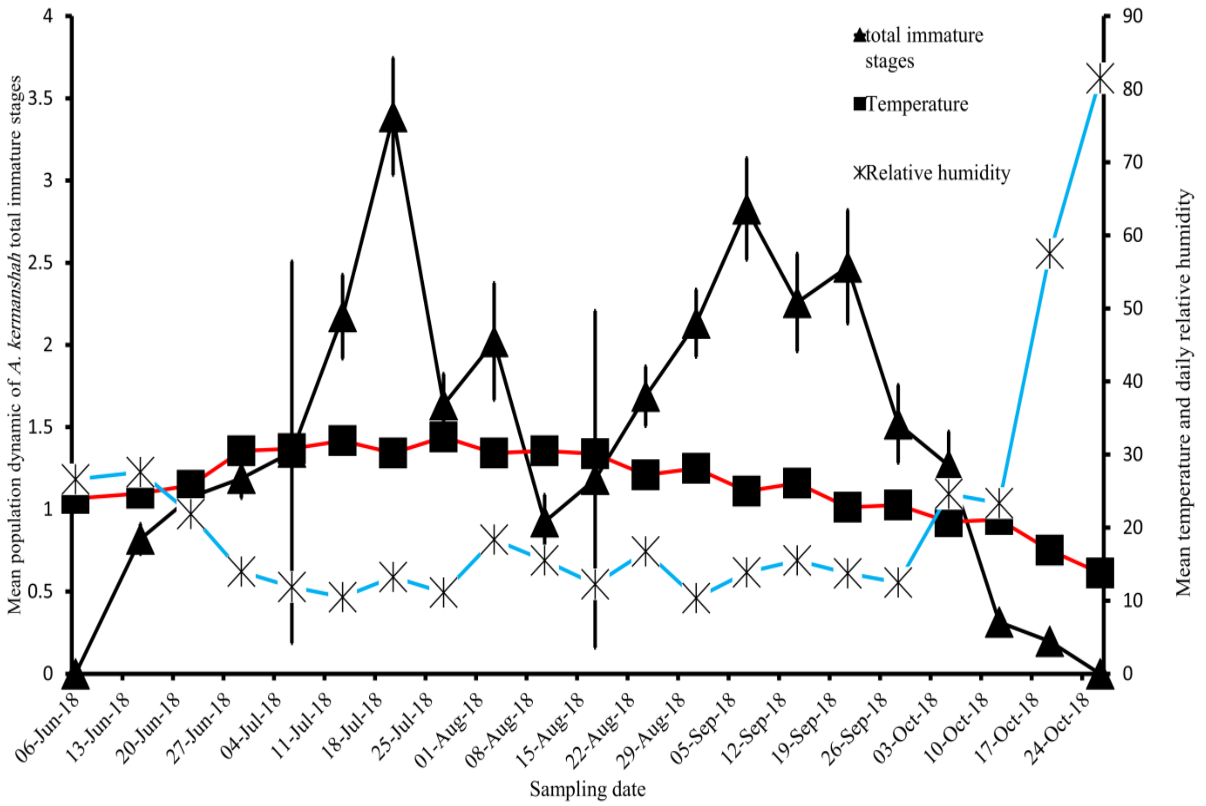

Fig. 9. Population fluctuation of the total immature stages of *Arboridia kermanshah*, temperature and relative humidity records of Kermanshah Meteorological Station during the sampling period in 2018.

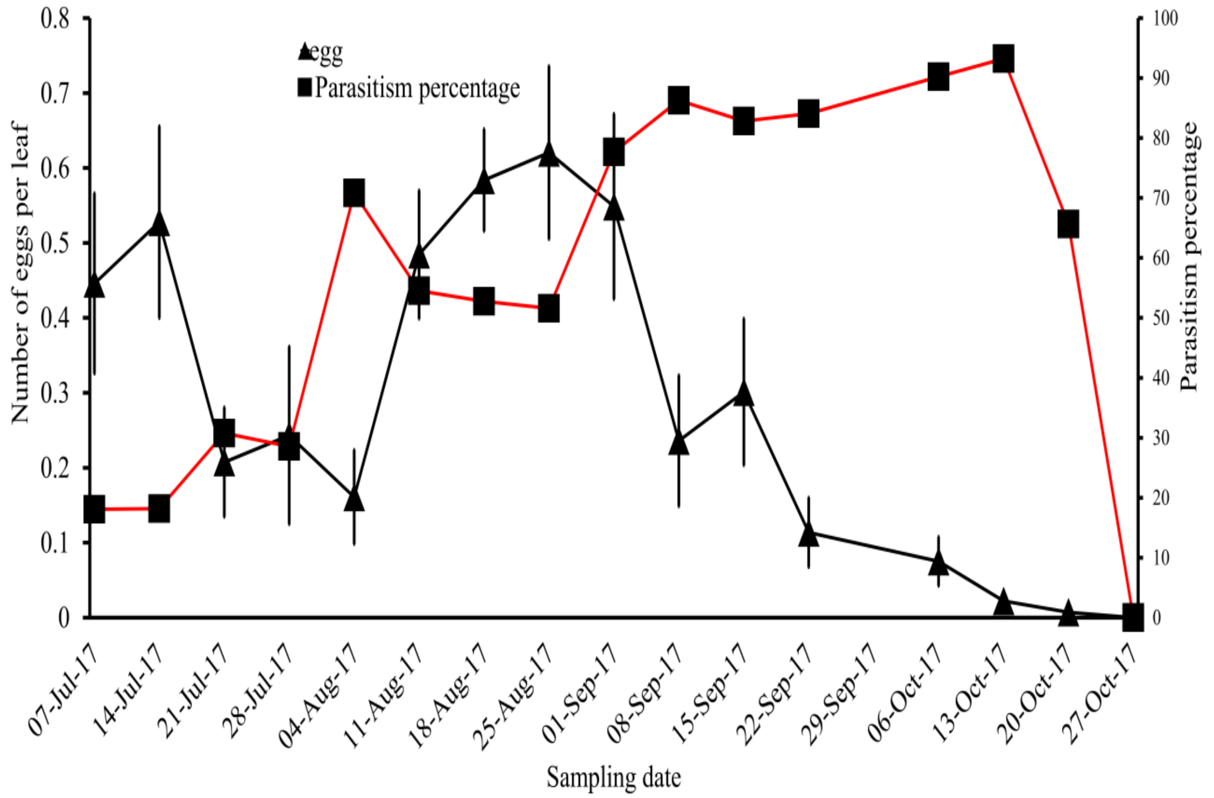

Fig. 10. Population fluctuation of egg and parasitism percentage of, *Arboridia kermanshah* the sampling period in 2017.

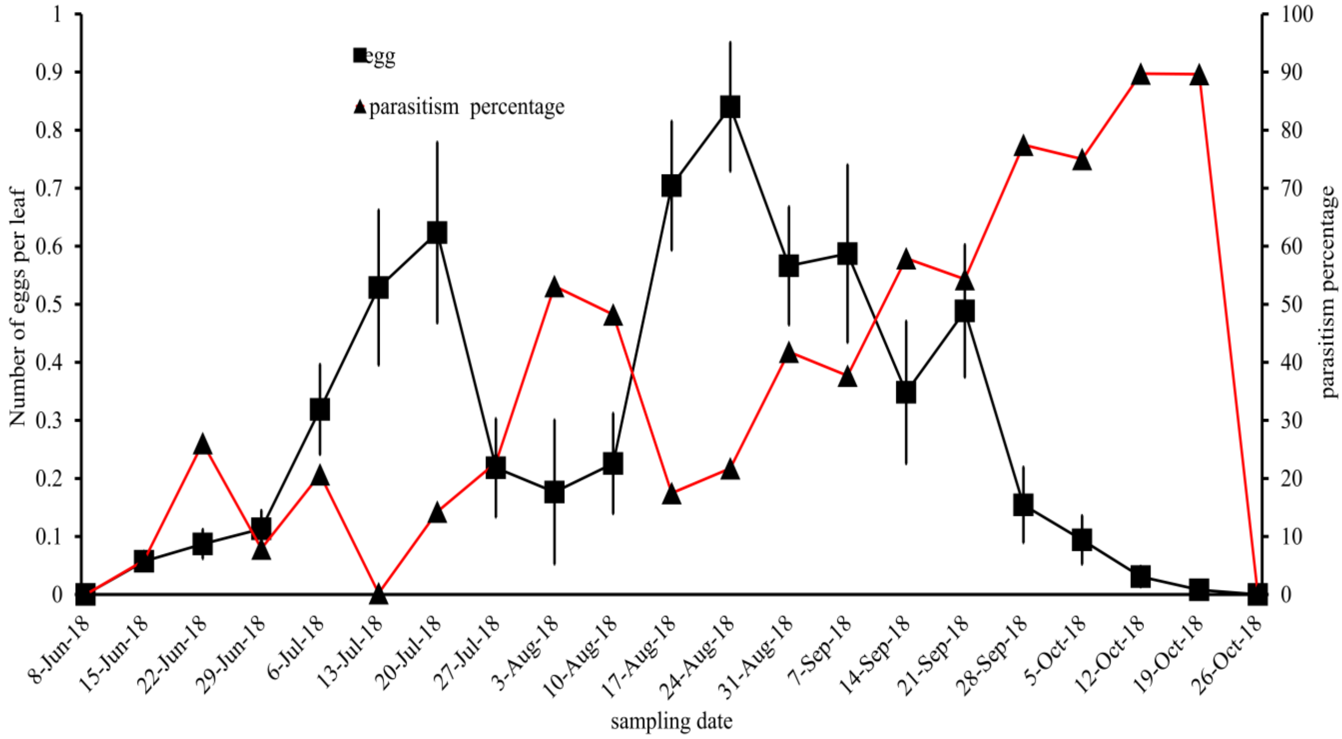

Fig. 11. Population fluctuation of egg and parasitism percentage of, *Arboridia kermanshah* in 2018.

Conclusion

The results of the present research indicate that the biological study of grape leaf hopper and the determination of its population peaks for all stages can help us predict the appropriate time of necessary actions and some potential natural enemies for the control of this pest.

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نوسانات جمعیت زنجرک مو، *Arboridia kermanshah* (Hemiptera: Cicadellidae)، در شرایط طبیعی در شهرستان کرمانشاه

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چکیده

زنجرک مو، *Arboridia kermanshah*،گونه مهم زنجرک در باغ‌های انگور ایران است. حشرات کامل و پوره‌های این زنجرک با سوراخ کردن بافت برگ و مکیدن محتویات سلولی خسارت وارد می‌کنند. اولین علایم خسارت به صورت نقاط سفید رنگ پراکنده روی برگ است. در این پژوهش زیست‌شناسی زنجرک مو *A. kermanshah*، در شرایط آب و هوایی کرمانشاه مورد بررسی قرار گرفت. جهت تخمین جمعیت پوره‌ها و تخم‌های زنجرک مو، برگ انگور به‌عنوان واحد نمونه‌برداری انتخاب شد. تله‌های چسبنده زرد رنگ برای تخمین جمعیت بالغین زنجرک مو استفاده شد. نمونه‌برداری‌ها به طور منظم و هفتگی طی دو فصل زراعی در سال‌های ۱۳۹۶ و ۱۳۹۷ از یک باغ انگور صورت گرفت. بررسی زیست‌شناسی این حشره نشان داد که در این منطقه سه نسل در سال و پنج سن پورگی دارد. زمستان‌گذرانی این آفت به‌صورت حشره کامل در میان بقایای ریخته شده و هم‌چنین زیر پوستک درختان مشاهده شد. در این پژوهش تغییرات جمعیت مراحل نابالغ *A. kermanshah*، در ارتباط با دما و رطوبت نسبی مطالعه شد. رابطه‌ی بین تغییرات جمعیت زنجرک مو با دما در سال ۱۳۹۶ با همه‌ی مراحل زیستی آفت به‌جز سنین پورگی دو و سه معنی‌دار و مثبت بود و رطوبت نسبی فقط با پوره سن پنج رابطه‌ی معنی‌دار داشت. در سال ۱۳۹۷ رابطه‌ی معنی‌دار بین دما و تغییرات جمعیت مراحل تخم، پوره سن پنج، مجموع مراحل نابالغ وجود داشت و تغییرات رطوبت نسبی با همه‌ی مراحل به‌جز سنین پورگی چهار و پنج رابطه‌ی معنی‌دار داشت. بیش‌ترین مقدار پارازیتیسم به‌دست آمده مربوط به پارازیتویید *Oligosita pallida* Kryger با ۹۳/۲۴±۱/۱ و ۸۹/۷۴±۴/۹ درصد به‌ترتیب در سال اول و دوم نمونه‌برداری محاسبه شد. با توجه به این‌که در نسل آخر، پارازیتویید تخم از فعالیت بالایی برخوردار است، کنترل شیمیایی با این آفت توصیه نمی‌شود.

کلمات کلیدی: Cicadellidae، چرخه‌ی زندگی، شرایط طبیعی، پارازیتیسم، تغییرات جمعیت

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