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Research article

Demographic parameters of two spotted ladybird *Adalia bipunctata* L. (Col.: Coccinellidae) on pomegranate aphid *Aphis punicae* (Hem.: Aphididae) under controlled conditions

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Abstract

Demographic parameters of *Adalia bipunctata* on *Aphis punicae* under controlled conditions were studied. Life table parameters of predator were studied on 100 newly laid *A. bipunctata* eggs. Daily larvae and adults of ladybirds were fed on fresh leaves of pomegranate which were infected by *A. punicae*. Fifteen pairs of *A. bipunctata* (24h-old) were selected to study the reproductive life history of *A. bipunctata*. The reproduction data were analyzed according to jackknife method. The results indicated that the incubation period was 30.22 days and adults emerged after 24.85 days. L_x was estimated 0.69 and the specific mortality age of larvae and adults of coccinellid increased gradually from day 46. Reproductive parameters of *A.bipunctata* showed that the gross fecundity rate, gross fertility rate, gross hatch rate, net fecundity rate and net fertility rate were 462.4±22.1, 405.3±23.4, 0.78651±1.058, 228.4±11 and 189.49±9.11 days, respectively. The mean numbers of eggs was estimated 11.8 egg/female/day and 13.21 egg/day. The net reproductive rate (R_0), mean generation time (T_c), doubling time (DT) and Finite rate of increase (λ) of *A.bipunctata* were 133.796, 25.27, 3.57, 1.21 days, respectively and intrinsic rate of natural increase (r_m) was 0.193 female/female/days. Also intrinsic rate of birth (b) and intrinsic rate of dead (d) were 0.2047 and 0.02851 female/ female/ day respectively. Hence, our findings may provide important information towards designing a comprehensive IPM program to control the pomegranate aphid in Iran. Up to date, no other published data are available concerning the demographic parameters of *A. bipunctata* on *A. punicae.*

Key words: Demographic parameters; *Adalia bipunctata*; *Aphis punicae, pomegranate*

1. Introduction

Aphids are major arthropoda pests in greenhouses and conservatories, feeding on a wide range of horticultural crops (McKeenzie 1967, Kole and Hennekam, 1990). *Aphis punicae* (Hem., Aphididae) is the most important aphid pest of pomegranate trees, it can damage plants by sucking the sap and producing honeydew, which is an excellent growth medium for black sooty mold fungi (Speare ,1922). Black sooty mold fungi are detrimental to plant because the dark fungal bodies cover leaves, thus limit photosynthesis and induce plant stress (Malais and Ravesberg, 1922). *A. punicae* is found in the most areas where pomegranate trees are cultivated. In many areas of Iran, this species is more abundant during March, April and May.

Many species have been successfully used in biological control programs against pest species in the field (Ramakers, 1989, van Steenis and EL-Khawass, 1955). The two-spotted ladybird, *Adalia bipunctata* L. (Col.: Coccinellidae) is a renowned predaceous ladybird distributed in Europe, Central Asia and North America (Sakuratani et al, 2000). *A. bipunctata* is a polyphagus Coccinellid that is widely distributed in tropical, subtropical and temperate regions. *A. bipunctata* feed on aphids, mealy bugs, mites also on eggs and immature stages of other insects. *A. bipunctata* is predator coccinellid that is used as a biological control agent of aphids especially in greenhouses in Western Europe. This coccinellid is introduced as a polymorphous species and it is the most important natural enemies of aphids that larvae and adults feed with different stages of aphids. *A. bipunctata* has three generations annually. The predator overwinters as adult and population peak reaches during April and May.
A. bipunctata is widespread in pomegranate orchards in Yazd (in the centre of Iran), and it is known as the most important enemy for the pomegranate aphid which can effectively control *A. punicae* (Shojaii, 1368). Demographic parameters of *A. bipunctata* were assessed under controlled conditions and understanding of these parameters will help the development of a comprehensive pest management program in pomegranate orchards in Iran. One of the most important steps in evaluating of the predator potential as a biological control agent is to study its life history on the target pest species. Demographic studies have the following applications: analyzing population stability and structure, estimating extinction probabilities, predicating life history evolution, predicting outbreak in pest species and examining the dynamics of colonizing or invading species (Vargas et al, 1997). In the present study, the development parameters were used to study the life table reproduction parameters and population growth parameters of *A. bipunctata* on *A. punicae*.

2. Materials and Methods

2.1 Rearing method and experimental conditions

Experiments were conducted in 2005 and 2006 under controlled conditions in growth chambers. *A. bipunctata* and *A. punicae* were originally collected from pomegranate orchards in Yazd province. The ensuing colonies of *A. punicae* as prey for ladybirds were maintained and reared on leaves of pomegranate at 27±2°C, 45±10% RH and 16 h photoperiod in large plastic boxes (30 × 40 × 15 cm) tightly covered with a mesh (hole: 0.3 × 0.4 cm). Adults of *A. punicae* were collected in early March 2004 from the pomegranate trees. Every 2 week, 10-15 aphids were transferred from an infested plant to a fresh plant in order to establish a new colony of aphid. To reduce the effects of plant age on aphid development and survivorship, the pomegranate leaves in Petri dishes were placed upside down on a water-saturated sponge daily. Adults and larvae coccinelid were reared in large cylindrical Plexiglas cages (50 cm height × 30 cm diameter) including an infested pomegranate leaves by *A. punicae* as food under controlled conditions. Newly emerged pairs of *A. bipunctata* adults from the stoke culture were placed separately in plastic Petri dishes (9 cm diameter × 1.6 cm height) which were covered with lids ventilated with a fine mesh net (2.5 cm in diameter). In order to evaluate the developmental time and mortality rate of each stage of ladybird, the newly laid eggs were collected from each treatment with a fine hair brush and were individually placed in Petri dishes.

2.2 Development and Survivorship of Immature

One hundred newly laid *A. bipunctata* (within 24-h old) eggs from cultures were placed individually in Petri dishes (7.5×1.5cm) and maintained to study development and survival of the immature *A. bipunctata* on *A. punicae* as prey. In order to investigate the developmental duration and mortality rate of each stage of ladybird, the newly laid eggs were checked daily. After hatching *A. bipunctata* developed through four larval stages, prepupal and pupal stage. Progress in development and survival was assessed every 24 hours and hatch, fecundity, mortality and molting to each immature instar and adult molt were recorded. The sex of each adult was also determined.

2.3 Pre-ovipositional period

The pre-ovipositional period (time interval required for ovary maturation and initiation of mature egg production) was measured for newly female coccinellids. Each female was isolated with a male in plastic Petri dishes with an excess of prey. Observations for initiation of egg laying were made every 24 h.

2.4 Biological cycle

The total time for completion of the biological cycle (time elapsed from egg stage until adult oviposition) was estimated by adding the period of immature stages with the respective pre-ovipositional period. Longevity of different stages and fecundity were observed in the laboratory condition . In order to determine the preovipostion and oviposition period, 15 pairs of coccinellid were introduced into Petri dishes with *A. punicae* and were observed daily. The eggs of each female were counted daily to record its fecundity. Developmental time of *A. bipunctata* was observed for eggs, different stages of larvae, prepupal and for pupal stages.

2.5 Reproduction and population growth parameters

For effective decision making in using natural enemies it is necessary to be aware of population growth parameters. The intrinsic rate of increase (r_m) of *A bipunctata* is a useful factor for describing population growth rate. Estimates of r_m have been used to assess the potential effectiveness of natural enemies. We studied population growth parameters of *A. bipunctata* on *A. punicae* was carried out under controlled conditions at temperature of 27±2 °C, 45±10% RH and 16: 8 h (L: D). Daily fertility of 15 ladybird females with both pre imagine developmental time and mortality were analyzed according to Jackknife method by using SAS ver.9 and MINITAB ver.14 statistical software. Different parameters such as pre-reproduction period, reproduction period, post-reproduction period, age specific fecundity, pre-adult mortality and female longevity were determined. From the fertility and survivorship schedules, the following population growth parameters were calculated using formula suggested by Carey (1993): intrinsic rate of natural increase (r_m), mean generation time (T), finite rate of increase (λ), net reproductive rate (R_0) and doubling time (DT). Also the intrinsic rate of natural increase (r_m) was estimated by iterative solving of the following equation (Brich, 1948).

$$\sum_{\alpha}^{\beta} e^{-rx} lxmx = 1$$

Where x is the age of individuals in days, r is the intrinsic rate of natural increase, l_x is the age specific survival, and m_x is the age specific number of female offspring. When r was computed for the original data (r_{all}), the jackknife method was applied to evaluate the differences in r_m values. The jackknife pseudo- value r_j was estimated for the n samples by using the following formula:

$r_i = n.r_{all}-(n-r)r_i$

2.6 Statistical methods

For statistical analysis, each mean value is given with its standard error (±SEM). Standard demographic parameters were calculated from daily records of mortality, fecundity and fertility of cohort of predator coccinellid females. The parameters symbols, formula and calculations were used according to Carey (1993, 2001). Statistical analysis was carried out using Minitab (MINITAB 2000) and SAS software. We used the Jacknife procedure to estimate the variance for r_m and the other population parameters (Meyer et al, 1986). It is used to quantify uncertainty associated with parameters estimates, as an alternative to analytical procedures, in cases for which the analytical methods required very complicated mathematical derivation (Maia et al, 2000). Similar procedures were used for the other parameters (R_0, T, λ and DT). The steps for the application of the method are as follows (Maia et al, 2000):

1) Estimation of r_m , R_0, T, λ and DT considering the survival and reproduction data for all the n females, referred to as true calculation. At this point, called step zero, estimates obtained are denoted as $r_{m(all)}$, $R_{0(all)}$, $T_{(all)}$, $\lambda_{(all)}$ and $DT_{(all)}$ (Maia et al, 2000).

2) Repeat the procedure described in part (1) for n times, each time excluding a different female. In so doing , in each step i , data of n-1 females are taken to estimate parameters for each step, now named $r_{m(i)}$, $R_{0(i)}$, $T_{(i)}$, $\lambda_{(i)}$ and $DT_{(i)}$ (Maia et al , 2000).

3) In each step i, pseudo -value are calculated for each parameter, subtracting the estimate in step zero from the estimate in step i , for instance, the pseudo value for r_m , $r_{m(j)}$, was calculated for the n samples using the following equation (Maia et al , 2000).

$$r_{m(j)} = n \, . \, r_{all} - (n\text{-}1). \, r_{m(i)}$$

4) After calculating all the n pseudo -values for r_m Jacknife estimate of the mean ($r_{m\ (mean)}$), by the following equations (Maia et al, 2000).

$$r_{m(mean)} = \frac{\sum_{1}^{n} r_m(j)}{n}$$

3. Results

3.1 Biology of Adalia bipunctata in pomegranate orchards:

The activity period of this ladybird in Yazd province is from the last days of March through the last days of May and also from the beginning of September through the last days of November. Ladybird lays its eggs in the form of colony on the surface of leaves. Larvae with different ages and adults are controlled through preying on pomegranate aphid. The ladybird prefers the different ages of *A. punicae* nymph to the winged and wingless adult. The feeding of ladybird adult influences in the number of *A. punicae* eggs. The hibernation of the ladybird is in the form of adults. The ladybird has 2-3 generations per year. In this study different forms of the ladybird were collected from pomegranate trees. The black speciesform (black ladybirds with red spots) in summer and autumn and bright species (red ladybirds with black spots) in spring proliferate saliently. Natural populations and sexual contacts of bright species are more than the black species. On the other hand, the black species had higher mortality during hibernation.

3.2 Development and Survivorship of Immature Insects

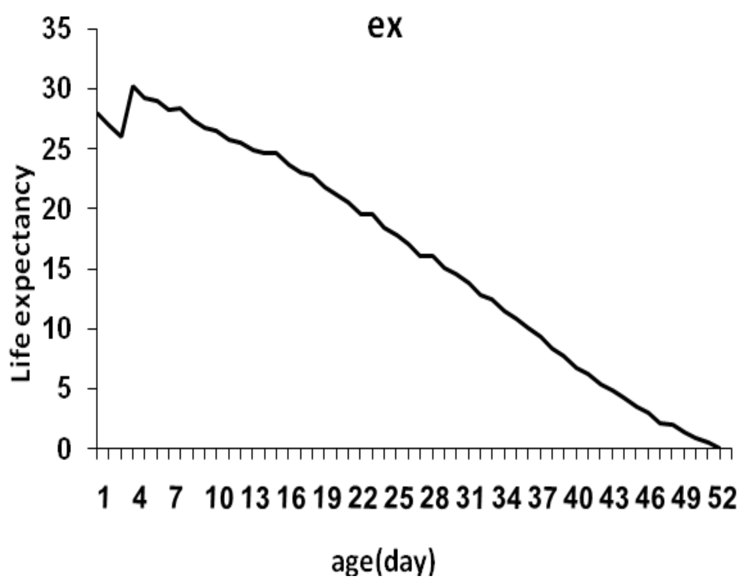
The mean duration of developmental stages of *A. bipunctata* from eggs to adult measured with feeding on *A. punicae* is shown in table 1.The highest mortality occurred during the egg stage. *A. bipunctata* successfully developed to adult in 14.29±0.08 days. Sex ratio of *A. bipunctata* on *A. punicae* under controlled conditions was determined as 1.5:1 (female/male).

Table 1: Development time for juvenile stages of *A. bipunctata* fed on *A. punicae* under controlled conditions.

Life Stage of *A. bipunctata*	Days ± SEM
Egg	2.49±0.06
1st instars' larvae	2.19±0.05
2nd instars' larvae	1.30±0.06
3rd instars' larvae	1.65±0.07
4th instars' larvae	2.37±0.06
Prepupae	1.07±0.02
Pupae	3.22±0.07

3.3 Reproduction and population growth parameters

For a life table, measurements and analysis of associated parameters used by Carey (1993) were employed. Basic parameters of life table were shown in figures 1, 2, 3 and 4. Life expectancy immediately following emerging of larvae from eggs increased to some extent and then decreased as in the normal process. Life expectancy after emerging of larvae from eggs was estimated to be 30.22 days (figure 1).



l_x at adults emerging time on *A. punicae* was 0.69, which shows that 0.31of individuals will be dead before becoming adult (figure 2).

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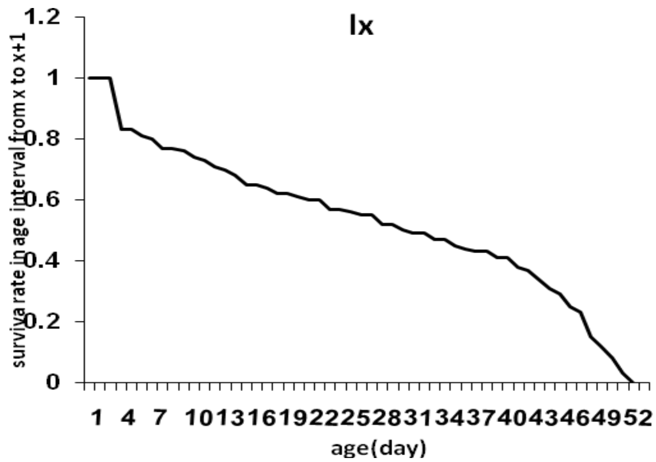
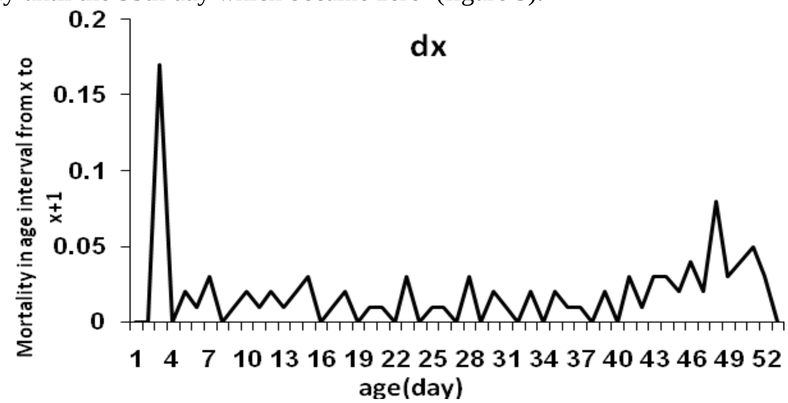
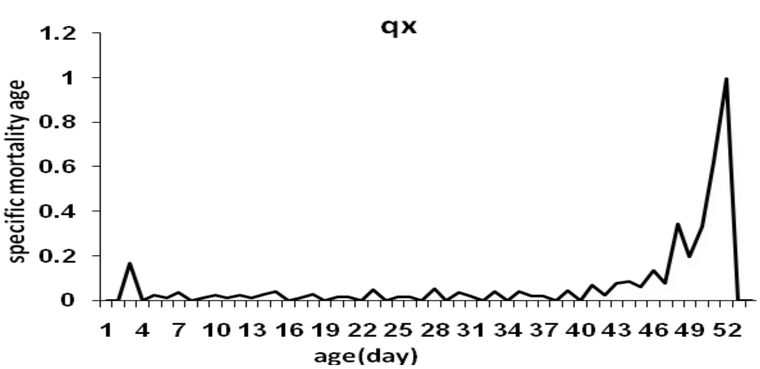


Mortality in age interval from x to x+1 in first day is zero and increased in third day and its value was 0.17 and then flacualted gradually until the 53th day which became zero (figure 3).



Beginning of specific mortality age (q_x) of larvae and adults of *A. bipunctata* feeding on pomegranate adult wingless aphid was almost steady for 39 days and from day 46 it increased gradually. Specific mortality age begins from day 3 with a value of 0.17 and with a little vibration increased gradually. Its value in the 52th day was 1 and in the 53th day it was 0 (figure 4).



Probability of adult remains alive until age x (P_x) in the first day of adult mortality was 1 which by increasing age in 47 day. Comparison of two parameters P_x and q_x with feeding on pomegranate adult wingless aphid showed that mortality of larvae and adult increased in 46^{th} and 47^{th} days. Females reared on *A. punicae* were tested for the length of pre-oviposition , oviposition ,post-oviposition periods and longevity (table 2). The females lived at least 4 days after egg laying and their recorded total longevity was 54 days. Mean total preimaginal developmental time from egg to adult emergence was 14.46±0.08 days. The oviposition period was the longest stage of reproductive period. Eggs were laid for about 48 days on ***A.*** *punicae.* Oviposition was depended on female age. Only one peak was observed in the process of fecundity. This was on day 28 which was recorded 400 eggs / female/ day for ***A.*** *punicae.* Oviposition began on 17th day, and all females oviposited by day 52. The eproductive parameters of *A. bipunctata* are showed in Table 2. The mean numbers of eggs per female per day was estimated 11.58 and the mean number of eggs per day was estimated 13.21. Values of population growth parameters of *A. bipunctata* fed on *A. punicae* under controlled conditions illustrated in Table 3.

Table 2: Reproductive parameters of *A. bipunctata* fed on *A. punicae* under controlled conditions.

Parameters	**Value**
Pre- oviposition	2.33 days
Oviposition	23.27 days
Post –oviposition	1.71 days
Female longevity	54.5 days
Gross fecundity rate	462.4±22.1
Gross fertility rate	405.3±23.4
Gross hatch rate	0.78651±1.058
Net fecundity rate	228.4±11
Net fertility rate	189.49±9.11

Table 3: Population growth parameters of A. bipunctata fed on A. punicae under controlled conditions.

Parameters	**Value**
Mean generation time (T)	25.266 ±0.031 days
Doubling time (DT)	3.58±0.05 days
Finite rate of increase (λ)	1.21± 0.003 days
Net reproductive rate (Ro)	133.796±0.546 female/female/generation
Intrinsic rate of population increase (r_m)	0.1937±0.002 female/female/ day
Intrinsic rate of birth (b)	0.2047 female/female/day
Intrinsic rate of dead (d)	0.02851 female/female/day

4. Discussion

At 27 °C the developmental time of *A. bipunctata* (14.76 days) in this study was lower than when reared on live *Acyrthosiphon pisum* (17.5 days), (or?) frozen *E. kuehniella* eggs (17.2 days), Frozen *E. kuehniella* eggs + frozen pollen (16.3 days) at 23 ºC based on De Clercq et al. (2005), *M. persicae* (on *Sinapis alba*=21.8 days, *Brassica napus*=22 days and *Vicia faba*=23.4 days as host plants) based on (Francis et al, 2000) and on *A. pisum* (17.8 days) by Obrycki and Tauber (1981), because the development rate is mainly dependent on food quality, food quantity, temperature and host plants (Wratten, 1973; Omkar and Pervez, 2005). Mills (1981) indicated that increase in temperature from 15.6 to 29.4 ºC cause decreases in the larval duration from 22.5 to 7.8 days, Also differences in host plants of prey, affect the fecundity and development of *A. bipunctata* (Francis et al, 2000 and 2001; Vanhaelen et al, 2002).

Vanhaelen et al., (2002) believed that, in order to choose a beneficial species, the prey host plant has a major influence on the potential efficacy of biological agent to control herbivore species such as aphids. Based on Lanzoni et al (2004) Mean developmental period for eggs, 1st, 2nd, 3rd and 4th instars larvae, and pupae of *A. bipunctata* reared on *Myzus persicae* at 25 ºC were obtained 2.3±0.1, 2.8±0.1, 1.6±0.1, 1.8±0.1, 3.4±0.2 and 6.5±0.1 days, respectively. Mean total preimaginal developmental time from egg to adult emergence was 18.4±0.2 days. These values are nearly equal to those found in the current study.

Values of The intrinsic rate of increase (r_m), net reproductive rate (R_0) and mean generation time (T_c) obtained by Lanzoni et al (2004) were, 0.081, 18.49, 40.06 days for *A. bipunctata*, 0.089 , 26.27 and 38.81 for *Harmonia axyridis* and 0.114, 52.75 and 41.88 for *Hippodamia variegate* respectively. Values for R_0 (133.796±0.546), Tc (25.266±0.031), DT (3.58±0.05), λ (1.21± 0.003) and r_m (0.1937±0.002) of *A.bipunctata* with feeding on *A. punicae* in experimental evidence in our investigation indicated that *A. bipunctata* is less adapted to *M. persicae* compared with *A. punicae*. On the other hand *A. bipunctata* is highly adopted and show greatest preference for *A. punicae* than *M. persicae*. Our findings showed that the potential for *A. bipunctata* (r_m=0.1937) is greater than that of two other species depending to prey host.

A comparison between the life table parameters of *H. axyridis* and those of other native ladybirds shows that the potential for *A. bipunctata* reared on *A. punicae* based on this study, is greater also for *Propylea quatuordecimpunctata* L. (r_m=0.15) (Obrycki et al., 1993), six cohorts of *A. bipunctata* (r_m=0.16) at 24 ºC by Pelicano and Folcia (2003) which supporting the ease in mass-rearing of *A. bipunctata* in the laboratory (Omkar and Pervez, 2005), and equal with *C. septempunctata* (r_m=0.19) (Phoofolo and Obrycki, 1995) and *Oenopia conglobata contaminate* (r_m=0.19) (Mehrnejad and Jalali, 2004). The value of rm in current study (0.19) was nearly equal the r_m value of its prey, the pomegranate aphid, *Aphis punicae* (r_m=0.21, 0.28, 0.32 and o.3 at 20, 22.5, 25 and 27.5 ^{oC} respectively) at 20^{oC}. In this regard, Van Lenteren and Woets (1988) suggested that natural enemies might be effective for biological control when, among other criteria, the intrinsic rate of increase of the natural enemy is equal to or larger than the intrinsic rate of increase of the pest. According to the results obtained in this study, *A. bipunctata* could be valuable as a biocontrol agent for *A. punicae* and at this stage conservation might be the right strategy for improving the efficiency of this species and other natural enemies of the aphid in the field. An integrated strategy of using *A. bipunctata* and honeydew has also been tested recently against *E. tiliae* infesting lime trees in France (Bardoux et al, 2003). According to Sakuratani et al. (2000) over-wintered adults of *A. bipunctata* appeared in March and laid eggs and adults emerged in spring. Our study indicated that the activity period of this ladybird in Yazd is from the last days of March through the last days of May and also from the beginning of September through the last days of November.

The results of this study provide information necessary to determine demographic parameters of *A. bipunctata* reared on *A. punicae.* Our results suggest that *A. bipunctata* seems to be a good candidate predator for control of pomegranate aphid from late winter to late spring. However, our experimental study indicates in order to evaluate *A. bipunctata* as a biological control agent of *A. punicae* , more information is needed to fully understand the general ecology of these species. Therefore, more research should be conducted to determine the effect of host aphid stages and other environmental factors on the development of *A. bipunctata*. Having these factors in mind, we will be able to develop suitable strategies for the biological control of *A. punicae*.

This study was undertaken to compare demographic parameters of *A. bipunctata* on *A.* punicae. Our findings may provide important information in designing a comprehensive program for IPM of the pomegranate aphid in Iran. No other published data are available concerning the demographic parameters of *A. bipunctata* on *A. punicae.* Results obtained in our laboratory can give important insights on the potential of this predator for biological control, developmental threshold and life history of *A. bipunctata* in pomegranate orchards. It will provide basic knowledge on their activity period and will find suitable time for controlling *A. punicae* when a study is conducted under field conditions.

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