

# Effect of different natural and factitious diets on development and life history parameters of *Chilocorus bipustulatus*

Haniieh Atrchian · Kamran Mahdian ·  
Shahnaz Shahidi

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**Abstract** The effects of five diets consisting of two natural prey (*Acanthococcus abaii* Danzig and *Agonoscena pistaciae* Burckhardt et Lauterer) and three factitious foods (frozen eggs of *Ephestia kuehniella* Zeller, a mixture of frozen corn pollen with eggs of *E. kuehniella* and a diet consisting of eggs of *Sitotroga cerealella* Olivier) on the development, survival and reproduction of *Chilocorus bipustulatus* L. were investigated. The results revealed that this predator successfully completed immature developmental procedures on the offered foods, but developmental duration was affected. Total larval survival of *C. bipustulatus* was higher on natural diets than factitious diets. Fresh weights of the newly emerged female and male coccinellids were also affected by the diet offered to this predator during their larval stage. The highest intrinsic rate of increase ( $r$ ), the net reproductive rate ( $R_0$ ) and the finite rate of increase ( $\lambda$ ) were obtained on *A. pistaciae*, and these values were significantly different from *A. abaii*.

**Keywords** Coccinellidae · Development · Factitious food · Fecundity · Intrinsic rate of increase · Natural prey

## Introduction

*Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae) is a predatory coccinellid that is widely distributed in Europe, the Eastern Palearctic region, Near East and North Africa. This coccinellid is a polyphagous predator that preys on aphids, armored scales and other scale families such as Eriococcidae, Coccidae, Asterolecaniidae and Pseudococcidae (Stathas et al. 2003, 2009; Eliopoulos et al. 2010) and more rarely on whiteflies (Yigit et al. 2003). This predator has been considered for augmentative release because of its wide host range (Gaillet 1967; Laudeho et al. 1970). However, its polyphagous nature may also include environmental risks when the insect is used in areas where it is not native (De Clercq et al. 2002; van Lenteren et al. 2003). The effects of different diets and temperatures on the development and reproduction of *C. bipustulatus* have received attention in several studies (Rosen and Gerson 1965; Hattingh and Samways 1993; Uygun and Elekcioglu 1998). However, information on the qualitative and quantitative effects of nutrition on the life table parameters of *C. bipustulatus* remains scarce. *Acanthococcus abaii* Danzig (Hemiptera: Eriococcidae) is a key pest of the saxaul plants *Haloxylon aphyllum* L., which is the main plant in desert areas of the Kerman, Sistan and Balouchestan provinces of Iran (Miller and Gimpel 2000; Moghadam and Tvakoli 2010). The common pistachio psylla, *Agonoscena pistaciae* Burckhardt et Lauterer (Hemiptera: Psyllidae), is an important pest

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H. Atrchian · K. Mahdian (✉) · S. Shahidi  
Department of Crop Protection, Faculty of Agriculture,  
Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran  
e-mail: kamranmahdian@vru.ac.ir

of pistachio trees that damages pistachio orchards every year in Iran (Mehrnejad and Copland 2006; Panahi et al. 2013).

It is necessary to know the effects of different diets on the growth and fecundity of the biocontrol agents to manage a successful biological control program. The nutritional value or food quality of prey or artificial diets is critical for the fitness of most predators. Parameters such as immature development, consumption capacity and egg production in response to feeding are used to define the food quality of prey. Therefore, a good understanding of the feeding habits and nutritional needs of a natural enemy is elementary for its implementation in biological control strategies (Hattingh and Samways 1993; Mahdian et al. 2005). There is much interest in food studies and their relationships with coccinellids as natural enemies of aphids, scales and mites among specialists of biological controls (Lucas et al. 2002; Villanueva et al. 2004; Evans 2009; Hodek and Honek 2009). The use of alternative prey may make the production of natural enemies for biological control more cost effective and reduce problems with space for the mass rearing of predators (De Clercq et al. 2005). Adult reproduction depends closely on prey suitability for larval development, but larvae and adults may differ in nutritional requirements (Michaud 2005). Eggs of the *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) are commonly used as alternative diets for the laboratory production of several coccinellids (Specty et al. 2003; Hamasaki and Matsui 2006; Berkvens et al. 2008b). A mixture of frozen, moist bee pollen and *E. kuehniella* eggs is a suitable alternative diet for the development and reproduction of lady beetles (Jalali et al. 2010). Evaluations of demographic parameters may be a useful tool for analyzing and understanding the effect of external factors such as food on the development, survival, reproduction, and intrinsic rate of increase of insect populations to provide a comprehensive description of the fecundity of predator populations (Chi and Su 2006; Farhadi et al. 2011). The present study evaluated the effects of different natural prey and factitious food on survival, development, longevity, reproduction and the intrinsic growth rate of *C. bipustulatus*. The results may improve rearing methods of *C. bipustulatus* as a biocontrol agent.

## Materials and methods

### Predator rearing

A laboratory colony of *C. bipustulatus* was established in May–June 2014 using individuals originally collected from pistachio orchards and *Haloxylon* shrubs near Rafsanjan, Kerman province, Iran. The food of the stock colony of predators primarily consisted of nymphs and adult *A. abaii*. Insects were reared in a growth chamber at  $25 \pm 1$  °C,  $60 \pm 5$  % RH, and a photoperiod of 16:8 (L:D) h. The coccinellids were maintained in transparent plastic containers (25 × 20 × 10 cm) covered by a lid with a fine mesh for ventilation. A piece of moist cotton in a 1-cm-diameter plastic dish served as a source of water for the ladybirds.

### Natural diets

Two natural diets including *A. abaii* and *A. pistaciae* were used as natural foods, which were collected daily from saxaul plants and pistachio orchards, respectively, and used in the experiments. Both prey were collected from a place with no insecticide treatment. The predators were supplied ad libitum with some branches of saxaul plants and two leaves of *Pistacia vera* infested with *A. abaii* and *A. pistaciae*, respectively.

### Factitious food

Mediterranean flour moth larvae were reared on wheat flour under laboratory conditions. The eggs of *E. kuehniella* were collected daily and stored in a deep freeze at  $-18$  °C for no longer than one month. Frozen eggs of *S. cerealella* were obtained from the Iranian Pistachio Research Institute, Rafsanjan, Iran, and were stored in a deep freeze at  $-18$  °C. All experiments were performed under laboratory conditions at  $25 \pm 1$  °C,  $60 \pm 5$  % RH, and a photoperiod of 16:8 (L:D) h.

### Effect of diets on larval development and survival of *C. bipustulatus*

Immature development of *C. bipustulatus* was monitored on each of five diets: female adults of *A. abaii*, fifth-instar nymphs and adults of *A. pistaciae*, frozen

eggs of *S. cerealella*, frozen eggs of the Mediterranean flour moth *E. kuehniella* and a mixture of frozen corn pollen with eggs of *E. kuehniella*. Monitoring of larval development began with first instar larvae. Batches of *C. bipustulatus* eggs (<24 h old) were collected from the stock colony for the experiments on developmental time. Thirty-five newly molted first instars of *C. bipustulatus* were randomly taken from laboratory cultures for each treatment and placed individually in Petri dishes (9 cm in diameter), covered with a fine mesh lid for ventilation and furnished with absorbent papers. A moist paper plug in a small cup (1 cm in diameter) supplied water. Each factitious diet was supplied ad libitum in a plastic cup (2.5 cm in diameter). Infested foliage of *H. aphyllum* and *P. vera* L. with *A. abaii* and *A. pistaciae*, respectively, was supplied ad libitum for the natural diets. The developmental time and survival of each *C. bipustulatus* larval instar were recorded, and adults were sexed and weighed on a Sartorius digital balance ( $\pm 0.01$  mg) upon emergence.

#### Effects of diets on *C. bipustulatus* reproduction

Reproductive capacities of the predators were evaluated on all diets that yielded development to the adult stage. Adults obtained from developmental tests upon emergence were placed together in rearing containers and were given the same food as in the larval stage. Twelve pairs of adults from each diet were collected from rearing containers after the first mating was observed. Mating pairs were isolated in Petri dishes (12 cm in diameter) furnished with an absorbent paper. A moist paper plug in a small cup provided water. Natural and factitious foods were presented in excess and replaced daily. Oviposition and survival were monitored daily until 41 days after the adult females began oviposition. Males that died before females were replaced by males reared under the same conditions, and females that died within five days were omitted from analyses. The experiments were performed in growth chambers at  $25 \pm 1$  °C with a RH of  $60 \pm 5$  % and a photoperiod of 16:8 (L:D) h.

#### Statistical analysis

The effect of different diets on developmental times and adult weights of *C. bipustulatus* was analyzed

using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ( $P = 0.05$ ). No reproduction was observed with factitious diets. Therefore, reproductive parameters of *C. bipustulatus* were compared between two natural diets using Student's *t*-test. Data were initially tested for normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene's test) prior to ANOVA. Sex ratios were analyzed using a  $\chi^2$  test. The raw life history data for *C. bipustulatus* were analyzed based on the theory of the age-stage, two-sex life table using TWOSEX-MSChart (Chi 2013). The standard errors of the life table parameters were estimated using the bootstrap technique (Ebrahimi et al. 2013; Akca et al. 2015). The age-stage specific survival rate ( $s_{xj}$ , the survivorship of the newborn eggs to age  $x$  and stage  $j$ ), age-stage specific fecundity ( $f_{xj}$ , the mean fecundity of individuals of age  $x$  and stage  $j$ ), age-stage total fecundity ( $Ft_{xj}$ , the total fecundity of all individuals of age  $x$  and stage  $j$ ), age-specific survival rate ( $l_x$ , the probability of a newly laid egg survives to age  $x$ ), age-specific fecundity ( $m_x$ , the mean fecundity of individuals at age  $x$ ), age-stage life expectancy ( $e_{xj}$ , the individuals of age  $x$  and stage  $j$  that are expected to be alive), age-specific maternity ( $l_x m_x$ ) and the life history parameters ( $r$ , the intrinsic rate of increase,  $\lambda$ , the finite rate of increase,  $R_0$ , the net reproductive rate and  $GRR$ , gross reproductive rate) were calculated. Student's *t*-test was used to compare the life history parameters of *C. bipustulatus* between the two different natural diets.

## Results

#### Effect of diets on developmental time, sex ratio and adult weight

The results revealed significant effects of all tested diets on developmental duration in *C. bipustulatus* ( $F = 37.37$ ;  $df = 4,126$ ;  $P < 0.05$ ). All instars successfully completed their life cycle on the foods offered. The mean total developmental time of *C. bipustulatus* from first instar to adult was  $22.92 \pm 0.4$ – $29.00 \pm 0.3$  days (Table 1). The longest and shortest total developmental times of *C. bipustulatus* were on eggs of *S. cerealella* and the diet of frozen corn pollen mixed with eggs of *E. kuehniella*, respectively ( $22.92 \pm 0.4$  vs.  $29.0 \pm 0.3$  days) (Table 1). Significant differences ( $F = 7.37$ ;  $df = 4,157$ ;

**Table 1** Developmental time (days) (mean  $\pm$  SE) for immature stage and sex ratio (% females  $\pm$  SE) of *Chilocorus bipustulatus* fed different factitious diets and natural prey

Developmental stage	Diets				
	<i>A. pistaciae</i> adult	<i>A. abaii</i> adult	Eggs of <i>E. kuehniella</i>	Eggs of <i>E. kuehniella</i> + pollen	Eggs of <i>S. cerealella</i>
Egg	5.06 $\pm$ 0.091 <sup>a</sup>	3.40 $\pm$ 0.084 <sup>b</sup>	–	–	–
First instar	3.68 $\pm$ 0.134 <sup>bc</sup>	4.97 $\pm$ 0.198 <sup>a</sup>	3.15 $\pm$ 0.120 <sup>c</sup>	3.31 $\pm$ 0.114 <sup>cd</sup>	3.94 $\pm$ 0.123 <sup>b</sup>
Second instar	3.24 $\pm$ 0.220 <sup>c</sup>	4.59 $\pm$ 0.145 <sup>a</sup>	4.20 $\pm$ 0.176 <sup>ab</sup>	2.90 $\pm$ 0.154 <sup>c</sup>	4.00 $\pm$ 0.127 <sup>b</sup>
Third instar	3.43 $\pm$ 0.202 <sup>b</sup>	4.73 $\pm$ 0.166 <sup>a</sup>	4.96 $\pm$ 0.189 <sup>a</sup>	3.41 $\pm$ 0.208 <sup>b</sup>	5.00 $\pm$ 0.131 <sup>a</sup>
Fourth instar	4.89 $\pm$ 0.209 <sup>b</sup>	5.42 $\pm$ 0.253 <sup>b</sup>	5.19 $\pm$ 0.147 <sup>b</sup>	5.00 $\pm$ 0.124 <sup>b</sup>	6.21 $\pm$ 0.159 <sup>a</sup>
Pre pupa	1.54 $\pm$ 0.144 <sup>a</sup>	1.66 $\pm$ 0.123 <sup>a</sup>	1.49 $\pm$ 0.150 <sup>a</sup>	1.49 $\pm$ 0.150 <sup>a</sup>	1.26 $\pm$ 0.166 <sup>a</sup>
Total developmental larvae	14.48 $\pm$ 0.823 <sup>ab</sup>	19.47 $\pm$ 0.917 <sup>d</sup>	16.00 $\pm$ 1.123 <sup>bc</sup>	13.38 $\pm$ 0.646 <sup>a</sup>	17.61 $\pm$ 0.808 <sup>cd</sup>
Pupa	7.11 $\pm$ 0.172 <sup>b</sup>	6.77 $\pm$ 0.223 <sup>b</sup>	6.93 $\pm$ 0.311 <sup>b</sup>	6.54 $\pm$ 0.149 <sup>b</sup>	7.86 $\pm$ 0.190 <sup>a</sup>
Total	24.33 $\pm$ 0.647 <sup>d</sup>	28.83 $\pm$ 0.332 <sup>b</sup>	26.73 $\pm$ 0.398 <sup>c</sup>	22.92 $\pm$ 0.400 <sup>c</sup>	29.00 $\pm$ 0.302 <sup>a</sup>
Sex ratio (% females)	62.93 $\pm$ 0.002 <sup>a</sup>	60.15 $\pm$ 0.001 <sup>a</sup>	50.48 $\pm$ 0.002 <sup>a</sup>	57.65 $\pm$ 0.002 <sup>a</sup>	50.00 $\pm$ 0.002 <sup>a</sup>

Means within a row followed by the same letter are not significantly different (Duncan's multiple range test or Student's *t*-test,  $P > 0.05$ )

Sex ratios followed by the same letter were not significantly different using  $\chi^2$  at 5 % for comparisons between diets

$P < 0.05$ ) in total developmental time of *C. bipustulatus* larvae were detected when fed on different natural and factitious diets. The results demonstrated that the diets had a significant effect on the development time of all different instar larvae from first to fourth instar. There was no significant difference in the developmental time of the prepupal stage ( $F = 0.981$ ;  $df = 4,170$ ;  $P > 0.05$ ), but the diets affected the duration of the pupal stage ( $F = 4.68$ ;  $df = 4,127$ ;  $P < 0.05$ ). The different diets did not affect the sex ratios of *C. bipustulatus* adults ( $\chi^2 = 2.21$ ;  $df = 4$ ;  $P > 0.05$ ) (Table 1). The fresh weights of newly emerged female and male coccinellids were affected by the diet offered to the predator during their larval stage (Male:  $F = 7.21$ ;  $df = 4,58$ ;  $P < 0.05$  and Female:  $F = 22.25$ ;  $df = 4,68$ ;  $P < 0.05$ ). Student's *t*-tests revealed significant differences ( $t = 4.78$ ;  $df = 107$ ;  $P < 0.05$ ) between the weight of adult males and females for all diets, and males exhibited lower weights than females (Fig. 1).

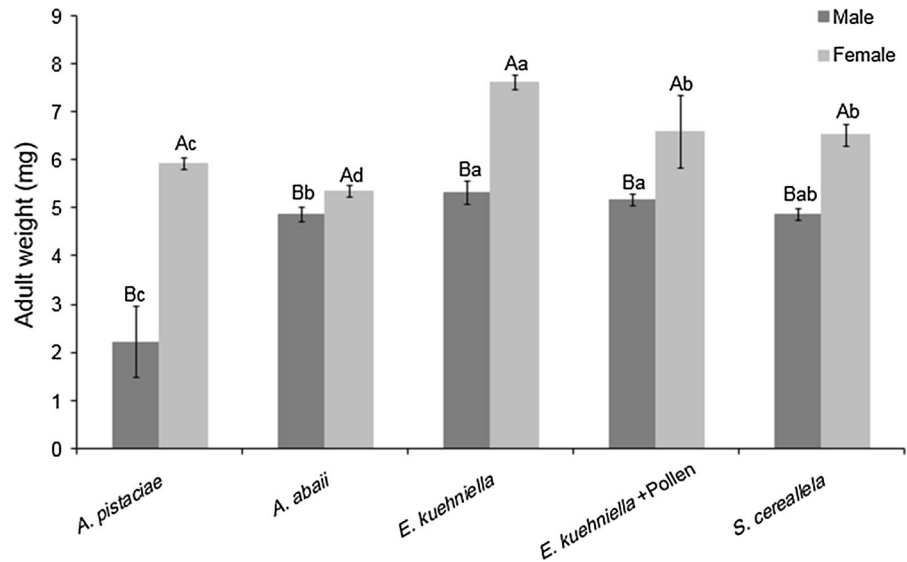
#### Effect of diets on larval, prepupal and pupal survival and longevity

The highest percentage of mortality was observed in the first instar larvae of *C. bipustulatus*. Total larval

survival of *C. bipustulatus* was higher on natural diets than on factitious diets. Survival of first instar of *C. bipustulatus* fed on *A. pistaciae* (88.57 %) was significantly lower than first instar fed on *A. abaii* (91.43 %), factitious diets consisting of *E. kuehniella* eggs, a mixture of frozen corn pollen or *E. kuehniella* eggs (91.43 %) and *S. cerealella* eggs (97.14 %) ( $F = 43.56$ ;  $df = 4,10$ ;  $P < 0.05$ ). No mortality was observed on any diet from pre-pupa to adult emergence. There were no significant differences in survival of *C. bipustulatus* on live prey used in our study. The survival rate of pre-pupa, pupa and adults was 100 %, and the lowest survival rate was observed in first instar larva on all diets. The significantly highest and lowest total survivals of *C. bipustulatus* were observed on *A. pistaciae* (77.14 %) and the diet of eggs of *S. cerealella* (62.86 %), respectively ( $F = 95.43$ ;  $df = 4,10$ ;  $P < 0.05$ ) (Table 2).

There were significant differences in adult longevity of *C. bipustulatus* fed different diets (male:  $F = 397.82$ ;  $df = 4,51$ ;  $P < 0.05$  and female:  $F = 589.21$ ;  $df = 4,68$ ;  $P < 0.05$ ), but no significant differences were observed between the different sexes. Longevity was significantly prolonged with factitious foods ( $F = 20.24$ ;  $df = 2,71$ ;  $P < 0.05$ ) when a mixture of frozen corn pollen and eggs of *E.*

**Fig. 1** Means of adult weights (mg) of *Chilocorus bipustulatus* fed different natural and factitious diets. Bars ( $\pm$ SE) with the same letter are not significantly different according to Duncan's multiple range test (for diets with lower case) ( $P > 0.05$ ) and Student's *t*-test (for sex with uppercase) ( $P > 0.05$ )



**Table 2** Age-specific survival (%) (mean  $\pm$  SE) of larval stages, pupa and pre-pupa of *Chilocorus bipustulatus* fed different diets and natural prey

Developmental stage	Diets				
	<i>A. pistaciae</i>	<i>A. abaii</i>	Eggs of <i>E. kuehniella</i>	Eggs of <i>E. kuehniella</i> + pollen	Eggs of <i>S. cerealella</i>
First instar	88.57 $\pm$ 0.586 <sup>c</sup>	91.43 $\pm$ 0.557 <sup>b</sup>	91.43 $\pm$ 0.577 <sup>b</sup>	91.43 $\pm$ 0.577 <sup>b</sup>	97.14 $\pm$ 0.588 <sup>a</sup>
Second instar	82.86 $\pm$ 0.534 <sup>d</sup>	82.86 $\pm$ 0.534 <sup>d</sup>	88.57 $\pm$ 0.606 <sup>c</sup>	85.71 $\pm$ 0.609 <sup>b</sup>	91.43 $\pm$ 0.549 <sup>a</sup>
Third instar	80.00 $\pm$ 0.885 <sup>c</sup>	80.00 $\pm$ 0.777 <sup>c</sup>	77.14 $\pm$ 0.777 <sup>d</sup>	85.71 $\pm$ 0.618 <sup>a</sup>	82.86 $\pm$ 0.473 <sup>b</sup>
Fourth instar	77.14 $\pm$ 0.618 <sup>b</sup>	80.00 $\pm$ 0.777 <sup>a</sup>	74.29 $\pm$ 0.585 <sup>c</sup>	74.29 $\pm$ 0.575 <sup>c</sup>	68.57 $\pm$ 0.729 <sup>d</sup>
Total	82.14 $\pm$ 0.658 <sup>c</sup>	83.57 $\pm$ 0.577 <sup>abc</sup>	82.85 $\pm$ 0.490 <sup>bc</sup>	84.28 $\pm$ 0.415 <sup>ab</sup>	85.00 $\pm$ 0.577 <sup>a</sup>
Pre-pupa	77.14 $\pm$ 0.658 <sup>a</sup>	74.29 $\pm$ 0.409 <sup>b</sup>	74.29 $\pm$ 0.662 <sup>b</sup>	74.29 $\pm$ 0.577 <sup>b</sup>	62.86 $\pm$ 0.496 <sup>c</sup>
Pupa	77.14 $\pm$ 0.658 <sup>a</sup>	74.29 $\pm$ 0.409 <sup>b</sup>	74.29 $\pm$ 0.662 <sup>b</sup>	74.29 $\pm$ 0.577 <sup>b</sup>	62.86 $\pm$ 0.496 <sup>c</sup>
Total	77.14 $\pm$ 0.658 <sup>a</sup>	74.29 $\pm$ 0.409 <sup>b</sup>	74.29 $\pm$ 0.662 <sup>b</sup>	74.29 $\pm$ 0.577 <sup>b</sup>	62.86 $\pm$ 0.496 <sup>c</sup>

Means within a row followed by the same letter are not significantly different (Duncan's multiple range test,  $P > 0.05$ )

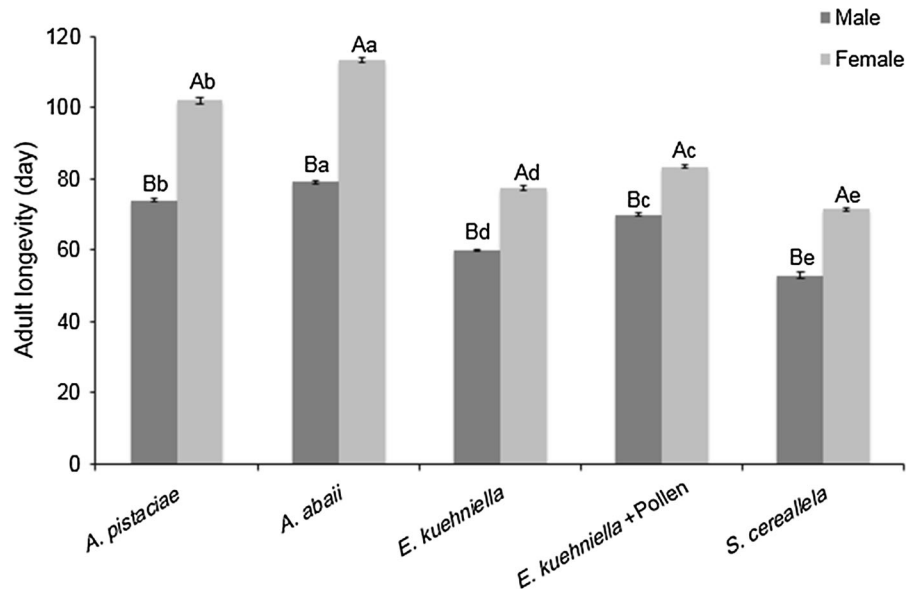
*kuehniella* were provided as food (Fig. 2). The longest and shortest longevities of male and female adults occurred in *C. bipustulatus* fed *A. abaii* and eggs of *S. cerealella*, respectively.

#### Effect of diets on egg hatching and oviposition

No reproduction was observed in *C. bipustulatus* fed factitious diets, but *C. bipustulatus* fed natural prey, including *A. abaii* and *A. pistaciae*, reproduced. The egg incubation period for *C. bipustulatus* fed *A. abaii* and *A. pistaciae* was significantly different ( $t = 4.45$ ;

$df = 68$ ;  $P < 0.05$ ) and ranged from  $5.06 \pm 0.09$  to  $3.40 \pm 0.08$  days, respectively (Table 1). The percentage of egg hatching of *C. bipustulatus* fed *A. pistaciae* was  $76.75 \pm 5.51$  %, which was significantly less than *C. bipustulatus* fed *A. abaii* ( $94.72 \pm 1.59$  %) ( $t = 17.05$ ;  $df = 68$ ;  $P > 0.05$ ). Student's *t*-tests revealed that the mean adult pre-oviposition period APOP of *C. bipustulatus* was significantly different in the *A. abaii* and *A. pistaciae* group ( $t = 7.20$ ;  $df = 26$ ;  $P < 0.05$ ). There was no significant difference between the means of total preoviposition period TPOP of *C. bipustulatus* reared

**Fig. 2** Means of adult longevity (day) of *Chilocorus bipustulatus* fed different diets at  $25 \pm 1$  °C. Bars ( $\pm$ SE) with the same letter are not significantly different according to Duncan's multiple range test (for diets with lower case) ( $P > 0.05$ ) and Student's *t*-test (for sex with uppercase) ( $P > 0.05$ )



on natural diets ( $t = 0.735$ ;  $df = 26$ ;  $P > 0.05$ ) (Table 3). The fecundity of *C. bipustulatus* fed *A. abaii* ( $473.07 \pm 13.26$  eggs) was higher ( $t = 19.09$ ;  $df = 26$ ;  $P < 0.05$ ) than the fecundity on *A. pistaciae* ( $425.98 \pm 10.4$  eggs) (Table 3). Daily oviposition of *C. bipustulatus* revealed that the maximum oviposition of females fed *A. abaii* occurred on day 15 with 17.07 eggs and day 17 with 19.06 eggs for *A. pistaciae* (Fig. 3).

#### Effect of diets on life table parameters

Table 4 shows the life table parameters of *C. bipustulatus* reared on two natural prey, *A. abaii* and *A. pistaciae*. The results indicated that diet affected all parameters (Student's *t*-test,  $P = 0.05$ ) except *GRR*.

Net reproduction rate ( $R_0$ ), intrinsic rate of increase ( $r$ ) and finite rate of increase ( $\lambda$ ) of *C. bipustulatus* on *A. pistaciae* were higher compared to predators fed *A. abaii* ( $R_0$ :  $t = 52.75$ ;  $df = 22$ ;  $P < 0.05$ ) ( $r$ :  $t = 45.00$ ;  $df = 22$ ;  $P < 0.05$ ) ( $\lambda$ :  $t = 427.00$ ;  $df = 22$ ;  $P < 0.05$ ). The age-stage fecundity ( $f_{xj}$ ) and age-stage total fecundity ( $Ft_{xj}$ ) of *C. bipustulatus* fed *A. pistaciae* began earlier (at 30 days) than *A. abaii* (33 days). The highest female fecundity of *C. bipustulatus* occurred on the 43rd and 45th day on *A. abaii* and *A. pistaciae*, respectively (Fig. 4).

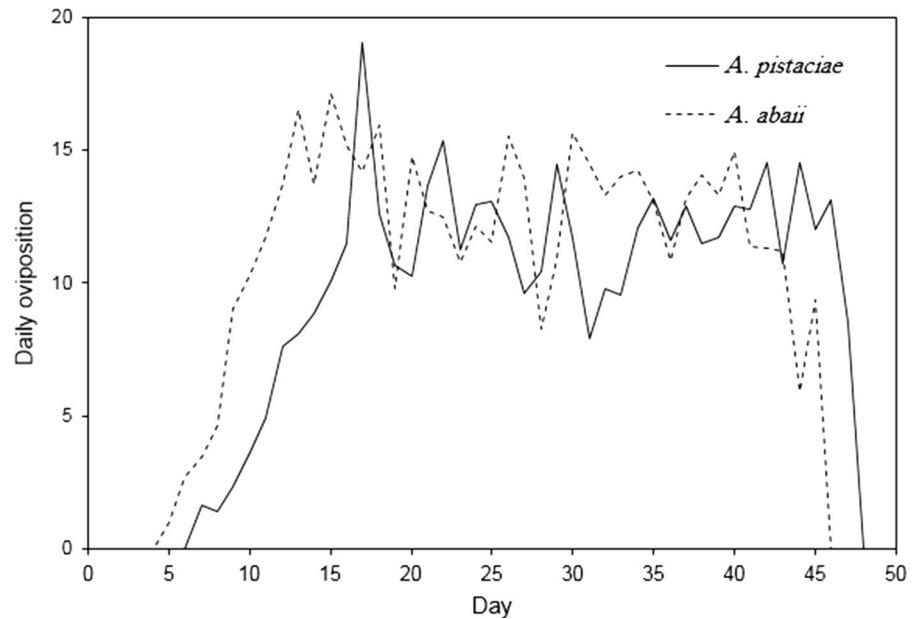
The variability of developmental rate between individuals was clearly observed in the overlaps of age-stage survival rates ( $S_{xj}$ ). The survival rate on both diets decreased with increasing age of *C. bipustulatus*. Age-stage survival rates ( $S_{xj}$ ) of *C. bipustulatus* were

**Table 3** Reproduction parameters (mean  $\pm$  SE) of *Chilocorus bipustulatus* fed different diets and natural prey at  $25 \pm 1$  °C

Parameters	Diets	Mean $\pm$ SE
Fecundity (eggs per female)	<i>A. abaii</i>	$473.07 \pm 13.260^a$
	<i>A. pistaciae</i>	$425.98 \pm 10.400^b$
Adult pre-oviposition period (days)	<i>A. abaii</i>	$4.54 \pm 0.144^b$
	<i>A. pistaciae</i>	$6.20 \pm 0.175^a$
Total pre-oviposition period (days)	<i>A. abaii</i>	$36.85 \pm 0.678^a$
	<i>A. pistaciae</i>	$36.00 \pm 0.894^a$

Means with the same letters are not significantly different (Student's *t*-test,  $P > 0.05$ )

**Fig. 3** Daily oviposition of *Chilocorus bipustulatus* fed two natural diets at  $25 \pm 1$  °C



**Table 4** Life history parameters (mean  $\pm$  SE) of *Chilocorus bipustulatus* on two natural prey, *Acantococcus abaii* and *Aganoscena pistaciae*, calculated by the age-stage, two-sex life table, at  $25 \pm 1$  °C

Food	$r$ ( $d^{-1}$ )	$\lambda$ ( $d^{-1}$ )	$R_0$ (offspring)	$GRR$ (egg)
<i>Acantococcus abaii</i>	$0.1034 \pm 0.005^b$	$1.1090 \pm 0.005^b$	$175.69 \pm 38.724^b$	$213.77 \pm 44.680^a$
<i>Aganoscena pistaciae</i>	$0.1081 \pm 0.005^a$	$1.1142 \pm 0.005^a$	$182.48 \pm 35.945^a$	$273.19 \pm 43.370^a$

Means within the same column followed by the same letter are statistically equivalent (Student's  $t$ -test,  $\alpha = 0.05$ )

$GRR$  gross reproductive rate

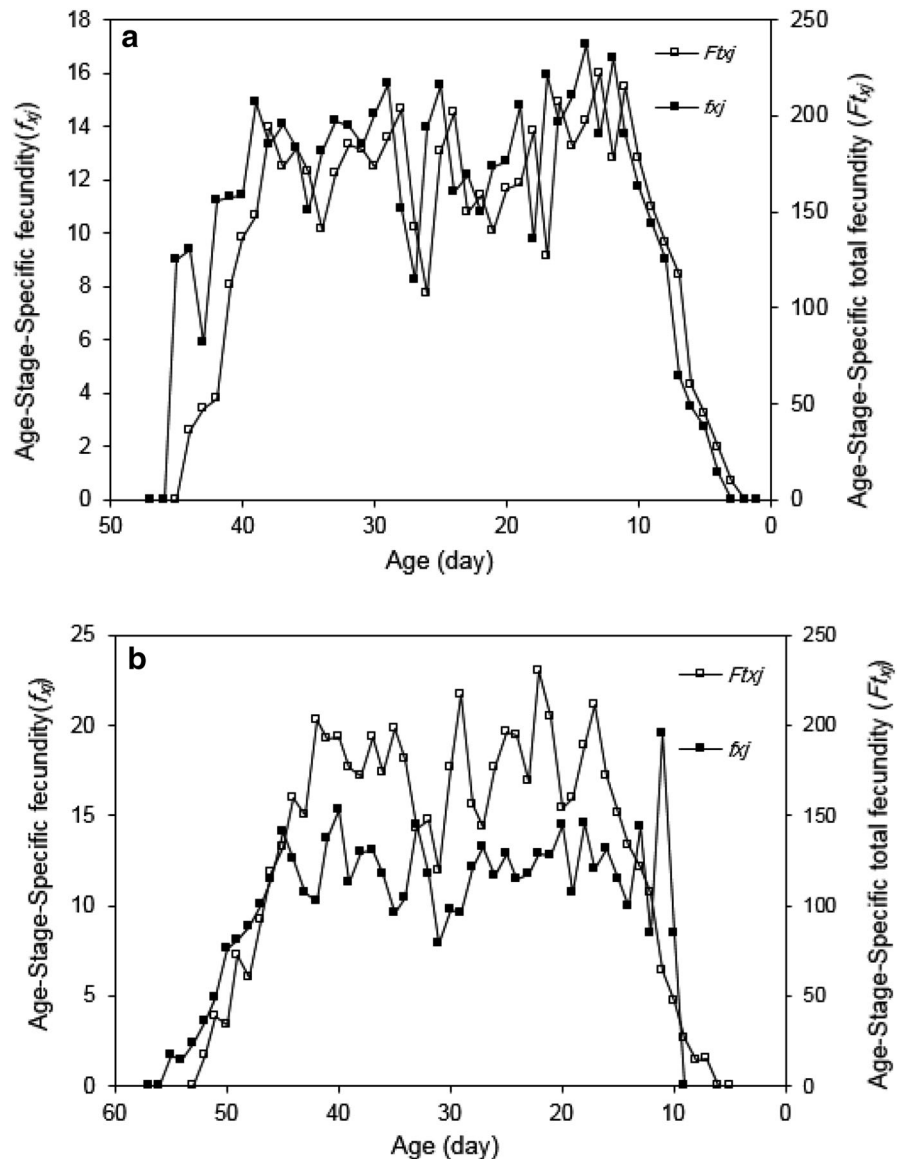
0.485 and 0.428 when feeding on *A. abaii* and *A. pistaciae*, respectively (Fig. 5). The highest age-specific fecundities ( $m_x$ ) of *C. bipustulatus* were 7.4 and 13 offspring on *A. abaii* and *A. pistaciae*, respectively. The highest fecundity was observed in  $l_x m_x$  and  $m_x$  curves in females reared on *A. pistaciae* (Fig. 6).

The life expectancy of a newly laid egg in the *A. abaii* and *A. pistaciae* groups was 64.11 and 57.51 days, respectively, and these expectancies decreased with increasing age. The life expectancy of *C. bipustulatus* was higher for females compared to males in both diets. The peak of reproductive values for both diets (*A. pistaciae* and *A. abaii*) occurred on days 39 and 40, respectively, and females of *C. bipustulatus* made the greatest contribution to the population.

## Discussion

The predatory coccinellid *C. bipustulatus* may be reared on live prey in the laboratory. Nymphs and adults of many diaspidids and pseudococcids were successfully used as prey for the laboratory rearing of *C. bipustulatus* (Uygun and Elekcioğlu 1998; Eliopoulos et al. 2010). Prey types for the Coccinellidae are classified as “essential” if the predators complete their larval development and reproduce on it or “alternative” if they provide only a source of energy for adult survival (Hodek and Honek 1996). *A. pistaciae* and *A. abaii* were essential prey for *C. bipustulatus* in the present study. Michaud (2005) suggested a difference between the nutritional requirements of larvae and adults. A diet that is suitable for the development of larval stages of insects may not be

**Fig. 4** Age-stage fecundity ( $f_{xj}$ ) and age-stage total fecundity ( $F_{txj}$ ) of *Chilocorus bipustulatus* fed two natural diets at  $25 \pm 1$  °C, *Acanthococcus abaii* (a) and *Aganoscena pistaciae* (b)



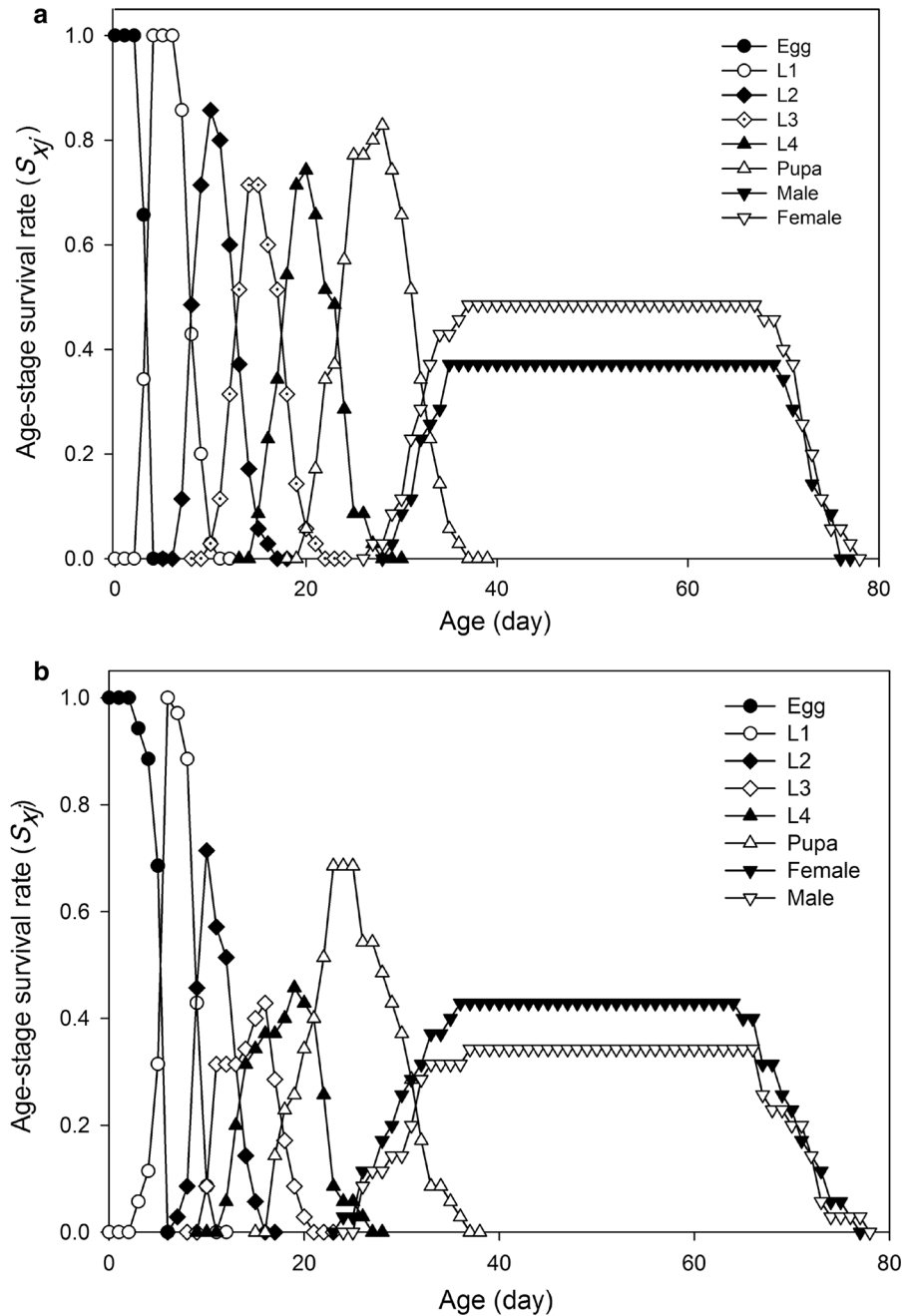
suitable for reproduction. *C. bipustulatus* completed its immature development on the alternative and natural prey diets at  $25 \pm 1$  °C in the current study. Our results demonstrated that different diets significantly influenced the developmental time. Uygun and Elekcioglu (1998) reported that the developmental time of *C. bipustulatus* fed *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) and *Aspidiotus nerii* (Bouche) (Hemiptera: Diaspididae) was  $27.8 \pm 0.40$  and  $26 \pm 0.41$  days, respectively. The development of *C. bipustulatus* in the current study was slower on *A. abaii* and faster on *A. pistaciae*. The

developmental times of *C. bipustulatus* on live prey in this study were close to the times reported by Uygun and Elekcioglu (1998), but they were shorter than *C. bipustulatus* reared on *A. nerii* and *Chrysomphalus aonidum* L. (Hemiptera: Diaspididae) from some previously published studies (Nadel and Biron 1964; Eliopoulos et al. 2010).

Eggs of *E. kuehniella* and a mixture of eggs of *E. kuehniella* and pollen were successfully used as factitious food for the laboratory rearing of several insect predators, including coccinellids (Berkvens et al. 2008a; Maes et al. 2014). *C. bipustulatus* fed a



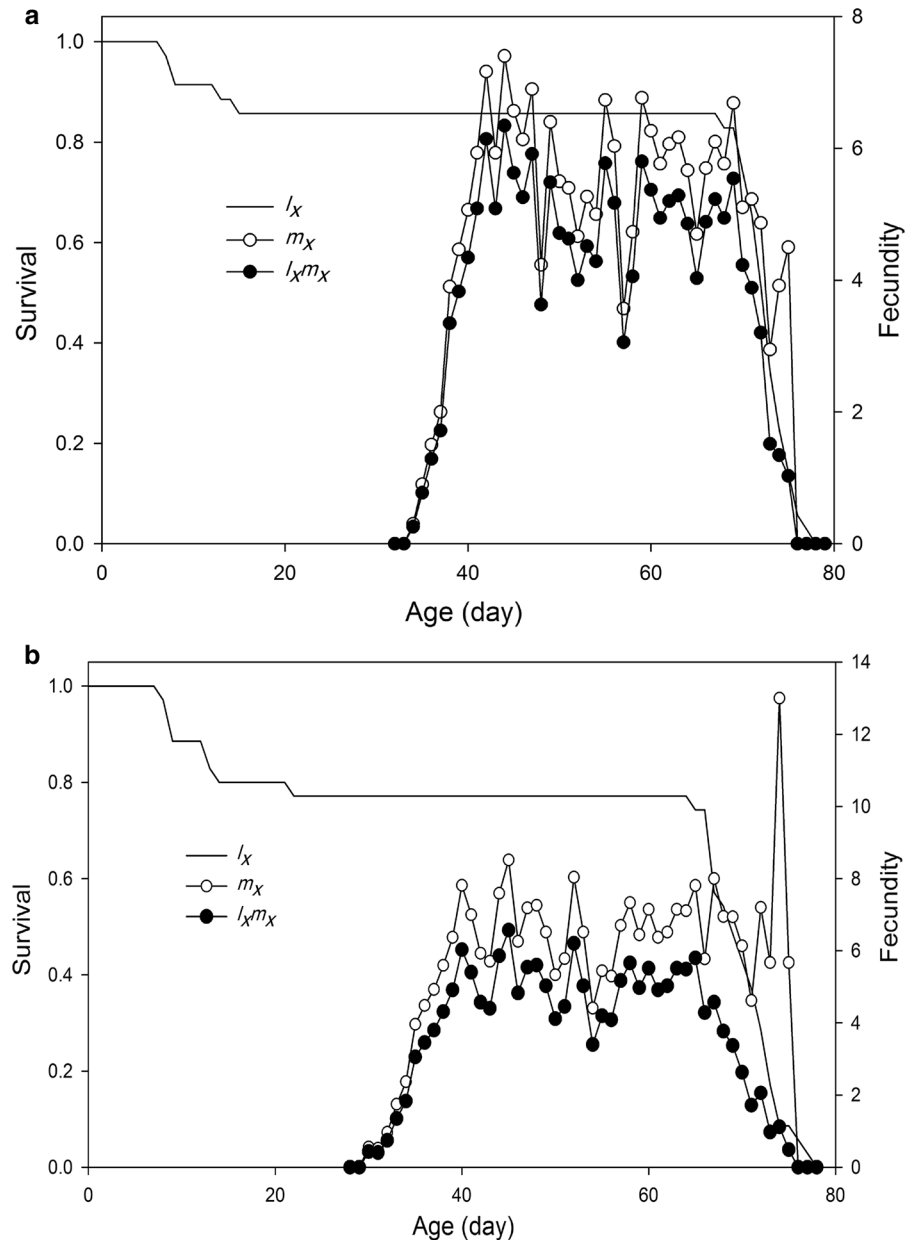
**Fig. 5** Age-stage survival rate ( $S_{xj}$ ) of *Chilocorus bipustulatus* fed two natural diets at  $25 \pm 1$  °C, *Acanthococcus abaii* (a) and *Aganoscena pistaciae* (b)



mixture of eggs of *E. kuehniella* and pollen developed faster than *C. bipustulatus* fed *E. kuehniella* eggs. *C. bipustulatus* reared on other diets did not successfully produce eggs. However, eggs of *S. cerealella* supported *C. bipustulatus* growth but not reproduction. This result may be explained by the fact that adults of *C. bipustulatus* have difficulty handling this type of

factitious food, likely because of the small egg size, and cannot extract nutrients at an appropriate rate to achieve reproduction. Females of *C. bipustulatus* produced more egg numbers on *A. abaii* than *A. pistaciae*. The current study revealed that the total developmental time of immature stages reared on eggs of *E. kuehniella* was significantly lower than *C.*

**Fig. 6** Age-specific survival rate ( $l_x$ ), age-specific fecundity ( $m_x$ ) and the age-specific maternity ( $l_x m_x$ ) of *Chilocorus bipustulatus* fed two natural diets at  $25 \pm 1$  °C, *Acanthococcus abaii* (a) and *Aganoscena pistaciae* (b)



*bipustulatus* reared on a mixture of frozen corn pollen and eggs of *E. kuehniella*. These findings are consistent with Bonte et al. (2010), who found that the developmental time of the immature stage of *Adalia bipunctata* L. (Coleoptera: Coccinellidae) on eggs of *E. kuehniella* was lower than *A. bipunctata* L. fed a mixture of pollen and eggs of *E. kuehniella*. The results of the present study of the effect of eggs of *E. kuehniella* and a mixture of pollen and eggs of *E. kuehniella* on adult weight and developmental time of

*C. bipustulatus* are consistent with earlier observations of De Clercq et al. (2005), who showed that *A. bipunctata* developmental time and adult weight were affected by these two diets. The authors indicated that the developmental time of *A. bipunctata* on eggs of *E. kuehniella* was significantly longer than *A. bipunctata* on a mixture of frozen corn pollen and eggs of *E. kuehniella*. They also demonstrated that the weight of adults of *A. bipunctata* was higher on a mixture frozen corn pollen and eggs of *E. kuehniella* than

*A. bipunctata* fed eggs of *E. kuehniella*. It is encouraging to compare the adult body weight and survival rate of *C. bipustulatus* in this study with the values found by Hattingh and Samways (1993), which were  $6.7 \pm 0.2$  mg and 69 % when *C. bipustulatus* fed on *A. nerii* and *Aspidiotus miliaris* L. (Hemiptera Diaspididae), respectively. Comparisons of the survival of *C. bipustulatus* that fed on *A. pistaciae* (77 %) and *A. abaii* (74 %) in the current study with survival in a previous study (Hattingh and Samways 1993) revealed that the survival of *C. bipustulatus* was higher. However, the mean weights of adults of *C. bipustulatus* reared on *A. pistaciae* ( $5.49 \pm 0.149$  mg) and *A. abaii* ( $4.86 \pm 0.729$  mg) were lower than adults that fed on *A. nerii* and higher than adults fed *A. miliaris*.

Life table analysis for estimating the demographic parameters is the most common basic procedure for investigations of insect population dynamics and predicting population growth (Carey 2001; Stark et al. 2007). Adult and total pre-oviposition periods of *C. bipustulatus* females in the current study were reduced when fed eriococcid *A. abaii*. This finding corroborates the results of Uygun and Elekcioglu (1998), who demonstrated that the pre-oviposition period of *C. bipustulatus* was the lowest ( $9.6 \pm 0.26$  days) when fed *A. nerii* compared to other diets (*A. aurantii*:  $12.8 \pm 0.43$  days and *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Hemiptera: Diaspididae):  $12.2 \pm 0.43$  days). The curves of age-stage specific survival rates ( $S_{xj}$ ) of *C. bipustulatus* revealed a survivorship differentiation and variable developmental rates. For example, the probability that a newborn egg of *C. bipustulatus* will survive to the adult stage was 0.428 for females on *A. pistaciae* and 0.485 for females on *A. abaii*. The intrinsic rate of increase ( $r$ ) is a useful indicator for comparing populations of different species or the same population on different diets (Gabre et al. 2005). The intrinsic rate of *C. bipustulatus* on *A. pistaciae* in the present study was higher than *C. bipustulatus* reared on *A. abaii*. The  $GRR$  is a simple summation of  $m_x$  at the beginning of reproduction, and  $m_x$  is calculated based on the fecundity of all surviving females. However,  $m_x$  at older ages is generally calculated based on the fecundity of a few surviving females, sometimes even a single female. Therefore,  $m_x$  at older ages contributes significantly less to the population. No significant difference was observed in the  $GRR$  of *C. bipustulatus* on both natural diets ( $t = 0.819$ ;  $df = 22$ ;  $P > 0.05$ ).

Nutrients and food preference play important roles in the developmental rate and fecundity of most species of Coccinellidae. However, generalist predators usually exhibit some degree of nutritional stringency and may display distinct food preferences in choice situations (Matthews and Matthews 1978; De Clercq 2002). Knowledge of life table parameters is necessary to provide a better understanding of predator population dynamics. The data of the current study suggest that all instar larvae of *C. bipustulatus* successfully completed their life cycle on the foods offered. However, the biological parameters of *C. bipustulatus* on *A. pistaciae* were inferior to *A. abaii*, and survival of *C. bipustulatus* on *A. pistaciae* was higher. Survival rate, developmental rate and fecundity describe the life history, and differences between stages are used to predict growth rate. The intrinsic rate of increase ( $r$ ) is a practical parameter to compare the potential of insect populations under different situations in pest management. In conclusion, our results demonstrate that the lady beetle, *C. bipustulatus*, is an effective biocontrol agent of *A. pistaciae* and *A. abaii*, and these beetles developed successfully to the adult stage and reproduced. This study aids the development of pest control management, and this type of laboratory study provides better insights into the management of the population dynamics of predators.

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**Haniieh Atrchian** This research is part of a MSc. project of Haniieh Atrchian Atrchian devoted to the analysis effects of

different diets on the development, survival and reproduction of *Chilocorus bipustulatus*.

**Dr. Kamran Mahdian** is studying biological control of arthropod pests, with emphasis on the potential of predatory insects, and non-target effects of pesticides.

**Dr. Shahnaz Shahidi-Noghabi** is involved in insect physiology and pest control with subjects in novel insecticides, integrated pest management (IPM), particularly in the integration of biological control with other control tactics including host plant resistance and transgenic crops.