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Research Article

Laboratory survey on biological and demographic parameters of *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae) fed on two mealybug species

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Abstract: Biological and demographic parameters of the mealybug ladybird, *Cryptolaemus montrouzieri* (Mulsant) were evaluated on citrus mealybug, *Planococcus citri* (Risso) and obscure mealybug, *Pseudococcus viburni* (Signoret) under 24 ± 2 ºC, 80 ± 5% relative humidity and a photoperiod of 16:8 (L: D) h. The mealybugs had a significant effect on developmental time and reproductive and demographic parameters of the ladybird. Life span (egg to adult death) was obtained 220.85 ± 5.78 and 119.44 ± 2.1 days when fed on *Pl. citri* and *Ps. viburni*, respectively. However, mean number of eggs per female (fecundity) and mean percent of egg hatching were significantly higher on *Ps. viburni* than on *Pl. citri*. In addition, values of intrinsic rate of increase (r_m), finite rate of increase (λ) and net reproductive rate (R_0) were significantly higher on *Ps. viburni* than on *Pl. citri*. The values of intrinsic rate of increase were estimated 0.081and 0.094 day^{-1} on *Pl. citri* and *Ps. viburni*, respectively. Results of this study suggested that the obscure mealybug is a more suitable prey than the citrus mealybug as the ladybird displayed shorter developmental time, and higher fecundity and growth rate when fed with obscure mealybug.

Keywords: Life table**,** Mealybug ladybird, Pseudococcidae

Introduction

Mealybugs (Hemiptera: Pseudococcidae) are an important group of plant pests worldwide (Williams, 1985; Ben-Dov, 1994; Miller *et al*., 2002; Miller *et al*., 2005) whose feeding on host tissues may cause leaf yellowing, defoliation, reduced plant growth, and in some cases, plant death (Hill, 1983). The citrus mealybug, *Planococcus citri* (Risso), is a polyphagous pest that attacks a wide range of crops and ornamental plants in tropical and subtropical regions (Hill, 1983; Ben-Dov, 1994; Miller, 2005). The obscure or tea mealybug, *Pseudococcus viburni* (Signoret) has been reported as a vineyard and pome fruit orchard pest s in New Zealand, United States and South Africa (Charles *et al*., 2010; Varela *et al*., 2006; Mudavanhu, 2009).

In Iran, mealybugs are prevalent greenhouse and orchard pests which cause significant damage and yield loss each year (Behdad, 1997). Mafi Pashakolaei (1997) reported the

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activity of five mealybug species on citrus and other host plants in northern Iran, among which *Pl. citri* was determined to be the dominant species. Abbasipour and Taghavi (2007) confirmed the occurrence and distribution of *Ps. viburni* in tea orchards of northern Iran, while in southern provinces of Iran, the spherical mealybug, *Nipaecoccus viridis* (Newstead), has been reported as the dominant mealybug species on host plants including citrus (Ghanbari *et al*., 2012). Despite increased concerns about the prevalence, damage, seasonal outbreak, and economic impact of mealybugs in Iran our information in this regard is very limited.

Recommended practices for controlling mealybugs using insecticides are complicated by the fact that they hide in bark crevices and other inaccessible places and secrete thick layers of protective wax (Hill, 1983; Gill *et al*., 2012). As an alternative, biological control by predators could act as an efficient method of control (Simmonds *et al*., 2000, Muştu *et al*., 2008). The mealybug ladybird, *Cryptolaemus montrouzieri* (Mulsant) is one of the most important biological control agents that preys upon a wide range of mealybug species in many parts of the world (Babu and Azam, 1987; Heidari and Copland, 1992; Simmonds *et al*., 2000). This predator, which has also been reported from northern Iran, has been shown to play an important role in the population suppression of mealybugs in citrus and tea orchards (Mafi Pashakolaei, 1997; Mafi Pashakolaei *et al*., 2011).

Life table study of natural enemies on various prey species provides useful information for understanding their ability to control pests and provides information for improving their mass rearing feasibility (Hassell, 1978; Carey and Vargas, 1985; Carey, 1993). There are various studies on life history, life table, and behavior of *C. montrouzieri*, feeding on citrus mealybug and pink mealybug, *Maconellicoccus hirsutus* Green as prey (*e.g*. Persad and Khan, 2002; Özgökçe *et al*., 2006; Elsherif *et al*., 2010; Ghorbanian *et al*., 2011; Villegas-Mendoza *et al*., 2012), but our knowledge about the biology and demography of *C. montrouzieri* feeding on obscure mealybug is scant. The main objective of this study is to construct the age specific life table and demographic parameters of *C. montrouzieri* and compare their biology on citrus and obscure mealybugs, under laboratory conditions.

Materials and Methods

Laboratory rearing and maintenance of mealybugs and ladybird

Pl. citri and *Ps. viburni* were originally collected from a citrus orchard in Amol (52° 22′ 50″ E and 36° 26′ 27″ N) and a tea orchard in Nashtarood (51° 0′ 37″ E and 36° 45′ 15″ N), Mazandaran, northern Iran, respectively and then reared on squash (*Cucurbita maxima* Duchesne) fruits and potato (*Solanum tuberosum* L.) tuber buds in a climate-controlled room, 26 ± 2 ºC, 80 ± 5% RH, and a photoperiod of 16L: 8D h.

The *C. montrouzieri* colony was originally provided by Tea Research Centre, Nashtarood, Mazandaran, Iran, and reared on infested squash fruits and potato tuber buds under the above-mentioned conditions. Prior to initiating feeding experiments on each prey (*Pl. citri* or *Ps. viburni*), the ladybird was reared for three generations on the corresponding prey to experience the same nutrient and physiological conditions.

Biological and demographic parameters

All experiments were conducted under similar conditions in an incubator at 24 ± 2°C, 80 ± 5% RH, and a photoperiod of L16:D8 h. Life table experiments were conducted using Carey's method (Carey, 1993). In order to obtain an egg cohort, 8 pairs of ladybirds were placed into two separate Petri dishes (10cm diameter) containing different developmental stages of mealybugs. One hundred eggs of *C. montrouzieri*, laid within a 24h period, were randomly chosen and transferred individually to a new Petri dishand provided with an *ad libitum* mixed diet of different developmental stages of mealybugs. Developmental and survival data of the ladybirds were recorded daily. After adult ladybirds emerged, females were paired with males of the same age and each pair was individually transferred to a separate Petri dish containing different developmental stages of the prey (37 and 41 pairs of adult ladybird in case of *Pl. citri* and *Ps. viburni*, respectively). Survival and fecundity data of the paired adults were recorded daily until the death of female individuals.

Statistical Analyses

The data obtained for durations of developmental stages of ladybird on two prey species were analyzed using Student's *t*-test (Proc *t*-test, SAS Institute, 2007). Demographic parameters were compared using the Jackknife method (Maia *et al*., 2000). The age-specific survival (l_x), fecundity (m_x), and life expectancy (e_x) and demographic parameters including intrinsic rate of increase (r_m), finite rate of increase (λ), net reproduction rate (R_0), doubling time (DT) and the mean generation time (T) were calculated according to Carey (1993, 2001). The intrinsic rate of increase was estimated by solving iteratively Euler's equation: $\sum_{\alpha=x}^{\beta} e^{-rx} l_x m_x = 1$ (Carey, 1993). The mean generation time was defined as the length of time required for a given population to complete one generation and calculated using $T = \ln R_0 / r_m$ equation (Carey, 1993).

Results

Mealybug species had a significant effect on developmental time of ladybird stages and instars including egg, larvae I, II and III, total larval, prepupa and pupae as well as on preoviposition, oviposition, and postoviposition periods (Table 1). In contrast, the duration of larval instar IV of the predator was not significantly different while feeding on the two mealybug species (t = 1.46, DF = 1,154, $P > 0.05$).

Mean developmental time for different stages or periods of ladybird fed on two mealybug species are illustrated in Table 2. Developmental times of most stages as well as periods of pre-oviposition, oviposition, and post-oviposition were significantly longer on *Pl. citri* than on *Ps. viburni*. Total life span (egg to adult death) was obtained 220.85 ± 5.78 and 119.44 ± 2.1 days on *Pl. citri* and *Ps. viburni*, respectively. Longevity of female ladybirds fed with *Pl. citri* (183.53 ± 5.78 days) was significantly higher than those that were fed with *Ps. viburni* (78.50 ± 2.1 days). Ovipositional period on *Pl. citri* (73.73 ± 5.72 days) was also longer than that on *Ps. viburni* (41.32 ± 0.23 days).

Table 1 The results of *t*-student analysis for durations of developmental stages/periods of *Cryptolaemus montrouzieri* fed on two mealybug species.

Developmental stage/period	*t*	$df_{t, e}$[1]	P_{value}
Egg incubation	159.96	1, 162	< 0.0001
Larva I	175.60	1, 156	< 0.0001
Larva II	56.36	1, 156	< 0.0001
Larva III	32.97	1, 155	< 0.0001
Larva IV	1.46	1, 154	< 0.2295
Total larval period	90.06	1, 156	< 0.0001
Prepupa	56.31	1, 154	< 0.0001
Pupa	703.75	1, 153	< 0.0001
Pre-ovipositional period	46.51	1, 152	< 0.0001
Ovipositional period	35.61	1, 76	< 0.0001
Post- ovipositional period	141.70	1, 73	< 0.0001

[1] $df_{t, e}$ indicates values of degree of freedom for treatment and error, respectively.

Means of reproductive parameters of *C. montrouzieri* fed on two mealybug species are presented in Table 3. Despite longer oviposition period, all reproductive parameters including mean number of eggs per female, mean number of hatched eggs, mean percent of egg hatching, mean number of male offspring, and mean number of female offspring were higher on *Ps. viburni* than on *Pl. citri*. Mean number of eggs laid during whole life time of a female ladybird was estimated to be 278.62 ± 0.21 and 385.33 ± 0.84 when fed with *Pl. citri* and *Ps. viburni*, respectively. The oviposition rate of *C. montrouzieri* fed on the two mealybug species are shown in Fig. 1. The majority of eggs on both prey species were laid on early days of oviposition period; the highest number of eggs was laid 3 and 7 days after oviposition began when ladybirds were fed with *Pl. citri* and *Ps. viburni*, respectively.

All demographic parameters of *C. montrouzieri* including intrinsic rate of increase (r_m), finite rate of increase (λ), net reproduction rate (R_0), mean generation time (T), and doubling time (DT) were significantly affected by mealybug species (Table 4). Values of r_m, λ and R_0 were statistically higher on *Ps. viburni* than on *Pl. citri.* Values of intrinsic rate of increase as the most important demographic parameter were estimated 0.081and 0.094 day^{-1} on *Pl. citri* and *Ps. viburni*, respectively. In contrast, values of T and DT were significantly higher on *Pl. citri* than on *Ps. viburni* and were estimated 57.11, 8.52 and 54.57, 7.37 days, respectively.

Age-specific survival (l_x) and life expectancy (e_x) rates of *C. montrouzieri* fed on two mealybug species are displayed in Fig. 2. According to the age specific life table constructed based on a cohort of 100 newly laid eggs, survival rate of egg, larval, and pupal stages of ladybird on *Pl. citri* and *Ps. viburni* were estimated 76.0, 96.05, 98.6 percent and 87.0, 95.4, 100 percent, respectively. In other words, 29.35 and 17.60 percent of the ladybird mortality (fed on *Pl. citri* than *Ps. viburni*, respectively) occurred during immature stages.

The maximum life expectancy (ex) of ladybirds, fed on both *Pl. citri* and *Ps. viburni*, was obtained by first instar larvae (196.13 and 100.52 days, respectively). Life expectancy of newly emerged fourth instar larvae fed on *Pl. citri* and *Ps. viburni* were estimated 187.13 and 93.63 days, respectively, while the values of life expectancy for newly emerged female ladybirds fed on these mealybugs were calculated 170.48 and 68.7 days, respectively.

Table 2 Developmental time of different stages of *Cryptolaemus montrouzieri* fed on two mealybug species, *Planococcus citri* and *Pseudococcus viburni*.

Stage/Period	Developmental time (days) (Mean ± SE)[1]	
	Planococcus citri	*Pseudococcus viburni*
Egg	6.66 ± 0.05**a** (*n* = 79)	5.58 ± 0.07**b** (*n* = 85)
Larva I	5.15 ± 0.08**a** (*n* = 73)	3.35 ± 0.10**b** (*n* = 85)
Larva II	3.33 ± 0.07**a** (*n* = 73)	2.62 ± 0.06**b** (*n* = 85)
Larva III	2.74 ± 0.06**b** (n = 73)	3.25 ± 0.06**a** (*n* = 84)
Larva IV	4.27 ± 0.07**a** (*n* = 73)	4.43 ± 0.10**a** (*n* = 83)
Total larval period	15.50 ± 0.06**a** (73)	13.64 ± 0.10**b** (82)
Prepupa	3.80 ± 0.04**a** (*n* = 73)	3.01 ± 0.09**b** (*n* = 83)
Pupa	11.34 ± 0.10**b** (*n* = 73)	18.70 ± 0.20**a** (*n* = 82)
Pre-ovipositional period	7.66 ± 0.10**a** (*n* = 72)	6.33 ± 0.16**b** (*n* = 82)
Ovipositional period	73.73 ± 5.72**a** (*n* = 37)	41.32 ± 0.23**b** (*n* = 41)
Post-ovipositional period	102.14 ± 5.78**a** (*n* = 36)	30.85 ± 2.14**b** (*n* = 39)
Adult longevity	183.53 ± 5.78**a** (*n* = 37)	78.50 ± 2.14**b** (*n* = 41)
Life cycle period	220.85 ± 5.78**a** (*n* = 37)	119.44 ± 2.14**b** (*n* = 41)

[1] Means followed by different letters in each row are significantly different (*t*-test, $p < 0.05$).

Table 3 Reproductive parameters of *Cryptolaemus montrouzieri* fed on two mealybug species, *Planococcus citri* and *Pseudococcus viburni*.

Parameter	Reproductive parameters (Mean ± SE)[1]	
	Planococcus citri	*Pseudococcus viburni*
Number of eggs per female	278.62 ± 0.21**b** (*n* = 37)	385.33 ± 0.84**a** (*n* = 41)
Number of hatched eggs	220.11 ± 0.16**b** (*n* = 37)	326.14 ± 0.71**a** (*n* = 41)
Percent of egg hatching	79.00 ± 0.02**b** (*n* = 37)	85.00 ± 0.01**a** (*n* = 41)
Number of male offspring	102.48 ± 0.08**b** (*n* = 37)	147.15 ± 0.32**a** (*n* = 41)
Number of female offspring	117.53 ± 0.09**b** (*n* = 37)	178.98 ± 0.40**a** (*n* = 41)

[1] Means followed by different letters in each row are significantly different (*t*-test, $p < 0.05$).

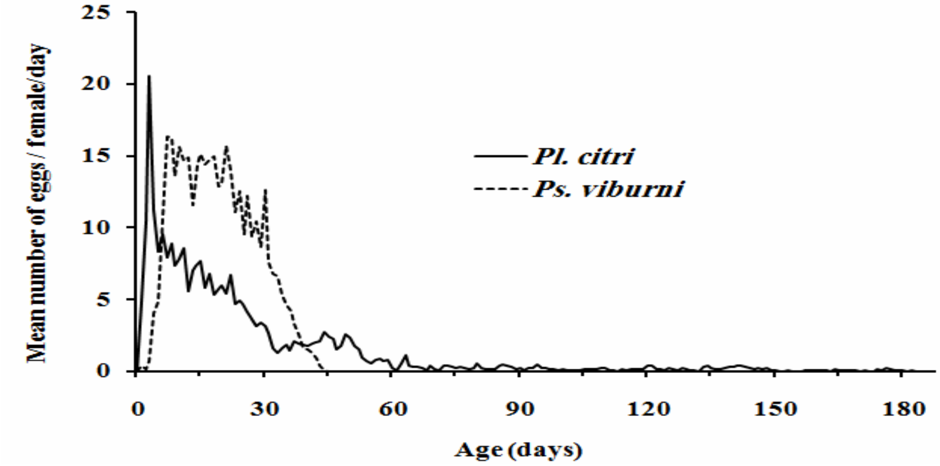

Figure 1 Age-specific oviposition rate of *Cryptolaemus montrouzieri* fed on two mealybug species, *Planococcus citri* and *Pseudococcus viburni*.

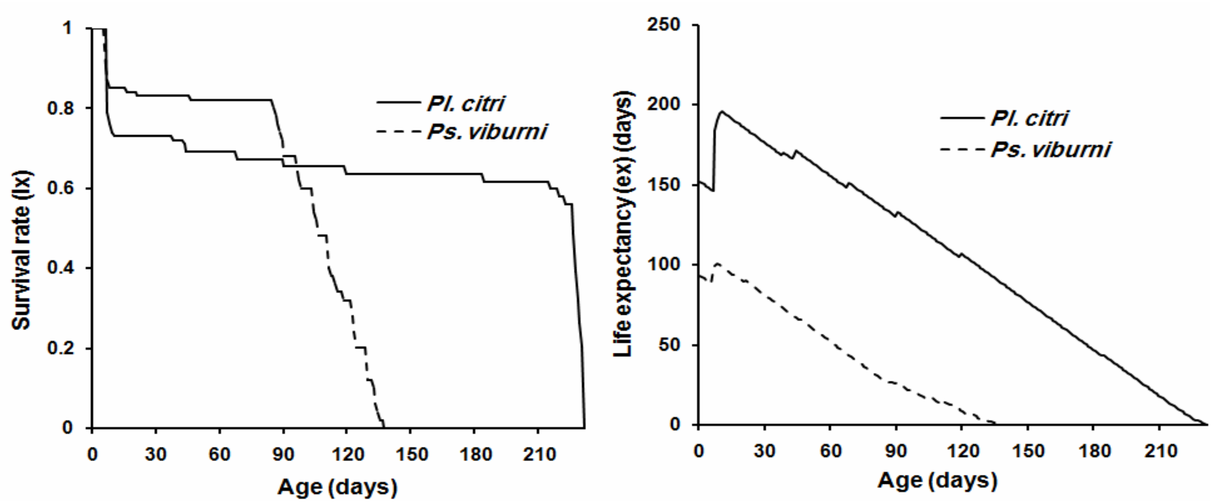

Figure 2 Age-specific survival (l_x), and life expectancy (e_x) rates of *Cryptolaemus montrouzieri* fed on two mealybug species, *Planococcus citri* and *Pseudococcus viburni*.

Table 4 Population growth parameters of *Cryptolaemus montrouzieri* fed on two mealybug species, *Planococcus citri* and *Pseudococcus viburni*.

Parameter	Population growth parameters (Mean ± SE)[1]	
	Planococcus citri	*Pseudococcus viburni*
Intrinsic rate of increase (r_m) (day^{-1})	0.081 ± 0.001**b** (n = 37)	0.094 ± 0.001**a** (n = 41)
Finite rate of increase (λ)	1.085 ± 0.001**b** (n = 37)	1.099 ± 0.001**a** (n = 41)
Net reproduction rate (R_0) (female /female/generation)	103.86 ± 5.73**b** (n = 37)	169.27 ± 5.98**a** (n = 41)
Mean generation time (T) (days)	57.11 ± 0.75**a** (n = 37)	54.57 ± 0.14**b** (n = 41)
Doubling time (DT) (days)	8.52 ± 0.12**a** (n = 37)	7.37 ± 0.05**b** (n = 41)

[1] Means followed by different letters in each row are significantly different (*t*-test, $p < 0.05$).

Discussion

Because of high risk of using conventional insecticides, mass rearing and releasing of *C. montrouzieri* has been recently considered as an effective and safe biological method for controlling mealybugs populations in tea and citrus orchards of northern and southern Iran (Mafi Pashakolaei, 1997; Mafi Pashakolaei *et al*., 2011; Mossadegh *et al*., 2008). Our findings showed that *C. montrouzieri* developed and reproduced well when fed on both citrus and obscure mealybug, even though a shorter life cycle and higher fecundity and intrinsic rate of increase (r_m) was observed when they fed on *Ps. viburni* compared to *Pl. citri*, Therefore obscure mealybug is suggested as a more suitable prey than citrus mealybug.

Effect of prey quality on biological and demographic parameters of ladybirds particularly aphidophagous species has been reported in many studies (*e.g.* Omkar and James, 2004; Tsaganou *et al*., 2004; Cabral *et al*., 2006). However, little information is available on the development and demography of *C. montrouzieri*, especially on obscure or tea mealybug, *Ps. viburni*. *C. montrouzieri* has been often known as a specialist predator on mealybugs and a lower developmental or reproductive rate has been reported when this predator fed on other non-specialist preys (Hodek and Honek, 2009).

Developmental and oviposition periods of *C. montrouzieri* obtained in our study are close to those values that reported for this predator feeding on *Pl. citri* (Baskaran *et al*., 2002; Özgökçe *et al*., 2006) and other mealybug species (Mani and Krishnamoorthy, 1997; Harmeet *et al*., 2010), but notably higher than the values that reported for other non-specialist preys. Larval developmental time observed in our study (13.64 days on *Ps. viburni*) was very close to 13.92 days that was reported for this ladybird when fed on *M. hirsutus* (Green) (Hemiptera: Pseudococcidae) (Parabal and Balasubramanian, 2000), but was notably lower than values that were reported for non-specialist preys such as *Aleurodicus dispersus* R. (Hemiptera: Aleurodidae) (Mani and Krishnamoorthy, 1999), *Aphis gossypii* (Glover) (Hemiptera: Aphididae) and *Dactylopius tomentasus* (Lamarck) (Hemiptera: Dactylopiidae) (Parabal and Balasubramanian, 2000; Baskaran *et al*., 2002). Ghorbanian *et al*., (2011) reported lower values for incubation, larval, prepupal and pupal periods of *C. montrouzieri* fed on *Pl. citri* compared to our study. We attribute these discrepancies to the higher temperature used in their study (27 ºC *vs*. 24 ºC), such temperature-dependent development and demography have been reported to many of other ladybird species (*e.g.* Kontodimas *et al*., 2004; Atlihan and Chi, 2008; Eliopoulos *et al*., 2010; Castro *et al*., 2011).

There are a few studies reporting the demographic parameters of *C. montrouzieri* feeding on different mealybugs species (Persad and Khan, 2002; Özgökçe *et al*., 2006; Elsherif *et al*., 2010; Ghorbanian *et al*., 2011), but there are apparently no reports published regarding the demography and life table of this predator feeding on *Ps. viburni*. Values of intrinsic rate of increase (r_m) in our study (0.081and 0.094 day^{-1} on *Pl. citri* and *Ps. viburni*, respectively) were in the range of 0.077 day^{-1} (Elsherif *et al*., 2010) to 0.135 day^{-1} (Persad and Khan, 2002) that have been reported on *Phenacoccus medeirensis* Green and *M. hirsutus*, respectively.

In conclusion, our findings can be helpful from both a biological control standpoint and a mass-rearing feasibility standpoint. We showed the high potential of *C. montrouzieri* to feed, develop, and reproduce successfully on both *Pl. citri* and *Ps. viburni* that could eventually suppress populations of these mealybugs in citrus and tea orchards of southern and northern Iran. We also showed that the ladybirds tend to feed more on *Ps. viburni* than on *Pl. citri*, which may have consequences on the efforts to mass rear this predator, especially in commercial insectaries.

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ارزیابی پارامترهای زیستی و جمعیتی کفشدوزک *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) با تغذیه از دو گونه شپشک آردآلود در شرایط آزمایشگاهی

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چکیده: پارامترهای زیستی و جمعیتی کفشدوزک *Cryptolaemus montrouzieri* (Mulsant) با تغذیه از شپشک آردآلود مرکبات، *Planococcus citri* (Risso) و شپشک آردآلود چای، *Pseudococcus viburni* (Signoret) در شرایط آزمایشگاهی (دمای ۲ ± ۲۴ درجه‌ی سلسیوس، رطوبت نسبی ۵ ± ۸۰ درصد و دوره‌ی نوری ۱۶ ساعت روشنایی به ۸ ساعت تاریکی) اندازه‌گیری شدند. گونه‌ی شپشک روی طول مدت نشوونما و نیز پارامترهای تولیدمثلی و جمعیتی کفشدوزک تأثیر معنی‌داری گذاشت. طول چرخه‌ی زندگی (تخم تا مرگ حشره‌ی کامل) کفشدوزک روی شپشک آردآلود مرکبات و شپشک آردآلود چای به‌ترتیب ۵/۷۸ ± ۲۲۰/۸۵ و ۲/۱ ± ۱۱۹/۴۴ روز به‌دست آمد. هم‌چنین، میانگین تخم‌گذاری به‌ازای هر کفشدوزک ماده (زادآوری) و میانگین درصد تفریخ تخم‌ها روی شپشک آردآلود چای نسبت به شپشک آردآلود مرکبات بیش‌تر بود. به‌علاوه، مقادیر نرخ ذاتی افزایش جمعیت (r_m)، نرخ متناهی رشد (λ) و نرخ خالص تولیدمثل (R_0) روی شپشک آردآلود چای به‌طور معنی‌داری از شپشک آردآلود مرکبات بیش‌تر بود. مقادیر نرخ ذاتی افزایش جمعیت روی شپشک آردآلود مرکبات و شپشک آردآلود چای به‌ترتیب ۰/۰۸۱ و ۰/۰۹۴ (بر روز) محاسبه گردید. نتایج این پژوهش نشان داد که شپشک آردآلود چای در مقایسه با شپشک آردآلود مرکبات برای این کفشدوزک شکار مناسب‌تری به‌شمار می‌رود زیرا طول دوره‌ی نشوونمای کفشدوزک روی آن کوتاه‌تر و مقادیر زادآوری و نرخ ذاتی افزایش جمعیت کفشدوزک روی آن بزرگ‌تر بود.

واژگان کلیدی: جدول زندگی، کفشدوزک کریپتولموس، Pseudococcidae