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Research Article

Effect of *Citrullus colocynthis* (Cucurbitaceae) agglutinin on the life table parameters of *Apomyelois ceratoniae* (Lepidoptera: Pyralidae)

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Abstract: A Castanea crenata agglutinin (abbreviated as CCA) was extracted and purified from bitter apple, *Citrullus colocynthis* L., to determine its effects on the demographic parameters of *Apomyelois ceratoniae* Zeller. Two groups of first larval instars were reared on an artificial diet containing 2% (w/w) of CCA and control diets until emergence of adults. Two Sex MS-chart life table software was used to analyze data and calculate life table parameters. Developmental times of larvae in control and CCA diets showed statistical differences for male, female except for third and fourth larval instars. It was found that *A. ceratoniae* larvae fed on control had a survival of 18 days but individuals fed on CCA diet survived for 12 days. The highest fecundity values of individuals fed on control and CCA diets were obtained at the age of 30 and 27 days, respectively. Adult longevity, pre-oviposition period, oviposition period and mean fecundity of *A. ceratoniae* fed on control were higher than those of individuals fed on CCA diet. The probabilities of a newborn egg surviving to age 28 days were 0.42 and 0.3 for control and CCA, respectively. Each female started egg laying on day 22 for control and 25 for CCA. Life expectancies of a newly laid egg were 29 days for control and 26 days for CCA treatment. Life table parameters except for mean generation time showed statistical differences between control and CCA treatments. These results demonstrated the negative effects of CCA on life table parameters of *A. ceratoniae* that might lead to a promising and alternative way to suppress population increase and damage caused by *A. ceratoniae*.

Keywords: *Apomyelois ceratoniae*, *Citrullus colocynthis*, Lectin, Life table

Introduction

Apomyelois ceratoniae Zeller (Lepidoptera: Pyralidae), known as carob moth, is the main destructive pest of pomegranate and pistachio in Iran, although it is considered as a cosmopolitan pest on carobs, almonds and dates (Gothilf, 1984). In California, *A. ceratoniae* is the most economically damaging pest of date industry (Baker *et al*., 1991). Adult females lay their eggs on crown of pomegranate or on any and all parts of other agricultural products. After hatching, larvae penetrate into inner parts of fruits and feed on tissues among pomegranate grains. Apart from

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larval feeding on fruits, fungal pathogens enter the fruits and destroy inner tissues making them useless. Since, larvae are inside the fruit, no chemical spraying could be effective against the pest.

Lectins are a group of carbohydrate-binding proteins in many organisms that bind reversibly to mono or oligosaccharides on the surface of cells. These proteins have a crucial role in defense of organisms against pathogens and herbivores (Peumans and van Damme, 1995). Different studies have shown reduced performance of insects after adding lectins to their diets that may confirm lectins as entomotoxic proteins (Janzen *et al*., 1976; Shukle and Murdock, 1983; Powell *et al*., 1993; Rahbé *et al*., 1995; Sauvion *et al*., 2004; Michiels *et al*., 2010; Shahidi-Noghabi *et al*., 2010). For example, GNA (*Gallanthus nivalis* agglutinin) affected development and fecundity of the peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Sauvion *et al*., 1996), the pea seed lectin reduced growth rate of pollen beetle larvae (Melander *et al.,* 2003) and elderberry lectin (SNA-I) exerted toxic effects on the larval growth and development of the beet armyworm, *Spodoptera exigua* (Hübner) (Shahidi-Noghabi *et al*., 2009).

Demographic studies are used to determine biological characteristics and reproductive capacity of an organism (Carey, 2001). Life table analyses are widely used in ecological studies to expand knowledge on age specific mortality and reproductive rates of insects (Carey, 2001). Apart from basic roles of life table studies, they could be useful to find suitability of a host or effect of xenobiotics (e.g., pesticides) on insects. It is also mandatory to determine growth, stage structure and fecundity of a pest in an environment to better understand its potential damages and population fluctuations. Life table analyses can provide comprehensive descriptions of the development, survival, and fecundity of a population (Chi and Liu, 1985). Chi and Liu (1985) developed an age-stage, two-sex life table theory to consider both sexes and variable developmental rates among individuals. This method is used in predation and parasitism interactions as well as effects of xenobiotics (Chi and Su, 2006). Bitter apple, *Citrullus colocynthis* L., is a medicinal plant of Cucurbitaceae family, containing bitter glycosides that are used as medicine for gut and liver disorders. In addition to anti-virus and anti-cancer properties, crude extract of fruits is effective to decrease blood sugar (Tavakkol-Afshari *et al*., 2005). An agglutinin (abbreviated as CCA) was extracted from the bitter apple, *Citrullus colocynthis* L., and purified to determine its effects on the demographic parameters of *A. ceratoniae*.

Materials and Methods

Insect rearing

Infested fruits of pomegranate were collected from pomegranate orchards in Yazd region, Iran. Larvae of *A. ceratoniae* were separated and fed on artificial diet (Lima *et al*., 2001) containing wheat bran (100 g), yeast (3 g), sugar (10 g), glycerin (40 ml) and water (40 ml) for at least three generations to have a homogeneous stock population, kept in a growth chamber at 27 ± 1 °C, a photoperiod of 16:8 h (L: D) and 70 ± 5% relative humidity (RH).

Preparation of Sepharose4B-Galactose column

To prepare the column, 20 ml of Sepharose 4B was suspended in 40 ml of 0.5 M Na_2CO_3 (pH 11.0). Two ml of divinylsulphone were added to the suspension, and the mixture was incubated for 70 min at room temperature with gentle shaking. After activation, 500 mg galactose in 50 ml of 0.5 M Na_2CO_3 (pH 11.0) were added and the suspension was re-incubated at room temperature for 12 h with gentle shaking. The sorbent was washed with water; the unbound arm was blocked with b-mercaptoethanol-containing buffer, and then packed into the column. The sorbent was equilibrated with Tris-HCl 0.1 M and it was used for the affinity purification of CCA (Bulgakova *et al*., 2004).

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Purification of CCA

Seeds of *C. colocynthis* were grounded to fine powder using a mill device. The dry powder was incubated in phosphate buffer (0.1 M pH 7.1) for approximately 20 h at 4 °C. The mixture was then centrifuged at 5000 rpm for 20 min and remaining debris removed by passing the supernatant through filter paper (Whatmann No. 4) (Hamshou *et al*., 2010). Supernatant was precipitated by 0-60% concentrations of ammonium sulfate and centrifuged at 5000 rpm for 20 min. Debris was eluted in Tris-HCl buffer (0.1 M, pH 7) and dialyzed in the same buffer overnight (de Oliveira *et al*., 2011). Affinity chromatography was performed on a Sepharose 4B-galactose column equilibrated with Tris-HCl buffer (0.1 M, pH 7). After loading the extract, the affinity column was washed with buffer. Then, the bound lectin was eluted with 20 mM 1,3-diaminopropane (DAP) (Hamshou *et al*., 2010). Fractions showing the highest protein content were pooled and used for forthcoming steps. The fractions of lectin obtained after the first affinity chromatography were loaded on an anion exchange chromatography column of DEAE-Cellulose fast flow, equilibrated with DAP (Hamshou *et al*., 2010). Then, the lectin was eluted using Tris–HCl (0.1 M, pH 7.0) containing 0.5 M NaCl. Finally, fractions were dialyzed against water and lyophilized and the purity of the lectin was analyzed by SDS-PAGE.

Life table study

For life table study, two artificial diets were prepared and 2% of CCA (W/W) were added to one of them. Sixty eggs (24 h old) were put on each diet in containers (7 × 5 × 3 cm) separately. Every day, eggs and larvae were checked and numbers of larvae were recorded. Sixty larvae were placed on each diet. After pupation, pupae were transferred to different containers until adult emergence. Larval development was recorded every 24 h to the adult stage. Survival, mortality and number of laid eggs by females were recorded daily. The emerged adults were paired and kept in covered plastic containers (20 × 10 × 5 cm). Survival and fecundity were recorded for each individual until death of all adults.

Data analysis

Life table data were analyzed using the age-stage, two-sex life table approach (Chi and Liu 1985, Chi 1988). The computer program, TWOSEX-MSChart (Chi, 2008), was used in this paper as available at http://140.120.197.173/Ecology/prod02.htm (National Chung Hsing University, Taichung, Taiwan). The age-stage specific survival rate (s_{xj}) (where x = age and j = stage), the age-stage specific fecundity (f_{xj}), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters (r_m), the intrinsic rate of increase; λ, the finite rate of increase, $\lambda = e^r$; R_0, the net reproductive rate; T, the mean generation time) were calculated accordingly. The age-specific survival rate included both male and female, and was calculated according to Chi and Liu (1985) as

$$l_x = \sum_{j=1}^{\beta} S_{xj}$$

$$m_x = \frac{\sum_{j=1}^{\beta} S_{xj} f_{xj}}{\sum_{j=1}^{\beta} S_{xj}}$$

where β is the number of stages.

The intrinsic rate of increase was calculated by using the iterative bisection method from

$$\sum_{x=0}^{\omega} l_x m_e e^{-r(x+1)} = 1$$

with age indexed from 0 to 50 (Goodman 1982). The mean generation time is the time length that a population needs to increase to R_0-times of its size as the stable age distribution and the stable increase rate are

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reached, i.e., $e^{rT} = R_0$ or $\lambda^{T} = R_0$. Thus, it was calculated as $T = (\ln R_0)/r$. The gross reproductive rate (*GRR*) was calculated as $GRR = \Sigma m_x$

$$T = \frac{\ln Ro}{r}$$

$$R_o = \sum_{x=0}^{\omega} l_x m_x$$

Results

In the current study, feeding of larvae on artificial diet containing 2% (w/w) of CCA revealed statistical differences for several demographic parameters of *A. ceratoniae* in comparison with control since majority of the results demonstrated negative effects of CCA. Table 1 shows mean developmental times (days) of control and *A. ceratoniae* fed on artificial diet containing 2% of CCA. Developmental times of larvae in control and treated *A. ceratoniae* showed statistical differences for male, female except for third and fourth larval instars (Table 1). Graphs regarding age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity of *A. ceratoniae* fed on control and CCA containing diets are shown in Figure 1. The age-specific survival rate (l_x) is the probability that a new egg will survive to age x and it is calculated by pooling all individuals of both sexes, meanwhile the m_x curve is plotted on the age from birth. The trend of adult survival rates were different on individuals fed on control and CCA diets (Fig. 1). In fact, it was found that larvae fed on control had a survival of 18 days but individuals fed on CCA diet had survived for 12 days (Fig. 1).The trend of age-specific fecundity (m_x) showed that reproduction began at the age of 22 days on control and 25 on CCA diets (Fig. 1). The highest fecundities of individuals fed on control and CCA diets were obtained at the age of 30 and 27 days, respectively (Fig. 1).

Table 2 shows adult longevity, pre-oviposition period, oviposition period and mean fecundity of *A. ceratoniae* fed on control and CCA diets showing higher amounts of these values in control reared individuals except for pre-oviposition period. The age-stage specific survival rates (S_{xj}) of *A. ceratoniae* fed on control and CCA containing diets are shown in Figure 2. The parameter is the probability that a newborn egg will survive to age x and stage j. Stage differentiation can be observed due to variable developmental rates among individuals on both diets. Because S_{xj} shows the variation among individuals in developmental rates, it can be detected as the stage overlapping in the development of a cohort and the survival curves of males and females. For example, the surviving probabilities of a newborn egg to age 28 of male adulthood were 0.42 and 0.3 for control and CCA (Fig. 2). In case of females, these values were found to be 0.3 and 0.25 (Fig. 2). Each female started egg laying on day 22 for control and its peak was observed on day 26, while in *A. ceratoniae* fed on CCA these times were on days 25 and 28 (Fig. 3). The life expectancy (e_{xj}) of each age-stage group fed on control and CCA diets are shown in Figure 4. The e_{xj} estimates the time individuals of age x and stage j are expected to live. For example, the life expectancies of a newly laid egg were 29 days for control and 26 days for CCA while the life expectancy decreased with age (Fig. 4). The reproductive value of control was higher than CCA treatment and it increased along with onset of reproduction (Fig. 5). Life table parameters including, net reproduction rate (R_0), intrinsic rate of population increase (r_m), finite rate of increase (λ) and gross reproduction rate (*GRR*) in control treatment were higher than those fed on CCA containing diet (Table 3). Mean generation time (T) of individuals fed on control and CCA diets had no statistical differences (Table 3).

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Table 1 Mean developmental times (days) of different stages of *Apomyelois ceratoniae* fed on artificial diet (control) and on diet containing 2% CCA.

Stages	Mean developmental time ± SE (days)					
	Male		Female		Total	
	Control	CCA 2%	Control	CCA 2%	Control	CCA 2%
Egg	2.57 ± 0.16	3.12 ± 0.08*	2.19 ± 0.11	3.13 ± 0.10*	2.40 ± 0.09	3.07 ± 0.05*
First instar	3.30 ± 0.10	3.71 ± 0.11*	3.29 ± 0.08	3.52 ± 0.11*	3.33 ± 0.06	3.62 ± 0.06*
Second instar	3.48 ± 0.11*	2.94 ± 0.06	3.23 ± 0.10*	3.04 ± 0.10	3.35 ± 0.07*	2.98 ± 0.05
Third instar	3.39 ± 0.12	3.59 ± 0.12	3.35 ± 0.09	3.70 ± 0.10	3.37 ± 0.06	3.58 ± 0.06
Forth instar	3.30 ± 0.10	3.47 ± 0.12	3.16 ± 0.08	3.83 ± 0.08	3.23 ± 0.06	3.53 ± 0.07
Fifth instar	3.91 ± 0.14	4.06 ± 0.1*	3.94 ± 0.10	4.43 ± 0.12*	3.93 ± 0.08	4.23 ± 0.08*
Pupa	4.96 ± 0.10	5.24 ± 0.11*	5.29 ± 0.08	5.30 ± 0.10	5.15 ± 0.06	5.28 ± 0.07*
Adult	4.43 ± 0.11*	3.76 ± 0.24	5.00 ± 0.00*	4.22 ± 0.14	4.76 ± 0.05*	4.03 ± 0.13

*. Means with asterisks in each row are significantly different at 5% (Independent t-student test).

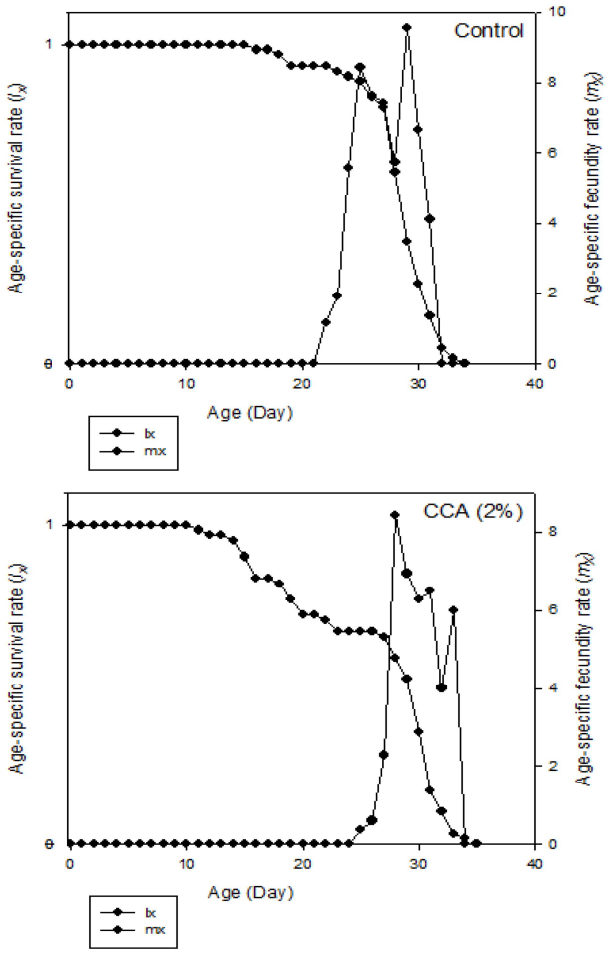

Figure 1 Age-specific survival rate and age-specific fecundity rate of *Apomyelois ceratoniae* fed on artificial diet (control) and on diet contain**.**

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Table 2 Some biological properties of *Apomyelois ceratoniae* fed on control diet and diet treated with CCA 2%.

Biological parameters	Control (Mean ± SE)	CCA 2% (Mean ± SE)
Longevity (total) (days)	28.45 ± 0.46*	26.23 ± 0.87
Adult Longevity (days)	4.76 ± 0.06*	4.03 ± 0.13
Pre-oviposition period (days)	24.45 ± 0.34	26.96 ± 0.26*
Oviposition period (days)	5.00 ± 0.00*	4.22 ± 0.14
Fecundity (eggs / female)	78.61 ± 1.19*	37.87 ± 2.83

*. Means with asterisks in each row are significantly different at 5% (independent t-student test).

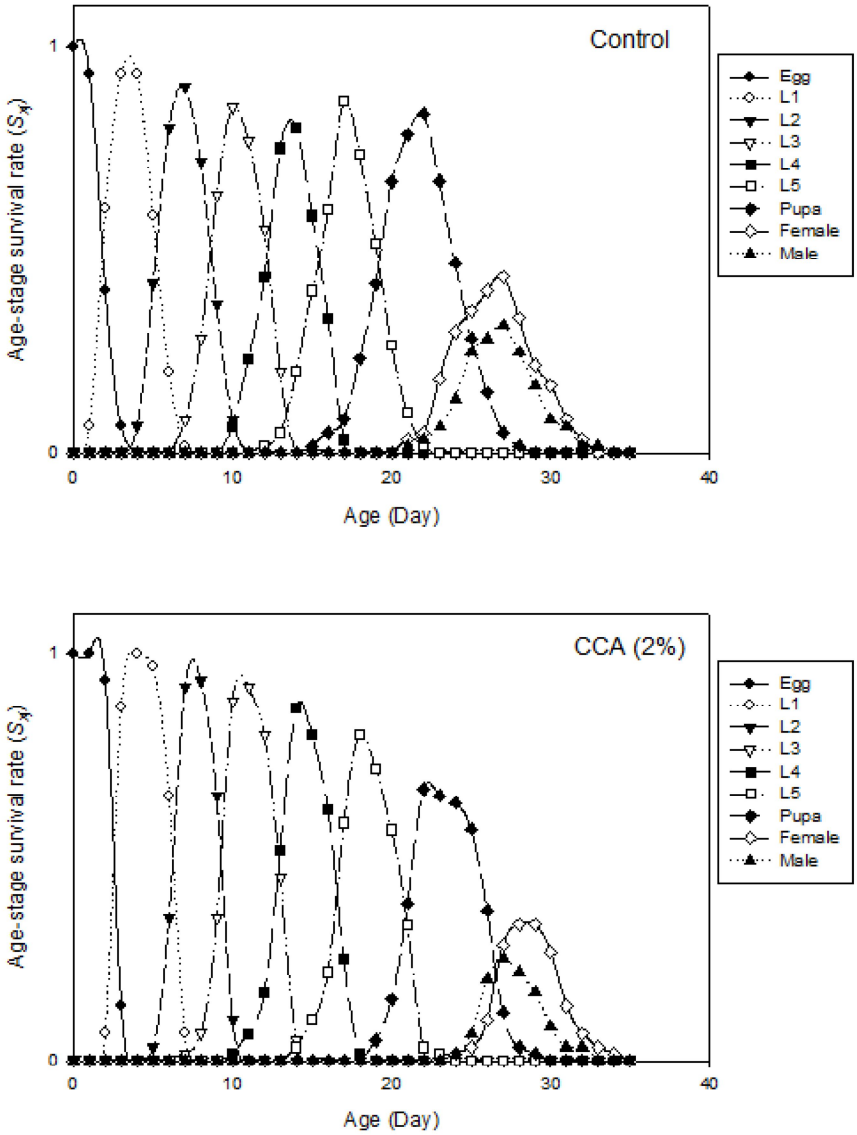

Figure 2 Age-stage survival rate of *Apomyelois ceratoniae* fed on artificial diet (control) and on diet containing 2% CCA.

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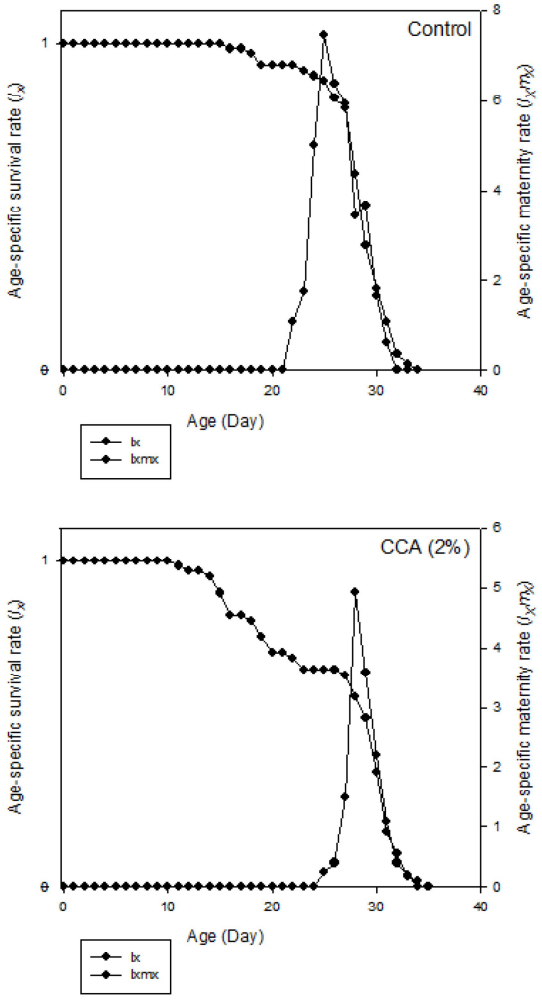

Figure 3 Age-specific maternity ($l_x m_x$) and age-specific survival rate of cohorts of *Apomyelois ceratoniae* fed on artificial diet (control) and on diet containing 2% CCA.

Discussion

Since lectins have anti-feedant effect against insects and bind to insect mid-gut epithelial cells, they could disrupt digestion and absorption of nutrients leading to possible mal-nutrition in treated individuals. The disruption occurs due to cytotoxicity of lectins on the midgut epithelial cells (Michielis *et al.,* 2010). These disruptions might affect insect demographic characteristics that have close relationship with feeding ability. Any limitation in ingested food is mandatory to population regulation of insects. Larval survival and development is reduced when fed on poor-quality diets (hosts) due to nutrient composition and potential inhibitors of digestion process (Michielis *et al*., 2010). Our results are in accordance with majority of studies showing reduced longevity and increased individual mortality when they are fed on lectin containing diet. Trębicki *et al*. (2009) exposed first nymphal stage of *Orosius orientalis* Matsumura (Hemiptera: Cicadellidae) to the PT-07 diet containing 0.1% (w/v) of *Gallanthus nivalis* L. (Amaryllidaceae) agglutinin, wheat germ agglutinin or Chickpea trypsin inhibitor. They found that insects fed on PT-07, as control, were alive for 43 days. Cowpea trypsin inhibitor showed no significant effects on survival or development of *O. orientalis*. However,

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agglutinin extracted from the plants had a significant anti-metabolic effect and as a result on survival and development of the leafhopper so that nymphal survival was reduced to 22 and 15 days as compared to control. Wakefield *et al*. (2006) reared larvae of *Lacanobia oleracea* L. (Lepidoptera: Noctuidae) on tomato leaves expressing *G. nivalis* agglutinin. It was found greater percentage of survival to both the pupal and adult stages than larvae de Oliveira *et al*. (2011) reported that a lectin from *Moringa oleifera* had no effect on survival of Mediterranean flour moth. Martinez *et al*. (2012) compared some ecological parameters of Mediterranean flour moth fed on artificial diet containing labramin (a lectin) with those of the moths in control treatment. They found a significant increase of larval, pupal and adult duration for individuals fed on labramin vesrus control. Meanwhile, mortality of these developmental stages on labramin was 2-to 4-fold higher than control (Martinez *et al*., 2012).

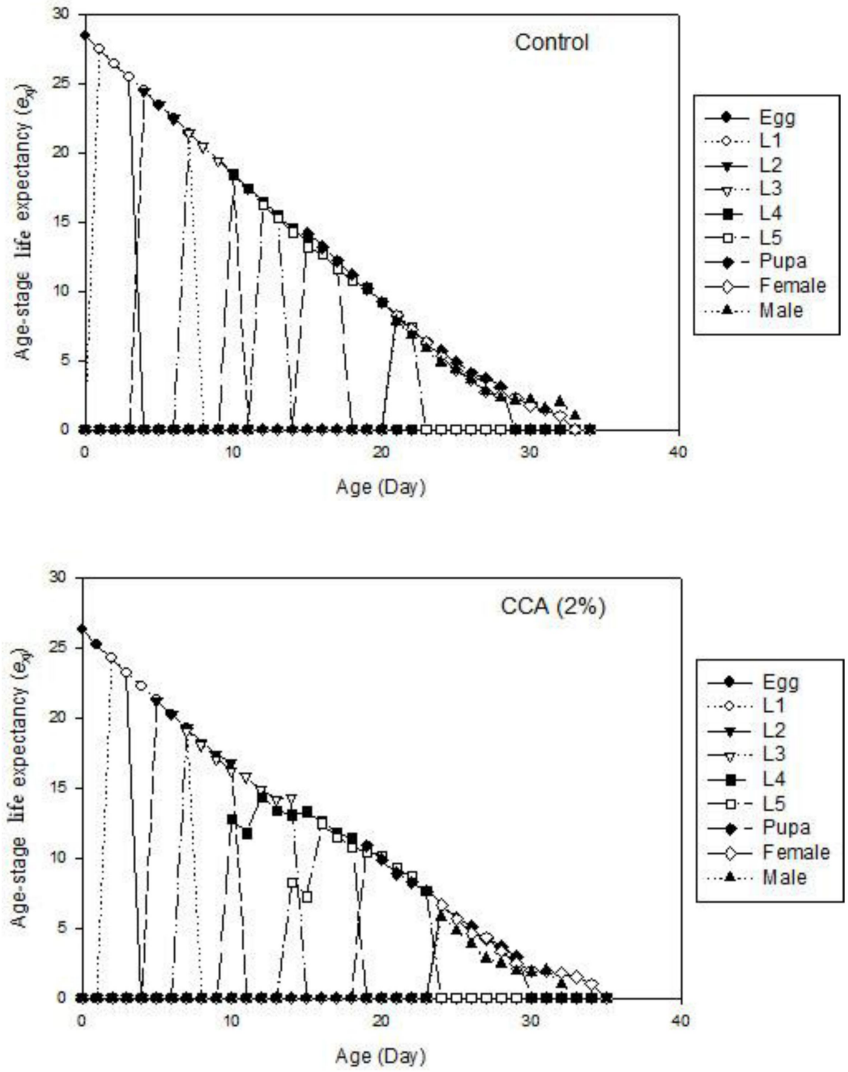

Figure 4 Age-stage life expectancy for each developmental stage of *Apomyelois ceratoniae* fed on artificial diet (control) and on diet containing 2% CCA.

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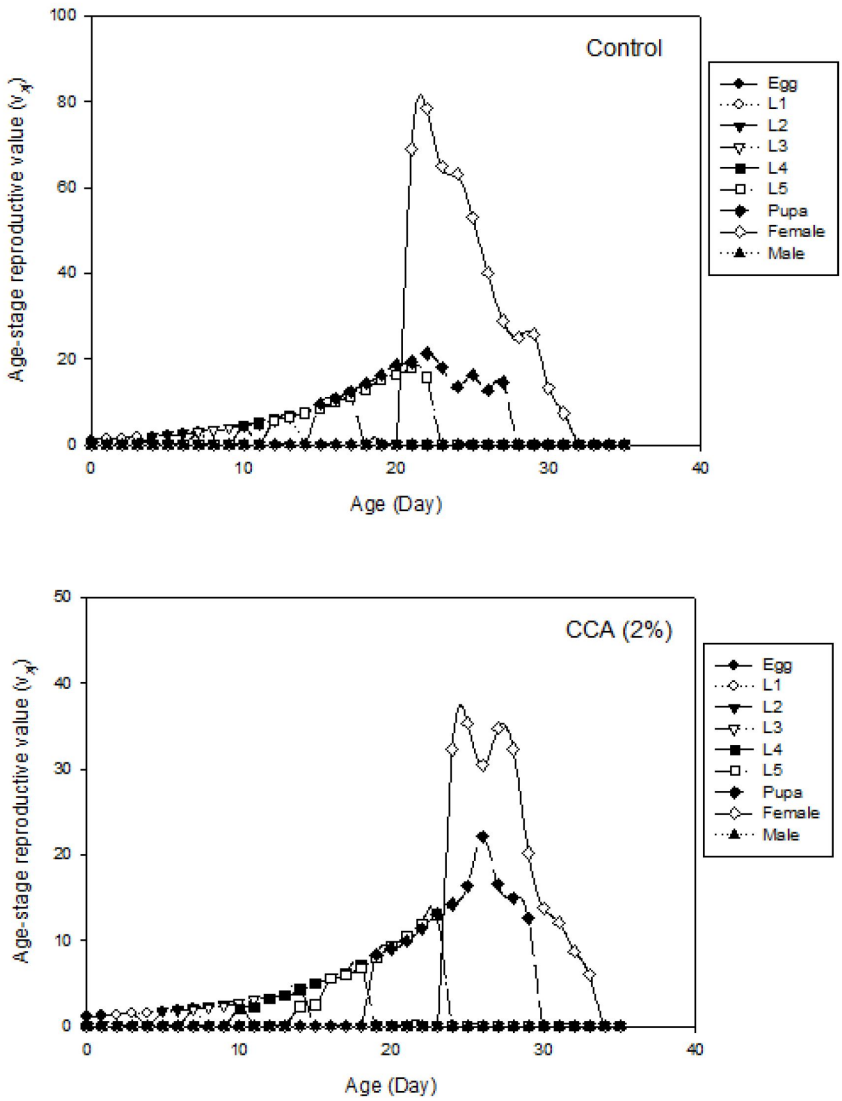

Figure 5 Age-stage specific reproductive value of *Apomyelois ceratoniae* fed on artificial diet (control) and on diet containing 2% CCA.

Table 3 Life table parameters of *Apomyelois ceratoniae* fed on control and treated artificial diet with CCA 2%.

Parameters	Control (Mean ± SE)	CCA 2% (Mean ± SE)
Net reproduction rate (R_0) (Offspring / female)	37.43 ± 4.67*	14.09 ± 2.71
Intrinsic rate of population increase (r) (d^{-1})	0.134 ± 0.004*	0.09 ± 0.006
Mean generation time (T) (days)	26.83 ± 0.350	29.62 ± 0.340
Finite rate of increase (λ) ($days^{-1}$)	1.14 ± 0.005*	1.09 ± 0.006
Gross reproduction rate (GRR) (Offspring / female)	56.94 ± 7.920*	41.44 ± 7.870

*. Means with asterisks each row are significantly different at 5% (independent t-student test).

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Fecundity had positive correlations with suitable feeding of larvae, the resulting pupal and adult masses and forewing development (Wakefield *et al*., 2010). Hence, lower fecundity of individuals fed on CCA containing diet could be attributed to discrepancies in food digestion and absorption caused by lectin. Wakefield *et al*. (2010) showed that longevity and reproductive characteristics of *Eulophus pennicornis* Nees (Hymenoptera: Eulophidae) were affected when it was reared on *L. oleracea* fed on *G. nivalis* lectin. Results revealed lower longevity, lower oviposition of ectoparasitoid reared on host fed on the diet containing lectin (Wakefield *et al*., 2010). The overlaps between different stages during a developmental period could be attributed to the various developmental rates among individuals that are more observable in control reared *A. ceratoniae* versus CCA fed ones. Fisher (1930) described the reproductive value as the contribution of an individual to the future population. In *A. ceratoniae*, the age-stage reproductive value (v_{xj}) means the contribution of an individual in age x and stage j to the future population. The reproductive value of a newborn (v_{01}) is exactly the finite rate of increase (Chi, 2005). Setamou *et al*. (2002) determined the life table parameters of *Eoreuma loftini* Dyar (Lepidoptera: Pyralidae) on Latin-expressing transgenic sugarcane. It was found that feeding on transgenic sugarcane caused lower net reproductive rate and total progeny versus non-transgenic and control diets. The intrinsic rate of increase was intermediate on nontransgenic diet, and was greater on transgenic versus control diet (Setamou *et al*., 2002). Also, it was obtained that generation times and finite rates of increase were similar between diets, and doubling time appeared slightly greater in transgenic relative to nontransgenic and control diets (Setamou *et al*., 2002). Except for mean generation time that showed no statistical differences between individuals fed on control diet and CCA containing diet, other parameters of life table in CCA fed individuals were statistically lower than those for control. Lower value of net reproductive rate (R_0) in individuals fed on CCA might indicate intervention of the lectin in digestive efficiency and mainly reproductive capability of the insect that leads to lower offspring and decrease in population over time. On the other hand, the higher r of individuals fed on control diet is due to faster development of immature stages, higher survivorship and higher fecundity rate. The parameter indicates the susceptibility of the individuals fed on CCA containing diet.

Providing resistant plants is a critical approach in IPM programs. So, identification of resistant host plants is the first step for an IPM program based on resistant varieties (Michiels *et al*., 2010). In case, introducing genes responsible for lectin synthesis to provide resistant (transgenic) varieties would be considered as the final goal of researches of this kind. These varieties act via antibiosis mechanism that adversely affects the life history or biology of the insect pests. Results of the current study clearly demonstrated that extracted lectin from *C. colocynthis* could significantly interrupt biology of *A. ceratoniae* by decreasing the life table parameters. It seems that CCA could be an alternative and promising method for the control of this pest when it was considered to provide resistant host plant. Determination of food quality is useful for management of insect pests since it influences biological performance of pests. Although lectins might be considered as a management tool and their negative effects on pests could increase efficiency of other control approaches. Additional studies are required to determine physiological processes involved in lectin effects on *A. ceratoniae*.

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تأثیر لکتین هندوانه ابوجهل، *Citrullus colocynthis* (Cucurbitaceae) بر پارامترهای جدول زندگی کرم گلوگاه انار، *Apomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae)

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چکیده: اثرات لکتین استخراج و خالص‌سازی از هندوانه ابوجهل بر پارامترهای دموگرافیک کرم کلوگاه انار مورد مطالعه قرار گرفت. دو گروه از لاروهای سن اول روی رژیم غذایی مصنوعی حاوی ۲ درصد لکتین و شاهد تا ظهور افراد بالغ پرورش داده شدند. نرم‌افزار جدول زندگی دوجنسی برای تجزیه و تحلیل داده‌ها و محاسبه پارامترهای جدول زندگی استفاده شد. زمان نمو لاروها در رژیم غذایی شاهد و لکتین تفاوت معنی‌داری را در افراد نر و ماده نشان داد. مشخص شد که لاروهای تغذیه شده روی رژیم غذایی شاهد ۱۸ روز بقا یافتند اما افراد تغذیه شده روی رژیم غذایی حاوی لکتین فقط تا ۱۲ روز زنده ماندند. بیش‌ترین مقدار باروری افراد تغذیه شده روی رژیم غذایی شاهد و لکتین به‌ترتیب در روز ۳۰ و ۲۷ به‌دست آمد. طول عمر افراد بالغ، دوره پیش‌تخم‌گذاری، دوره تخم‌گذاری و میانگین باروری در افراد تغذیه شده روی رژیم غذایی شاهد بیش‌تر از رژیم غذایی لکتین بود. احتمال این‌که یک تخم تازه گذاشته شده تا سن ۲۸ روزگی زنده بماند به‌ترتیب ۰/۴۲ و ۰/۳۰ برای شاهد و لکتین بود. هر فرد ماده تولید تخم را در روز ۲۲ برای شاهد و روز ۲۵ برای لکتین آغاز کرد. امید به زندگی تخم‌های تازه گذاشته شده برای شاهد و لکتین ۲۹ و ۲۶ روز بود. جز متوسط زمان نسل، همه پارامترهای جدول زندگی تفاوت معنی‌داری را بین شاهد و لکتین نشان دادند. این نتایج اثرات منفی لکتین هندوانه ابوجهل را روی پارامترهای جدول زندگی کرم گلوگاه انار نشان داد که ممکن است به راهی امیدوارکننده و جایگزین برای کاهش جمعیت و خسارت این آفت منجر شود.

واژگان کلیدی: *Citrullus colocynthis Apomyelois ceratoniae*، لکتین، جدول زندگی

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