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Effect of some diets on demographic parameters of *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) *in vitro*

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Abstract: The carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), is a cosmopolitan species widely distributed in different habitats worldwide. This moth is a well known pest of many fruits as well as dried fruits in storage. In this study, the biological parameters of the carob moth were studied in a growth chamber under controlled conditions (30±2°C, 65±5% and 16L : 8D). The studied moths were on four diets including pistachio, and pomegranate (both are referred to as the natural diets), and semi-synthetic, and synthetic food. The results demonstrated that survival rate of the carob moth on the two natural diets was higher than the survival rate of the carob moth on the semi-synthetic, and synthetic diets (also referred to as the artificial diets). The highest and lowest amount of life expectancy (e_x) were obtained for moths on the pistachio diet (38.82), and the pomegranate diet (29.32), respectively. The highest age-stage specific reproductive value (v_{xj}) was observed for moths on the pistachio diet (181.1). The intrinsic rate of increase (r) and finite rate of increase (λ) were calculated to be highest when the pomegranate diet was used (0.091 and 1.096) and lowest when the synthetic diet was used (0.06) and (1.062). The highest amounts of net reproductive rate and mean fecundity were obtained for moths fed the pistachio diet and the lowest were for moths fed the synthetic diet. Our results also showed that the mean generation times were 48.32, 44.42, 37.19, and 42 days for moths fed synthetic food, pistachio, pomegranate, and semi-synthetic food, respectively. The outcome of this research can be used to effectively select the most useful rearing of carob moths for using them in natural pheromone traps as Integrated Pest Management programs.

Key words: carob moth, demographic parameters, *Ectomyelois ceratoniae*, fruit pest, *in vitro*, two-sex life table

Introduction

The pomegranate (*Punica granatum* L.) is a member of the Punicaceae family native to Asia (from Iran to northern India) and cultivated throughout the Mediterranean, the East Indies, Africa, Southeast Asia, and the United States (Jurenka 2008). Pomegranates have been grown as an important widespread crop for centuries in the central regions of Iran. In recent years, there has been an increased interest in the commercial production of the pomegranate fruit (18,555 tons in a year) in this region (Shakeri 2008). The carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), is one of the most significant pests of pomegranate trees due to the carob moth's widespread distribution in all pomegranate-producing regions of Iran (Shakeri 2008). The carob moth lays its egg in the calyx of the flower of the pomegranate, at or immediately after flowering, and the larva bores into the fruit. Infested fruit either rots and drops, or remains on the trees until the end of the season. But larva feeding can encourage the growth of saprophytic fungi.

Because the carob moth's development takes place in fruit, there has been a considerable amount of research on various aspects of the carob moth's developmental biology. Research has been done on laboratory rearing of the moth on synthetic or semi-synthetic foods. Jansen and de Groot (2004) noted that the physiological and biological growth of insects is effected by various factors such as: humidity, temperature, food quality, and quantity.

There are different reports concerning the rearing of carob moths on diets such as: soy flour (Gothilf 1969), almond (Gothilf 1984), cracked shell (Navarro *et al*. 1986), pomegranate (Ghavami 2002), sweet corn, and almond (Hung *et al*. 2003), pistachio, pomegranate, fig, and walnut (Mozaffarian *et al*. 2008), pistachio (Barkhordar and Goldansaz 2006), wheat bran, sucrose, yeast, lysine, glycerin (Mediouni and Dhouibi 2007), and wheat flour, honey, glycerin, yeast, and water (Zolfaghaieh *et al*. 2009).

In this study, an age-stage, two-sex life table to analysis both sexes and the variation in the growth rate among individuals have been used (Chi and Liu 1985; Chi 1988). Researchers have introduced the quality of the host as the average of the intrinsic rate of increase (Dixon 1987). The effects of diets on demographic parameters have been evaluated in several studies. Norouzi *et al*. (2008) studied the effect of four host plants on the demographic parameters of the carob moth. There were significantly higher the

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intrinsic rate of increase (r) and the net reproductive rate (R_0) values for insects reared on pomegranate and pistachio than for insects reared on dates and figs. Such results indicate the suitability of pomegranate and pistachio for pest population growth in comparison to fig and date.

The aim of this study was to determine the effects of four diets consisting of two natural foods (pomegranate and pistachio), one synthetic diet, and one semi-synthetic diet (referred to as artificial diets) on the age-stage, two-sex life table parameters of *E. ceratoniae* feeding on these diets under laboratory condition.

Materials and Methods

Insect rearing

Fruits infested by carob moth larvae were originally collected from Yazd Agricultural Research Centre orchards (31°55′N, 55°16′E, 3,975 ft). The fruits were transferred to a growth chamber with controlled conditions [30±2°C, 65±5% relative humidity (RH) and 16L : 8 D]. New emerged adults from infested fruits were moved to mating cages (50 × 50 × 80 cm) for one night (Gothilf 1968; Navarro *et al*. 1986). After mating, each mated female was released separately into a plexiglass cylindrical container (25 × 15 cm) for laying eggs. During the reproduction period a piece of cotton wool, which had been soaked with a 5% honey water solution, was placed in a container for the moths. The carob moths on each diet were raised for five generations in the laboratory before the main experiments. Newly laid eggs were divided up and each group put on one of the four different diets (synthetic, and semi-synthetic diets, pomegranate; Meykhosh cultivar, and pistachio; Kaleghoochi cultivar). The semi-synthetic diet is not commercially available and is prepared by the user. The synthetic diet was based on a diet developed by Mediouni and Dhouibi (2007). Our semi-synthetic preparation was made from: wheat bran (432 g), pistachio (400 g), yeast tablets (6 g), aureomycin (7 g), glycerin (60 ml), honey (72 ml), and distilled water (30 ml). The synthetic diet contained: wheat bran (600 g), sucrose (120 g), yeast (23 g), salt mixture (20 g), vitamin C (6.7 g), aureomycin (6.7 g), methyl parabenzen (1.3 g), lysine (3 g), glycerin (150 ml), and distilled water (250 ml).

Effect of food on the biology of carob moth

To determine the life table parameters, 120 newly hatched eggs (described in the first section), in Petri dishes (6 cm in diameter and 0.5 cm in depth), were placed separately on one of the four different diets, so that there were eggs on each diet. The number of eggs hatched and not hatched was recorded daily and new larvae were separately placed in transparent containers (8 × 6 × 4 cm). To facilitate ventilation, a 5-cm-diameter opening was cut on top of the container and covered with nylon mesh. Approximately, 3–4 g of each diet were placed in each container. Larval developmental and mortality periods (based on head capsule size and moulting) were checked daily. Body length of the last instar larvae, pupal period, pupae weight and sex ratio were recorded. Newly emerged (less than 24 h old) females and the same number of males were randomly selected and placed into mating cages (50 × 50 × 80 cm) for one night (Gothilf 1968; Navarro *et al*. 1986). Then, each mated female was released separately into a plexiglass cylindrical container (25 × 15 cm). Oviposition rate, survival time, percentage of hatched eggs for each treatment and the female and male mortality rates were calculated. During the mating and laying period, adult moths were fed an emulsion of a 5% honey and water solution. This procedure was recorded daily until the death of the last female. Necessary factors for the age-stage, two-sex life table included: daily fecundity of females, developmental stages (egg, larvae, pupa, and adult), and sex of individuals which were noted and recorded in Chi software (Chi 1988). Regardless of the male population and growth rate changes, the simulation of population growth creates a curve of the female population without grouping stages that this kind of simulation is not suitable for applied research and theory.

Ignoring the developmental changes may lead to inaccurate simulations (Chi 1988). According to Chi and Liu (1985), the total size of the population is obtained using the equation:

$$N_t = \sum_{i=1}^{n}\sum_{j=1}^{m} n_{ij},$$

where: n – the number of age groups, m – the number of stages, i – age group, j – stage, n_{ij} – the number of individuals in age group i and stage j

The total number of individuals in stage j (N_j) is obtained using the equation:

$$N_j = \sum_{i=1}^{n} n_{ij},$$

Thus, in simulation according to the age-stage, two-sex life table, there can be curves obtained for each stage and for the total population (Chi 1988). The age-stage specific survivorship (s_{xj}) (where: x = age, j = stage), the age-stage specific fecundity (f_{xj}), the mean fecundity (F), the age-stage specific reproductive value (v_{xj}), the age-specific survival rate (l_x), the age-specific fecundity (m_x), and the population parameters (r – 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 (Chi 1988).

The intrinsic rate of increase [r, the number of added females (female per day) to the population] was calculated with the iterative bisection method using the following formula with an age index of zero (Goodman 1982):

$$l_x = \sum_{j=1}^{\beta} s_{xj}, \qquad m_x = \frac{\sum_{j=1}^{\beta} s_{xj} f_{xj}}{\sum_{j=1}^{\beta} s_{xj}},$$

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1.$$

where: β – the number of stages, $l_x m_x$ – age-specific maternity

Data analysis

The collected data were analysed by SPSS16 software and the means compared using Duncan's test. Data were analysed using an age-stage, two-sex life table theory. Therefore, developmental time of all individuals and female daily fecundity were analysed according to the age-stage, two-sex life table model (Chi and Liu 1985). In Chi and Liu's model (Chi and Liu 1985) population parameters were calculated based on data for all of the same age groups in both sexes, and variations in growth rates among individuals. Parameters and standard errors of r, λ, R_0 and T were calculated by using an age-stage, two-sex life analysis table and based on the Bootstrap method. The life-table analysis and the bootstrap method were used, using the computer program, TWOSEX-MS Chart (Chi 2011) in Visual Basic for the Windows operating system (available at http: //140.120.197.173/Ecology/download/Twosex-MSChart.zip). Curves and graphs were plotted using Sigmaplot 11.0 software.

Results and Discussion

The effects of diet on two-sex life table parameters

According to the analysed two-sex life table parameters (Chi and Liu 1985; Chi 1988), the survival rate for each age-stage interval (s_{xj}) of carob moth was shown in figure 1 which indicates the survival probability of a newly laid egg to age x and stage j. The survival curves and differences of stages, show that there is an overlay of the stages and changes of growth rate among individuals (Yang and Chi 2006). On the synthetic diet and pistachio treatments, males and females emerged in one day but on the semi-synthetic and pomegranate treatments, males emerged earlier than females. In all the treatments, females survived longer than males. Since the age-stage, two-sex life table has been used for growth rate changes among individuals, a significant overlay among stages was noted (Yang and Chi 2006) (Fig. 1).

Age-specific survival rate (l_x), female age-specific fecundity (f_{x9}, female is the ninth life stage), the age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of the carob moth are shown in figure 2. Age-specific survival rate is the survival probability of a newly laid egg to age x, calculated by the unite of the survival of all individuals of both sexes and those who had died prematurely. The l_x curve is a simplified version of the curves in figure 1. The mortality in the following diet treatments: the synthetic diet, pistachio, pomegranate, and semi-synthetic diet, began on the 7th, 7th, 6th, and 7th days, respectively. Also the life duration was 58, 55, 46, and 54 days, respectively. The highest and lowest survival rates were 58 and 46 days when using the artificial and pomegranate diets, respectively. The average number of offspring produced by individuals carob moths at age x and stage j per day, is shown as an age-stagespecific fecundity (f_{xj}) shown in figure 2. Since only females produce offspring, there is only one f_{x9} curve (Yang and Chi 2006). The results showed that the survival of adult females on synthetic diet treatments, and pistachio, pomegranate, and semi-synthetic diet treatments, was 19, 19, 17, and 19 days and the oviposition period for the above-mentioned treatments was 18, 18, 16, and 18, respectively. Therefore, the lowest survival and oviposition periods were observed when the pomegranate treatment was used.

Age-stage specific life expectancy (e_{xj}) of the carob moth is shown in figure 3. Age-stage specific expectation of life is the expected survival time of each individual from age x to stage j. To predict the survival of a population (Yang and Chi 2006), the age-stage specific expectation of life was calculated by using the survival rate for each s_{xj}, without assuming that the population obtains a stable age-stage distribution. The duration of life from the first day after the adult's emergence (on the 39th day) were 11.19 and 8.92 days for females and males, respectively who were fed the synthetic diet, while there were 9.89 and 6.88 on the 36th day when the pistachio diet was used, 10.48 and 5.71 on the 29th day when the pomegranate diet was used, and 9.25 and 7.4 on the 35th day when the semi-synthetic diet was used.

The age-stage specific life-expectancy based on the age-stage, two-sex life table determine differences among individuals of the same age but in different stages or of different sexes (Chi 1990). Life expectancy (e_x) means the remaining life expectancy for an individual to reach age x, is shown in figure 4. With the use of the synthetic diet treatment as well as the pistachio, pomegranate, and semi-synthetic diet treatments, the life expectancies (understood as starting from the beginning of life) were: 33.57, 38.82, 29.32, and 33.19 days, respectively. Based on these results, life expectancy was the highest when the pistachio treatment was used (38.82) and the lowest when the pomegranate treatment was used (29.32).

Fisher (1999) determined the reproductive value as a contribution of an individual to the next population. The age-stage specific reproductive value (v_{xj}) means the expected proportion of the individuals in reproduction from age x to stage j. This value is shown in figure 5. The reproductive rate of a newborn individual ($v_{0,1}$) is exactly the finite rate of increase. The reproductive rate increases significantly when reproduction begins. Females on the synthetic diet, and on the pistachio, pomegranate, and semi-synthetic diets, showed a main peak in reproductive parameters at the 40th day ($v_{40,9}$ = 136.77), 37th day ($v_{37,9}$ = 181.1), 31th day ($v_{31,9}$ = 94.78), and the 36th day ($v_{36,9}$ = 106.82), respectively. If a female does not produce offspring, her reproductive value becomes zero but her survival curve may continue (Yang and Chi 2006).

Effects of diet on population parameters

The results of the Bootstrap method showed a significant difference between the intrinsic rate of increase ($F_{3,116}$ = = 358.89, P = 0.00), the net reproductive rate ($F_{3,116}$ = 142.04, P = 0.00), the mean generation time ($F_{3,116}$ = 1924, P = 0.00), the finite rate of increase ($F_{3,116}$ = 357.49, P = 0.00), and mean fecundity ($F_{3,116}$ = 191.69, P = 0.00). The effects of the different treatments on population parameters based on the Bootstrap method, are presented in table 1. These results showed that the highest amount of r and λ were obtained for individuals on the pomegranate diet treatment

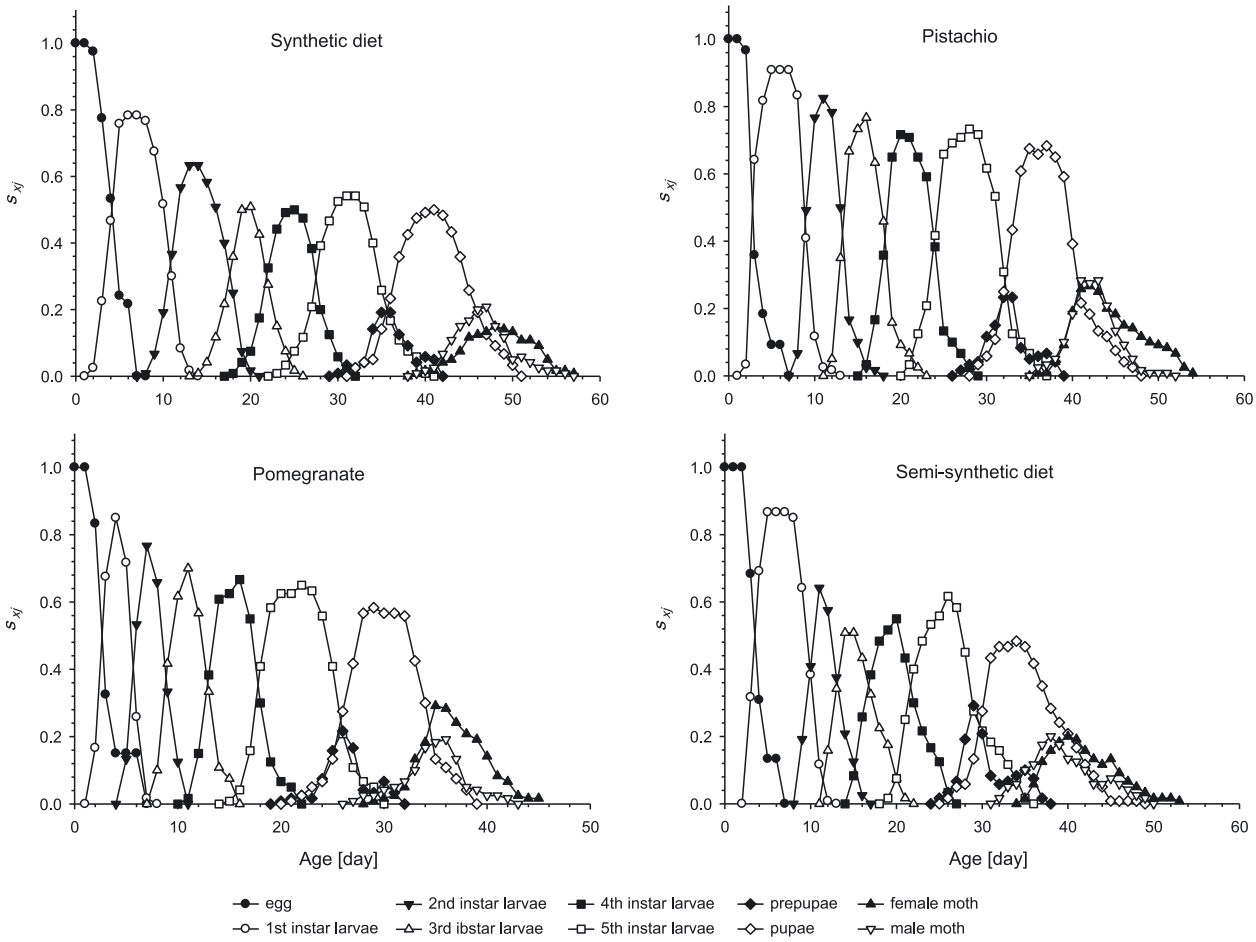

Fig. 1. Age-stage specific survivorship (s_{xj}) of *Ectomyelois ceratoniae* in which the moths had been grouped and each group had been fed one of four diets

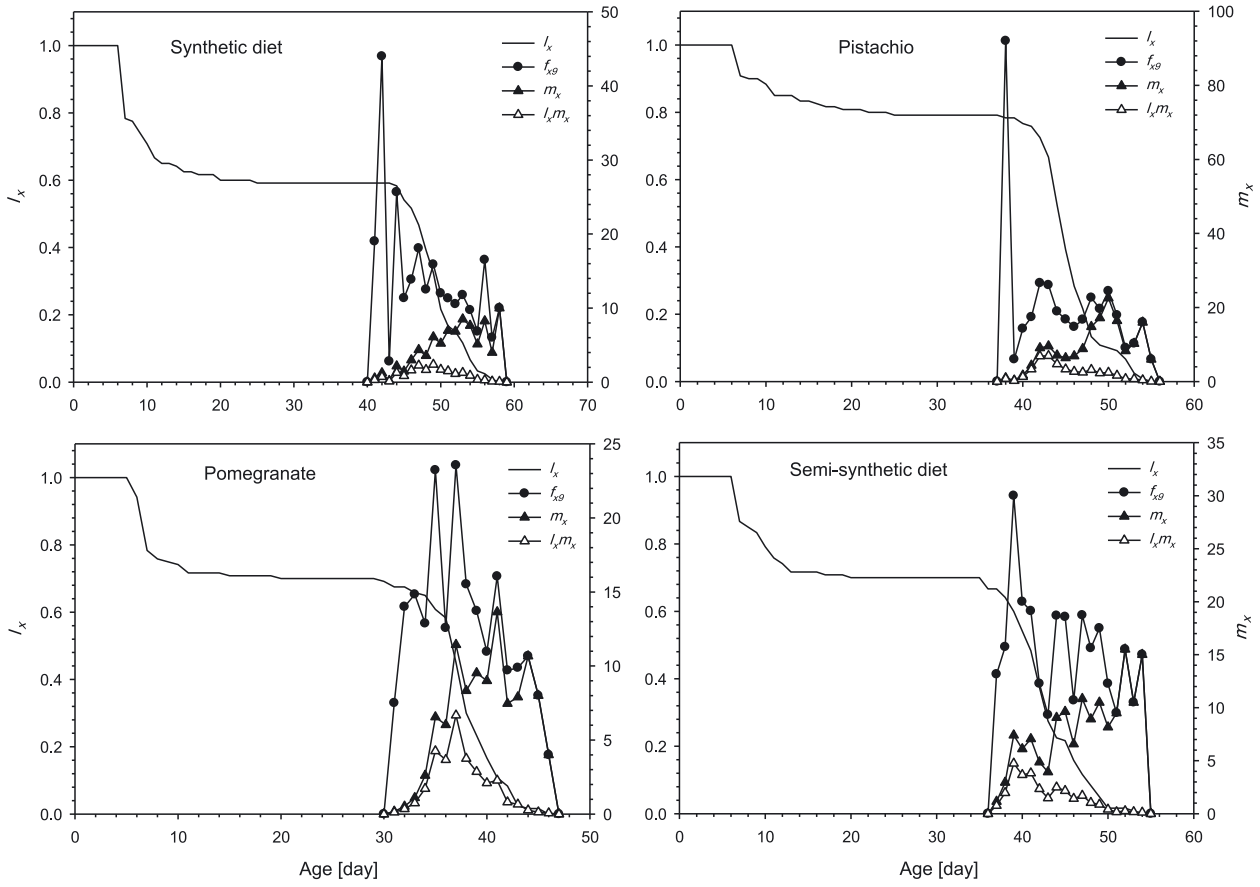

Fig. 2. Age-specific survival rate (l_x), female age-specific fecundity (f_{x9}) (eggs/female), and age-specific maternity (l_xm_x) of *Ectomyelois ceratoniae* in which the moths had been grouped and each group had been fed one of four diets

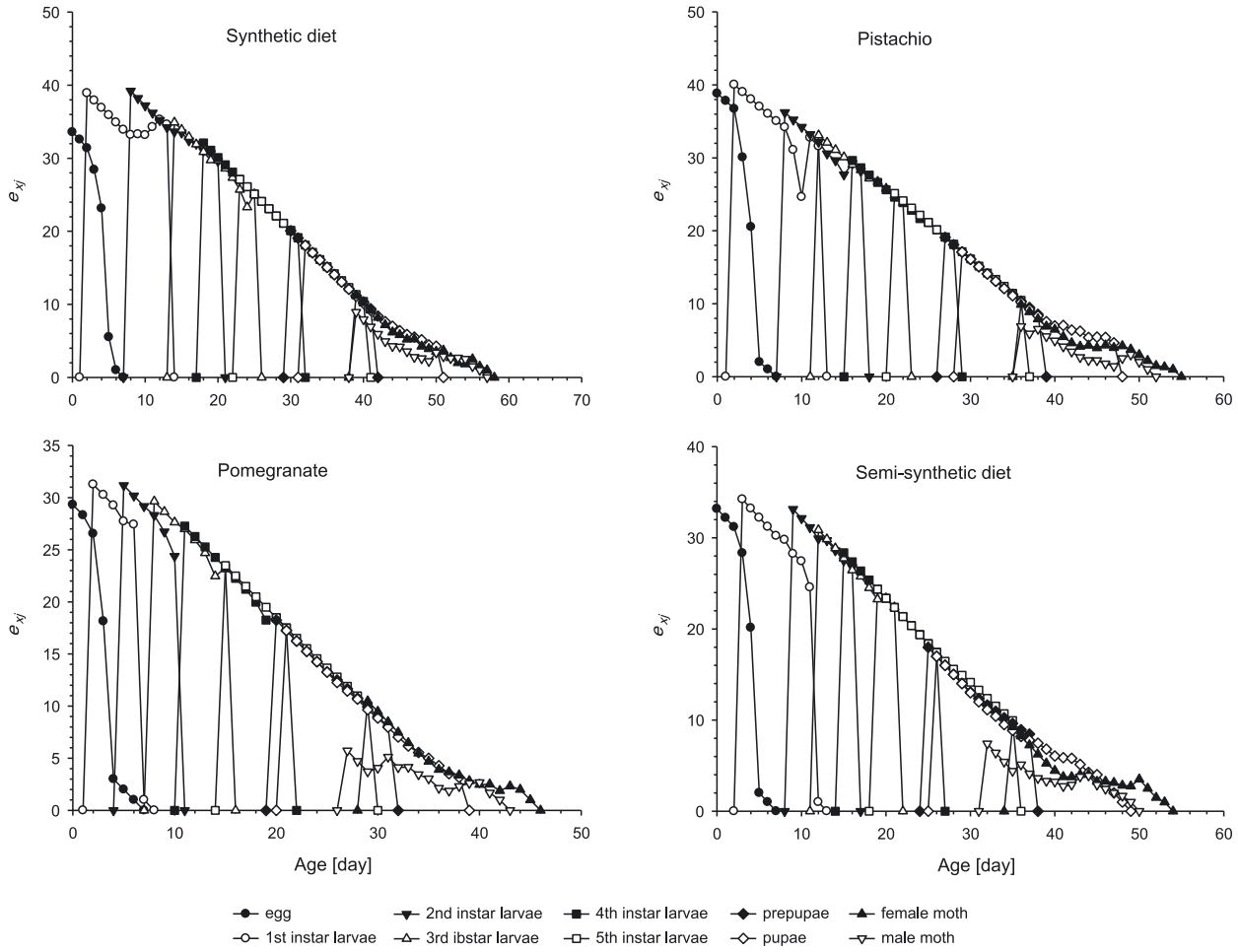

Fig. 3. Age-stage specific life expectancy (e_{xj}) of *Ectomyelois ceratoniae* in which the moths had been grouped and each group had been fed one of four diets

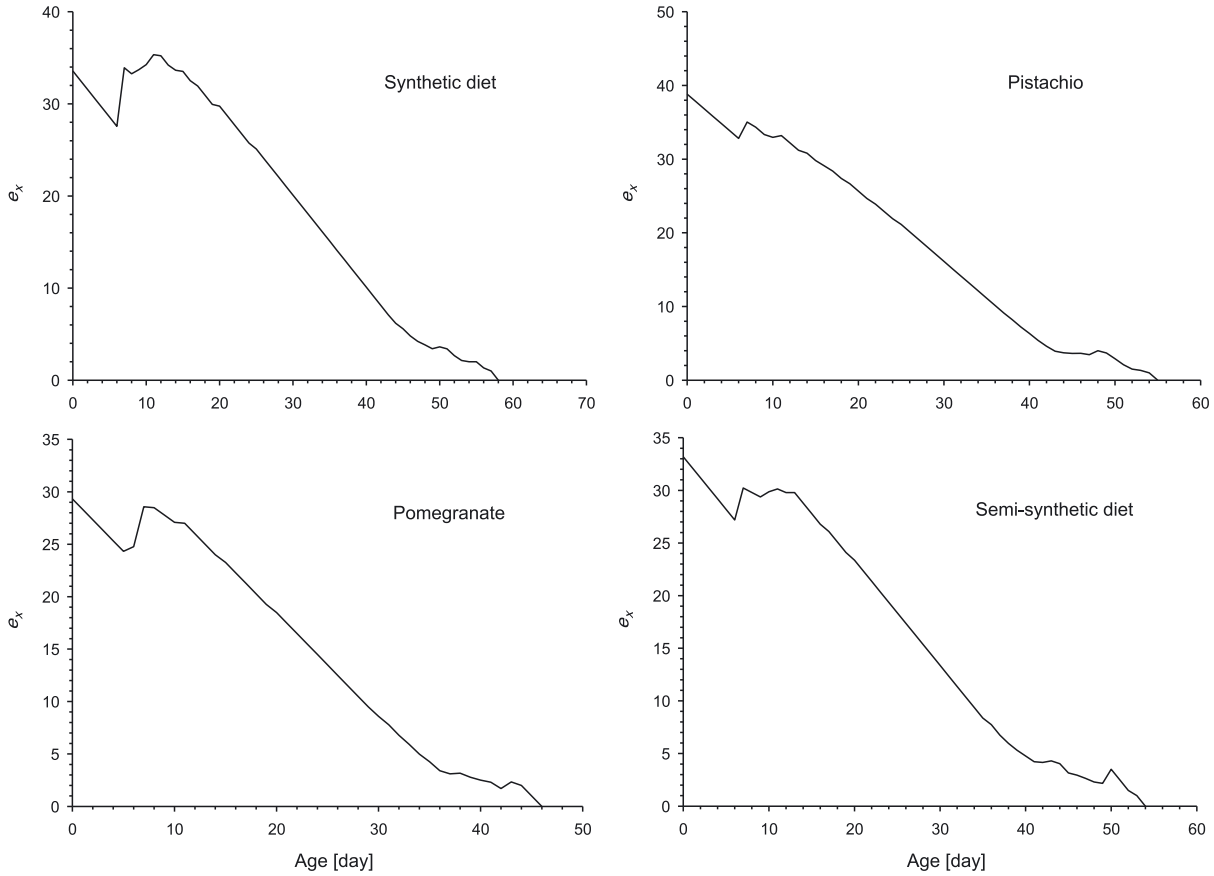

Fig. 4. Life expectancy (e_x) of *Ectomyelois ceratoniae* in which the moths had been grouped and each group had been fed one of four diets

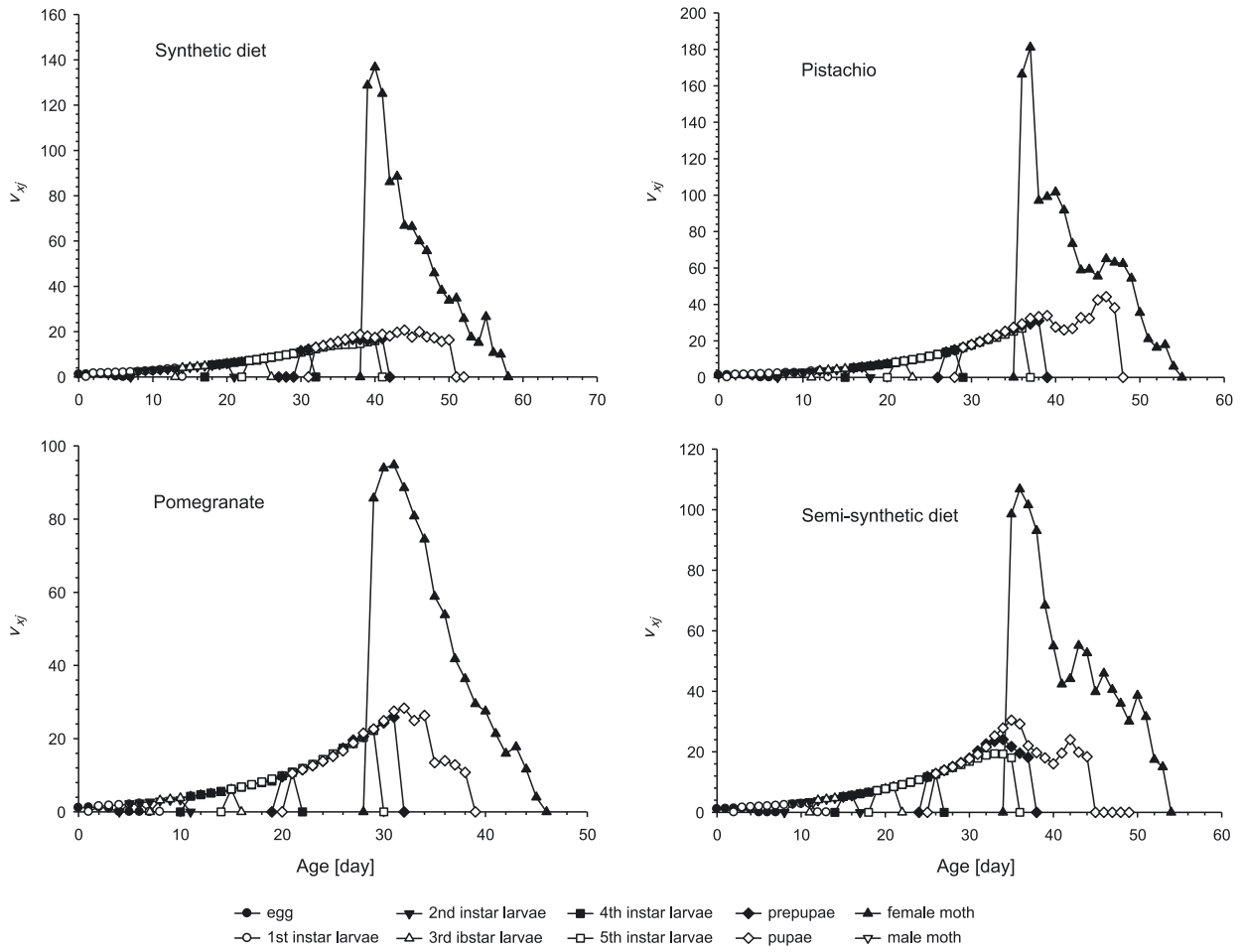

Fig. 5. Age-stage reproductive value (v_{xj}) of *Ectomyelois ceratoniae* in which the moths had been grouped and each group had been fed one of four diets

Table 1. Population parameters (mean ±SE) of *Ectomyelois ceratoniae* on four diets, based on the Bootstrap method

Parameteres	Diets			
	synthetic	pistachio	pomegranate	semi-synthetic
Intrinsic rate of increase	0.0605±0.0008 d	0.0850±0.0006 b	0.0914±0.0007 a	0.0799±0.0007 c
Finite rate of increase	1.0624±0.0008 d	1.0888±0.0007 b	1.0957±0.0007 a	1.0832±0.0007 c
Net reproductive rate	18.90±0.63 c	44.21±1.24 a	30.15±0.70 b	28.98±0.79 b
Mean generation time	48.32±0.14 a	44.42±0.10 b	37.19±0.05 d	42±0.10 c
Mean fecundity	76.63±1.20 d	114.5±1.20 a	84.04±0.84 c	98.45±1.50 b

Different letters in the rows indicate significant ($p < 0.05$) differences within various diets

and the lowest values were observed for those on the synthetic diet. The highest amount of R_0 was observed when the pistachio diet was used and the lowest for when the synthetic diet was used. Net reproductive rate and mean fecundity had the highest value for those on the pistachio diet and the lowest value for those on the synthetic diet. The highest and lowest mean generation time were observed when the synthetic and pomegranate diet were used, respectively.

The intrinsic rate of increase is the most important parameter of population growth. This statistic is a standard indicator for describing the population growth rate. According to the definition, it is the highest rate of increase for a species of a specified biological and physical condition. The calculation of r can be useful for prediction of a pest condition. This calculation also can be as a quantitative tool or ecological indicator to compare the responses of different species to environmental conditions and several factors such as temperature, humidity, food quality, plant morphology, and secondary chemical compounds of plants (Andrewartha and Birch 1954; Messenger 1964; Samih and Izadi 2006). Various factors, such as plant volatile material, leaf morphology, the nutritional quality of the host or the combination of these factors have an effect

on the *r* value and other demographic parameters (Sarfraz *et al*. 2006). A low nutritional value of the host plant causes a decrease in regeneration or an increase in the developmental time in vegetarians (Michaud 1999). Effects of the insect's diet on demographic parameters have been evaluated in several studies. Norouzi *et al*. (2008) investigated the effects of four host plants on the demographic parameters of the carob moth. The *r* value of the pomegranate, pistachio, fig and date, were: 0.107, 0.1, 0.055, and 0.018, the R_0 value on those were: 38.49, 45.74, 13.45, and 3.64, and the mean generation time on those were: 34, 38.06, 47.7, and 72.13, respectively. These findings show that *r* and R_0 values in insects reared on pomegranates and pistachios are significantly higher than insects reared on dates and figs. The indication is that the pomegranate and pistachio are suitable for growth of the pest population for use in fig and date comparison. The highest and lowest mean generation-time were observed with the use of the date and pomegranate, respectively. In the study *r* and R_0 values in insects reared on the pomegranate and pistachio are significantly higher than insects reared on semi-synthetic and synthetic diets. These results indicate the suitability of the pomegranate and pistachio for growing the pest population as compared to the two other diets. Nay and Perring (2008) reported that the amounts of the intrinsic rate of increase, the finite rate of increase, and the net reproductive rate were: 0.0928, 1.0972, and 38.1 for ripe date. These values are lower than the results from the insects reared on kimri and khalal dates. Zare (2011) showed the effects of three pomegranate cultivars on demographic parameters of carob moth. He reported that *r* values on Malase Daneh Siah, Gabri, and Shahvar cultivars were: 0.079, 0.088, and 0.105, the finite rate of increase: 1.083, 1.092, and 1.11, the net reproductive rate: 23.71, 36.26, and 51.01, and the mean generation time: 41.2, 40.79, and 37.43, respectively. He stated that the Malase Daneh Siah cultivar had the longest period of generation time and the lowest rate of increase and reproductive rate. Therefore, the Malase Dane Siah cultivar is less sensitive to the carob moth than the other two cultivars. Because of differences in pomegranate cultivars, experimental conditions, and accuracy of measurements, there are differences between his results and the results of our study.

Hung *et al*. (2003) reared carob moth on a synthetic diet containing agar, sweet corn, yeast, cholesterol, ascorbic acid, aureomycin, formalin, phosphates, and water. They also reared the moths on a natural diet which included almond slices. Rearing took place where conditions were: temperature 25±1°C, RH 70±5%, and a photoperiod of 12L : 12D. The developmental periods of the eggs, larvae, female and male pupae of carob moths reared on almond slices were: 4±0.3, 68±15.7, 10.6±5.2, and 16.5±3.4 days. The developmental periods for those reared on an artificial diet of corn were: 3.9±0.3, 21.4±4.9, 11.4±4.5, and 9.6±4.8 days, respectively, compared with use of the artificial diet of our study (4.7, 31 and 8) larval period in Hung's study was shorter and pupal period was longer. The difference is due to the different diets and experimental conditions. Mediouni and Dhouibi (2007) prepared an artificial diet for rearing the carob moth. Their diet was composed of wheat bran, yeast, sucrose, a salt mixture, vitamin C, aureomycin, and glycerin. The laboratory rearing conditions were: temperature 28±1°C, RH 75±5%, and a photoperiod of 15L : 9D. Based on the incubation period, the 1st, 2nd, 3rd, 4th, and 5th instar larvae and pupal period were obtained at: 3.14, 5.01, 4.58, 4.56, 4.67, 4.89, and 7.33, respectively. This synthetic diet was similar to the synthetic diet of our study which was based on the incubation period where the 1st, 2nd, 3rd, 4th, and 5th instar larvae and pupal period were obtained at: 4.75, 6.9, 6.61, 4.37, 5.61, 7.58, and 8.09 days, respectively. The difference may be due to differences in the experimental conditions. Ghavami (2002) used three diets including an artificial diet (soybean meal and oil), a semi-artificial diet (soybean and freeze-dried pomegranate), and a natural diet (pomegranate) for rearing this pest in the laboratory. The average of growth periods including the egg, larvae, prepupae and pupae periods were obtained at: 3.1, 22.8, 2.31, and 8.75 days on the artificial diet; 4.02, 18.67, 1.61, and 6.29 days on the semi-artificial diet; and 4.88, 17.93, 1.43, and 6.39 days on pomegranate, respectively. In our present study, the results on pomegranate were: 3.61, 22.83, 1.22, and 7 days which are somewhat similar and only the larval period is slightly longer. Ghavami (2002) said the semi-artificial diet is the most suitable food for rearing this pest in the laboratory because a semi-artificial diet provides all the nutrient requirements. In our study, however, pomegranate seeds had the best results. In one study, carob moth was reared on pomegranate, fig, pistachio, and walnut, in the laboratory. The study was meant to compare the shape and size of the wings in populations on four host plants. The larger-sized moths on hosts other than pomegranate showed that some host plants, such as fig, pistachio, and walnut, can provide more nutritional reserves for larvae which results in more successful overwintering and higher fecundity in adults (Mozaffarian *et al*. 2008). In another study, a comparison was done of the calling behavior and some biological parameters of the carob moth reared on pistachio, among three populations under experimental conditions. The developmental periods from egg to adult were evaluated 39, 40.1, and 41.3 days in the Saveh, Kerman, and Arsanjan populations, respectively (Ziaadini *et al*. 2010).

In summary, since the natural pheromone traps with female moths and sterilization of males are two effective methods for pest control, thus it is necessary knowing which diet is better for mass rearing of carob moth *in vitro*. Based on this research, we know that the most desirable food for this pest is pomegranate but synthetic diet is more affordable, economically.

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