

Research Article

## Effect of some diets on biological parameters of *Apomyelois ceratoniae* in laboratory and efficiency of natural pheromone traps in pomegranate orchards

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**Abstract:** Pomegranate fruit moth (PFM), *Apomyelois ceratoniae* Zeller is a major pest of pomegranate in Iran. In this study, the effect of four diets including pistachio, pomegranate, semi-artificial and artificial diets were studied on biological parameters of PFM in a growth chamber under controlled conditions at  $30 \pm 2$  °C,  $65 \pm 5\%$  RH and 16:8 (L: D) h. The results demonstrated that the longest incubation period, larval developmental time and preimaginal development time was observed on artificial diet and the shortest was on the pomegranate. The highest and lowest body length of fifth instar larvae were observed on pistachio and artificial diet, respectively. The heaviest weight of third day pupae was recorded for the larvae that were reared on pistachio ( $0.0275 \pm 0.0004$  g) while the lightest was on artificial diet ( $0.0216 \pm 0.0004$  g). In the next experiment, the effect of four diets was considered to assess the efficiency of pheromone traps in a twenty year old pomegranate orchard cultivated with Malasse Yazdi cultivars. The effect of food on catch of traps showed a significant difference. The virgin PFM that were fed with pomegranate attracted more males. Also the traps caught the males mostly from 11:00 pm until 4:00 am, with the peak time between 23:00 and midnight. No significant difference was seen between diet and time of catch, indicating that starting or ending time and the maximum time of catch was not affected by the diets.

**Keywords:** biological parameters, catch time, Pomegranate fruit moth, Malasse Yazdi cultivar, artificial diet

### Introduction

Pomegranate, *Punica granatum* (Punicaceae), is one of the important horticultural products throughout the Indian subcontinent, Central Asia, the drier parts of southeast Asia (e.g. China) and the Middle East (e.g. Iran). Pomegranate is renowned as an important fruit because of its high

economic value (Vozarayi, 1987). The pomegranate tree is attacked by several insect species that decrease the quality and quantity of its product, among which pomegranate fruit moth is considered to be the most serious one (Shakeri, 1992). The Pomegranate fruit moth (PFM), *Apomyelois ceratoniae* (Zeller), is the major pest of pomegranate in Iran (Shakeri, 2004). The damage starts in early May, when the fruits are small and continues during the rest of growing season. Besides consuming the fruit, it opens an avenue for penetration of saprophytic fungi and other pests into the fruit in the orchards and

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storage. Chemical control cannot be effective on the population of this pest as the eggs are laid in the calyx and all stages develop inside the fruit (Shakeri and Sadatakhavi, 2004). Various methods are used to control the PFM including, collecting infested fruits from orchards as a mechanical control (Shahrokhi and Zare, 1994; Shakeri, 1992), pomegranate calyx stuffing (Mirkarimi, 2002), removing pomegranate stamens (Farazmand, 2009) to avoid egg laying, use of biological control agent *Trichogramma* spp egg parasitoids (Mirkarimi, 2002), resistant varieties (Shakeri and Sadatakhavi, 2004), and insect sterile technique (Tabatabaai, 2011; Tan, 2000; Zolfaghariéh *et al.*, 2009).

Sex pheromones are considered to play an important role in the integrated pest management. Species pheromone communication system is affected by different geographical conditions and diet (Noldus and Potting, 1990). In addition host plant has a significant effect on the physiological and behavioral parameters associated with pheromone production (Landolt and Phillips, 1997). For example, secondary plant compounds such as pyrrolizidine alkaloids, in male of some species of Nymphalidae butterflies and Arctiidae moths could be used as a pheromone precursor (McNeil and Delisle, 1989).

The importance of natural diet for rearing has been documented in different studies such as using almond with cracked shell (Navarro *et al.*, 1986), pomegranate (Ghavami, 2006; Mozaffarian *et al.*, 2008; Norouzi *et al.*, 2008), pistachio, walnut (Mozaffarian *et al.*, 2008), fig (Norouzi *et al.*, 2008; Mozaffarian *et al.*, 2008) pistachio (Ziaadini *et al.*, 2010; Norouzi *et al.*, 2008) and date (Norouzi *et al.*, 2008). Since rearing of PFM for the whole year on natural host plant would be, use of artificial diet becomes necessary for mass rearing in laboratory. Pomegranate fruit moth was reared in the laboratory in 1968, for the first time (Gothilf 1970). In addition, artificial diets also have been addressed by using soy flour, casein and lysine (Al-Izzi, 1988), ground whole wheat, date syrup and glycerol (Alrubeai, 1987); agar, sweet corn and yeast (Hung *et al.*, 2003); wheat bran, sucrose, yeast, lysine, glycerine (Mediouni and

Dhouibi, 2007); soybean meal and oil (Ghavami, 2006); wheat flour, honey, glycerin, yeast and water (Zolfaghariéh *et al.*, 2009); X-soy protein, sucrose, multivitamin, linoleic acid,  $\beta$ -sitosterol and salt mixture (Levinson and Gothlif, 1965).

In this study the effect of four diets including, two natural plant foods, one artificial and one semi-artificial diet on biological parameters and efficiency of virgin females' pheromone traps were assessed in order to find suitable diet for rearing of PFM and also increasing the efficiency of pheromone traps.

## Materials and Methods

### Insect culture and experimental condition

The infested fruits were collected from Yazd Agricultural Research Center orchards. Fruits were transferred to a growth chamber with controlled condition at  $30 \pm 2$  °C,  $65\% \pm 5\%$  RH and 16:8 (L: D) h. Adults emerging from infested fruits were transferred to the mating cages ( $50 \times 50 \times 80$  cm) for one night (Gothilf, 1968; Navarro *et al.*, 1986). After mating each mated female was released separately into a Plexiglas cylindrical container ( $25 \times 15$  cm) for laying eggs. During the reproduction period a cotton wool, which was soaked with 5% honey water solution, was placed in the container as a diet for female moth. The PFM was reared on each diet for five generations in the laboratory before being used in the main experiments.

### Diet preparation

In this test, four diets were prepared as following:

1- Artificial diet: wheat bran (600 g), sucrose (120 g), yeast (23 g), salt mixture (20 g), vitamin C (6.7g), aureomycin (6.7 g), methyl paraben (1.3 g), lysine (3 g), glycerine (150 ml) and distilled water (250 ml) (Mediouni and Dhouibi, 2007). 2- Natural fruit diets: pistachio nut (Kaleghoochi cultivar). 3- Natural fruit diets: fresh pomegranate (Meykhosh cultivar). 4- Semi-artificial diet: wheat bran (432 g), pistachio (400 g), yeast (6 g), aureomycin (7 g), glycerine (60 ml), honey (72 ml) and distilled water (30 ml).

Wheat bran and pistachio were disinfected in the microwave before diet preparation.

### **The effect of diets on biology of the PFM**

The new (one day old) eggs of the PFM were separately placed in Petri dishes (60 mm in diameter) on the four different diets. For each treatment, 120 eggs were used as three replicates. Developmental stages were checked daily by a scaled binocular with measuring head capsule according to Dyar's rule (Dyar, 1890). Developmental periods and mortality of eggs, larvae, pupae and adults were recorded. Also, the length of last wandering larvae, the weight of the pupae and sex ratio of moths were determined during the test.

### **Evaluation of moths produced from diets as natural pheromone trap**

This study was conducted in the orchard of Yazd Agricultural Research Center where pomegranates are the predominant fruit trees. The selected pomegranate orchards were cultivated with Malasse Yazdi cultivar of similar age (20 years old) and horticultural operations. The natural pheromone traps were constructed of PVC tubes (11 × 25 cm) the inner surface of which was covered with a thin layer of sticky aluminum sheet. An oval container (size of salt-sprinkler 4×8 cm) containing a one-day old virgin female moth, fed on one of the four rearing diets, was placed in the top and middle of tube in hanging traps (1.5 m. height). The catches were counted during trapping, then the average of daily catch was calculated for each treatment and analysis was conducted according to these data.

The experiment was conducted according to a randomized complete block design with four blocks and four treatments of moth pheromone traps containing females fed on four diets, as described above (16 traps: 4 traps for each diet). The four blocks were situated at a distance of 50 meters from each other. Treatments were set at a distance of 30 meters from each other in every block.

### **Effects of diet on diurnal catch**

This experiment was conducted according to a previous test with three blocks and five treatments including one-day old virgin female moths fed on the four different diets (two natural

diets, one artificial and one semi-artificial diet) and moths which were at darkness some hours earlier than other treatments (fed on semi-artificial diet), as fifth treatment. All traps were checked and the caught moths were counted at 10, 11, 12 pm and 2, 3, 4, 5, 6 and 7 am.

### **The effect of darkness on trap catches**

The experiment was conducted with three blocks and two treatments containing female moths fed on semi-artificial diet in normal condition and female moths fed on semi-artificial diet that were exposed to treatment in the dark a few hours earlier. This experiment was continued for four consecutive nights. Specifications of blocks and distance between blocks and treatments were same as in the previous experiment.

### **Statistical analysis**

The analysis of data was performed using SPSS 16. The post-test analysis of variance (ANOVA) was used to compare means by Tukey's test among treatments. Independent T- student test was used to compare significant differences between two treatments. Differences among means were considered significant at  $P \leq 0.05$ .

## **Results**

### **Dietary effects on biological parameters**

The results showed that different diets affected the biological parameters including the egg incubation ( $F_{3,8} = 56.93$ ,  $P = 0.0001$ ,  $CV = 10\%$ ), the 1<sup>st</sup> instar larvae ( $F_{3,8} = 482.8$ ,  $P = 0.0001$ ,  $CV = 26\%$ ), the 2<sup>nd</sup> instar larvae ( $F_{3,8} = 1219$ ,  $P = 0.0001$ ,  $CV = 29\%$ ), the 3<sup>rd</sup> instar larvae ( $F_{3,8} = 44.54$ ,  $P = 0.0001$ ,  $CV = 9\%$ ), the 4<sup>th</sup> instar larvae ( $F_{3,8} = 19.24$ ,  $P = 0.001$ ,  $CV = 5\%$ ), the fifth instar larvae ( $F_{3,8} = 7.15$ ,  $P = 0.012$ ,  $CV = 2\%$ ), prepupal ( $F_{3,8} = 19.72$ ,  $P = 0.0001$ ,  $CV = 13\%$ ), pupal ( $F_{3,8} = 47.23$ ,  $P = 0.0001$ ,  $CV = 6\%$ ), larval development time ( $F_{3,8} = 161.72$ ,  $P = 0.0001$ ,  $CV = 11\%$ ), and preimaginal development time ( $F_{3,8} = 194.86$ ,  $P = 0.0001$ ,  $CV = 10\%$ ), body length of 5<sup>th</sup> instar larvae ( $F_{3,8} = 68.1$ ,  $P = 0.0001$ ,  $CV = 4\%$ ) and weight of the 3<sup>rd</sup> day pupae ( $F_{3,8} = 23.96$ ,  $P = 0.0001$ ,  $CV = 8\%$ ) significantly but, there were no significant differences between sex ratios ( $F_{3,8} = 0.968$ ,  $P =$

0.454, CV = 16%). Average of variables was grouped using Tukey's test at 5% level (Table 1). The results showed that the eggs of moths fed with artificial food and pomegranates had the longest and the shortest incubation period, respectively. The first and second instar larvae had the maximum period on artificial diet and the minimum period on pomegranate. The longest third instar larvae developmental time was observed on pistachio and the shortest on pomegranate and semi-artificial food. The longest fourth instar larvae development time was obtained on pistachio and artificial food and the shortest on pomegranate and semi-artificial food. The fifth instar larvae had the maximum developmental time on pistachio and minimum on semi-artificial food. Prepupal period was the longest on the artificial and semi-artificial diet and the shortest on pistachio and pomegranate. Pupae that were fed with artificial food during their larval stages had the longest pupal period and those fed on the pomegranate and semi-artificial food had the shortest. The longest and shortest larval development time and also preimaginal development time were observed on artificial diet and pomegranate. Body size of 5<sup>th</sup> instar larvae and

weight of third day pupae were maximum on pistachio and minimum on artificial diet.

The effect of diets on mortality of different growth stages revealed the following results: The averages of treatment effects on stage specific mortality are shown in Table 2. Stage mortality is obtained using equation: Stage mortality =  $\frac{\text{The number of alive in previous level} - \text{The number of alive in present level}}{\text{The number of alive in previous level}}$  (Carey, 2001). The highest and lowest mortality of 1<sup>st</sup> instar larvae was observed on artificial diet and pistachio, respectively. Totally, the 1<sup>st</sup> and 2<sup>nd</sup> instar larvae had the maximum mortality, whereas the 5<sup>th</sup> instar larvae had the minimum. The stage specific mortality of the 1<sup>st</sup> instar larvae were significantly different ( $F_{3,8} = 10.38$ ,  $P = 0.004$ , CV = 35%) but, no significant differences were observed among the other stages including: the 2<sup>nd</sup> instar larvae ( $F_{3,8} = 2.54$ ,  $P = 0.13$ , CV = 36%), the 3<sup>rd</sup> instar larvae ( $F_{3,8} = 1.13$ ,  $P = 0.395$ , CV = 36%), the 4<sup>th</sup> instar larvae ( $F_{3,8} = 0.48$ ,  $P = 0.705$ , CV = 37%), the 5<sup>th</sup> instar larvae ( $F_{3,8} = 1.83$ ,  $P = 0.22$ , CV = 31%) and total mortality prepupal period ( $F_{3,8} = 0.75$ ,  $P = 0.553$ , CV = 32%) and pupal developmental time ( $F_{3,8} = 0.75$ ,  $P = 0.553$ , CV = 32%).

**Table 1** Developmental time, body size, 3<sup>rd</sup> day pupal weight and sex ratio of *Apomyelois ceratoniae* on four diets.

Parameters	Artificial	Pistachio	Pomegranate	Semi-artificial
Development time (days)				
Embryonic period	4.75 ± 0.09 <sup>a</sup>	3.69 ± 0.04 <sup>c</sup>	3.61 ± 0.04 <sup>c</sup>	4.26 ± 0.08 <sup>b</sup>
1 <sup>st</sup> instar larvae	6.90 ± 0.12 <sup>a</sup>	6.18 ± 0.07 <sup>c</sup>	3.15 ± 0.05 <sup>d</sup>	6.47 ± 0.04 <sup>b</sup>
2 <sup>nd</sup> instar larvae	6.61 ± 0.01 <sup>a</sup>	4.40 ± 0.02 <sup>b</sup>	3.32 ± 0.07 <sup>d</sup>	3.47 ± 0.02 <sup>c</sup>
3 <sup>rd</sup> instar larvae	4.37 ± 0.04 <sup>b</sup>	4.85 ± 0.08 <sup>a</sup>	4.02 ± 0.08 <sup>c</sup>	3.86 ± 0.01 <sup>c</sup>
4 <sup>th</sup> instar larvae	5.61 ± 0.09 <sup>a</sup>	5.67 ± 0.05 <sup>a</sup>	5.00 ± 0.06 <sup>b</sup>	5.07 ± 0.09 <sup>b</sup>
5 <sup>th</sup> instar larvae	7.582 ± 0.002 <sup>ab</sup>	7.678 ± 0.002 <sup>a</sup>	7.331 ± 0.001 <sup>bc</sup>	7.15 ± 0.001 <sup>c</sup>
Prepupal period	1.69 ± 0.05 <sup>a</sup>	1.42 ± 0.03 <sup>b</sup>	1.22 ± 0.09 <sup>b</sup>	1.84 ± 0.05 <sup>a</sup>
Pupal period	8.09 ± 0.06 <sup>a</sup>	7.49 ± 0.07 <sup>b</sup>	7.00 ± 0.09 <sup>c</sup>	6.85 ± 0.08 <sup>c</sup>
Larval development time	31.08 ± 0.37 <sup>a</sup>	28.78 ± 0.26 <sup>b</sup>	22.83 ± 0.29 <sup>d</sup>	26.02 ± 0.15 <sup>c</sup>
Preimaginal development time	45.61 ± 0.44 <sup>a</sup>	41.38 ± 0.31 <sup>b</sup>	34.66 ± 0.23 <sup>d</sup>	38.96 ± 0.28 <sup>c</sup>
Body length of 5 <sup>th</sup> instar wandering larvae (cm)	1.20 ± 0.01 <sup>d</sup>	1.354 ± 0.005 <sup>a</sup>	1.31 ± 0.003 <sup>b</sup>	1.27 ± 0.009 <sup>c</sup>
3 <sup>rd</sup> day pupal weight (g)	0.022 ± 0.0004 <sup>c</sup>	0.028 ± 0.0004 <sup>a</sup>	0.025 ± 0.0002 <sup>b</sup>	0.025 ± 0.0007 <sup>b</sup>
Sex ratio (female / total)	43.04 ± 3.49 <sup>a</sup>	50.39 ± 2.94 <sup>a</sup>	52.43 ± 4.99 <sup>a</sup>	45.11 ± 5.84 <sup>a</sup>

Means with different letters in a row indicate significant differences (Tukey's test,  $P < 0.05$ ).

**Table 2** The effect of diets on stage specific mortality of *Apomyelois ceratoniae*.

Stages	Mortality $\pm$ SE (%) <sup>1</sup>			
	Artificial	Pistachio	Pomegranate	Semi-artificial
1 <sup>st</sup> instar larvae	0.22 $\pm$ 0.01 <sup>a</sup>	0.09 $\pm$ 0.00 <sup>c</sup>	0.15 $\pm$ 0.01 <sup>b</sup>	0.13 $\pm$ 0.02 <sup>bc</sup>
2 <sup>nd</sup> instar larvae	0.17 $\pm$ 0.02 <sup>a</sup>	0.06 $\pm$ 0.02 <sup>a</sup>	0.09 $\pm$ 0.02 <sup>a</sup>	0.15 $\pm$ 0.04 <sup>a</sup>
3 <sup>rd</sup> instar larvae	0.05 $\pm$ 0.01 <sup>a</sup>	0.02 $\pm$ 0.00 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>
4 <sup>th</sup> instar larvae	0.04 $\pm$ 0.02 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	0.02 $\pm$ 0.02 <sup>a</sup>	0.01 $\pm$ 0.0 <sup>a</sup>
5 <sup>th</sup> instar larvae	0.00 $\pm$ 0.00 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	0.01 $\pm$ 0.01 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
prepupae	0.32 $\pm$ 0.09 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>a</sup>	0.30 $\pm$ 0.03 <sup>a</sup>	0.29 $\pm$ 0.04 <sup>a</sup>
pupae	0.32 $\pm$ 0.09 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>a</sup>	0.30 $\pm$ 0.030 <sup>a</sup>	0.29 $\pm$ 0.04 <sup>a</sup>

<sup>1</sup> Means with different letters in a row indicate significant differences (Tukey's test,  $P < 0.05$ ).

### Effect of diet on catch of moths used as natural pheromone trap

#### Effect of food on catch of traps

The result showed that the effect of diets on traps from 30 July to 4 August ( $F_{3,12} = 5.52$ ,  $P = 0.013$ ,  $CV = 49\%$ ) and from 26 September to 1 October 2012 ( $F_{3,12} = 5.93$ ,  $P = 0.01$ ,  $CV = 51\%$ ) and also in general attractant traps for these durations ( $F_{3,28} = 7.07$ ,  $P = 0.001$ ,  $CV = 57\%$ ) were significant. The averages of variables are shown in Table 3. The most catch was recorded in traps with females that were fed by pomegranate, for both durations.

#### Effect of diets on trap catches at different night hours

There were no significant relation between interaction of diet and time of catch ( $F_{3,9} = 0.55$ ,  $P = 0.93$ ). It means that different diets had no effect on time of catch. Also no significant difference was observed between catch of traps in different hours in treatment containing moths that were fed by semi-artificial diet and were at darkness a few hours earlier than the other treatments ( $F_{8,18} =$

0.926,  $P = 0.512$ ) (Table 4). The maximum catch was recorded at 23 and 24 o'clock on artificial diet, 24 o'clock for pistachio, at 23 and 24 o'clock for pomegranate and at 24 o'clock for semi-artificial diet treatments. There were no significant differences between catch of traps at different hours on semi-artificial diet treatment in darkness condition, although the most catch was recorded at 23 o'clock. However, there was significant difference between catch of traps at different hours in treatments including artificial diet ( $F_{8,18} = 3.8$ ,  $P = 0.009$ ), pistachio ( $F_{8,18} = 2.68$ ,  $P = 0.039$ ), pomegranate ( $F_{8,18} = 3.03$ ,  $P = 0.024$ ) and semi-artificial diet ( $F_{8,18} = 4.02$ ,  $P = 0.007$ ).

#### Effect of darkness on general catch of traps

General catch of traps were counted for four consecutive nights and mean catch of treatments were analyzed. There was no significant differences in the number of insects in the trap when the adults were kept in the dark compared with the insects were kept in the light ( $t = -1.308$   $df = 2$   $p = 0.204$ ) (Table 5).

**Table 3** The effect of food on catch of *Apomyelois ceratoniae* males for each duration dates.

Date	No. of males / trap / five days (Mean $\pm$ SE) <sup>1</sup>			
	Artificial	Pistachio	Pomegranate	Semi-artificial
30 July to 4 August	30.25 $\pm$ 7.79 <sup>b</sup>	45.50 $\pm$ 5.00 <sup>b</sup>	74.00 $\pm$ 5.60 <sup>a</sup>	35.25 $\pm$ 12.65 <sup>b</sup>
26 September to 1 October	14.50 $\pm$ 3.28 <sup>b</sup>	28.25 $\pm$ 3.90 <sup>ab</sup>	43.00 $\pm$ 7.27 <sup>a</sup>	22.00 $\pm$ 4.45 <sup>b</sup>
Total	22.37 $\pm$ 4.90 <sup>b</sup>	36.87 $\pm$ 4.39 <sup>b</sup>	58.50 $\pm$ 7.24 <sup>a</sup>	28.62 $\pm$ 6.69 <sup>b</sup>

<sup>1</sup> Means with different letters in each row indicate significant differences (Tukey's test,  $P < 0.05$ ).

**Table 4** Effects of diets on catch of *Apomyelois ceratoniae* males at different hours.

Treatment	No. of males / trap (Mean $\pm$ SE) <sup>1</sup>								
	10 pm	11 pm	12 pm	2 am	3 am	4 am	5 am	6 am	7 am
Artificial diet	0 <sup>b</sup>	6.60 $\pm$ 4.40 <sup>a</sup>	7.00 $\pm$ 4.27 <sup>a</sup>	1.30 $\pm$ 1.10 <sup>b</sup>	0.33 $\pm$ 0.33 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>
Pistachio	0 <sup>b</sup>	2.00 $\pm$ 1.15 <sup>ab</sup>	4.16 $\pm$ 1.30 <sup>a</sup>	2.60 $\pm$ 2.10 <sup>ab</sup>	0.33 $\pm$ 0.33 <sup>b</sup>	0.33 $\pm$ 0.33 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>
Pomegranate	0 <sup>b</sup>	6.00 $\pm$ 3.05 <sup>a</sup>	6.80 $\pm$ 3.60 <sup>a</sup>	6.16 $\pm$ 1.36 <sup>ab</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0.66 $\pm$ 0.66 <sup>b</sup>	0.33 $\pm$ 0.33 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>
Semi-artificial diet	0 <sup>b</sup>	2.30 $\pm$ 2.30 <sup>b</sup>	5.50 $\pm$ 1.40 <sup>a</sup>	2.00 $\pm$ 0.57 <sup>b</sup>	0.33 $\pm$ 0.33 <sup>b</sup>	0.33 $\pm$ 0.33 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>
Semi-artificial diet and darkness condition	2.60 $\pm$ 0.33 <sup>a</sup>	7.00 $\pm$ 6.50 <sup>a</sup>	6.33 $\pm$ 5.36 <sup>a</sup>	1.60 $\pm$ 1.60 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

<sup>1</sup> Means with different letters in each row indicate significant differences (Tukey's test,  $P < 0.05$ ).

**Table 5** Comparison of conditioning of females in light and dark in catch of males for four consecutive nights.

Diet	No. of males / trap (Mean $\pm$ SE) <sup>1</sup>	
	Lightness <sup>2</sup>	Darkness <sup>2</sup>
Semi-artificial diet	2.87 $\pm$ 1.03	9.00 $\pm$ 4.56 <sup>ns</sup>

<sup>1</sup> Means of catch for four consecutive nights.

<sup>2</sup> Females were kept in light or dark few hours before use in trap

<sup>ns</sup> non-significant using independent t-student test at  $P > 0.05$ .

## Discussion

The results showed that developmental time and pupal weight are affected by the four different diets but not the mortality. Preimaginal development time was 45.61, 41.38, 34.66 and 38.96 days on artificial diet, pistachio, pomegranate and semi-artificial diet, respectively. Since the short life cycle indicates suitability of the food source, it can be concluded that pomegranate is the most suitable food, whereas semi-artificial diet, pistachio and artificial diet are the next suitable diets for mass rearing of the PFM, respectively. Considering that pomegranate is not always available, artificial and semi-artificial diets have to be used for mass rearing. Even though artificial diet is undesirable in comparison to natural and semi-artificial diets, it is easily accessible and more economical. The pupae that were fed with pistachio were heavier than those on other

diets and their adult moths were quite bigger. Al-Izzi *et al.* (1987) reared PFM on artificial diet containing casein, soy protein, dextrose, cellulose, agar, olive oil, vitamins and other minerals at  $27 \pm 2$  °C,  $65 \pm 5\%$  relative humidity and 16:8 (L: D) h. They reported 5, 20, 1 and 8 days for stages: egg, larval, prepupal and pupal, respectively. Our results under similar environmental condition for artificial diet were 4.7, 31, 1.7 and 8 days. Hung *et al.* (2003) used two diets for rearing of PFM. The first diet had been made by artificial material including: agar, sweet corn, yeast, cholesterol, ascorbic acid, aureomycin, formalin, phosphates and water. The second diet consisted of almond slices. The results of life table parameters including eggs, larvae and female and male pupae for artificial diet were  $3.9 \pm 0.3$ ,  $21.4 \pm 4.9$ ,  $11.4 \pm 4.5$  and  $9.6 \pm 4.8$  days, respectively and for natural diet were  $4 \pm 0.3$ ,  $68 \pm 15.7$ ,  $10.6 \pm 5.2$  and  $16.5 \pm 3.4$  days respectively. In their tests the period of larval stage was longer but the pupal stage was shorter than findings of our test. These differences in results could be due to types of food and different experimental conditions. Mediouni and Dhouibi (2007) used an artificial diet for rearing of the PFM that contained wheat bran, yeast, sucrose, salt mixture, vitamin C, aureomycine and glycerin ( $28 \pm 1$  °C,  $75 \pm 5\%$  R.H and 15 L:9 D photoperiod)). The results of biological parameters data based on egg incubation, the first, second, third, fourth, fifth instar larval

and pupal stages were 3.14, 5.01, 4.58, 4.56, 4.67, 4.89 and 7.33, respectively. This artificial diet was similar to artificial diet in this study. The slight difference may be due to differences in experimental conditions. Ghavami (2006) used pomegranate (peel and seed) for laboratory rearing. She reported 3.61, 22.83, and 1.22 and 7 days respectively for egg incubation, larvae, prepupae and pupae stages for natural diet which are similar to our data. Norouzi *et al.* (2008) reported rearing of PFM on four diets including pomegranate, pistachio, fig and date. His results for using pomegranate and pistachio were similar to ours. Zare (2011) studied developmental parameters of this pest on three cultivars of pomegranate including Malass Dane Siah, Gabri and Shahvar at  $30 \pm 1$  °C,  $70 \pm 5\%$  RH and 16L: 8D. His results on Shahvar cultivar were similar to our results on Meykhosh cultivar. Fakharzadeh (2004) found that some substrate like protein (wheat flour, soy flour and corn flour), and syrup (honey and date syrup) are very effective on biological parameters like larval period, pupae weight, adult longevity and fertility rate. Mozaffarian *et al.* (2008) reported that the moths that were fed with fig, pistachio and walnut have higher fecundity and overwintered more successfully than those that were fed with pomegranate. Since the short life cycle on the food source indicates its suitability for the pest, it can be concluded that pomegranate is the most suitable food for PFM and this factor can increase the release of sex pheromone and males attraction. On the other hand, some insects use host plant compounds to synthesize sex pheromones or sex pheromone precursor Chemicals from host plants often intensify insect responses to sex pheromones (Landolt and Phillips, 1997). When PFM was fed with pomegranate and placed in the pomegranate orchard and received chemicals of host, it released sex pheromones in response to particular cues from host plant, so that catch was more in comparison with other treatments. Researches conducted on the

European corn borer in France showed that in wind tunnel experiments males fed on maize (*Zea mais* L.) were attracted to females fed on maize and males fed on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.) were attracted to females fed on mugwort and hop (Pélozuelo *et al.*, 2004). Therefore more catch of traps containing female moths fed on pomegranate is also likewise justified. Some parts of the calling behavior like the starting time of pheromone release and duration of pheromone secreting are quite specific and depend on limited time of day or night (Calatayud *et al.*, 2007). In present study, catch time started from 23 o'clock to 4 but the maximum catch was recorded between 11 to 12 pm. Whereas when the moth encountered darkness before being attached to trap, calling behavior started immediately at 10 p.m. without any delay. Vetter *et al.* (1997) reported that female PFM reared at photoperiod of 16:8 (L: D) started their mating behavior from 4-6 hours after darkening time. This result is consistent with the time of catch onset in our study. Ziaadini *et al.*, (2010) studied the calling behavior of the PFM in three different geographical populations. They found that in all 3 population calling behavior started five hours after darkening phase and then it gradually increased. We found that the efficiency of female moths on trap increased if they were kept for four hours in darkness

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 to find a suitable diet for mass rearing of the PFM. Based on this research, we know that the most desirable food for this pest is pomegranate but it is not affordable, while it can be replaced with artificial diet which is applicable and economic. In addition, details about pheromone traps catch can be helpful to improve the efficiency of catching. A suitable diet (and keeping the PFM females in the dark for a few hours before being used in the traps, can increase the attraction of males.

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## بررسی اثر چند رژیم غذایی کرم گلوگاه انار، *Apomyelois ceratoniae* Zeller (Lep.: Pyralidae) روی پارامترهای زیستی و کارآیی تله‌های فرمونی طبیعی در باغ‌های انار

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**چکیده:** کرم گلوگاه *Apomyelois ceratoniae* Zell. (Lep.: Pyralidae) مهم‌ترین آفت انار در ایران است. در این پژوهش اثر چهار رژیم غذایی شامل پسته، انار، غذای نیمه‌مصنوعی و مصنوعی روی پارامترهای زیستی کرم گلوگاه در دمای  $20 \pm 2$  درجه سلسیوس، رطوبت نسبی  $5 \pm 65$  درصد و دوره نوری روشنایی: تاریکی ۸:۱۶ ساعت بررسی شد. نتایج نشان داد که بیش‌ترین طول دوره انکوباسیون تخم، دوره لاروی و دوره پیش از بلوغ روی غذای مصنوعی و کم‌ترین آن روی انار مشاهده شد. بیش‌ترین طول بدن لارو سن ۵ و وزن شفیره سه روزه روی پسته و کم‌ترین آن روی غذای مصنوعی بود. هم‌چنین اثر این چهار رژیم غذایی بر کارآیی تله‌های فرمونی در باغ انار رقم ملس یزدی ۲۰ ساله بررسی شد. نتایج نشان داد که اثر غذا روی شکار حشرات نر در تله‌ها معنی‌دار بود. زمانی که شب‌پره‌های ماده کرم گلوگاه انار که با رژیم غذایی دانه‌های انار تغذیه شدند در تله‌های فرمونی استفاده شد بیش‌ترین شکار ثبت شد. به‌طور کلی شکار تله‌ها از ساعت ۲۳ تا ۴ صبح بود و حداکثر شکار تله‌ها در ساعت‌های ۲۳ و ۲۴ ثبت شد. بررسی اثر رژیم غذایی و ساعت بر شکار تله‌ها معنی‌دار نبود یعنی رژیم‌های غذایی مختلف اثری روی ساعت شروع و پایان و ساعت حداکثر شکار تله‌ها نداشتند. دستاورد این پژوهش برای انتخاب و پرورش مناسب کرم گلوگاه انار برای برنامه مدیریت تلفیقی مبارزه با آفت می‌تواند مورد استفاده قرار گیرد.

**واژگان کلیدی:** پارامترهای زیستی، زمان شکار، کرم گلوگاه انار، رقم ملس یزدی، رژیم غذایی مصنوعی