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Original Research Article

The First Attempt to Rear Olive Leaf Moth [(*Palpita unionalis* (Hübner) (Lepidoptera:Pyralidae)] on Artificial Diet

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ABSTRACT

The olive leaf moth, *Palpita unionalis* (Hübner) (Lepidoptera:Pyralidae) is an important pest in olive orchards. To facilitate planning of pest management strategies, it is important to develop an artifical diet for laboratory rearing. Several artifical diets previously developed to rear lepidopteran larvae were tested in this study. The artifical diet developed for *Spodoptera* spp. based on pinto bean, wheat germ and torula yeast was the most adequate diet to rear *P. unionalis* among the diets tested. Addition of host plant material in the artificial diet showed no positive effect on larval growth and survival. The larval and pupal recovery was similar for artificial diets as well as for its natural food, olive leaves. The diet is nutritionally suitable to rear olive leaf moth in the laboratory.

Key Words: *Palpita unionalis*, Artifical Diet, Rearing, Olive leaves

Zeytin fidan tırtılının [(*Palpita unionalis* (Hübner) (Lepidoptera:Pyralidae)] yapay besin üzerinde ilk yetiştirilme durumu

ÖZET

Zeytin fidan tırtılı, *Palpita unionalis* (Hübner) (Lepidoptera:Pyralidae) zeytin bahçelerinde görülen önemli bir zararlıdır. Zararlılarla mücadele stratejilerinin planlanmasında, zararlıların laboratuvarda yetiştirilmesi için yapay besinin geliştirilmesi önemlidir. Bu çalışmada, daha önce lepidopter larvalarının beslenmesi için geliştirilen yapay besinler test edildi. *Spodoptera* spp. beslenmesi için geliştirilen barbunya unu, buğday ruşeyni, torula mayası içeren yapay besin, *P. unionalis*'in beslenmesinde test edilen besinler içinde en uygun ve yeterli besin olduğu tespit edildi. Larva gelişmesi ve canlı kalma oranı üzerinde, besine ilave edilen konukçu bitki materyalinin pozitif etkisinin olmadığı belirlendi. Larva ve pupa gelişmesi bakımında konukçu bitki zeytin yapraklarında ve yapay besin üzerinde benzer sonuçlar elde edildi. Laboratuvarda zeytin fidan tırtılının yetiştirilmesinde test edilen besinin uygun olduğu belirlendi.

Anahtar Kelimeler: *Palpita unionalis*, Yapay Besin, Yetiştirme, Zeytin Yaprakları

INTRODUCTION

The olive leaf moth, *Palpita unionalis* (Hübner), (Lepidoptera: Pyralidae: Pyraustinae) is an important lepidopteran pest species, reported in Turkey (Kovancı et al., 2006; Kumral et al., 2007; Yılmaz and Genç, 2012), Egypt (El-Kifl et al., 1974), Greece (Vassilaina-Alexopoulou and Santorini, 1973), Israel (Avidov and Rosen, 1961) and Italy (Triggiani, 1971, Fodale ve ark. 1990, Antonelli and Rossi, 2004). It is originated in the Mediterranean Basin (Tzanakakis, 2003; Noori and Shirazi, 2012). The pest has been reported on Oleaceae genera *Oleae, Jasminum, Ligustrum, Fraxinus* and *Phillyrea* (Athanassiou et al., 2004). Larvae of this moth is increasingly damaging young shoots, causing severe damage to olive trees and nursery trees, and damaging ripening olive fruits (Athanassiou et al., 2004; Kumral et al., 2007). As olives, ornamentals such as jasmine cultivated for perfume production in France (Gargani, 1999) are damaged from larval attacks on leaves and flower buds.

An extensive study on the biology of the pest revealed that it has six instars based on head capsule measurements in the laboratory on olive leaves (Yılmaz and Genç, 2012). Athanassiou et al (2004) studied male trapping techniquies in the field. Pinto and Salerno (1994) recorded *Apanteles syleptae* as an effective parasitoid. Khaghaninia (2002) reported a tachinid pupal parasitoid, *Carcelia* sp. in the Tarome Olia region.

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Arambrourg (1986) recommended the use of *Bacillus thuringiensis* var. *kurstaki* as a biological control. Organophosphate and pyrethoid insecticides like dimethoate, deltamethrin, cypermethrin are used for chemical control in olive cultivation (López-Villalta, 1999) in some countries, but there is no registrated insecticide or active compound in Turkey. However, there are increasing reports of olive leaf moth damage in Turkey, and there is no precise method to control this pest in olive orchards. Many factors contribute to olive leaf moth population fluctuations, including distribution, alternative hosts, natural enemies and insecticides resistance.

It is important to know the basic biology of the pest so as to understand factors involved in population fluctuations. Several studies have been published on the biology of olive leaf moth (Noori and Shirazi 2012; Yılmaz and Genç, 2012), life tables (Kumral et al., 2007), population fluctuations, and some of the enviromental factors that may affect it (Kovancı et al., 2006). Rearing on an artificial diet has not been examined. To develop and improve IPM strategies, studies should be done to understand its natural life cycle, pest behavior, possibilities to use *Bacillus thuringiensis* and insecticides, augmentation of natural enemies and other control approaches. To conduct these studies, it is important to have large numbers of healthy and uniformly developed insects.

Rearing of *P. unionalis* mainly depends on natural host plants such as olive leaves. Availability of host leaves, transfering larvae from old leaves to fresh leaves, and susceptibility of larvae to pathogen infections are important issues to be considered, and such rearing is costly in time and labor. A laboratory rearing method and artificial diet for *P. unionalis* needs to be developed.

In the present study, we tested different artificial diets, determined the effects of some biological parameters, and maintained an olive leaf moth colony on an artificial diet in the laboratory.

MATERIALS AND METHODS

Biological Material

Olive leaf moth larvae were obtained from infested olive orchards in Çanakkale province, Turkey. They were reared for about 10 generations on olive leaves, *Oleae europaea* (Yılmaz and Genç, 2012) at 24 ± 1 °C, 65% RH and 16:8 h L:D in the growth chamber. The adults were fed a 10% honey solution. The eggs were collected daily and placed in a sealed petri dish for 3-4 days before hatching. Neonates from these eggs were used throughout this study.

Ingredients of Experimental Artificial Diets

Larval diets investigated were based on known formulations previously used in rearing lepidopterans. The composition of the experimental diets and their quantity in a diet mixture, are shown in Table 1. Six artificial diet treatments were offered to newly hatched olive leaf moth larvae. *Bicyclus* sp.(Lepidoptera:Nymphalidae) (Diet I) (Bergomaz and Boppre, 1986; Holloway et al., 1991), *Mussidiae nigrivenella* Ragonot (Lepidoptera:Pyralidae) (Diet II) (Poitout and Bues 1970; Moyal and Tran, 1991), *Cactoblastis cactorum* (Berg) (Lepidoptera:Pyralidae) (Diet III) (Kfir, 1992; Zimmermann, 2003; Marti et al., 2008), *Trichoplusia ni* Hübner (Lepidoptera:Noctuidae) and *Spodoptera* spp. (Lepidoptera:Noctuidae) (Diet IV) (Guy et al.,1985; Genc and Nation, 2004). We added 10 % of freeze dried olive leaves into Diet IV as a phagostimulant (Diet V). The positive control diet was olive leaf shoots. Experimental diets were freshly prepared before each experiment, and olive leaf shoots were obtained from olive orchards in Çanakkale, Turkey (Figure 1). All ingredients were weighed and stirred into the mixture until the diet ingredients appeared to be homogeneously mixed. Vitamins and mold inhibitors were added at the end. The prepared diet was transferred to plastic boxes. After the diet solidified, it was stored at 4 ºC until used.

Rearing Procedures

Diets were sliced into small pieces, weighing 30-35 g and placed into plastic containers (10 x 15 cm or 8 cm in diameter, 10 cm in length) for the biological study. Newly hatched (n=30) larvae were transferred with a fine hair brush on each of 4 replicates of each diet. Larval development was monitored daily under an Olympus SZX9 stereozoom microscope. Larval development period was measured from egg hatch to the first day of

pupation. Petri dishes with diet and larvae were kept at 24±1°C, 16:8 photoperiod and 65% R.H, until larval development was completed. When the pupae were observed on the surface of the diet, they were recorded and harvested daily and kept in the laboratory, and sexed by examination of the ventral of last abdominal segments (based on abdomen tip) before adult emergence. Newly emerged adults were paired and kept in cages containing olive leave shoots for oviposition. Ten percent honey solution was provided in adult cages. Leaves with egg masses were collected and replaced daily with fresh leaves.

For each diet, the following biological parameters were recorded: (1) larval recovery (%) (total number of larvae produced from 30 neonates), (2) pupal recovery (total number of pupae produced from the number of original larvae), (3) percentage of adult emergence, (4) larval development time (from hatching until first larvae started to crawl out of diet to pupation , (5) pupal development time (from the onset of pupation until the emergence of the first adults), adult fecundity and longevity. Diets were evaluated against the control (natural) diet. The most appropiate diet with the best larval recovery in the previous test was used in further experiments along with by addition of 10% freeze dried olive leaves and olive leave shoots to rear the next generation, with 4 replicates of diet.

Data Analysis

Descriptive statistics as Means ± Standard error (SE) were calculated. Data were transformed. The differences among means were determined by analysis of variance (ANOVA) and Tukeys's test was used for larval recovery, pupation and adult emergence (P = 0.05). The larval recovery (%), pupal weight (mg), pupation percentage, adult emergence were analyzed with JMP version 5.01 statistical software (SAS, 1999).

Table 1. Composition of the diets previously developed for lepidopteran species and tested for *Palpita unionalis*.

Diet Ingredients	**Diet I**	**Diet II**	**Diet III**	**Diet IV**
Pinto Bean	18.7 g	-	63 g	19 g
Brewers Yeast	4.4 g	2 g	18.6 g	-
Agar	3.77 g	3 g	4.5 g	6.83 g
Wheat germ	-	-	-	14 g
Torula yeast	-	-	-	8 g
Corn meal	-	22.4 g	-	-
Sucrose	-	-	10 g	-
Methyl paraben	0.125 g	0.2 g	0.6 g	0.5 g
Sorbic acid	0.125 g	-	0.42 g	0.3 g
Cholestrol	0.125 g	-	-	-
Ascorbic acid	0.875 g	-	0.96 g	1 g
Streptomycin	0.1 g	0.04 g	-	0.01 g
Acid mix	-	-	-	0.3 ml
Sodium benzoate	-	0.25 g	-	-
Formaldehyde	-	-	-	1 ml
Vitamin mix (BioServ #F8095)	-	-	-	0,56 g
Olive oil	7 ml	-	-	-
Water	100 ml	120 ml	250 ml	182 ml

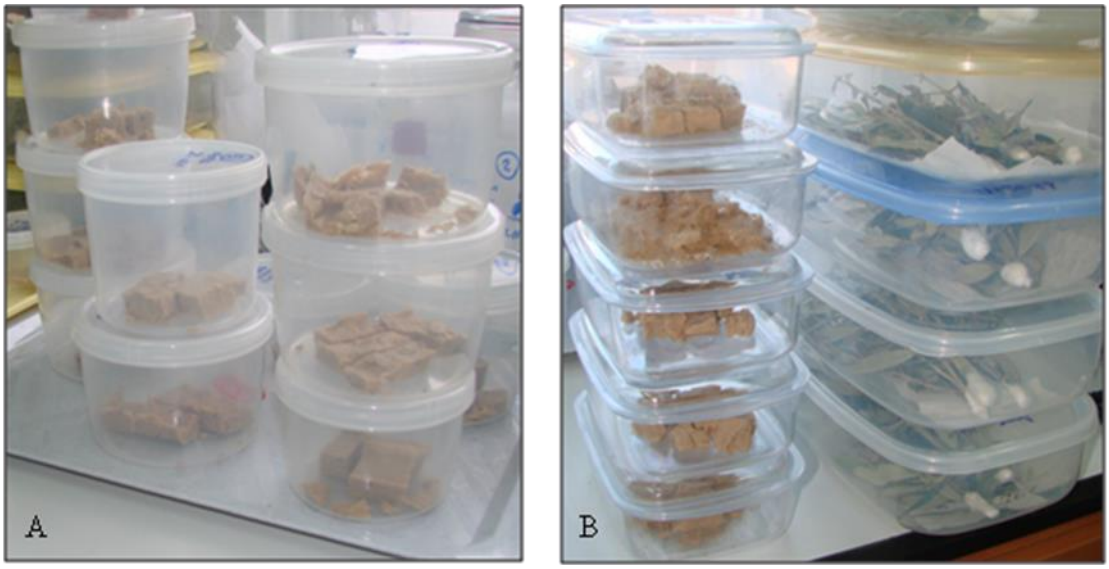

Figure 1. A view of experimental larval diets Diet IV (A), Diet V and control (B)

RESULTS AND DISCUSSION

Five different artificial diets along with a control diet of fresh olive leaves were tested in this study. Larvae refused to feed on Diet I and survived only 3 days. Diet II was not suitable for olive leave moth larvae (Figure 2A), as larvae fed only 7-10 days on this diet in the laboratory. They prepared silken filament and attached themselves on the diet to feed on (Figure 2A) and completed their first instar. However, they all died after 10 days of feeding. Larval mortality was 100 % on Diet I and Diet II.

Larvae survived on Diet III and completed their development, with larval survival ranging from 10 to 15% (Figure 2B), and 3-5% pupation; however, there was no adult emergence. Therefore, Diet III was not suitable for rearing olive leaf moth larvae.

Out of the five artificial diets evaluated, Diet IV and Diet V were more suitable for larval development of olive leaf moth along with control (Diet VI) diet (Figure 2C and 2D). Comparative performance based upon larval recovery (%), pupal recovery (%), adult emergence (%), duration in larval and pupal stages, and adult fecundity is given in Table 2. Larval recovery from previously formulated diets tested ranged from 55.8 to 65.0 %. No significant differences in larval and pupal recovery were detected between artificial diets and natural host plants (control), but larval development time on artificial diets was longer compared with the control diet (Table 2) ($F=9.15; df=2,11; P<0.05$). Pupal duration on artificial diets was significantly longer than control ($F=4.71; df=2,18; P<0.05$), but no significant differences were found for pupal duration between artificial diets (Diet IV and Diet V). Both diets have a consistency that allows olive leaf moth larvae to move and feed easily and pupate. Pupae measured 12.9 ± 0.9 mm long, 3.2 ± 0.2 mm wide, and weighed 60.5 ± 2.1 mg on Diet IV (Figure 3A), and measured about 12.9 ± 0.2 mm long, 2.9 ± 0.2 mm, and weighed 49.4 ± 2.5 mg on Diet V. On Diet VI, pupae measured 13.6 ± 0.1 mm long, 3.1 ± 0.1 mm wide, and weighed 63.9 ± 2.4 mg (Figure 3B). The comparisons indicated that the highest pupal weight was obtained on the control diet. The lowest average was observed with Diet V, while Diet IV presented an intermediate average ($F=12.73; df=2,33; P<0.05$). Adult emergence was significantly different between artificial diets and the control diet, but not between artificial diets. The comparable performance of olive leaf moth on artificial diet indicated that Diet IV was good enough for the development and survival of the pest in the laboratory. Addition of host plant material to the artificial diet (Diet V) did not improve overall performance, but natural host plant was stimulatory and promoted feeding activity. The larvae might possible have difficulty in using some nutrients from artificial diet that do not need to be digested and caused delay in larval development (Table 2).

The natural diet of fresh olive leaves is clearly the best diet in the tests, but Diet IV (Guy et al., 1985) (Fig. 3A) is nearly as good as the control diet (Fig. 3B), with only minor, but significant, variations in evaluated parameters (Table 2 and Figure 3).

Table 2. Comparative performance of *P.unionalis* larvae reared on artificial diets and natural host plant (olive leaves) (mean±SE)

Parameters	**Diet Type**		
	Diet IV	**Diet V**	**Control (olive leaves)**
Larval recovery (%)	56.3±6.4A	65.5±7.1A	63.7±2.8 A
Pupal recovery (%)	54.5±7.2 A	48.5±1.7 A	60.2±2.8 A
Adult emergence (%)	27.2±3.9 B	27.7±2.9 B	43.7±5.3 A
Larval duration (day)	33.1±4.8A	33.7±3.1A	23.3±2.1 B
Pupal duration (day)	11.5±1.9A	11.2±1.8A	9.8±1.1 B

Means within a row followed by the same letter do not differ significantly by Tukey test (P <0.05).

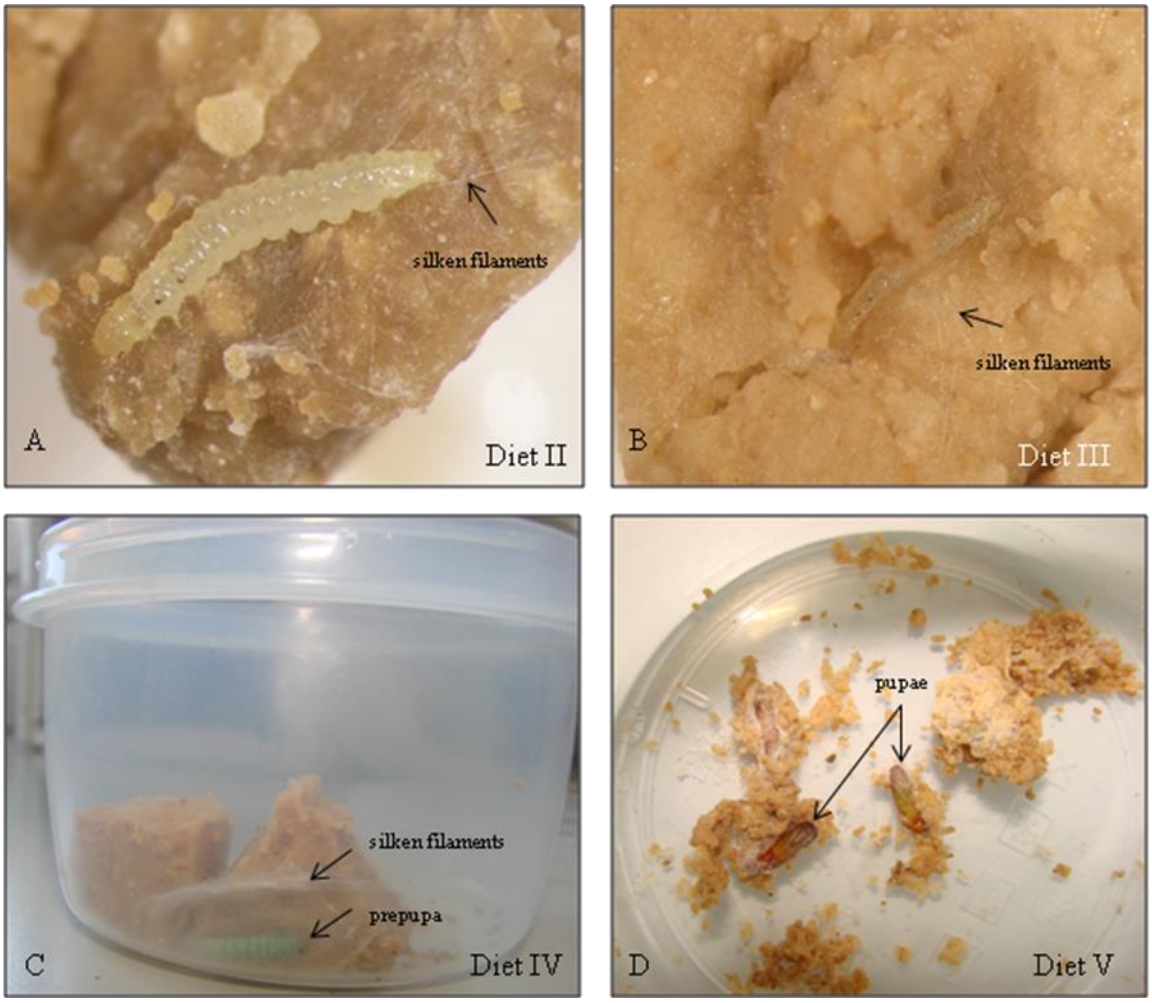

Figure 2. Rearing olive leaf moth larvae on different artificial diets

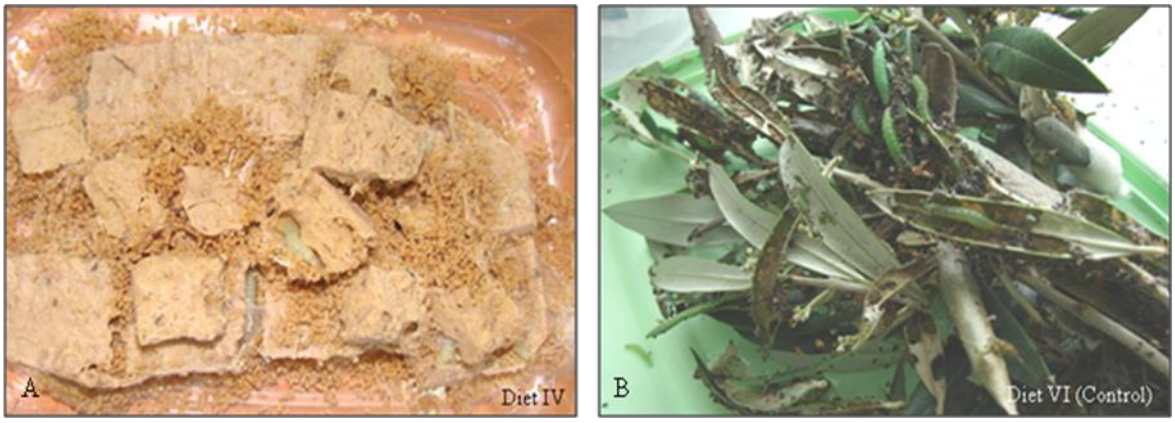

Figure 3. *Palpita unionalis* larvae feed on Diet IV (A) and olive leave shoots (B) as control

Data on oviposition, pre and postoviposition, adult longevity, fecundity and egg hatching are given in Table 3. The preoviposition period lasted about 1.5 to 1.8 days for olive leaf moth adults exposed to different larval diets (Table 3). Oviposition period ranged from 5.2 to 8.9 days and postoviposition lasted about 1.8-2.3 days. The longevity values of 8.6 and 11 days for the males and females and a female laid 385 eggs in her lifespan (Table 3). The lowest mean fecundity was 272 eggs on Diet V, longevity with 9 and 10.10 days for males and females respectively. The highest fecundity mean was 412 eggs on control, along with 3.9 and 14.9 days longevity for males and females respectively. Egg hatching was 70 % on control diet however, 42% and 43% of eggs hatched on Diet IV and Diet V respectively.

The mean total developmental period of the olive leaf moth reared under laboratory conditions (24±1°C, 65% and 16:8 photoperiod) ranged from 55 to 59 days on Diet IV and 45 to 54 days on olive leave shoots. However, total development period on olive was reported as as 27.52 days by Kumral et al. (2007) and 27 days by Shehata et al (2003), 26 days by Vassilaina-Alexopoulou and Santorini (1973). Biological parameters depend on the rearing conditions, host plants, temperatures, humidity, and even the suitability of host plant parts etc. The adults survival also can be extended by feeding both flowering plants and honey solutions in the laboratory.

The olive leaf moth larvae were tested on Diet IV for two generations. There were no difference on fitness parameters between generations. This study clearly demonstrated that olive leaf moth performed well on Diet IV. Addition of host plant leaves into artificial diet (Diet V) did not promote larval survival. The fitness parameters on the diet were very similar to those on natural host and there were no statistical differences between artifical diets (Diet IV and V). These results suggest that Diet IV can provide sufficient nutrition for growth, survival and fecundity. This indicates that Diet IV which have been previously used to rear *Spodoptera* spp. (Guy et al., 1985) and *Phyciodes phaon* (Genc and Nation, 2004) can be used succesfully for *P. unionalis* and possibly will be an alternative to natural host for laboratory rearing purposes.

There are many factors that affect larval feeding on artifical diet such as proportion and balance of nutrients, moisture level and texture of diet (Nation, 2002). Addition of some host plant materials in artificial diets often promotes growth, survival and fecundity, and may act as necessary stimulants for oviposition and successful rearing (Genc and Nation, 2004). In this study, the content of natural host material was 10% of the total weight of dry ingredients and we expected that to produce higher viability and fecundity, but it did not have any positive effect.

Table 3. Some biological parameter of adult *Palpita unionalis* exposed to different larval diets regimens (n=10) (mean±SE)

Biological Parameter	**Diet Type**		
	Diet IV	Diet V	Control (olive leaves)
Preoviposition (day)	1.5 ± 0.52 A	1.8 ± 0.22 A	1.8 ± 0.63 A
Oviposition (day)	7.9 ± 2.23 A	5.2 ± 3.64 B	8.9 ± 2.42 A
Postoviposition (day)	1.9 ± 0.36 A	2.3 ± 0.56 A	1.8 ± 0.63 A
Adult longevity (♂)	8.6 ± 2.06 B	9.0 ± 2.90 B	14 ± 3.72 A
Adult longevity (♀)	11 ± 2.90 B	10.1 ± 4.93 B	15 ± 2.47 A
Fecundity (number)	385± 44.10 AB	272 ± 88.10 B	412 ± 102.10 A
Hatched eggs (number)	118± 68.04 C	159 ± 36.64 B	290 ± 47.10 A

Means within a row followed by the same letter do not differ signifiantly by Tukey test (P <0.05).

CONCLUSIONS

This study is the first to record the rearing of olive leaf moth on artificial diet, although the data refers to two generations only. The tested artifical diet IV proved sufficient growth, development and decent survivorship. This can be prepared easily and has a good texture supporting larval development of olive leaf moth. Further studies should be conducted to determine the possibility of rearing larvae on the artifical diet for successive generations.

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