

Laboratory Rearing of Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on Artificial Diet

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Abstract

The tomato leaf miner, *Tuta absoluta* (Meyrick) is a crucial tomato pest distributed from South America to Europe and introduced in Turkey in 2009. Since then it is one of the most important tomato pest in our country. Studies on laboratory adaptation and rearing on artificial diet are important to develop new control approaches for the insect pests. In this study, some biological parameters such as larval viability, larval duration, pupal recovery and adult emergence of *T. absoluta* were determined on six different artificial diets and tomato leaves. The larval viability was the highest on tomato leaves followed by Diet 5, originally developed for *Plutella xylostella* and Diet 6 or HG diet, formulated here for this study. Larval mortality was high on the first instars for all tested diets. The tomato leaf miner was reared on both Diet 5 and 6 for four consecutive generations in the laboratory. *Plutella xylostella* diet was tested for the first time for tomato leaf miner and indicated that it's suitable for laboratory rearing and maintains.

Keywords: Artificial diet, *Tuta absoluta*, tomato leaf miner, tomato borer

Domates Yaprak Güvesi *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'nın Laboratuvarda Yapay Diyetle Geliştirilmesi

Özet

Domates yaprak güvesi, *Tuta absoluta* (Meyrick) domates bitkisinin kritik öneme sahip zararlısıdır. Güney Amerika'dan Avrupa'ya yayılmıştır ve 2009 yılında Türkiye'ye ilk kez saptanmıştır. O zamandan itibaren ülkemizde domatesin en önemli zararlılarından biri haline gelmiştir. Laboratuvar adaptasyonu ve yapay diyet çalışmaları zararlı böceklerle yeni mücadele yaklaşımlarının geliştirilmesi için oldukça önemlidir. Bu çalışmada, domates yaprak güvesinin altı farklı yapay diyet ile domates yaprakları üzerinde larva canlılık oranı, larva gelişme süresi, pupa olma oranı ve oluşan ergin sayıları gibi bazı biyolojik gelişme parametreleri çalışılmıştır. Domates yapraklarında larvaların canlılık oranlarının en yüksek olduğu belirlenmiş ve bunu orijinal olarak *Plutella xylostella* için geliştirilen Diet 5 ve bu çalışma kapsamında oluşturulan Diyet 6 ya da HG diyeti takip etmiştir. Test edilen tüm yapay diyetlerde en fazla larva ölümlerinin ilk larva döneminde olduğu kaydedilmiştir. Domates yaprak güvesi hem Diyet 5 hem de Diyet 6 da devamlı olarak dört generasyon boyunca laboratuvarda yetiştirilmiştir. *Plutella xylostella* yapay diyetinin ilk kez laboratuvarda domates yaprak güvesinin yetiştirilmesinde kullanılmasının uygun olduğu tespit edilmiştir.

Anahtar Kelimeler: Sunni besin, *Tuta absoluta*, domates yaprak güvesi, domates güvesi

Introduction

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is commonly known as tomato leaf miner or tomato borer first recorded in 2009 in Turkey (Kılıç, 2010). It is a non-indigenous pest and originating from South America that devastates

mainly tomato plants, *Lycopersicum esculentum* Mill (EPPO, 2010). It is distributed in the Mediterranean region, Iberian Peninsula in 2006 (Urbaneja et al., 2009; 2012), France, Italy, Greece, and the United Kingdom (Desneux et al., 2010). The pest was outbreak in 2009 in many countries

along with Turkey (Kılıç, 2010; Erler et al., 2010; Unlu, 2011, Unlu, 2012; Öztemiz, 2012; Urbeneja et al., 2012; Garzia et al., 2012; Cocco et al., 2013).

It is a microlepidopterous and oligophagous (Siqueira et al., 2000) attacks on potato (*Solanum tuberosum* L.) (Pastrana, 1967; Galarza, 1984; Fernandez and Montagne, 1990; Unlu, 2012), eggplant (*Solanum melongena*) (Galarza, 1984; Viggiani et al., 2009), tobacco (*Nicotiana tabacum* L.) and some non-solanaceous cultivars such as *Solanum nigrum* (Vargas, 1970), *S. eleagnifolium* L., *Datura ferox* L. *D. stramonium* L. (Garcia and Espul, 1982) and *Solanum americanum* (Fernandez and Montagne, 1990). A weed called as *Chenopodium album* L. is also recently recorded as a new host (Ögür et al., 2014).

The tomato leaf miner is a multivoltine species, mines all aerial structures of plants mainly on leaves and fruits, causing 80-100% of crop losses (Desneux et al., 2010; Urbaneja et al., 2012; 2013). Females lay eggs on the host leaves and neonates bores the leaf mesophyll and damage caused by larvae, mining leaves reducing photosynthesis and yield (Desneux et al., 2010).

There are studies regarding life cycle, biology and development (Pereyra and Sanchez, 2006; Krechmer and Foerster, 2017; Cuthbertson et al., 2013; Erdoğan and Babaroğlu, 2014), biological control (Luna et al., 2007; Bajonero et al., 2008; Doğanlar and Yiğit, 2011; Öztemiz, 2012) and granulovirus Phop GV (Mascarin et al., 2010). It is important to rear large numbers of larvae in health and easily with low cost to conduct these studies. Rearing of tomato leaf miner in the laboratory mostly depends on its primary host mostly tomato leaves. There are some important issues need to be considered in mass or continuous rearing in the laboratory such as maintaining of host plants in large numbers, availability of fresh host leaves, transferring larvae to new leaves, preventing chemical applications from host plants used in rearing, a space to keep plants, rearing cost and labor (Yılmaz and Genç, 2013; Zou et al., 2015; Nation, 2016).

Artificial diets have been known as crucial to studies on pest's biology, development, ecology, biological control and management (Nation, 2016). The understanding of pest nutritional requirements and ecology could allow developing appropriate artificial diets and also accelerate laboratory adaptation. Tomato leaf miner was reared on artificial diet previously (Greene et al. 1976; Mihsfeldt and Parra, 1999; Marin et al., 2002; Garcia-Suabita et al., 2012; Bajonero and Parra, 2017). In order to find an appropriate diet to rear an insect in the laboratory, firstly test specifically known diets if any and secondly start with

modification of some diets, formulated for closely related species or having similar nutritional requirements (Nation, 2016; Bajonero and Parra, 2017).

The aim of the present work was to test different artificial diets for laboratory rearing of tomato leaf miner.

Materials and Methods

Tuta absoluta colony rearing

The laboratory colony was established from the stock population of tomato leaf miner maintained in Akdeniz University, Department of Plant Protection, Antalya, Turkey. Tomato leaf miner was reared continuously on a local tomato cultivar for about a year. The larvae were reared in Tupperware plastic container (30 cm x 21 cm x 7 cm) having a paper towel at the bottom and ventilated lid with chiffon. After larval development, pupae were harvested and transferred in a Petri dish (9 cm diameter). Adults were kept in rearing cages (45 cm x 45 cm x 45 cm) having tomato plants and a honey-water solution (10%). Infested tomato plants were removed and checked for eggs daily then replaced with new tomato plants. Eggs were collected with a fine brush from the tomato leaflets and incubated in the moisturized black filter paper, placed in a Petri dish. Embryonic developments were monitored daily under Olympus SZX9 stereo zoom microscope until the larvae hatching. Newly hatched (neonate) larvae were used in the experiments. All experiments were taken in the laboratory conditions, at 23±2°C, 65% RH and 16:8 (light:dark) photoperiod.

Tested Artificial Diets

Several artificial diets were tested to rear larvae of tomato leaf miner in the laboratory (Table 1). Diet 1, used for tomato leaf miner previously (Mihsfeldt and Parra, 1999 modified from Greene et al. 1976; Bajonera and Parra, 2017), diet 2 artificial medium developed for noctuid species (Shorey and Hale, 1965), diet 3 (Jha et al., 2012) and diet 4 (Hamed and Nadeem, 2008) were used to rear *Helicoverpa armigera*, diet 5, proposed by Shelton (2012) for the development of *Plutella xylostella*, diet 5, formulated for this study. The natural larval host of tomato leaves was included as positive control for all the tests. All experimental diets were freshly prepared and kept at +4 °C until used. The artificial diet mixtures were composed of agar, cellulose, casein, yeast, soy flour, pinto bean, dextrose, salt mixture, methyl paraben, sorbic acid, vitamine mixtures and ascorbic acid. Some tested diet formulations were included freeze-dried tomato leaves (10% of dry

weight of diet ingredients) as feeding stimulants (Genc and Nation, 2004).

Rearing procedure

Newly hatched larvae were transferred on a small cube of tested diet (4 cm x 2 cm x 1 cm) with a fine brush under the microscope. For each diet, 100 neonate larvae were used with three replications. Larval viability, pupal recovery and

adult emergence were evaluated. The diet was changed if needed. In addition, as positive control, 100 neonate larvae were also reared on tomato leaflets with moisturized cotton wrapped around the petiole. Data were analyzed through ANOVA (SAS 1999). The data were normalized and LSD test was used to determine separation and significance of means ($P \leq 0.05$) (SAS 1999).

Table 1. Composition of tested artificial diets for *Tuta absoluta*

INGREDIENTS	Diet (1)	Diet (2)	Diet (3)	Diet (4)	Diet (5)	Diet (6)
White beans	75 g	426 g	15g	60 g	-	-
Wheat germ	60g	-	5.5 g	-	175 g	8 g
Soybean flour	30 g	-	-	-	-	-
Casein	37.5 g	-	-	-	126 g	8 g
Yeast	37.5g	64g	6 g	6 g	-	6 g
Ascorbic acid	3.6 g	7 g	0.6 g	1.2 g	14 g	2 g
Sorbic acid	1.8 g	2 g	0.15 g	0.45 g	-	0.5 g
Methyl paraben	3 g	4 g	0.17 g	0.75 g	5.4 g	0.3 g
Antibiotic	113mg	-	-	0.01 g	4 g	-
Formaldehyde (10%)	3.6 ml	4 ml	-	1.5 ml	-	-
Vitamin mixture	9 ml	-	-	0.06 g	36 g	2 g
Agar	23 g	25 g	3.75 g	4.5 g	96 g	7.5 g
Water	1200 ml	1280 ml	130 ml	100 ml	3000 ml	250 ml
L-cysteine	-	-	0.06 g	-	-	-
Sucrose	-	-	-	6 g	135 g	6 g
Choline chloride (20%)	-	-	-	3 ml	-	-
Cholesterol	-	-	-	0.06 g	-	0.5 g
Wheat germ oil	-	-	-	0.06 ml	-	-
Wesson Salt Mix	-	-	-	-	36 g	2.5 g
Potassium sorbate	-	-	-	-	4 g	-
Cellulose	-	-	-	-	25 g	3 g
Propyl gallate	-	-	-	-	0.8 g	-
Raw linseed oil	-	-	-	-	30 ml	-
KOH (43.6%)	-	-	-	-	9 ml	-
Formaldehyde (37%)	-	-	-	-	3 ml	0.2 ml
Soy hydrolysate	-	-	-	-	-	4 g
Raw olive oil	-	-	-	-	-	2.5 ml
KOH (4 M)	-	-	-	-	-	2.5 ml
Tomato leave powder	-	-	-	-	5%	5%

Result and Discussion

The first attempt was to rear larvae of tomato leaf miner on Diet 1 which was not successful and proved insufficient and no survivorship. The mortality of the first instars was about 90% on Diet 1 during the first three days. Then Diets 2, 3 and 4 were tested but they were also not supported larval growth and development. Neonate larvae were not kept alive on these diets on the second day of the experiments. However, tomato leaf miner larvae were reared on the natural host, tomato leaflets as positive control along with tested artificial Diets 1, 2, 3 and 4 (Figure 1 and Table 2). Larvae fed on tomato leaves (Figure 1) and the percentage of

larval viability was 65.70 ± 8.39 , larval period lasted about 11.85 ± 2.75 days, the percentage of pupal recovery was about 58.77 ± 6.71 and 52 adults were emerged (Table 2 and Figure 1). Adult longevity was about 14-18 days.

After the negative results obtained in the previous diet experiments, Diet 5 was tested (Table 2) which was used for rearing *Plutella xylostella* previously (Shelton, 2012). Diet 5 was yielded about $59.42 \pm 8.25\%$ larval viability (Table 2 and Figure 2). The larval duration was 15.81 ± 3.31 days and pupal recovery was about $49.82 \pm 7.19\%$ (Table 2). The number of adult emergence was 46 (19♀:27♂) on Diet 5. Emerged adults were mated in adult rearing cages and mated females laid eggs

on tomato leaflets. The egg viability was about 83%. The following generation was set up with F1 eggs on Diet 5. So, tomato leaf miner was continuously reared on Diet 5 for four generations. The larval feeding and their feeding behavior (making feeding galleries and mines) were shown

in Figure 2. They made tunnels during feeding and left frasses indicating that larvae feed on diet and also the diet was supported larval growth and development adequately (Figure 2).



Figure 1. A view of tomato leaf miner larvae reared on tomato leaflets as positive control (a) larval rearing container (b) tomato leaflets with moisturized cotton with parafilm (c) larval tunnels and (d) larval silk during feeding

Another diet was formulated in the laboratory according to lepidopteran nutritional requests and called as HG diet or Diet 6 (Table 1). Larval viability was about $55.53 \pm 7.66\%$ with 19.26 ± 3.01 days larval duration on Diet 6 (Table 2). After larval development was completed on Diet 6, the pupal viability was $43.16 \pm 8.27\%$. The number of adult emergence was 40 (15♀:25♂) as first generation on Diet 6. Females were able to lay

eggs and the egg viability was about 80%. The results indicated that performance of Diet 6 was also successful when compared to all tested parameters. The larval feeding was indicated in Figure 3. The opening holes and tunnels on diet 6 were visible. Larvae were feeding inside the loose-silky area and the larval frasses were observed. Mature larva was also shown in Figure 3C.

Table 2. Larval performances of tomato leaf miner on Diet 5, Diet 6 and tomato leaves (Mean \pm SD)

Diets	Larval Viability (%)	Larval duration (days)	Pupal recovery (%)	No. Adult emergence
Diet 1	-	-	-	-
Diet 2	-	-	-	-
Diet 3	-	-	-	-
Diet 4	-	-	-	-
Diet 5	59.42 ± 8.25 b	15.81 ± 3.31 b	49.82 ± 7.19 b	46 b
Diet 6	55.53 ± 7.66 c	19.26 ± 3.01 c	43.16 ± 8.27 c	40 c
Tomato leaves	65.70 ± 8.39 a	11.85 ± 2.75 a	58.77 ± 6.71 a	52 a

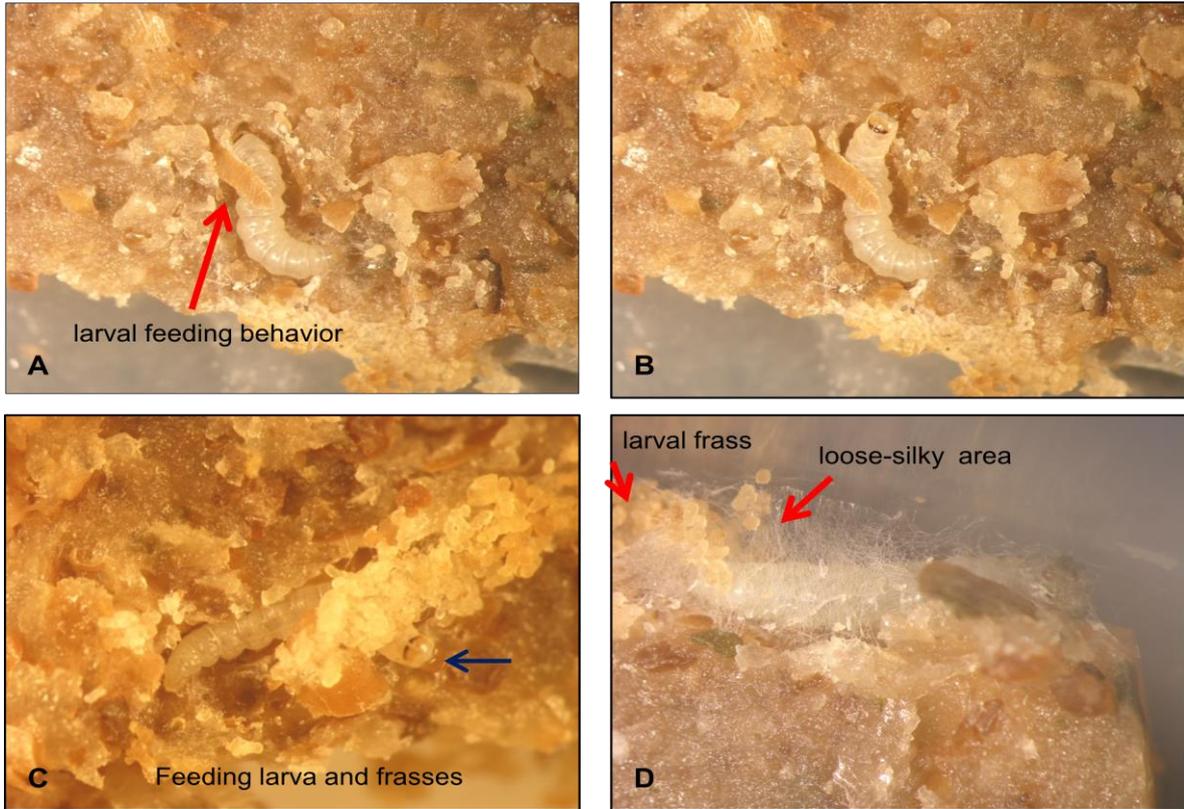


Figure 2. Tomato leaf miner larvae on Diet 5, (a) larval feeding, (b) tunneling behavior (c) larval frasses and (d) feeding larvae inside the loose-silky area



Figure 3. Tomato leaf miner larvae on Diet 6, (a), (b) larval feeding, (c) mature larva on diet and (d) feeding larvae inside the loose-silky area with observed frass

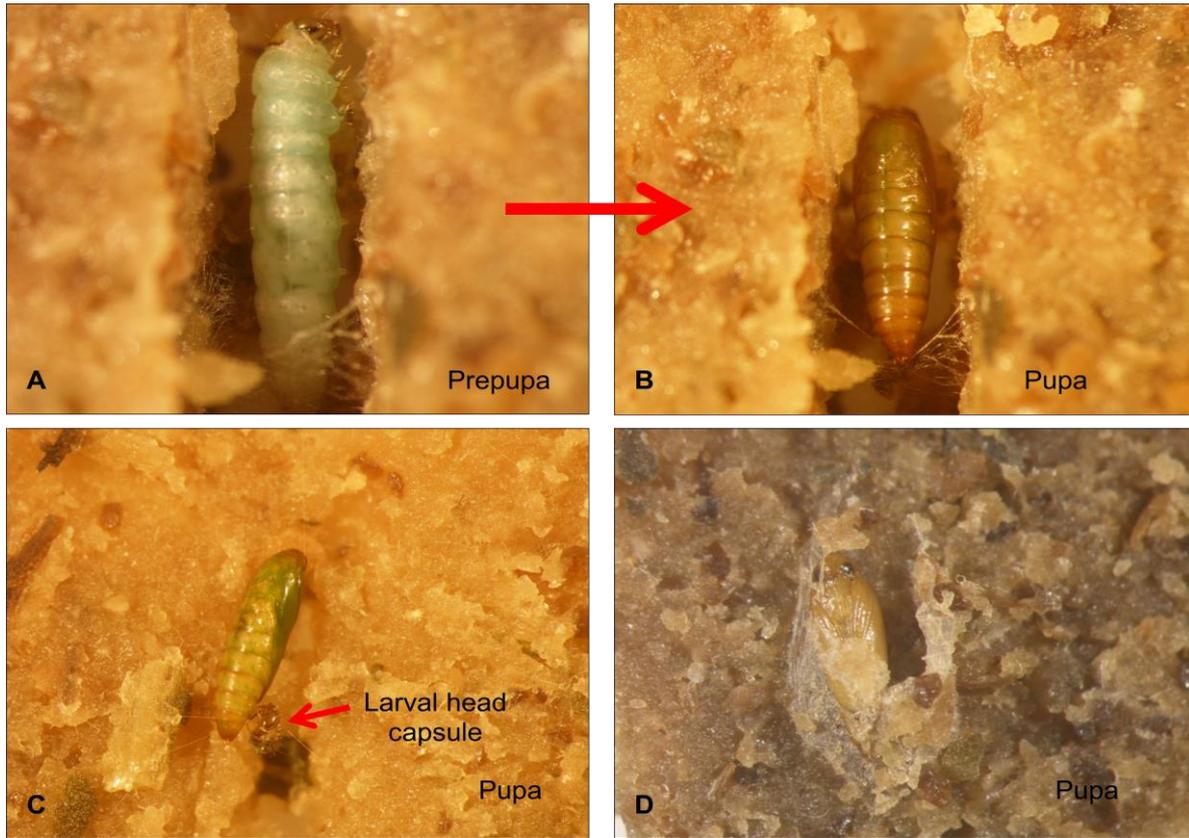


Figure 4. The prepupal and pupal stages of tomato leaf miner, (a) prepupa, (b) pupa, (c) pupa with the shredded larval head capsule and (d) pupa in silken tunnel

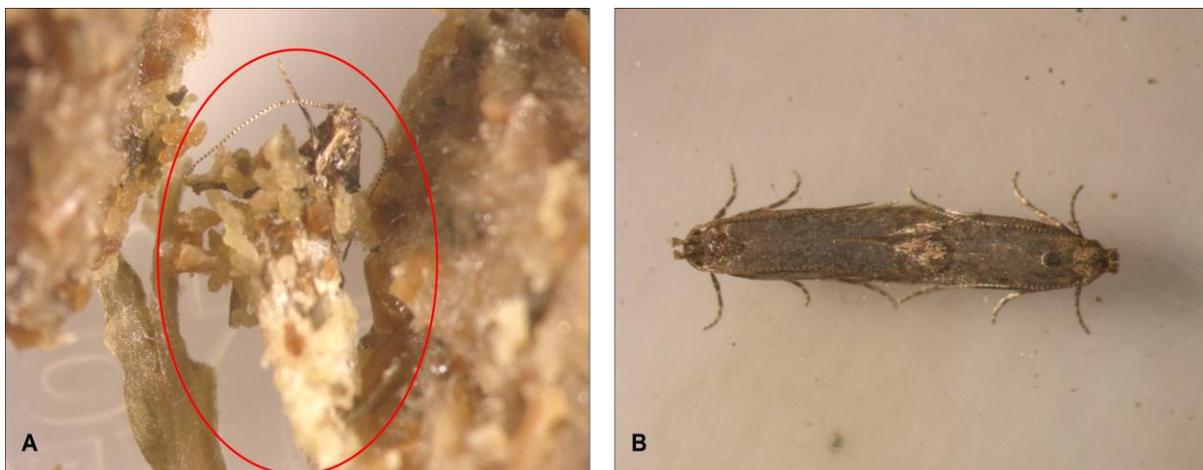


Figure 5. Tomato leaf miner, the emerged adult in diet larval rearing container (a) and (b) mated individuals

In the present study, several artificial diets were tested for larval growth of tomato leaf miner but only two artificial diets (Diet 5 and Diet 6) were supported larval developments. Diet 5 and Diet 6 were used to rear tomato leaf miner continuously four generations in the laboratory.

Bajonero and Parra, (2017) recently reared the tomato leaf miner on a modified diet from Berger (1963) for eight generations with about

75% overall viability, having cellulose, wheat germ and casein. Diet 1 was tested previously (Mihsfeldt and Parra, 1999) and formulated for tomato leaf miner, having 18.52 days of larval duration with a success of 70.56% larval viability (Mihsfeldt and Parra, 1999). According to Bajonero and Parra (2017), larval duration of tomato leaf miner was 15.83 ± 4.25 days and larval viability was $35.33 \pm 3.91\%$ on the same diet (Diet 1). This diet

formulation tested and tried different times but it did not work in this study to rear tomato leaf miner. It is important to get the neonate larvae feeding on the diet, because high mortality usually occurred at this stage (Zalucki et al., 2002; Mihsfeldt and Parra, 1999).

There are several reasons for an artificial diet to be accepted or rejected by larvae such as the nutritional value of the diet, diet texture or structure, cohesion and phagostimulants (Vanderzant et al., 1957; Vanderzant and Richardson, 1963; Mihsfeldt and Parra, 1999; Genc, 2006; Genç, 2008; Yılmaz and Genç, 2013). Lyophilized host plant materials play a role as a phagostimulant which were usually added about 10% of the tested diet's dry ingredients into the lepidopteran artificial diets (Genc and Nation, 2004; Nation, 2016) just to get the larvae start feeding on the diet. Here, there were added about 5% of lyophilized tomato leaves on Diet 5 and Diet 6 (Table 1). Cellulose supports the success of the artificial diets even though not having any nutritional value but plays an important role in diet's textures when it is added as a jelling agent in dipteran (Tsitsipis and Kontos, 1983) and lepidopteran pests (Mihsfeldt and Parra, 1999; Shelton, 2012). Another important issue is diet's surface moisture, causing trapped neonates not to crawl around the diet (Anthon et al., 1971). It is also known that antimicrobial agents in artificial diets may cause feeding inhibition (Dunkel and Read, 1991).

Conclusion

In this study, the diet formulated originally for *Plutella xylostella* was tested successfully for the first time to rear larvae of tomato leaf miner, *Tuta absoluta*, as Diet 5. In order to develop or test an artificial diet for a larva, one approach is started with simple modifications of known diets and selecting some formulations based on nutritional and behavioral aspects of closely related species (Damos et al., 2009). Diet 6 was formulated here and similar results to Diet 5 were obtained in terms of larval viability, larval duration, pupal recovery and adult emergence (Table 2). Life cycle duration was 22-30 days on tomato leaves (Barrientos et al., 1998) and was about 31-34 days on Diet 5 and 30-35 days on Diet 6. Tomato leaf miner was reared on Diet 5 and Diet 6 and laboratory colony was maintained successfully.

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