

**LIFE CYCLE AND DETERMINATION OF LARVAL INSTARS OF
Leucinodes orbonalis GUENEE (LEPIDOPTERA: CRAMBIDAE)**

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ABSTRACT

The eggplant shoot and fruit borer, *Leucinodes orbonalis* Guenee is a significant and destructive pest of eggplant causing considerable damage to shoot and fruits in South and Southeast Asian countries including Malaysia. Therefore, it is important to understanding the life cycle if this species in order to implement the best management strategy. The objective of this study was to investigate the life cycle *L. orbonalis*. The result on the biology of *L. orbonalis* recorded that the incubation period was 5.5 ± 0.09 days; the total larval period was 12.07 ± 0.19 days; and underwent five larval instars stages which are characterized by the size of the cephalic capsule. The pupal stage took 10.37 ± 0.10 days. The mean lifespan of adult females and males moths were recorded at 4.75 ± 0.17 days and 3.4 ± 0.23 days respectively. The duration of the total life cycle of the females and males moths were 32.67 ± 0.55 days and 31.32 ± 0.61 days respectively. On average, each female produced 174 ± 22.01 eggs with the sex ratio of female to male was almost 2:1. The eggs were measured at 0.66 ± 0.01 mm in length and 0.31 ± 0.01 mm in width. The full-grown larvae were measured at 15.49 ± 0.50 mm body length and 1.37 ± 0.04 mm head capsule. Pupae were measured at 7.79 ± 0.25 mm in length with 2.49 ± 0.07 mm body width, females were on average of 9.63 ± 0.24 mm in length with 21.41 ± 0.35 mm wing width whereas the mean value for male was measured 7.43 ± 0.13 mm body length with 16.28 ± 0.36 mm wing width. Result of this research may be helpful to develop an effective pest management strategy.

Keywords: Pest, eggplant shoot, fruit borer, *Leucinodes orbonalis*, life cycle

ABSTRAK

Pengorek pucuk dan buah iaitu *Leucinodes orbonalis* Guenee merupakan perosak yang signifikan bagi tanaman terung di Asia Tenggara dan Asia Selatan termasuk Malaysia. Oleh yang demikian, ianya adalah penting untuk mengetahui kitar hidup spesies ini bagi memastikan implmentasi strategi pengurusan yang terbaik dapat dilaksanakan. Objektif kajian ialah menentukan kitar hidup lengkap *L. orbonalis*. Hasil kajian menunjukkan tempoh inkubasi direkodkan selama 5.5 ± 0.09 hari. Jangka masa larva ialah 12.07 ± 0.19 hari yang terdiri daripada 5 peringkat instar yang ditentukan melalui saiz kapsul sefalik. Peringkat pupa mengambil masa

selama 10.37 ± 0.10 hari. Jangka masa hayat betina dan jantan dewasa ialah masing-masing 4.75 ± 0.17 hari and 3.4 ± 0.23 hari. Manakala kitar hayat lengkap betina dan jantan dewasa adalah masing-masing 32.67 ± 0.55 hari and 31.32 ± 0.61 hari. Secara puratanya, betina dewasa menghasilkan 174 ± 22.01 telur dengan nisbah betina:jantan ialah 2:1. Saiz telur adalah 0.66 ± 0.01 mm in panjang dan 0.31 ± 0.01 mm in lebar. Saiz larva peringkat akhir ialah 15.49 ± 0.50 mm panjang dengan saiz kapsul kepala ialah 1.37 ± 0.04 mm. Saiz pupa adalah 7.79 ± 0.25 mm panjang dan 2.49 ± 0.07 mm lebar. Betina dewasa berukuran 9.63 ± 0.24 mm panjang dengan kelebaran sayap 21.41 ± 0.35 mm, manakala jantan dewasa pula berukuran 7.43 ± 0.13 mm panjang dengan kelebaran sayap 16.28 ± 0.36 mm. Hasil kajian ini dijangkakan dapat membantu dalam membangunkan strategi pengurusan perosak ini dengan lebih efektif.

Kata kunci: Perosak, pucuk terung, pengorek buah, *Leucinodes orbonalis*, kitar hayat

INTRODUCTION

Eggplant, *Solanum melongena* L. is one of the most important vegetable cultivated in many temperate and tropical regions around the world (FAO 2003). The color, shape and size of the fruit varies, but the most widely cultivated is purple color cultivar type (Musa et al. 2020). Eggplant has been reported to contain phytochemical composition that is acknowledged as a nutraceutical (Scalzo et al. 2016). The total eggplant cultivation area in Malaysia was substantially smaller with 2407 hectares yielding 39311 tonnes. It is cultivated in all states of Malaysia nevertheless Johor and Pahang are the major producers of eggplant, covering an area of 479.64 hectares and 447.77 hectares, respectively (DOA 2018). However eggplant and many other vegetables are largely produced by smallholder farmers and this industry provides thousands of jobs for people, particularly rural dwellers (Sani et al. 2020).

Insect pest and diseases are the major constraints of vegetable production. During growth, several species of insect pests, such as armyworms, caterpillars, beetles, aphids, whiteflies, mites and thrips, cause severe damage to various vegetable crops (Sani et al. 2020). Eggplant shoot and fruit borer, *Leucinodes orbonalis* (Lepidoptera: Crambidae) is an insect pest that causes major economic losses to eggplant (Latif et al. 2009; Rashid et al. 2008). According to Lal (1975), eggplant shoot and fruit borer is widely distributed in South and Southeast Asia. The *L. orbonalis* is found mostly in areas with hot and humid climate (Srinivasan 2008). The damage is estimated as high as 70% in India (Singh et al. 2008). It is prevalent throughout the year and considered as a major constrain to the production of eggplant across the globe. The damages were caused by the larvae which feed the inside of the fruits, thereby leaving a large exit hole upon pupate. Consequently, the damages inflicted by the infestation of the larvae on eggplant fruits, thus reducing the market values of the fruits, thereby rendering them unfit for human consumption (Alam et al. 2003).

The life cycle of *L. orbonalis* has been widely investigated by researchers and the findings were highly varied. For instance, in India Rohokale et al. (2018) reported that *L. orbonalis* completes its life cycle in 25.65 days. Meanwhile, studies by Onekutu et al. (2013), Yadav et al. (2015), Laichattiwat et al. (2017), and Singla et al. (2018) found that *L. orbonalis* completes its lifecycle within 28.17, 29.23, 27.43, and 36.72 days respectively. Studies also showed that the larvae underwent five instars stages (Bindu et al. 2013) while some studies (FAO 2003; Prabhat & Johnsen 2000) reported that larvae underwent six larval instars stages. Considering the above different findings in the life cycle, the present investigation attempted to determine the complete life cycle and to describe the morphology of *L. orbonalis* on eggplant in laboratory conditions. Furthermore, the life cycle of *L. orbonalis* in Malaysia is yet to be

reported and these findings are necessary for the adoption of appropriate control measures towards this significant pest species.

MATERIALS AND METHODS

Insect Sampling

The infested fruits with *L. orbonalis* were collected from the organic research farm of the Faculty of Agriculture, Universiti Putra Malaysia (UPM) (2°59' 02.2" N, 101°44' 04.8" E) with the elevation of 60 m from the sea level. Additionally, some infested fruits were also obtained from the vegetable market of Pasar Borong Selangor, Malaysia. The infested fruits were kept in a rearing cage (20.0×15.0×15.0 cm) that was covered with a piece of muslin cloth. The fruits were replaced daily in the morning hours to avoid fungal contamination until pupation. Proper hygienic conditions were sustained throughout the trial time. The late instar that emerged from the fruits pupated in spun cocoons on the edge of muslin cloth covers were collected. These pupae were kept individually in a plastic container (7.5×7.5×8.0 cm). All samples were brought to the Entomology laboratory, Department of Plant Protection, Faculty of Agriculture, UPM, Selangor, Malaysia for rearing purpose.

Culturing and Rearing of *Leucinodes orbonalis*

The life cycle of *L. orbonalis* was studied in laboratory conditions from September to December 2019 for one generation using eggplant as source of food. The techniques of laboratory cultures, morphometric measurement, and rearing of *L. orbonalis* in this study were adopted from Sandanayake (1987); Wankhede et al. (2009); Mohd Noor et al. 2014 and Roseli et al. (2019). Whole trials were maintained in the laboratory under the settings; the temperature of 28±2°C, the relative humidity of 70±10%, and 12:12h photoperiod.

After the emergence, pairs of the male and female adults were transferred to a separate cage (20.0×12.0×12.0 cm) supplied with 30-day-old potted seedling that was installed in the cage for the mating and oviposition process. Adults *L. orbonalis* were fed with a 10% sugar solution soaked in cotton swabs. The opening of the cage was covered with a piece of muslin cloth and tightened with its cover. The cotton swabs were replaced daily to avoid fungus growth.

Determination of Incubation, Larval and Pupal Period

The eggs were laid singly or in batches on the foliage, most often on the lower surface of young leaves and sometimes laid on muslin cloth. The leaves with eggs were cut into small pieces with a scissor and then transferred from the rearing cage into glass petri dishes (10.0×10.0 cm). Ten eggs per petri dish were replicated five times to study the life cycle. The observation was done separately on each petri dish and then recorded.

After hatching, the larvae were reared individually in a plastic container (7.5×7.5×8.0 cm). In each plastic container, the first instar larva was padded with moistened tissue paper and provided with twigs, soft leaves, and tender buds of the eggplant. The container was labelled as the date of the first instar. For second and third instar larvae, soft fruits of eggplant were given as they feed inside the fruits by making tunnels in the fruit. The larval period was recorded from hatching until the initiation of pupation. When larvae pupated, they were kept undisturbed until the completion of the cycle and then observed daily in the morning hours (7:00-8:00 am) to study the pupal period during the months of the experiment. The pupation period was determined from the pupal formation until the emergence of the adult.

Determination of Larval Instars Stages

Morphometric determination of larvae began with newly hatched larvae (one-day-old first instar larvae). Ten individuals were taken out daily and then soaked in hot water for one minute. Then, the dead larvae were put on filter paper to be dried for two minutes before the morphometric measurement was taken. The determination process was extended until all larvae in the plastic container pupated. Head capsule width was measured at the largest diameter of the head capsule and the body length of each larva was measured from the edge of the head capsule until the edge of the posterior end. All measurements were done using a Dino-eye microscope eyepiece (ANMO electronics corporation, Taiwan) connected to a Wild Herrburgg Microscope (M3Z Switzerland). Photos of specimens were captured using Dino-capture 2.0 software.

Determination of Longevity, Reproduction, and Sex Ratio

Twenty pairs of newly emerged adults (one male with one female) were placed in separate rearing cages (20.0×12.0×12.0 cm) containing a 30-day-old potted seedling of eggplant for egg-laying. The adult moths were fed with 10% sugar solution soaked in cotton. The mouth of each cage was coated with a piece of muslin cloth and tightened with a cover. The number of eggs produced by each pair, the adult's life span, and the ratio number of males to females were recorded. Additionally, morphometric measurements of the adult were conducted at this stage.

Morphological Description of *Leucinodes orbonalis* at Every Stage

External morphology was observed and described based on colour, shape, and marks on the body. The morphometric determination was taken from 10 individuals for each stage. The measurements include i) width and length of egg, ii) width of cephalic capsule size and body length of the larvae, iii) width and length of the pupae, iv) wingspan of adult moths, v) wing length of adults, vi) the body length and breadth of adults. As the egg's shape is about oval and not round, the breadth of the egg was measured at the broadest part, which is perpendicular to the longest part (length).

The determination of breadth for pupae were measured at the widest part of the body while body length was measured from the tip of the cephalic capsule until the edge of the posterior end. The adults wingspan were measured between the two forewing tips in a straight-line on the wingspread position. The determination of wing length was taken from the base of the forewing at thorax to the tip of the forewing in a straight-line. In addition, for adults body length measurement, the distance from the edge of the head until the edge of the abdomen.

Data Analysis

Data on the width of head capsule and body length of larval instars, male and female lifespan, the adult morphometric measurement (wingspan width, wings length, and body length) were subjected to one-way ANOVA and the means were separated with LSD test at 0.05 level of significance. All data were analyzed using SAS version 9.4.

RESULTS

Developmental Time of *Leucinodes orbonalis*

Incubation period

In this experiment, it was found that the incubation period of *L. orbonalis* varied between five to six days. The mean value was recorded as 5.5 ± 0.09 days (Table 1). The eggs hatched into light brown larvae.

Table 1. The mean duration of eggs, larvae, pupae, and life span adult

Phase		Range (day)	Mean±SE (day/No)	Total adult lifespan (day)
Egg (Incubation period)		5-6	5.5±0.09	
Larvae		10-13	12.06±0.19	
Pupae		9-11	10.36±0.10	
Adults (mated)	female	3-6	4.75±0.17	32.67±0.55
	male	2-5	3.4±0.23	31.32±0.61
Adults (un-mated)	female	4-6	5.40±0.15	33.32±0.53
	male	4-5	4.45±0.11	32.37±0.49
Fecundity		40-369	174±22.01	
Sex ratio (female: male)			2:01	

Larval Period and Instars Determination

It was found that the larval period ranged from 10 to 13 days with an average of 12.06 ± 0.19 days (Table 1). The mean duration of the first, second, third, fourth, and fifth instar larvae were recorded as three, two, two, two, and three days, respectively. The body length and cephalic capsule size of each instar are summarized in Table 2.

Table 2. Measurement of the width of the head capsule and body length of different larval instars

Larval instars	Mean±SE (mm)			
	Range(day)	Width of cephalic capsule	Range(day)	Body length
First instar	0.14-0.24	0.21±0.00e	1.06-2.52	1.63±0.08e
Second instar	0.24-0.42	0.36±0.01d	2.08-3.75	2.89±0.12d
Third instar	0.63-1.25	0.81±0.04c	4.92-9.79	7.52±0.32c
Fourth instar	0.71-1.27	1.09±0.03b	6.72-14.76	10.41±0.53b
Fifth instar	1.13-1.73	1.37±0.04a	10.90-19.89	15.49±0.50a

Means with the same letter within the column are not significantly different at $p = 0.05$

The size of the cephalic capsule varied with each instar and increased between instars. A substantial increase in size of the cephalic capsule happened four times: from day three (0.20 ± 0.03 mm) to four (0.34 ± 0.04 mm), day five (0.37 ± 0.02 mm) to six (0.85 ± 0.19 mm), day seven (0.77 ± 0.16 mm) to eight (1.07 ± 0.17 mm) and from day nine (1.10 ± 0.11 mm) to 10th (1.27 ± 0.06 mm), with a corresponding increase of 0.14, 0.48, 0.30 and 0.17 mm. The head capsule for all larval instars were significantly different ($F = 281.23$; $df = 4, 95$; $P < 0.05$). The mean value for the width of head capsule for first, second, third, fourth, and fifth instar were 0.21 ± 0.00 mm, 0.36 ± 0.01 mm, 0.81 ± 0.04 mm, 1.09 ± 0.03 mm, and 1.37 ± 0.04 mm, respectively. Similarly, the length of body among all larval instars were significantly different ($F = 245.91$; $df = 4, 95$; $P < 0.05$), with values of 1.63 ± 0.08 mm (first instar), 2.89 ± 0.11 mm (second instar), 7.52 ± 0.32 mm (third instar), 10.41 ± 0.52 mm (fourth instar) and eventually, 15.49 ± 0.49 mm (fifth instar).

Pupal Period, Longevity of Adult, and Fecundity

The average duration of the pupal stage was recorded at 10.36 ± 0.10 days, which ranged from 9-11 days. The results showed significant differences in the longevity between male and female ($F = 21.28$; $df = 1, 38$; $P < 0.05$). Overall, female moths survived longer than males. The mean

longevity of adult female moth was observed as 4.75 ± 0.17 days, ranging from 3-6 days whereas, in the case of the male, it varied between 2 to 5 days with an average of 3.4 ± 0.23 days. Similarly, there was a significant difference ($F = 24.94$; $df = 1, 38$; $P < 0.05$) in the longevity between un-mated male and female moths. The longevity of un-mated adults was recorded differently from mated adult moths; for male, it ranged between 4-5 days with an average of 4.45 ± 0.11 days, whereas for female, it varied from 4 to 6 days with an average of 5.40 ± 0.15 days. The fecundity ranged between 40 and 369 eggs/female, and the mean fecundity value was recorded as 174 ± 2.01 (Table 1).

Total Life Span and Sex Ratio

Table 1 shows the mean duration of eggs, larvae, pupae, and adult life span. The overall lifespan of *L. orbonalis* (mated) was recorded as 32.67 ± 0.55 days for females and 31.32 ± 0.61 days for males. Meanwhile, the life span for unmated adults were 33.32 ± 0.53 for female and 32.37 ± 0.49 for male. The number of emerged male and female showed that the sex ratio (proportion of male to a female) during one generation was 1:2.

Morphological Descriptions of *Leucinodes orbonalis* at Every Stage

Eggs

Female moths deposited eggs in batches of five or six, or singly glued on the lower surface of leaves, green stems, flower buds, and on the surface of muslin cloth. Freshly laid eggs were oval-shaped and creamy-white. The mean length varied from 0.59-0.78 mm, and the mean width varied from 0.25-0.41 mm. The length was measured 0.66 ± 0.01 mm, and the width was measured 0.31 ± 0.01 mm. When the embryos matured, the eggs gradually became orange and eventually, turned to black with a prominent black spot at the tip of the egg which was the developing head of the larvae (Figure 1).



Figure 1. Batches of eggs laid by female adult

Larvae

The freshly hatched larva was glabrous, light brown, and the body colour of the larvae subsequently changed from whitish to dark pinkish. Five larval instars were observed after eggs emergence. Various instars of larvae had similar morphology and varied mainly in size as measured in micrometer. A full-grown larva was pinkish with a cylindrical shape. The larvae's head was dark brown and had powerful mandibles for chewing food. The thorax part of the body showed three separate segments with a pair of well-developed thoracic legs on each segment. Five pairs of prolegs were distributed in ten segments of the abdomen (Figure 2).



Figure 2. Larva

First instar

After the completion of the incubation period, the freshly hatched larvae were glabrous, light brown. During this instar, it was observed that larva fed on eggplant twigs, soft leaves, tender buds, and freshly sliced eggplant. The width of the cephalic head capsule ranged from 1.06 to 2.52 mm with an average of 1.63 ± 0.08 mm and the body length varied from 0.14 to 0.24 mm with an average of 0.21 ± 0.00 mm (Figure 3).



Figure 3. First instar larva

Second instar

The second instar larvae produced regular holes in the eggplant slices filled with exuviae. Few minutes before molting, the larva stopped its feeding. It was observed that the size of the second larva was larger than the first instar larva with the minimum and maximum length was measured 2.08-3.75 mm with an average body length of 2.89 ± 0.12 mm, whereas the head capsule width varied from 0.24 mm to 0.42 mm with an average head capsule width of 0.36 ± 0.01 mm (Table 2). The head was narrower than prothorax with brownish color. The mesothorax was seen obvious with black colour compared to the first instar larva (Figure 4).

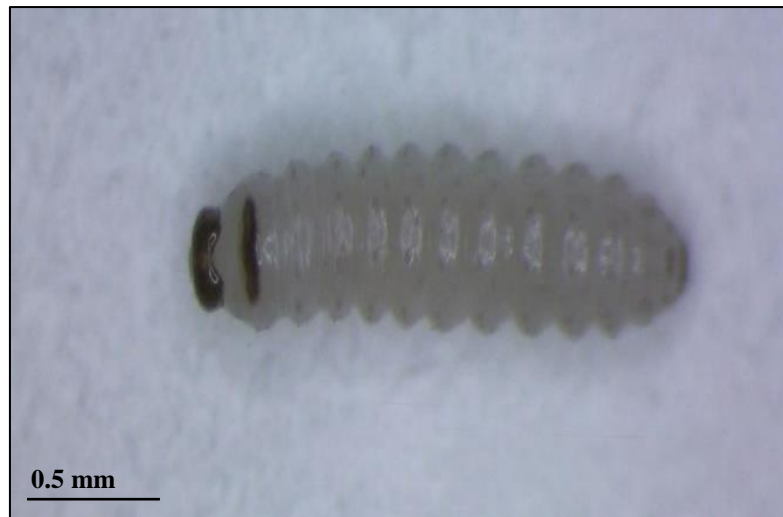


Figure 4. Second instar larva

Third instar

In this stage small brown spots were visible on the dorsal and ventral sides of the body with the more spots observed on the dorsal side. Fine spine was observed from the middle of each spot (Figure 5). The larvae were 7.52 ± 0.32 mm in body length, ranging from 4.92 to 9.79 mm while the average width of the head capsule was 0.81 ± 0.04 mm, ranging from 0.63 to 1.25 mm (Table 2).

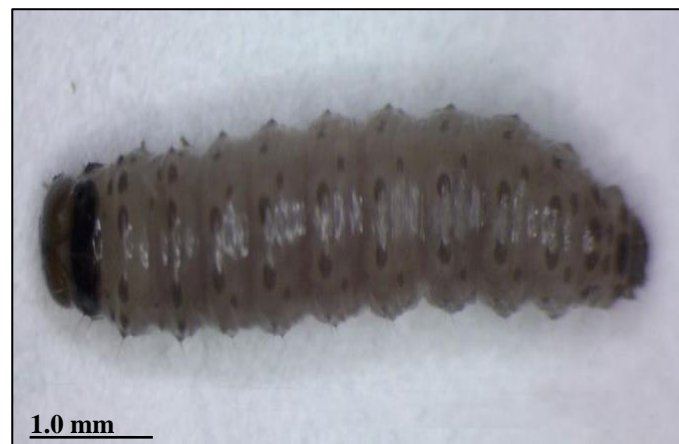


Figure 5. Third instar larva

Fourth instar

The larvae were brownish and fed voraciously compared to the previous instars (Figure 6). The body length was 10.41 ± 0.53 mm with the head capsule width of 1.09 ± 0.03 mm. The body length ranged from 6.72 to 14.76 mm, and the width of the head capsule ranged from 0.71 to 1.27 mm (Table 2).



Figure 6. Fourth instar larva

Fifth instar

The morphology of the larva was almost similar with previous instar, except there was a commensurate addition in the size of different parts of the body. The pupating behavior was more obvious at this stage. Larvae at this stage were observed to have a cylindrical shape, pinkish-brown with five pairs of well-developed prolegs and distinct segments of the thorax. In contrast with the previous instars during this stage, the larvae did not seem to have a voracious appetite (Figure 7). The larvae were measured with an average body length of 15.49 ± 0.50 mm, and the mean width of the cephalic capsule was 1.37 ± 0.04 mm (Table 2).



Figure 7. Fifth instar larva

Pupae

Pupation usually took place on muslin cloth, on soil, around the periphery of the rearing cage, and sometimes on the sepal of the fruit. The freshly developed pupae were pinkish, and whenever they are near to emergence, their colour changed into dark brown. They were elongated oval in shape, gradually tapering posteriorly with almost straight abdomen; wing margins extended up to the posterior margin of the abdominal segment. Male and female can be distinguished at the pupal stage based on the genital slit. The distance between the last

abdominal segment and the genital slit was wider in males while the distance in the case of females, the distance between the two was narrower (Figure 8). The results showed that the length of the pupae varied between 6.64 and 9.80 mm, with an average body length of 7.79 ± 0.25 mm, whereas the body width ranged between 2.15 and 3.09 mm with an average body width of 2.49 ± 0.07 mm, and the head capsule was recorded as 1.38 ± 0.05 mm (Table 3).



Figure 8. Pupa

Adult stage

The adult moths were smaller in size with whitish wings, blackish-brown head and thorax. The wings had brown and dark markings which were bigger on the forewings. Hind wings were seen with black dots and angled margin. The female moth had a swollen abdomen that seemed to be ovate in structure, whereas in the male moth, it was cylindrical and thinner (Figure 9). The abdominal tip of the females was tapering and pointed towards the end (Figure 10), whereas in the males' moth, it was blunt with some white-coloured hairy structures.

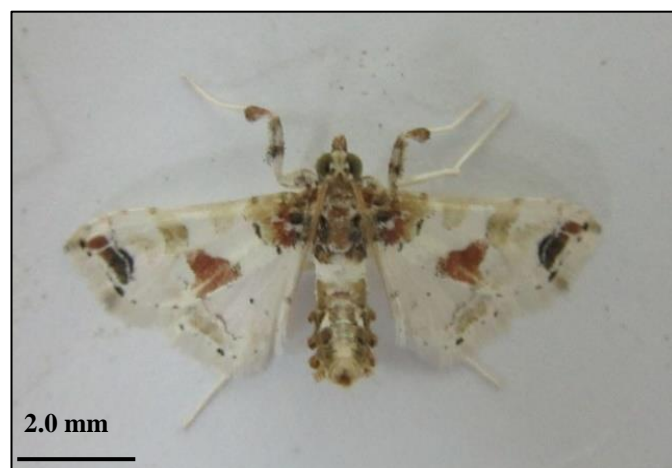


Figure 9. Male adult moth



Figure 10. Female adult moth

The results showed that there was a significant difference between males and females body length ($F= 64.59$; $df = 1, 28$; $P < 0.05$). For males, they ranged between 6.61–8.43 mm with an average body length of 7.43 ± 0.13 mm, and for the females, they ranged between 8.19–11.71 mm with an average mean body length of 9.63 ± 0.24 mm. Similarly, the body width between males and females showed a significant difference ($F = 102.67$; $df = 1, 28$; $P < 0.05$). They ranged from 1.18–1.87 mm with a mean value of 1.47 ± 0.05 mm for males, whereas for females, they were 1.81–2.62 mm with an average body width of 2.24 ± 0.06 mm.

Table 3. The mean numbers of body measurement of adult male and female

Gender	Male (mm)		Female (mm)	
	Mean±SE	Range	Mean±SE	Range
Body length	7.43 ± 0.13 b	6.61-8.43	9.63 ± 0.24 a	8.19-11.71
Body width	1.47 ± 0.05 b	1.18-1.87	2.24 ± 0.06 a	1.81-2.62
Wings width	16.28 ± 0.36 b	13.60-18.55	21.41 ± 0.35 a	18.78-22.87
Wings length	7.60 ± 0.17 b	6.66-8.72	9.89 ± 0.22 a	8.05-10.98

Means with the same letter within the row are not significantly different at $p = 0.05$

The wings width between male and female moths showed a significant difference ($F = 103.95$; $df = 1, 28$; $P < 0.05$). They ranged from 13.60 to 18.55 mm with an average of 16.28 ± 0.36 mm for males, while for females, it ranged from 18.78 to 22.87 mm with an average of 21.41 ± 0.35 mm. Similarly, the result for wings length between males and females showed a significant difference ($F = 66.41$; $df = 1, 28$; $P < 0.05$). The wing length ranged from 6.66 to 8.72 mm with an average wing length of 7.60 ± 0.17 mm for males, whereas they varied for female moths from 8.05–10.98 mm and the mean wing length of 9.89 ± 0.22 mm (Table 3).

DISCUSSION

The biological study is a fundamental pillar to understand the life cycle of an insect pest. Laboratory investigations can contribute valuable knowledge on the development, survival, longevity, and reproduction of insects. The members of order Lepidoptera, the butterflies and moths complete the life cycle through four-stage that is egg, larva, pupa, and adult (Hadley 2018). The egg's incubation period obtained from this study is relatively longer compared to the studies by Bindu et al. (2013) and Natikar et al. (2019) who have reported 3.40 days and

3.8 days respectively while Laichattiwar et al. (2017) reported that the incubation period was recorded 4.10 days.

The present investigation showed that larvae pass five instars which found similar with studies by (Laichattiwar et al. 2017; Rohokale et al. 2018; Singla et al. 2018; Yadav et al. 2015). However, some studies (FAO 2003; Prabhat & Johnsen. 2000) also reported that larvae underwent six larval instars. Interestingly, previous studies performed by Maravi et al. (2013), Ambhure et al (2016) reported that the larval period ranges from 9 to 18 days under different laboratory conditions. It was found that the larval period was the longest, followed by the pupal and incubation period.

A substantial increase in size of the cephalic capsule produced steep gradients that indicate the occurrence of the molting process during that particular period (Ghafoor 2011). According to the graph, the larvae have undergone five different larval instars before entering the pre-pupa stage. The trend in daily variation of body length was directly proportionate with the breadth of the cephalic capsule size, but it did not provide a distinct morphological difference between instars. Therefore, the size of the cephalic capsule can be used as an accurate indication of each instar compared to body length (Figure 11). Furthermore, the use of head capsule breadth measurement in determining larval instars in the order of Lepidoptera have been described in former studies (Onekutu et al. 2013; Sandanayake & Edirisinghe 1992). Changes in the width of the head capsule are due to the molting process or ecdysis. The rigid exoskeleton was shed and then replaced, which resulted in the increasing size of the head capsule (Esperk et al. 2007). Hence, the number of larval instars of *L. orbonalis* was five, which is similar to the findings from previous studies (Bindu et al. 2013; Onekutu et al. 2013; Singla et al. 2018).

The duration of the pupal stage of *L. orbonalis* was longer than that previously reported by Laichattiwar et al. (2017). The results from the present study confirm the longevity of adults reported by Natikar et al. (2019) who recorded male longevity as 3.32 days and female 4.58 days. It was observed that the emergence of adult moth started during the first half of the night. Maximum mating occurred at late night, with 90.80% mating occurred on the first night of adult emergence and oviposition of females begun on the second night of emergence (Mannan et al. 2015). The fecundity ranged between 40 and 369 eggs/female, and the mean fecundity value was recorded as 174 ± 22.01 . Singh & Singh (2001) recorded an average egg-laying of 174.95 eggs per female. There was a significant difference in the number of eggs laid by a female as compared to previous studies done by Onekutu et al. (2013); Rohokale et al. (2018) and Singla et al. (2018) who recorded that a single female lays, on an average of 123, 74 and 93 eggs, respectively.

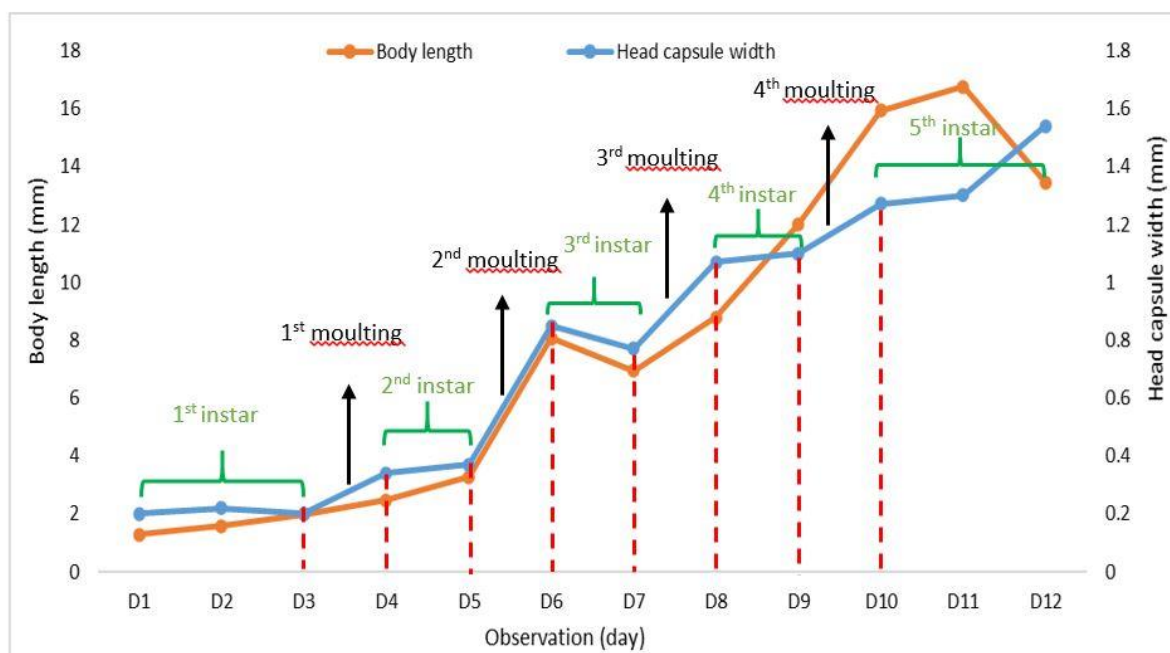


Figure 11. Determination of larval instars characterized by body length and head capsule width

Based on present investigations, it was found that *L. orbonalis* completed its life cycle within 32.67 ± 0.55 days. In contrast to the present finding Rohokale et al. (2018) reported that total developmental periods of *L. orbonalis* was 25.65 ± 2.75 days in India. The development of *L. orbonalis* is influenced by location, altitude, and agro-ecological characteristics. It differs from season to season and area with another area. There were some variations between the life cycle of *Arthroschista hilaralis* in different countries it was recorded 25 days in Malaysia (Thapa 1970) and that of *A. hilaralis* in India (Thapa & Bhandari 1976) which is 23.5 days and that of *A. hilaralis* in Indonesia which is 25.1 days (Susanty et al. 2017). The increase in temperature and humidity will contributed to the increase of fecundity and shorten the duration of the life cycle (Onekutu et al. 2013). These changes in the life cycles of each type of insects are influenced by temperature, air humidity, nutritional food, and so on during the growth of insects (Mavi & Tupper 2004; Wigglesworth 2012). The number of emerged male and female showed that the sex ratio (proportion of male to a female) was 1:2, which is very similar to the studies done by (Singla et al. 2018 and Ambhure et al. 2016) who have reported that male-female ratio varies from 1:1.07 to 1:2.

The measurement of eggs in this study was different to that observed by (Natikar et al. 2019) who reported the mean length of eggs varied between 0.73-0.85 mm and the mean width ranged from 0.56-0.62 mm. The larval period is the most important stage of *L. orbonalis* life cycle, which is responsible for damaging fruits.

The mean duration of the first, second, third, fourth, and fifth instar larvae was recorded as three, two, two, two, and three days, respectively which was in contrast to that recorded by Laichattiwar et al. 2017 who reported the duration of first, second, third, fourth, and fifth larval instars was 1.33, 2.77, 2.56, 3.03 and 3.50 days, respectively. The duration of second instar larvae was almost similar with Singla et al. (2018) who reported that the mean duration was two days and Rohokale et al. (2018) who stated that the fifth instar larvae lived between 3-4

days. On the other hand, the measurement of larval instars in terms of body length and width of cephalic capsule was different to that previously reported by Onekutu et al. 2013.

They recorded the body length of first, second, third, fourth and fifth instar larvae as 4.19 ± 0.58 mm, 6.98 ± 2.16 mm, 11.10 ± 2.32 mm, 16.58 ± 1.75 mm and 18.44 ± 0.28 mm respectively. While the width of cephalic capsule of first, second, third, fourth and fifth instar larvae were 0.45 ± 0.05 mm, 0.74 ± 0.06 mm, 1.06 ± 0.11 mm, 1.44 ± 0.08 mm and 1.73 ± 0.15 mm respectively.

The result shows a significant difference in body length, body width and wings width as compared to the previous studies done by Onekutu et al. (2013), however, reported that the male wingspan was measured 21.59 mm, an average body length of 13.26 mm, and a body width of 4.20 mm. Whereas for an adult female, the wingspan was 24.33 mm, a body length of 14.17 mm and the average body width was measured 4.59 mm. Other researchers have found slightly different results. For instance, Bindu et al (2013) observed that the males have wingspan of 21.59 mm, body length of 13.26 mm, and the body width were measured 4.20 mm. For females, they showed wingspan of 24.33 mm, body length of 14.17 mm and the average body width measured 4.59 mm. From the results it was found that the female moths were usually bigger than the male and had a bunch of hair at the end of the abdomen.

CONCLUSION

In this study, the results showed that *L. orbonalis* underwent five larval instars. The incubation time of eggs recorded as 5.5 ± 0.09 days, larvae was recorded as 12.06 ± 0.19 days; pupal period was observed as 10.36 ± 0.10 days; the mean lifespan of adult females and males were recorded at 4.75 ± 0.17 days and 3.4 ± 0.23 days respectively and completed its life cycle within 32.67 ± 0.55 days. On average, each female produced 174 ± 22.01 eggs with the sex ratio of female to male almost 2:1. Result of this research may be helpful to plan an effective pest management strategy.

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