Indian Journal of Entomology 86(2): 606-610 (2024) DoI. No.: 10.55446/IJE.2023.638

A SIMPLE MASS REARING METHOD FOR BRINJAL SHOOT AND FRUIT BORER *LEUCINODES ORBONALIS* GUENEE

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ABSTRACT

A simple, effective and economically cheaper method for mass rearing of the brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee was developed using both modified artificial diet fortified with brinjal powder and natural diet - potato. A comparison of the various biological parameters such as egg incubation period, larval period, larval weight (12-day old), pre-pupal period, pupal period, pupal weight (1-day old), adult longevity, fecundity, oviposition period and sex ratio of different life stages of the test-insect were studied for both the food sources in this investigation. The larval and pupal periods on natural (potato) diet were 14.58± 1.51 and 6.7± 1.22 days respectively, with a mean fecundity of 165.55± 36.26 per female and a mean life cycle of 26.91days. The larval and pupal period of shoot and fruit borer reared on modified diet were 15.97± 1.24 and 6.25± 1.23 days respectively with a mean fecundity of 149.45± 25.83 per female and a mean life cycle of 25.26 days. Provision of a suitable site for adult mating and oviposition was attained by designing a separate mating chamber along with a suitable egg laying substratum. Thus, an easy and economical rearing technique could successfully support the rearing to six generations of target-insect in laboratory.

Key words: *Leucinodes orbonalis,* artificial diet, biological parameters, head capsule width, larval period, pupal period, potato, mating chamber, morphometry, rearing technique

The brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee is one of the most destructive lepidopteran pests infesting brinjal worldwide (Karyanna et al., 2020). It gained notoriety status in the tropics and subtropics (Srinivasan, 2009), resulting in severe loss up to 60% (Mall et al., 1992). The larvae of *L. orbonalis* bore into tender shoots and fruits, reducing the plant vigour (Alam et al., 2003; Srinivasan, 2009) and fruits become unfit for human consumption (Sagarbarria et al., 2018). Varied losses caused by this pest vary from 55.66 to 80% (Singh et al., 2000; 2001; 2009). Owing to its cryptic nature, management of the pest is difficult and application of insecticides is the prime management practice (Alam et al., 2003). The pesticide sprays are often ineffective due to the difficulty to attain contact with insect larvae which are concealed. Usually, farmers spray twice a week, applying 15 to 40 sprays, or more, in one season depending on infestation levels (Alam et al., 2003; Shelton, 2010). However, the overuse and misuse of pesticides, results in insecticide resistance (Rahman et al., 2011; Kodantharam et al., 2015; Shirale et al., 2017; Kariyanna et al., 2019). Notably, a feasible mass rearing technique is essential to critically examine majority of basic laboratory based studies on *L. orbonalis*. Several food materials including artificial diets have been tried in India for mass rearing of *L. orbonalis* (Alam et al., 2003; Rahman et al., 2011; Sethi et al., 2016; Laichattiwar et al., 2017 and Hegde et al., 2018). The present study was formulated to develop a most suitable and economically cheaper method for mass rearing of *L. orbonalis*.

MATERIALS AND METHODS

The insect culture was initiated with 47 larvae collected from infested brinjal fruits (var. Pusa Kranti) obtained from vegetable farm, ICAR - Indian Agricultural Research Institute, New Delhi (28.3823" N, 77.0727" E) during 2017-18. A potato-based artificial diet prepared with slight modifications in the existing protocol was used as larval food (Sethi et al., 2016). Rearing was done in a controlled environment rooms (Walk-in chamber 25± 1^{0}C, 60± 5% RH; Light: Dark 12: 12 W). Double distilled water (150 ml) was added to agar to decrease its viscosity during dispensing of finished diet. Instead of using 84 g of bengal gram, a mixture of coarse grounded bengal gram (60 g) with brinjal powder (30 g) (slightly modified AVRDC method) was used as the base ingredient. Approximately 200 g of hot diet was dispensed into diet plates till it gets cooled. Another modification was in quantity of agar

powder- a 5 g additional agar powder (16 g) was given. The dried yeast granule was replaced with purified yeast extract. Diet plates (75 x 12 mm) were removed from refrigerator 24 hr prior to use, and exposed to room temperature. The lid of diet plates was kept half open to remove excess moisture. Approximately 2.5 g of diet cut pieces were transferred to plastic containers. Five neonate larvae were transferred per container of diet using a fine camel hair brush.

On natural dieting, medium sized fresh potatoes were procured; washed thoroughly under tap water to remove dried scales, dirt, mud, etc.; and soaked in formaldehyde solution (10%) for surface sterilization for 10 minutes, and air dried afterwards. Potato-tubers were halved and placed in sterilized (with 70% alcohol) rearing glass jars (150 mm diameter x 200 mm high). Two paper discs made out of blotting sheets were placed at the bottom of jar in order to absorb excess moisture from the potatoes. The freshly emerged neonate larvae were transferred from egg jars to cut ends of each potato with a very fine camel brush. Neonates (5-7) were transferred to each potato depending on the size of halved piece. Care was taken not to harm the delicate neonate larvae while transferring. Around eight to ten pieces of halved potatoes along with larvae were transferred to each rearing jars as the cut ends facing upwards. Further, the open end of the jar was fitted with a moistened double layered muslin cloth to prevent the escape of neonates. Once the larvae pupated, they were transferred to fresh jars with blotting paper layer at the bottom to support pupation. Pupae were collected from blotting papers and muslin cloth and disinfected with 0.002% sodium hypochlorite solution. Pupae were kept in separate glass jars/ containers (200×150 mm). The bottom of containers were covered with thin layer of blotting sheet, the pupae were kept in this setup till adult emergence.

Oviposition was done in controlled conditions (27± 1^0C, RH 65± 5 % and 06: 18 hr of light and dark phenophase). An oviposition container cum mating jar/ cage (200x150 mm) was prepared by assembling a set of plastic containers of equal size one above the other. Mating cages consists of a set of translucent plastic containers of equal size. Bottom end of upper container was modified with 10 mm perforations (8-10 holes/ jars). The lower part of mating cage consists of another translucent plastic jar of equal size but the bottom is non-perforated. During assembly, un-perforated container was filled with distilled water up to its bottom 1/5^{th} level, to ensure the humidity. The perforated container was lined by a double layered paper discs at the bottom to prevent escape of adult moths through the perforation to bottom container. A transparent airtight sterile container with modified lid (230 x 230 mm) was used as a support for holding egg laying substrata. Two to three leaved succulent twigs of brinjal were used as egg laying substratum. This container was filled with water to extend the freshness of twigs up to four days. The adult feeding cup containing 10% fortified honey solution was placed in the mating jar. Ten pairs of freshly emerged adults of *L. orbonalis* were released into the container; fine black sheet was placed over the top of the container secured with rubber bands to prevent escape of adults. It also provides additional space for egg laying.

The egg laying substratum was changed on every second day till the death of adult *L. orbonalis*. The freshly laid eggs were collected and stored in surface sterilized clear jars till hatching. A round transparent 500 ml plastic container (80×90 mm) with airtight lid was used as egg storage container. It was prepared by placing tissue paper in the bottom of a container. The black sheet was cut into small pieces without damaging eggs. The leaves containing eggs and black sheet pieces were placed on the tissue paper facing upwards. The lid was tightly fitted and eggs were incubated at 25± 1^0C and 65± 5% RH. The data collected on egg, larval and pupal duration, moth emergence, male and female emergence, male: female ratio, fecundity were subjected to the statistical analysis ANOVA using R software (R Studio version 1.3.1056).

RESULTS AND DISCUSSION

The data obtained under laboratory condition on certain biological parameters of *L. orbonalis* reared using both slightly modified artificial diet and unpeeled halved potato are presented in the Table 1. Adult mated females usually preferred to lay eggs on tender succulent brinjal shoots than on black muslin cloth/ nylon nets, usually lays pale yellow coloured eggs singly. The adults occasionally lay eggs in groups of 3-5 adhered to the egg laying substratum. Viable eggs usually hatch within 3-6 days with the mean of 3.05± 0.83 and 3.20± 0.77 days for potato and diet respectively. These results corroborate with the earlier studies (Laichattiwar et al., 2017; Ambure et al., 2016; and Bindu et al., 2013; Kumar et al., 2011; Wankhede et al., 2009; Jat et al., 2003 and Pal et al., 2003) who observed the egg incubation period last for 3-6 days under various laboratory conditions. Newly emerged larvae are dirty whitish and bore into the potato/diet and stays inside until become mature to pupate. Each

larva passes through five instars in order to become deep pink colored matured larva. This is in conformity with Ambure et al. (2016). The larval duration was longest with artificial diet, with a mean of 15.97± 1.24 days and 14.58±1.51 days on potato. This finding was in accordance with Rahman et al. (2011) who observed that the larval duration on potato was 14.6± 0.40 days whereas, on the artificial diet, the larval period was 15.3± 0.70 days. *L. orbonalis* larvae (larva age) reared on diet weighed 1.43 times heavier than those larvae grown on potato. This finding did not support the earlier findings of Sethi et al. (2016) who had reported the larval weight of 53.29± 3.07 g on artificial diet.

Pupation took place outside the food source and healthy adults emerged depending on quality of foods source. In this study, pre-pupal period was at par for the larvae grown on both food sources. Laichattiwar et al. (2017) observed similar result with the prepupal duration ranged between 1-2 days. Length and width of pupa varied from 8.15-10.45 mm and 2.55-3.54 mm with an average of 9.20± 0.69 and 2.98±0.30 mm, respectively. The pupal period was shorter on diet than those on potato. The pupal periods were 6.25± 1.23 and 6.7± 1.22 days on diet and potato respectively. Rahman et al. (2011) reported the pupal periods on potato and artificial diets were 13.6± 0.45 and 12.6± 0.9 respectively, which did not match with our study. On the potato, pupae were 1.23 times heavier than those on diet. This is in conformity with findings of Sethi et al. (2016). On potato, male and female adult lived 1.01 and 1.07 times longer respectively than those on diet. Many workers reported that adult longevity of *L. orbonalis* varied between 2-7 days (Jat et al., 2003; Harit et al., 2005; Wankhede et al., 2009; Kumar et al., 2011; Pramanik et al., 2012; Maravi et al., 2013; Onekutu et al., 2013; Ambure et al., 2016; Sethi et al., 2016 and Laichattiwar et al., 2017) Pre oviposition duration ranged from 1 to 2 days on both food sources with an average of 1.35± 0.49 and 1.25± 0.44 days on potato and diet respectively. This observation agrees with the findings of Onekutu et al. (2013) and Laichattiwar et al. (2017) who had reported 1.19 and 1.17 days respectively. On potato, oviposition period was 1.04 times shorter than those on diet. This is in agreement with the findings of Sethi et al (2016). On

Table 1. Biological attributes of *L. orbonalis* as affected by the natural (potato) diet

Biological attributes	Diet/ potato	n	Duration (days)/ mg	
			Range	Mean± SD
Egg incubation period	potato	20	3-5	4.30± 0.73
	diet	25	4-6	4.89± 0.69
Larval period	potato	50	12-17	14.58± 1.51
	diet	43	14-18	15.97± 1.24
Larval weight (12-day old)	potato	20	31.4-51.4	39.17± 0.01
	diet	20	31.1-58.7	43.7± 0.01
Pre-pupal period	potato	50	1-2	1.26± 0.44
	diet	20	1-2	1.2± 0.4
Pupal period	potato	20	5-9	6.7± 1.22
	diet	20	5-8	6.25± 1.23
Pupal weight (1-day old)	potato	20	24.5-99.2	57.7± 0.02
	diet	20	37.7-62.6	46.84± 0.01
Adult longevity				
Male	potato	20	2-4	3.10± 0.91
	diet	20	2-4	3.05± 0.69
Female	potato	20	3-7	5.30± 1.03
	diet	20	3-6	4.95± 0.78
Sex ratio	potato	20	--	1:1.95
	diet	20	--	1:1.90
Pre-oviposition period	potato	20	1-2	1.35± 0.49
	diet	20	1-2	1.25± 0.44
Oviposition period	potato	20	2-4	3.05± 0.83
	diet	20	2-4	3.20± 0.77
Fecundity/ female	potato	20	104-207	165.55± 36.26
	diet	20	110-172	149.45± 25.83

Table 2. Morphometrics of life stages of *L. orbonalis* on potato (27± 1^{0}C)

Stages	Length (mm)	Width (mm)	Head capsule width (mm)
Egg	0.55± 0.03(0.51-0.59)	0.31± 0.02 (0.27-0.33)	---
I instar	3.40± 0.43 (2.88-3.94)	0.45± 0.06 (0.32-0.56)	0.21± 0.02(0.18-0.24)
II instar	6.14± 0.56(4.55-6.58)	0.43± 0.07(0.34-0.54)	0.43± 0.04(0.26-0.40)
III instar	10.60± 1.01(9.11-12.56)	2.28± 0.39(1.62-2.63)	0.75± 0.07(0.45-0.65)
IV instar	13.31± 1.11(12.2-15.26)	2.36± 0.19(2.10-2.69)	1.22± 0.04(1.19-1.25)
V instar	18.18± 2.37(15.73-23.21)	3.84± 0.51(3.41-4.57)	1.35± 0.06(1.22-1.42)
Pupa (without cocoon)	9.20± 0.69(8.15-10.45)	2.98± 0.30(2.55-3.54)	---
Adult male	12.11± 1.03(10.56-13.50)	20.06± 1.30(18.10-22.25)	---
Adult female	15.345± 0.81(14.25-16.8)	23.793± 1.9(20.30-26.40)	---
Mean± SD. (n=10) (Range)			

both food sources, females slightly dominated the male with a sex ratio of 1:1.95 and 1:1.90 on potato and diet respectively. Here on potato, adult females produced 1.1 times more eggs than those larvae reared on diet. Similar observations were also recorded by Ambure et al. (2016). Fecundity ranged between 104 to 207 and 110-172 eggs/ female on potato and diet respectively. These results are in conformation with the findings of Kumar et al. (2011); Onekutu et al. (2013); Ambure et al. (2016) and Laichattiwar et al. (2017).

Various morphometric measurements including head capsule width (Table 2) were combined for both diet and potato reared larvae as there was no significant difference occurring between the two treatments. The approximate 1.5 times increase in head capsule width between instars follows Dyar's law (Dyar and Rhinebeck, 1890). The present observations were in conformity with Bindu et al. (2015) and Laichattiwar et al. (2017). The potato and modified diet which gave similar results in important biological parameters viz., larval and pupal lengths, larval and pupal weight, adult longevity, oviposition length and fecundity was considered as the best one for mass rearing of *L. orbonalis* at very low cost.

ACKNOWLEDGEMENTS

The first author acknowledged the award of DST-INSPIRE Fellowship (2016/IF160820). The facilities extended by various Entomology Labs, Division of Entomology, ICAR-IARI, New Delhi are duly acknowledged.

AUTHOR COTRIBUTION STATEMENT

ML and GKM conceived and designed research. ML conducted experiments. ML analyzed data. ML and GKM wrote the manuscript. Both the authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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(Manuscript Received: October, 2022; Revised: March, 2023;
Accepted: March, 2023; Online Published: March, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23638