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Dinesh Rajaram Hegde
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

SJ Nelson
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

N Natarajan
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Mohan Kumar
Department of Biotechnology Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

T Arumugam
Department of Vegetable Science, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Dinesh Rajaram Hegde
Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

A Study on growth and development of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenee) on different Artificial Diets

Dinesh Rajaram Hegde, SJ Nelson, N Natarajan, S Mohan Kumar and T Arumugam

Abstract
A laboratory experiment was conducted to assess the suitability of four artificial diets viz., A, B, C and D for the growth and development of brinjal shoot and fruit borer (*Leucinodes orbonalis* Guenee) and these diets were compared with natural (brinjal) and laboratory (potato) host of the insect. Five larvae selected from each first three larval instars were tested on the artificial diets plus brinjal and potato for their growth and development. Brinjal and potato served best for the insect in terms of growth, development, longevity of larvae and pupae and fecundity of adult female moth. The larval survival and adult emergence in potato is 96.00 percent in case of all larval instars which in case of brinjal, it is 92.00 per cent (I and II instars) and 100.00 (III instars) per cent. The fecundity rate was high in both the cases with more than 90 eggs/female observed. The artificial diet also performed better and gave satisfactory results. The diet D which employed brinjal pulp had shown better larval survival and adult emergence rates (64.00 per cent in case of III instars) and also better larval and pupal periods (13.00 and 9.20 days respectively for III instar) and fecundity of 64.5 eggs on an average per female moth. The next best diet appeared promising was diet C followed by Diet B and the diet A had shown very poor result in terms of larval survival and adult emergence (44.00 percent), higher larval and pupal period (14.00 days and 9.60 days respectively) and lowest fecundity rate (43.60 eggs/female) when tested with III larval instar. Therefore, with notable satisfactory results, the diet D can be used as an alternate diet other than Brinjal and Potato for mass rearing of *Leucinodes orbonalis* in the laboratory.

Keywords: Brinjal, Potato, Brinjal shoot and fruit borer (BSFB), natural and artificial diet

Introduction
Brinjal or egg plant (*Solanum melongena* Linn.) is one of the most important, nutritious and popular vegetable among all classes of people, grown exclusively in South East Asia mainly India, Bangladesh, Pakistan, China, Philippines and in South East Europe [1]. India is recognized as centre of origin of Brinjal and also leading producer in the world in 2015 after China with 12.76 million tones annual production grown in an area of 0.68 million hectare [2]. Among several insect pests causing damage on brinjal both in terms of its production and marketable quality, brinjal shoot and fruit borer (BSFB) (*Leucinodes orbonalis* Guenee) holds its key as number one till today owing to its internal mode of feeding rendering the management practices ineffective [3].

The infestation appears soon after transplanting of the crop and continues till the harvest. At initial stage, the larvae bore into the shoot choking the flow of plant sap causing shoot drying and at later stages, it enter inside the fruit and devour its internal content. This kind of internal mode of feeding makes this pest much evasive from the reach of even most powerful insecticides.

The damage is estimated as high as 70 percent [4] and the pest has received much attention in its management aspects such as biological control, insecticides, use of pheromones, genetically modified crops etc. Bioassay is an important technique used to assess the effectiveness of management aspects requiring year round availability of the insect and necessitating the observation of its biological parameters such as larval instars, pupal stage, adult longevity etc.

Synthetic diet meeting out the nutrient requirement for the proper growth of insect larvae is considered as best way to rear the insect in the laboratory which will be economical and less time consuming. It enables oneself to know about the biology of insects under controlled condition. So far, different artificial diets have been developed and proposed for the

maintenance and consistent rearing of economically important insect pests mostly lepidopterous pests [5, 6]. Though some success in efforts to rear succeeding generations of these insects entirely on an artificial diet was reported, in many cases there is loss of both fitness and reproductive potential, extended developmental period and lower fecundity rate were encountered leading to low economics [7]. For those who work on life and fecundity tables of insects, it is important to know on what type of diet the insect has been reared.

As like with other insects may be with the exception of *Helicoverpa armigera* Hubner, the report on artificial diet for successful rearing of *L. orbonalis*, is very scarce. Considering the above perspective, an attempt was made to standardize the laboratory mass rearing method for *L. orbonalis* on the artificial diet and to assess the effect of different food materials on the development of different stages of brinjal shoot and fruit borer.

2. Materials and Methods

Pure culture of *Leucinodes orbonalis*, was established at Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu by collecting large number of infested fruits from the nearby farmer's market and also from the farmer's field adjacent to the Coimbatore city.

2.1. Maintaining pure culture: The infested fruits were kept in a plastic container (30 cm dia x 15 cm ht) containing layer of sand to facilitate for pupation. The healthy pupae adhering to the infested fruit and sand were collected in a petridish brushing out sand particles carefully and treated with 0.1 % of sodium hypochlorite for disinfection before placing them inside the adult rearing cage (45 X 45 X 68cm) for eclosion. Upon the emergence of moths, they were sexed out (male: female, 1:1), adults were transferred to small plastic container. Cotton ball soaked in 20 per cent honey solution along with few drops of multivitamin syrup (Zicovit™) was provided for adult feeding. The top of the container was covered with muslin cloth for oviposition. Each day, the muslin cloth containing eggs were removed and replaced with new cloth along with fresh feeding syrup for adult moths. The muslin cloth containing eggs were kept for incubation for hatching. The different stages of insect cultured in the laboratory are shown in Figure 1 (a –d).

2.2. Evaluation of Natural and Artificial diets

Brinjal fruit was selected as it is essential natural host of the insect and potato which is considered as suitable laboratory host for *L. orbonalis* [8]. Uninfested and fresh brinjal fruits were taken and kept inside the plastic container. The different stage of larval instars (which were cultured upon brinjal fruits separately) are released and observed for their pupation and subsequent lifecycles. In case of potato as host, the medium sized rigid potato bulbs of approximately 50g in weight were taken and gently peeled to remove the outer skin cover. Later, different larval instars in separate containers were released and observations were made as said earlier.

Four different artificial diets were prepared and evaluated which were modified from the existing study reports on the artificial diets [9, 10]. The compositions of diets employed are as follows.

1. Diet A

Ingredients	Quantity	Ingredients	Quantity
*Chickpea flour and Black gram flour	75.00 g	Formaldehyde (10%)	1.00 ml
L-ascorbic acid	1.50 g	Vitamin solution -Zincovit™	3.00 ml
Sorbic acid	0.50 g	Brinjal fruit powder	25.00 g
Methyl-4-hydroxy benzoate	1.00 g	Agar Agar	12.00 g
Streptomycin sulphate	1.50 g	Water	500ml
Yeast	8.00 g		

2. Diet B: Composition was same as that of diet A except black gram flour instead of chick pea flour.

Preparation of the diet: The chickpea flour/Black gram flour was added to the vessel containing 150ml of water. Brinjal powder (prepared by cutting by grinding of sun dried brinjal fruit pieces) was added and stirred well. In another container, sorbic acid, methyl-4-hydroxy benzoate, streptomycin sulphate, vitamin solution and formaldehyde were added to 250ml of water. In third part, agar gar was boiled in 250ml of water along with brewer's yeast for 10 minutes and later kept for cooling for 5 minutes. All three preparations were finally mixed and the contents were poured to the plastic trays. The contents were kept for drying and then placed in the refrigerator at 4ºC for further use.

2. Diet C

Ingredients	Quantity	Ingredients	Quantity (g)
Black gram powder	75.00 g	Formaldehyde (10%)	1.00 ml
Wesson's salt	2.50 g	Vitamin Solution - Zicovit™	3ml
Methyl-4-hydroxy benzoate	1.00 g	Brewer'sYeast	6.00
Sorbic acid	0.50 g	Agar	12.00 g
Ascorbic acid Sucrose	1.50 g 1.50 g	Water	500ml

The preparation of diet is as same as that of diet A and B except for any content of brinjal powder in it.

3. Diet D

Ingredients	Quantity (g)	Ingredients	Quantity (g)
Black gram flour	40.00 g	Vitamin solution (Zincovit)	3ml
Brinjal fruit pulp	100.00 g	Formaldehyde 10%	1.00 ml
Ascorbic Acid	1.50 g	Agar agar	12 g
Sorbic Acid Wesson's salt	0.50 g 2.50 g	Yeast	6.00 g
Methyl-P- hydroxybenzoate	0.50 g	Water	500ml

Preparation of diet: Healthy brinjal fruits were cut into pieces and later grind with little quantity of water to make it pulp. It was later boiled in 150 ml of water until the content turned little darker with appropriate consistency. In a another glass vessel, Black gram flour, ascorbic acid, sorbic acid, methyl – P- hydroxybenzoate, vitamin solution, formaldehyde are added in afore said quantity to 150ml of water and properly stirred**.** In the third part, Yeast is added to 200ml of water which is later boiled along with agar agar for 10 minutes. After 5 minutes of cooling down, the remaining above two parts were added to it with proper stirring. The contents were poured into plastic trays and kept for cooling. The trays were later kept in refrigerators at 4^0C for further use. The artificial diet along with emerged moths are shown in Figure2.

2.3. Observation and statistical analysis

Three larval instars such as I, II and III were tested. The appropriate larval instars were selected from laboratory culture on brinjal. The larvae were released at the rate of 5 larvae per diet in five replications. The parameters such as percentage survival of larvae and pupae, larval and pupal periods, adult emergence rate, fecundity were recorded. The data's were statistically analyzed and significance was tested by DMRT.

3. Results and Discussion

The study dealt with two natural and four artificial diets to rear the larvae of *L. orbonalis* under laboratory conditions. The efficiency of the diets is measured with three different instars of the larvae viz., I, II and III were assessed with respect to the larval survival rate, percent pupation or adult emergence, larval period, pupal period and fecundity rate of the emerged female moths [11].

Larval survival was highest with more than 90 per cent survival rate was observed in case of potato and brinjal when compared to any diets irrespective of their larval stage. In case of diets B, C and D, the survival rate came closer to 50 per cent in case of I instar larvae and the diet D had touch even good significant rate with the natural hosts with 64 per cent survival rate in case of II and III instars survival percentage. The diet A with chick pea flour was found to be less preferred by the larvae over black gram flour ingredient with the I instar larval survival in the former was as low as 24 percent which barely touched as high as 44 percent in case of III instar larval survival.

The reduction in the survival of larvae in the first two diets viz., A and B can be attributed to the absence of minerals that was being supplied in the other two diets viz., Diet C and Diet D. Islam (1973) has observed that larvae of brinjal shoot and fruit borer usually required a light amount of sodium, potassium, calcium and phosphorus derived from the host plants [12]. On the second part, the preference of black gram flour over bengal gram, flour is may be due to some of the antinutritional factors such as trypsin inhibitors and their relative quantities in the latter such as trypsin inhibitors. Saeed *et al.* (1997) have reported about the influence of trypsin inhibitor on reduction of larval development and subsequent adult emergence when incorporated in diet against *H. armigera* [13].

The percent adult emergence was significant in case of natural hosts i.e., brinjal and potato with again more than 90 percent survival of pupae and subsequent adult emergence rate. In case of artificial diet, Diet D was found to be good in case of II and III instar larvae with the adult emergence rate 64.00 per cent and 56.00 per cent respectively. However, the test conducted with first larval instar showed that none of the artificial diet found to cross 50 per cent adult emergence rate with the highest in case of diet B (44.00 per cent). The lowest adult emergence was observed in diet A and diet C with second instar larva.

Das (1984) has studied the mass rearing on brinjal fruits and found that the adults emerging from such rearing were quite normal with usual fertility and about 98% of them mated successfully [14]. Alam *et al.* (2003) have reared brinjal shoot and fruit borer on potato, brinjal, whole potato and sliced potato and found that adult emergence and rate of development after the 3rd generation was higher in potato than its original host [15].

As far as the larval period is concerned, the natural hosts brinjal and potato were significantly different from rest of the artificial diets. The larval period in case of I instar larval test was found to be 12.40 and 12.60 days in brinjal and potato respectively (Table 1). The diet C and D shown 14.60 days whereas it was 14.00days in case of diet A and 13.00 days in diet B. Overall, it is clear to note that the artificial diet took on an average of 13-14 days whereas it was 12 days on an average in case of natural hosts (Table 2 and 3). The l*a*rval period was shorter and

the larval weight was higher when reared on brinjal cultivars having higher percentage of protein contents[16, 17, 18].

The pupal period had shown varied results. In case of I instar larval rearing, it was 8.20 and 9.00 in case of brinjal and potato respectively (Table 1) which however found to be slighter lower with average around 7.5 days in case of II and III larval instars (Table 2 and 3). In case of artificial diet, the diet A had longer pupal period with 10.60, 10.40 and 9.60 respectively in case of I, II and III larval instars. Diet D shown next higher pupal period in days in increasing trend with the instars of larvae i.e., I, II and III with the value of 8.60, 9.00 and 9.20 days respectively. The other diets viz., diet B and diet C had shown pupal period on an average of 8.5 days (Table 1, 2 and 3).

Brinjal and potato which had shown remarkable rate in their larval survival and adult emergence had also shown fecundity rate which is statistically significant with the rest of the diets. In case of I instar larvae, it is 78.00 and 81.20 respectively in brinjal and potato which showed an increasing trend with 91.40 and 99.20 in III instar larval trial (Table 1 and Table 3). The diet A is found to support lowest fecundity rates with 47.60, 46.40 and 43.60 and eggs/female in I, II and III instar larval trial. The diet D has found to perform consistently with an approximate average fecundity rate of 64.50 eggs /female in all larval trials and the diet performance was on par with diet C which has got average fecundity rate of 63.00 eggs/female. The next best diet was diet B which in case of I larval trials shown lowest fecundity of 47.60 eggs/female which however showed an increasing trend with II and III larval instars with the fecundity of 59.80 and 69.40 eggs/female respectively (Table1, 2 and 3).

The natural host brinjal which has shorter period of development, higher per cent of adult emergence, higher growth index and lower percentage of larval mortality, was considered as the best one for mass rearing of *L. orbonalis* [19, 20]. In this experiment, the growth and development of larval to adult stages were compared with those fed on natural food (brinjal) along with potato as alternative host under laboratory condition. Among the diets tested, potato was found as the best alternative for the growth and development as well as the reproductive performances of BSFB apart from brinjal. On

the other hand, potato is available throughout the year with an affordable price.

4. Conclusion

Among the four artificial diets tested, the new method which employed brinjal pulp instead of brinjal powder had shown some promising results interms of its considerable support for rearing BSFB. It also saves time of preparation of diet since making of brinjal pulp is easier than brinjal powder. Though the artificial diet is a costlier in nature and bit time consuming compare to potato, in some experiments concerning bio assay studies, they have their immense role in yielding better results. The diet has to be further standardized with varying compositions to meet the appropriate growth requirement of larvae.

Table 1: Growth of I instars larvae on artificial diet

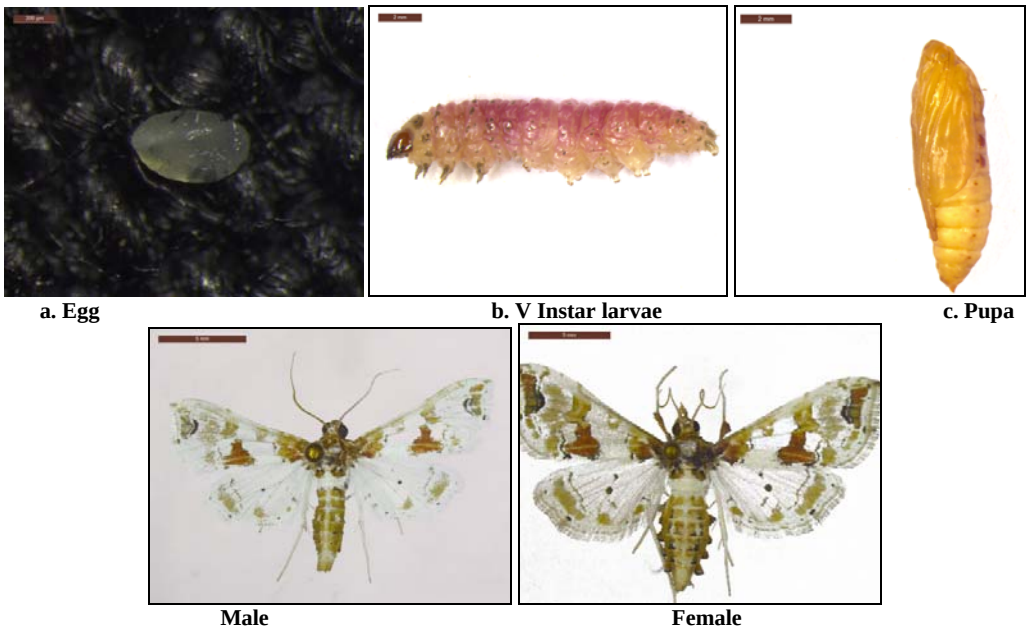
Diet	Growth parameters				
	Larval survival (%)	Adult emergence (%)	Larval period (Days)	Pupal period (Days)	Fecundity (No. of eggs)
Brinjal	92.00 (12.37)^{a}	92.00 (12.37)^{a}	12.40 (20.62)^{c}	8.20 (16.64)^{b}	78.00 (8.80)^{a}
Potato	96.00 (12.64)^{a}	96.00 (12.64)^{a}	12.60 (20.78)^{c}	9.20 (17.59)^{b}	81.20 (9.00)^{a}
Diet A	24.00 (6.22)^{c}	16.00 (4.85)^{c}	14.00 (21.97)^{ab}	10.60 (18.98)^{a}	47.60 (6.82)^{b}
Diet B	56.00 (9.55)^{b}	44.00 (8.50)^{b}	13.00 (21.12)^{bc}	9.20 (17.63)^{ab}	47.60 (6.82)^{b}
Diet C	48.00 (8.70)^{b}	40.00 (7.50)^{b}	14.60 (22.46)^{a}	8.80 (17.25)^{b}	61.60 (7.80)^{ab}
Diet D	44.00 (8.39)^{b}	32.00 (7.17)^{bc}	14.60 (22.46)^{a}	8.20 (16.62)^{b}	65.40 (8.02)^{ab}

Table 2: Growth of II instars larvae on artificial diet

Diet	Growth parameters				
	Larval survival (%)	Adult emergence (%)	Larval period (Days)	Pupal period (Days)	Fecundity (No. of eggs)
Brinjal	92.00 (12.36)^{a}	92.00 (12.36)^{a}	12.00 (20.25)^{b}	7.20 (15.55)^{c}	97.20 (9.82)^{ab}
Potato	96.00 (12.64)^{a}	96.00 (12.64)^{a}	12.40 (20.61)^{b}	7.40 (15.77)^{c}	90.40 (9.44)^{a}
Diet A	36.00 (7.65)^{bc}	32.00 (7.17)^{c}	13.00 (21.12)^{ab}	10.40 (18.80)^{a}	46.40 (6.73)^{d}
Diet B	44.00 (7.86)^{bc}	40.00 (7.50)^{bc}	13.00 (21.12)^{ab}	8.20 (16.61)^{bc}	59.80 (7.70)^{cd}
Diet C	44.00 (7.86)^{bc}	32.00 (6.65)^{c}	13.60 (21.63)^{a}	8.20 (16.61)^{bc}	67.40 (8.14)^{bc}
Diet D	64.00 (10.23)^{ab}	56.00 (9.60)^{b}	13.80 (21.79)^{a}	9.00 (17.44)^{b}	60.60 (7.71)^{cd}

Table 3: Growth of III instars larvae on artificial diet

Diet	Growth parameters				
	Larval survival (%)	Adult emergence (%)	Larval period (Days)	Pupal period (Days)	Fecundity (No. of eggs)
Brinjal	100.00 (12.92)^{a}	100.00 (12.92)^{a}	12.00 (20.26)^{bc}	7.80 (15.99)^{d}	91.40 (9.95)^{a}
Potato	96.00 (12.64)^{a}	96.00 (12.64)^{a}	12.60 (20.26)^{c}	7.60 (16.20)^{d}	99.80 (9.49)^{ab}
Diet A	44.00 (8.50)^{bc}	44.00 (8.50)^{c}	14.00 (21.96)^{a}	9.60 (18.04)^{a}	43.60 (6.58)^{d}
Diet B	52.00 (9.18)^{bc}	40.00 (7.86)^{c}	13.40 (21.46)^{ab}	8.20 (16.63)^{cd}	69.40 (8.30)^{bc}
Diet C	52.00 (9.24)^{bc}	52.00 (9.24)^{bc}	13.40 (21.46)^{ab}	8.60 (17.04)^{bc}	60.00 (7.70)^{cd}
Diet D	64.00 (10.23)^{b}	64.00 (10.23)^{b}	13.40 (21.47)^{ab}	9.20 (17.65)^{ab}	67.20 (8.14)^{c}



a. Egg **b. V Instar larvae** **c. Pupa**

Male **Female**

d. Adult moths

Fig 1: Different life stages of *Leucinodes orbonalis*

Fig 2: Culturing of *L. orbonalis* on artificial diet

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