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In vitro rearing of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenée) (Lepidoptera: Crambidae) on artificial diet

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ABSTRACT: The brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* (Guenée) (Lepidoptera: Crambidae) is a key pest of brinjal. Studies were carried out on the development of its *in vitro* rearing on artificial diet on a large scale. An artificial diet without natural ingredients of brinjal is found to support the growth and development as good as or better than natural brinjal fruits. The BSFB takes 26.25 days to complete life cycle on artificial diet as compared to 25.42 days on natural brinjal fruits (var. Pusa Kranti). On the contrary, other traits viz., 13 day old larval weight, 2 day old pupal weight, % pupation, % adult emergence, adult longevity and fecundity of the BSFB on artificial diet are either better or is at par with those on the natural brinjal fruits. The artificial diet has a shelf life of 75 days at 4 ^{0}C. Thus, the artificial diet is useful for quality and economic production of insects under aseptic conditions on the basis of rearing at 27 ^{0}C, 60-75% rh and 13 hr photophase for more than 56 generations without fortification with field populations. The prospects of this method being useful for studies on various aspects of BSFB management including insect resistance management in insect protective transgenic brinjal are discussed.

KEYWORDS: Brinjal, *Leucinodes orbonalis*, artificial diet, rearing.

INTRODUCTION

Brinjal (*Solanum melongena* L.), also called aubergine or egg plant, is an important vegetable crop grown in India and many other parts of the world. It is tasty and nutritious vegetable rich in minerals (Choudhary and Gaur, 2009). India is the second leading producer of brinjal crop in the world after China with an annual production of 12.2 million tons from about 7,00,000 ha (FAO, 2012, 2013). Brinjal has a wide spectrum of insect pests (Srinivasan, 2009). The brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* (Guenée) (Lepidoptera: Crambidae) is the key pest that causes heavy damage throughout the crop life (Atwal, 1976). The pest is primarily monophagous in nature, but sometimes has been reported on some other crops (Dhankar, 1988; Srinivasan, 2009; Onekutu *et al*., 2013). The infestation starts from the seedling stage till the final harvest. Within few hour of the egg hatching, BSFB larvae bore into and feed on the tender shoots and the fruits of the brinjal plant. It damages the young shoots, reducing plant growth thereby adversely affecting productivity. The boring nature of BSFB, with a barely visible sign during early infestation is the

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major reason that impairs the effective control of the pest (Butani and Verma, 1976). Despite a large scale use of insecticides (15-40 applications in a crop season), an annual loss of 60-70% in production was reported (MoEF, 2009). The pest is perennial in nature and its incidence varies with temperature, relative humidity (rh) and other environmental factors (Katiyar and Mukharji, 1974; Singh, 1977; Mahyco, 2006; Shukla and Khatri, 2010). Hence, the knowledge of its biology, ecology and susceptibility to insecticides is an essential requirement for integrated pest management including deployment of insect protective transgenic crops. The basic need for these studies is the availability of adequate number of high quality insects throughout the year. Rearing of BSFB is carried out on the natural fruits to study its biology and also elucidate the insect plant relationship including crop resistance (Wankhede *et al*., 2009; Maravi *et al*., 2013; Onekutu *et al*., 2013). However, non-availability of fruits of suitable varieties could be constraint in its rearing, as brinjal varieties are also known to be resistant to some extent (Dhankar, 1988; Ranjithkumar *et al*., 2013; unpublished). Besides continuity of regular supply of brinjal fruits; storage and associated contamination will adversely affect its rearing. Hence, Talekar *et al*. (1999) developed an artificial diet for its rearing using 1part of dried brinjal fruits and 9 parts of cotton bollworm artificial diet. Similar artificial diets with brinjal constituents with varying degree of success were also developed for BSFB rearing (Mahyco, 2006; Rahman *et al*., 2011; Ghante, 2012; Ranjithkumar *et al*., 2013). However, the use of natural fruits or its ingredients will limit the efforts to identify the suitable allelochemics responsible for resistance to BSFB, as the pest will adapt to such chemicals on their rearing over generations. Hence, the development of artificial diet with all nutrients in right proportion without natural ingredients of brinjal is essential (Patil, 1990; Cohen, 2004).

The present study reports the *in-vitro* rearing of BSFB *en masse* based on the development of artificial diet devoid of any nutrients of brinjal fruits for mass rearing over generations along with biological traits.

MATERIALS AND METHODS

(i) Collection and maintenance of test insects

Infested brinjals were collected from the wholesale vegetable market from Delhi (India) in 2010 and sliced to collect different stages of BSFB larvae. The collected larvae (69 larvae) were kept singly in sterile plastic containers (2 cm h × 5 cm dia) with brinjal slice at 27±2 °C, 60-75% rh and 13L: 11D photoperiod. The newly emerged adults were transferred to the mating jars (20 cm h × 15 cm dia), having a fresh brinjal twig dipped in water for egg laying and 10% honey solution fortified with multivitamins for feeding. Twigs containing the eggs were collected and kept in plastic jar (11 cm h × 7.5 cm dia) for hatching. Twig was replaced every 2nd day for more egg laying. The insect culture was maintained on the brinjal (var. Pusa Kranti) for first generation at 27±2 °C and 60-75% rh and 13 hr photophase for multiplication. These environmental conditions were maintained throughout studies.

(ii) Preparation of artificial diet

The key ingredients of the test diet were divided into three fractions (Table 1). Fraction A (chickpea, dried yeast powder, casein, L-ascorbic acid, methyl parahydroxybenzoate, sorbic acid, streptomycin sulphate, cholesterol, Wesson salt mixture), Fraction B (multivitamins, tocopherol, formaldehyde), Fraction C (Agar) and double distilled water. All the dietary ingredients used were of high quality and weighed accurately.

Diet was prepared by weighing the different fractions separately. The fraction A of the diet was mixed in one part of double distilled water (300 ml) and blended for 2 min in a one liter blending jar. Meanwhile, Fraction C was mixed with other part of double distilled water (150 ml) and boiled for 1-2 min in a microwave. The boiled fraction was allowed to cool down to 50±5 °C in order to reduce degradation of heat sensitive ingredients of artificial diet. Fraction B (except formaldehyde) was added to the finely mixed Fraction A and blended for 2-3 min. The cooled Fraction C and formaldehyde was added to the mixture of fraction A and B in the

Table 1. Cost of production of 450 ml of artificial diet for brinjal shoot and fruit borer

Fractions	Diet ingredients	Quantity (g)	Price (INR) per Unit pack	Cost (INR) as per quantity required
	Distilled water	450 ml	70 (5000 ml)	6.80
A	Bengal gram	84	23.25 (500 g)	4.20
	Dried Yeast granules	11	110 (500 g)	2.24
	Casein	5	781.5 (500 g)	7.82
	L-Ascorbic acid	3	2280 (500 g)	27.36
	Methyl parahydroxy benzoate	2	1015 (500 g)	6.10
	Sorbic acid	1	1008 (500 g)	2.02
	Streptomycin sulphate	0.2	4950 (500 g)	1.96
	Cholesterol	0.5	12190 (500 g)	12.19
	Wesson salt mixture	0.5	4035 (500 g)	8.07
B	Multivitamins#	1 ml	29.90 (15 ml)	1.90
	Vitamin E##	1 capsule	17 (10 capsule)	1.70
	Formaldehyde (10%)	1 ml	194 (500 ml)	0.39
C	Agar	11	4080 (500 ml)	89.76
				172.51 (Total)

#Each ml contains approximately: Energy (0.66 Kcal), Carbohydrate (0.15 g), Protein (0 g), Fat (0.007 g), Vitamin C (40 mg), Zinc sulphate (13.3 mg), Vitamin B_3 (10 mg), Lysine Hydrochloride (10 mg), Vitamin B_5 (1 mg), Vitamin B_2 (1 mg), Vitamin B_1 (1 mg), Vitamin B_6 (0.5 mg), Pine bark extract (500 mg), Vitamin A (2500 I.U.), Vitamin D_3 (200 I.U.) and Vitamin E (2.5 I.U.).
Vitamin E capsule USP: Tocopheryl acetate IP (400 mg each).

blender, and blended again for 2-3 min. The diet was poured in the sterile Petri plates (17 cm dia × 3 cm h) and allowed to solidify at the room temperature. After solidification, the diet was stored in the refrigerator (at 4 °C) until use.

(iii) Evaluation of the artificial diet vis-à-vis brinjal fruits

The artificial diet was evaluated in comparison with natural brinjal oblong fruits and fruit slices (var. Pusa Kranti) at 27±2 °C, 60-75% rh and 13L: 11D photoperiod. The neonates of BSFB, five in number, were transferred on the artificial diet in cuboid form (3.4 cm length × 1 cm width× 1.5 cm h) weighing about 7 ± 1 gm in a sterile plastic container (2 cm h × 5 cm dia) or on to the brinjal fruit slice of ca. 7 gm or a fruit with a soft bristle brush. At least 30-40 replicates were maintained for each treatment. The brinjal fruit slice or fruit, as above, was replaced every alternate day to avoid decay while the artificial diet was added for growing larvae every 7th day. The observations of larval and pupal periods, % pupation and adult emergence, 13 day old larval and 2 day old pupal weights were recorded. The per cent pupation or adult emergence was estimated on the basis of the number of respective pupae formed or adults emerged out of neonates. The sex ratios were noted. The newly emerged adults were kept in the mating jars (19 cm h × 14.5 cm dia) with fresh brinjal twigs dipped in water for egg laying and 10% honey solution fortified with multivitamins for feeding of moths. Ten pairs of adults were kept per mating jar and the mouth of the jar was covered with rough cotton cloth. The eggs laid on the twig and rough cotton cloth were collected and kept in plastic container (11 cm h × 7.5 cm dia) until they hatch. The observations on various biological traits including adult longevity, oviposition and incubation periods, fecundity and total life cycle for each treatment were noted and subjected to analysis of variance (ANOVA) using software (SAS, 1999).

In another set of experiments related to the effect of neonate density on insect growth and development; 2, 5 and 10 neonates (belonging F_{18} generation) were carefully transferred separately to each artificial diet (of the size as above) treatment in the plastic container of uniform size (2 cm h × 5 cm dia); with 30 replicates per treatment. The observations on various biological traits were recorded after every 24 hr without disturbing or removing the diet. The larvae were allowed to grow and pupate in the same container. The pupae formed in each treatment of different neonatal density were counted and collected in plastic Petri plates (11 cm dia × 2.5 cm h), lined with blotting paper at the bottom, until adult emergence. Observations on larval and pupal periods, % pupation and % adult emergence were subjected to ANOVA.

(iv) Evaluation of shelf life of artificial diet

Shelf life of the test diet was evaluated with neonates of BSFB belonging to $F_{21\text{-}22}$ generations. The test diet was prepared, as mentioned above. The freshly prepared diet was poured in 4 aliquots (100 gm each) in small Petri plates (11 cm dia × 2 cm h). The aliquots were labeled and date of diet preparation was mentioned on the covering lid. Once properly solidified, the 4 plates were covered, sealed with cling film and stored at 4 °C. One aliquot, labeled as 0 day prepared, was used for evaluating the biological traits viz. larval period, pupal period, % pupation and % adult emergence. The stored diet aliquots were taken out at different intervals (days) of 8, 18, 32, 75 and fed to the neonates as mentioned. Ten to twelve replicates were made for each shelf life treatment, with 5 neonates per diet replicate in a each plastic container (2 cm h × 5 cm dia). The 0 day prepared diet was kept as control and first observations were recorded on the 7^{th} day. The rest of the stored diet was brought to room temperature before use. The rearing method was same as described above, and each time, 50-60 neonates were used to evaluate the stored diet quality by rearing of BSFB and analyzing their biological traits.

(v) Evaluation of microbial contamination

Prior to the evaluation of shelf life of diet, the stored diet aliquots were tested for contamination. A small fraction of the diet was diluted in autoclaved double distilled water and streaked on the nutrient agar (NA) medium. The plates were kept at 27 °C and 37 °C. Observations were recorded at 24 hr, 48 hr and 72 hr. Every time, six plates were streaked.

(vi) Biology of BSFB during different generations on artificial diet

BSFB was reared on the artificial diet for successive generations and in conditions as mentioned, except, that the brinjal twig was no longer used for egg laying as these were laid on the rough cotton cloth. The artificial diet was often not more than a week old, stored at 4 ^{0}C. The rough cotton cloth containing eggs was moistened with distilled water using spray bottle and stored in the plastic container. The biological traits were estimated the same way as described above. The larval density was 5 neonates on each diet cuboid in a plastic container (11 cm h × 7.5 cm dia) until they pupated at 27±2 °C, 60-75% rh and 13 h photophase.

(vii) Cost of artificial diet

Cost of artificial diet in Indian Rupees (INR) was calculated at the rates at which the ingredients (from the reputed supplier of chemicals) were purchased during the month of May 2013 and for the F_{40} generation. The rate of brinjal fruits were ascertained from the market.

(viii) Statistical analysis

The significance of difference in various traits of BSFB growth and development was determined by one way ANOVA at 95% degree of confidence using SAS software Enterprise guide 4.2 and Tukey's HSD ($p < 0.05$) test. The per cent data on pupation and adult emergence were subjected to angular transformation prior to ANOVA. The cost of the diet was calculated in INR.

RESULTS

(i) Evaluation of artificial diet

The biological traits of BSFB on artificial and brinjal fruit diets showed differences (Table 2). The larval durations of BSFB fed on the artificial diet and the control diet (brinjal) differed ($F_{1,171}$ = 34.25, p < 0.0001). The pupal period was more on artificial diet than on control ($F_{1,142}$ = 4.91, p < 0.05). The per cent pupation was higher on artificial diet ($F_{1,59}$ = 46.65, p < 0.0001) and adult emergence was at par on either foods ($F_{1,59}$ = 23.38, p < 0.0001). There was no significant difference in the 13th day old larval weight on either diets ($F_{1,27}$ = 0, ns). The pupal weight of BSFB reared on brinjal fruit was significantly higher than that on artificial diet ($F_{2,27}$ = 53.13, p < 0.0001). Male and female longevities were at par on either diet, so were oviposition and incubation periods (Table 2). Female moths were more in number than male moths on either food source. The total life cycle of BSFB was shorter on brinjal than on artificial diet ($F_{1,40}$ = 5.51, p < 0.05). However, there were no differences in the fecundity of insects reared on either diets (Table 2).

(ii) Effect of Larval rearing density

The larval density affected all biological traits significantly (larval period, $F_{2,156}$ = 51.12 p < 0.0001; pupal period, $F_{2,131}$ = 5.89, p < 0.005; % pupation: $F_{2,89}$ = 10.14, p = 0.0001; % adult emergence, $F_{2,89}$ = 13.23, p < 0.05) (Table 3). The neonates grew and pupated in the same plastic container. The treatment of larval density of 5 neonates per diet cube (ca. 7±1 gm) in the plastic container (2 cm h × 5 cm dia) was the best in terms of % pupation and adult formation.

(iii) Effect of Shelf life of the diet

The F_{20} and F_{21} generation neonates acclimatized and adapted to the test artificial diet at 27±2 °C, 60-75% rh and 13L: 11D were used for evaluation of shelf life of the artificial diet (Table 4). The larval and pupal periods were significantly affected due

Table 2. Biological traits of brinjal shoot and fruit borer reared on artificial diet and natural brinjal fruits

Development parameters		Artificial diet	Brinjal*	F	P
Development duration (days)	Larval	15.89±0.08^a	15.08±0.12^b	34.23	<0.0001
	Pupal	7.21±0.09^a	6.88±0.11^b	4.91	0.03
% Survival	%Pupation	54.99±1.11^a	43.81±1.21^b	46.65	0.000
	%Adult emergence	46.61±1.45^a	38.58±1.48^b	23.38	0.000
Body weight (mg/insect)	Larva#	53.29±3.07^a	53.07±1.52^a	0	0.95
	Pupa##	37.50±1.02^b	53.80±1.99^a	53.13	<0.0001
Adult Longevity (days)	Female	3.95±0.15^a	4.00±0.16^a	0.05	0.824
	Male	3.35±0.15^a	3.45±0.14^a	0.25	0.62
Oviposition period (days)		2.6 ± 0.16^a	2.5 ± 0.17^a	0.18	0.67
Incubation period (days)		3.86 ± 0.18^a	3.75 ± 0.16^a	0.19	0.67
Fecundity (eggs/♀)		152.25±2.65^a	147.95±1.62^a	1.91	0.17
Total life cycle (days)**		26.25±0.25^a	25.42±0.25^b	5.51	0.024
Sex ratio (♂:♀)		1:1.5	1:1.5		

*Values (mean±s.e.) within the row, followed by same letter are not significantly different; # 13 day old larva and ##2 day old pupa; *Brinjal was offered in form of slices of ca. 7 gm each; **Egg to adult stage*

Table 3. Effect of larval density on the biological traits of brinjal shoot and fruit borer

Development Parameters	No. of neonate/container			F	P
	2	5	10		
Total neonates	60	150	300		
Larval period (days)	14.96±0.15[b]	15.90±0.08[a]	14.33±0.12[c]	51.12	<0.0001
Pupal period (days)	6.71±0.14[b]	7.21±0.09[a]	7.5±0.10[a]	5.89	0.003
% pupation	42.27±4.78[b]	54.99±1.11[a]	36.85±1.25[b]	10.14	0.001
% adult emergence	35.45±4.68[b]	48.61±1.45[a]	26.19±2.19[c]	13.23	0.000

Values (mean±S.E.) within the row, followed by same letter are not significantly different.
n=number of replicates for each treatment

Table 4. Biological traits of brinjal shoot and fruit borer on artificial diet stored for different periods at 4 ^{0}C

Biological traits	Diet shelf life (days)					F	P
	0	8	18	32	75		
Larval period (days)	15.62±0.08[b]	14.22±2.22[d]	14.64±0.13[c]	15.33±0.18[b]	17.74±0.15[a]	81.45	<0.0001
Pupal period (days)	6.67±0.09[c]	6.58±0.09[c]	6.44±0.10[c]	7.52±0.09[b]	8.39±0.10[a]	65.77	<0.0001
% pupation	57.80±4.69[b]	58.09±3.63[b]	58.09±3.63[b]	64.14±4.07[a]	52.26±2.84[c]	1.21	0.32
% adult emergence	57.08±0.30[b]	57.86±1.07[b]	57.49±0.71[b]	62.91±0.52[a]	59.58±1.99[ab]	4.79	0.02

Values (mean±S.E.) within the row, followed by same letter are not significantly different.

to use of the artificial diets stored for different durations at 4 ^{0}C (larval period: $F_{4,175} = 81.45$, $p < 0.0001$; pupal period: $F_{4,126} = 65.77$, $p < 0.0001$). The % pupation and adult emergence were differently affected with dietary treatment (pupation: $F_{4,49} = 1.21$, $p > 0.05$; adult emergence : $F_{2,14} = 4.79$, $p < 0.05$). The BSFB rearing is unaffected on the diet stored until 32 days and possibly even more at 4 °C.

(iv) Biological fitness of BSFB of different generations on artificial diet

The biological traits of the BSFB in 2nd, 10th, 37th and 56th generation on the artificial diet at 27 ^{0}C, 60-75% rh and 13 h photophase differed in terms of larval and pupal periods and %pupation and adult emergence (Table 5). However, the larval and pupal weights were the highest in the 37th generation (larval weight: $F_{3,40}$ =5.7, $p = 0.003$; pupal weight: $F_{3,42} = 7.84$, $p = 0.0003$), suggesting that inbreeding did not affect adversely these traits.

(v) Cost of the artificial diet

The artificial diet produced significantly more adults per 100 gm diet than sliced or oblong brinjal fruits ($F_{2,16} = 436.89$, $p < 0.0001$)). Although sliced brinjal per INR cost produced more insects; nevertheless, artificial diet was much better in terms of higher number of adults, less drudgery and better insect quality (Table 6).

Table 5. Biological parameters of brinjal shoot and fruit borer reared on the artificial diet over 56 generations

Biological traits	Generations				F	P
	2	10	37	56		
Larval period	15.25±0.08^{b}	15.36±0.09^{b}	15.31±0.08^{b}	15.88±0.11^{a}	10.22	<0.0001
Pupal period	6.21±0.08^{b}	6.32±0.09^{b}	6.52±0.09^{a}	6.97±0.13^{a}	10.41	<0.0001
% pupation	48.57±2.38^{b}	54.57±1.94^{a}	53.30±1.69^{ab}	55.84±2.07^{a}	2.43	0.00001
% adult emergence	43.85±1.88^{b}	47.42±2.54^{ab}	49.73±2.15^{ab}	50.99±2.55^{a}	1.88	0.00001
Larval weight (mg)	48.5 ± 1.51^{b}	53.1 ± 2.21^{b}	59.3 ± 0.75^{a}	56.63±2.53^{ab}	5.70	0.003
Pupal weight (mg)	37.5 ± 1.02^{b}	43.5 ± 0.95^{a}	45.9 ± 0.79^{a}	42.92±1.59^{a}	7.84	0.0003

Values (mean±S.E.) within the row, followed by same letter are not significantly different.
[#] 13-day old larva and [##] 2 day old pupa

Table 6. Cost of production for each 100 gm diet and its equivalent natural food

Food Source (100 g)	Cost of diet/ 100 g (INR)	Adult production /100 g	Insect/ per INR	F value	Pr > F
Artificial diet	38.33	50.2 ± 1.5^{a}	1.3	436.89	<0.0001
Brinjal (oblong)*	3.00	4.2 ± 0.4^{b}	1.4		
Brinjal (sliced)*	3.00	6.8 ± 0.8^{b}	2.3		

Values (mean±S.E.) within the column, followed by same letter are not significantly different.
** Pusa Kranti*

(vi) Contamination test

The NA medium plates streaked with diluted stored diet aliquots did not show any microbial growth even at 37 °C.

DISCUSSION

The development of insect protective Bt brinjal and its consequent failure to get approval for commercialization in India and the successful commercialization in Bangladesh during 2013-14 has revived the need for studies on its biology, ecology and integrated pest management (http://www.barc.gov.bd/bt_brinjal.php). The rearing of BSFB has become a necessity to develop baseline susceptibility data for xenobiotics and also toxins present in insect protective transgenic brinjal, besides other studies (Rao *et al*., 1999; Wankhede *et al*., 2009; Ghante, 2012; Kalia *et al*., 2013; Ranjithkumar *et al*., 2013). The European corn borer, *Ostrinia nubilalis* was the first lepidopteran reared on artificial diet under controlled environment (Bottger, 1942), followed by many economical important insects (Singh, 1977; Cohen, 2004). The development of artificial diet for insect rearing is better than the use of natural diet in view of large scale availability round the year, economy of cost, less labour need and production of insects of uniform quality. The rearing of BSFB has been reported for limited generations on the natural fruits for studies on development of artificial diet as well as varietal screening for resistance in the laboratory (Kumar, 2004; Wankhede *et al*., 2009; Rahman *et al*., 2011; Maravi *et al*., 2013; Onekutu *et al*., 2013). The limitation in rearing on natural host or its parts is of a short shelf life and consequent decay (Maravi *et al*., 2013). Patil (1990) reported only 20-25% pupation of BSFB when reared on brinjal fruit slices (variety, not mentioned) which were changed every alternate day. We in the present studies observed 43% pupation on brinjal fruit of var. Pusa Kranti. Further, the artificial diet was proved to be the best alternative to brinjal fruits or its slices for mass rearing of BSFB under controlled conditions (27±2 °C, 60-75% rh, 13L; 11D). Patil (1990) developed the mass rearing technique for BSFB and observed larval period of 18.50 days, 53.3% normal adult emergence and 66.7% pupation on artificial diet. Talekar *et al*. (1999) has reported the artificial diet comprising of one part of dried eggplant powder and 9 parts of the *Spodoptera exigua/Helicoverpa armigera* diet (commercially prepared, sold by BioServe Inc., USA) for rearing of BSFB. Our artificial diet appeared to be more successful in terms of % pupation and % adult emergence on the basis of neonates.

The larval density is also important constraint for their normal growth and development and consequently on mass rearing. The crowding of larvae leads to shortage of food, cannibalism and poor biological traits of the insects (Morton, 1979; Bhavanam *et al*., 2012). In the present studies, the cannibalism was observed in the treatment wherein 10 neonates/container were kept resulting in drastic reduction in % adult emergence. The cannibalism has been reported in other lepidopterans like cotton bollworms (Morton, 1979; unpublished). Hence, 5 neonates per plastic container was optimum density for efficient rearing of BSFB under controlled conditions. The artificial diet showed a good shelf life at 4 ^{0}C and without microbial contamination. Thus, we developed artificial diet and associated rearing techniques for successful rearing of BSFB for more than 56 generations without any infusion of wild population and without use of natural brinjal constituents based up the basic principles of nutrition and environment (Katiyar and Mukharji, 1974; Morton, 1979; Cohen, 2004). Interestingly, we did not observe any inbreeding depression, unlike in cotton bollworms (unpublished). At present, the rearing is in its F_{56} generation for more than 4 years. Although a100 gm artificial diet costs INR 34.70/-, it successfully supported 50 neonates to reach adult stage while the same amount of natural of brinjal fruit pieces, although cheaper, produced only 4-7 adults. This *in vitro* rearing technology may provide further impetus to the perspective studies on various aspects of biology, ecology, physiology and toxicology of xenobiotics.

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