The Journal of Basic & Applied Zoology (2016) 73, 9–12

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The Egyptian German Society for Zoology

The Journal of Basic & Applied Zoology

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Viable mass production method for cotton pink bollworm, *Pectinophora gossypiella* (Saunders)

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Received 29 July 2015; accepted 25 September 2015

KEYWORDS

Cotton pink bollworm;
Pectinophora gossypiella;
Mass production;
Artificial diet

Abstract Cotton seed based artificial diet has been standardized for continuous rearing of pink bollworm *Pectinophora gossypiella* (Saunders) at the Central Institute for Cotton Research, Regional Station, Coimbatore. The ingredients of the diet are easily available and are cost effective. Basic ingredients of the diet are cotton seed flour (processed) and chick pea flour, Carbohydrate, Protein, Fat sources, multi vitamin, antimicrobial agents and agar as thickening agent are used as other ingredients. Micro centrifuge tubes with lid were used as rearing containers. Individual neonate larvae were released on each piece of the diet inside the micro centrifuge tube and the lids were closed. This prevented larval escape, retaining them inside the tubes and also prevented diet dehydration. The recovery of insect reared on diet was recorded as 95.56%. Egg hatchability and adult emergence were 100% while pupal malformation was nil. Eggs, larval and pupal periods were recorded as 4.8 ± 0.632, 25.10 ± 0.994 and 7.9 ± 0.88 days, respectively. Larval and pupal weights were recorded as 21.40 mg ± 3.63, 18.00 mg ± 2.73, respectively.

Introduction

Cotton is attacked by many species of lepidopterous insects in different stages of crop growth and the pink bollworm, *Pectinophora gossypiella* (Saunders) is the most damaging pest and it is a widespread pest in almost all cotton growing countries of the world (Curl and White, 1952). Moths of this pest are very active fliers, whereas larvae mostly remain inside the fruiting bodies (squares, flowers and bolls) and cause severe damage. They web the cotton flower petals, imparting a characteristic 'rosette' appearance. Feeding within the boll results in malformation, rotting, premature or partial boll opening, reduction in fibre length and overall reduction in quality of the cotton due to staining of the lint.

After China, India is the largest producer and consumer of cotton, the country accounting for a little over 21% of the global cotton production in 2008–09. India is the largest producer covering an area of 103.29 lakh ha with a production of 295.00 lakh bales and 486 kg/ha productivity during 2014–15 (www.cicr.org.in).

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Peer review under responsibility of The Egyptian German Society for Zoology.

http://dx.doi.org/10.1016/j.jobaz.2015.09.004

Among the bollworms, the pink bollworm assumed a major pest status in the recent past (Ghosh, 2001). World over, pink bollworm *P. gossypiella* has become economically the most destructive pest of cotton and has known to cause 2.8–61.9% loss in seed cotton yield, 2.1–47.10% loss in oil content and 10.70–59.20% loss in normal opening of bolls (Patil, 2003). Bt cotton (Bollgard®) offered a high level of resistance against the cotton bollworm complex i e., *Helicoverpa armigera* (Hubner), *Earias vittella* (Fabricius) and *P. gossypiella* both in laboratory as well as field conditions (Ghosh, 2002; Kranthi et al., 2002; Kranthi and Kranthi, 2004). In 2009, the Genetic Engineering Appraisal Committee (GEAC) approved 248 new Bt cotton hybrids for commercial cultivation in the 2009 season, in addition to the 274 Bt cotton hybrids approved for sale in 2008, for a total of 522 hybrids (Choudhary and Gaur, 2010). Currently more than 1000 Bt hybrids are available for cultivation (GEAC, 2012).

But in the present scenario of Bt cotton cultivation, resistance monitoring for Bt toxin in bollworms is essential. As a resistant management measure, the susceptibility of geographic populations of *H. armigera* and *E. vitella* was studied for 2 years before the commercial approval of Bt cotton in India. However, such studies were found to be difficult to conduct primarily because of the difficulties in mass rearing facility of *P. gossypiella* in the laboratory. In India some of the efficient parasites such as *Bracon greeni* Ashmead and *Rogas aligharensis* Quadri could not be mass multiplied and used in the release programmes for want of a simple artificial diet for the rearing of the pink bollworm. The lima-bean diet developed by Patana (1977) and the wheat germ diet developed by Adkinsson et al. (1960) and Raulston (1971) for *P. gossypiella* could not be used under Indian conditions due to non-availability of some of their ingredients. This necessitated an artificial diet prepared from locally available ingredients with easily adoptable methodology by researches, by which importation of expensive diet premixes can be avoided. The aim of the present study was to develop a mass rearing diet for *P. gossypiella* using locally available ingredients.

Materials and methods

Starter culture

Larvae of the pink bollworm were collected from the infested cotton bolls from the fields of the Central Institute for Cotton Research, Regional Station, Coimbatore and reared in the laboratory. The larvae were fed with locules of green bolls and

Table 1 Ingredients and cost economics of the diet.

Fraction A*		Quantity of the ingredients	Cost (INR)
1	Cotton seed flour	50 g	6.00
2	Chickpea flour	35 g	2.10
3	Sucrose	15 g	17.70
4	Distilled water	200 ml	3.00
*Fraction B***			
1	Agar-agar	19 g	79.04
2	Distilled water	200 ml	3.00
*Fraction C****			
1	Dried yeast powder	8.0 g	17.84
2	Ascorbic acid	1.2 g	5.60
3	Methyl 4-hydroxy benzoate	1.6 g	2.70
4	Multivitamin	1.0 ml	2.49
5	Streptomycin sulphate	0.2 g	2.18
6	Bavistin	2.0 g	1.54
7	Casein	10 g	14.02
8	Cystiene	0.1 g	2.65
9	Wessons salt	2.5 g	159.59
10	Cholesterol	0.5 g	12.37
11	Sorbic acid	0.5 g	0.76
		Total cost	332.58

* Sucrose (99% purity, HimediaLaboratories™).

** Agar agar (Ultra pure, Sd Fine Chemicals).

*** Ascorbic acid (99% purity, Sd Fine Chemicals); Methyl 4-hydroxyl benzoate (99% purity, Loba Chemicals); Multi vitamin with minerals (MULTIGATES®, Each ml contains Vitamin C, 4 mg; Zinc sulphate, 13.3 mg; Lysine hydrochloride, 24 mg; Nicotinamide, 2.4 mg; Flax seed oil, 3 mg; D-panthenol, 1 mg; Vitamin B1, 0.8 mg; Vitamin B2, 0.1 mg; Vitamin B6, 0.5 mg; Vitamin A, 500 IU; Vitamin, D 3, 100 IU; Tocopheryl acetate 2.5 IU; Choline bitartrate, 25 mcg; Biotin, 10 mcg; Vitamin B 12, 1 mcg; Folic acid, 100 mcg; Ferric ammonium citrate, 5 mcg; Manganese sulphate, 500 mg; Copper sulphate, 10 mcg; Potassium chloride, 1 mcg; Chromiun chloride, 1 mcg; Sodium chloride, 10 mcg; Sodium selenate, 5 mcg; Nickel sulphate 1 mcg; Stannous chloride, 1 mcg; Sodium terta borate, 1 mcg; Carbohydrates, 595 mg; Energy, 1.9 cal; Fat, 1.5 mg); Strepomycine sulphate (HimediaLaboratories™); Bavistin (Bavistin® DF-fungicide); Casein (95% purity, HimediaLaboratories™); Cystiene (98% purity, HimediaLaboratories™); Wessons salt (Calcium carbonate, 2.63 g; Ferric phosphate, 0.183 g; Magnesium sulphate, 1.125 g; Potassium chloride, 1.50 g; Potassium Phosphate monobasic, 3.875 g; Sodium chloride, 1.312 g; Tricalcium phosphate, 1.860 g; Copper sulphate, 4.48 mg; Manganese sulphate, 2.50 mg; Potassium aluminium sulphate, 1.13 mg; Potassium iodide, 0.63 mg; Sodium fluride, 7.13 mg); Cholesterol (98% purity, Loba Chemicals); Sorbic acid (98.5% purity, Sd Fine Chemicals).

maintained at 27 ± 0.5 °C until pupation. Sex differentiation of the pink bollworm was done in the larval stage as mentioned by Dharajothi et al. (2010) for pairing of adult moths. After adult emergence, 20 pairs of moths were released per egg laying cage and provided with honey (20% of honey solution mixed with 1 ml multivitamin drop and proteinex) kept with adult food (20% honey solution + 1 ml multivitamin drop + proteinex) and cotton twigs (having terminal leaves and buds) inserted in a conical flask with water for egg laying. The bottom of the twigs was in water to retain the moistness of the tissue. Cotton twigs were changed once in two days and they were transferred to transparent plastic containers covered with white muslin cloth and tightly secured with a rubber band for egg hatching.

Diet preparation

Basic ingredients of the diet consisted of cotton seed flour (processed), Chick-pea flour with carbohydrate, protein, fat sources, multi vitamin, anti microbial agents and agar as thickening agent constituted other ingredients. The ingredients and cost economics are presented in Table 1. All the ingredients were weighed accurately and ingredients of fraction A were added to 200 ml of distilled water, heated to 60 °C, stirred well, cooled, transferred to a blender and mixed thoroughly. Fraction B containing agar-agar was boiled in 200 ml of distilled water up to beading consistency and then added to fraction A in the blender and mixed well. Finally the ingredients of fraction C were added to fraction A in the blender and mixed thoroughly. The diet was poured in glass petriplates to a depth of 2 cm under aseptic conditions and sliced (0.533 g) into 2 cm × 0.2 cm × 0.5 cm sized pieces.

Rearing of P. gossypiella

Micro centrifuge tubes of 1.5 or 2 ml size were used as rearing containers. Individual neonate larvae were released on each piece of the diet inside the tube and the lids were closed. This prevented larval escape. Though the larva was able to complete its total developmental period inside the tube, to provide a bigger space for the developing IV instar larvae, the larvae were shifted to penicillin vials (glass) from the tube and secured tightly with a rubber cork with a hole in the centre. Glass ware used in the preparation and storage of diet were thoroughly washed with water and sterilized.

Composition of artificial diet

The test diets used and some of the ingredients used tested by previous researchers Paul et al. (1987) and Muralimohan et al. (2009) and the development were compared with the present study.

Observations

Observations on the biological parameters were recorded by taking 20 larvae from cohort egg hatching. Biological parameters like egg, larval, pupal period, egg hatchability, generation time, pupal malformation, adult emergence, % hatching, sex ratio and percent recovery were recorded.

Results and discussion

Egg, larval and pupal periods were recorded as 4.8 ± 0.632, 25.10 ± 0.994, 7.9 ± 0.88 days respectively. Larval and pupal weights were recorded as 21.40 ± 3.63, 18.00 ± 2.73 mg, respectively (Table 2). Throughout the rearing methodology, fungal contamination of the diet, pupal and adult malformations were not observed. The pink bollworm was reared up to five generations and developmental parameters were compared with the earlier reported diets. Paul et al. (1987) and Muralimohan et al. (2009) are the two available reporters for artificial rearing of the pink bollworm. However, these techniques have demerits in the developmental parameters and recovery percentage of the pink bollworm. Also, the diet ingredients, covered in this study, were distinctly different from the two reported diets.

In general weight gained in the pupal stage is an indicator of the food conversion efficiency during the larval stage. Pupal weight of the *P. gossypiella* reared on the artificial diet by Paul et al. (1987) was lower than the present study under report. Glass vials secured with non-absorbent cotton, have the major disadvantage of rapid dehydration of diet. This makes the diet to dry out relatively quickly and requires replacing with fresh diet very frequently which is a time consuming process and adds an extra step in the procedure which in turn affects the larval development. But in the present study, it was refined by securing the vials with rubber cork with a hole.

The larval period was the shortest and pupal weight was the highest, respectively than in the diet reported presently. Since the diet reported by Muralimohan et al. (2009) is a biphasic diet which includes an artificial diet and bhendi (*Abelmoschus esculentus* (L) Moench.) cut pieces, it necessitates change over from diet to cut pieces of bhendi and it is an extra step in the procedure to be adopted which makes the methodology lengthy. As reported by the authors, while rearing on bhendi cut pieces, fungal contamination is a frequently encountered problem. Moreover pupal and adult malformation was noticed in the biphasic diet which directly reduced the percentage recovery of the insect. Adult emergence was also lower (91.66%) than the diet (100%) being presented herein. In the present study, throughout the rearing methodology, fungal contamination of the diet, pupal and adult malformations were not observed. Out of the ingredients mentioned in

Table 2 Performance of artificial diet in the rearing of *P. gossypiella*.

Sl. No	Biological parameters	Mean*
1	Insect recovery (%)	95.56
2	Egg hatchability (%)	100
3	Egg period (days)	4.80 ± 0.63
4	Larval period (days)	25.10 ± 0.99
5	Pupal periods (days)	7.90 ± 0.88
6	Larval weight (mg)	21.40 ± 3.63
7	Pupal weight (mg)	18.00 ± 2.73
8	Pupal malformation	Nil
9	Adult emergence (%)	100
10	Sex ratio	2:1 (female: male)

* Mean of 20 observations.

Table 1 and 1200 cubes (2 cm × 0.2 cm × 0.5 cm sized pieces.) of the diet can be made in which 1200 larvae can be reared successfully at a cost of Rs. 333.

Conclusions

The present study facilitated to develop an artificial diet for *P. gossypiella* prepared from locally available ingredients and simple adoptable methodology by researches. Monitoring for Bt toxin resistance is highly important to sustain the transgenic technology in cotton and the current methodology developed is highly useful for conducting monitoring bioassays for Bt toxin.

Acknowledgments

The authors are very grateful to The Director, Central Institute for Cotton Research, Nagpur and the Project Coordinator and Head, Central Institute for Cotton Research, Regional Station, Coimbatore for valuable guidance and adequate facilities provided.

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