Growth, development, reproductive competence and adult behaviour of *Spodoptera litura* (Lepidoptera: Noctuidae) reared on different diets

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Abstract. *Spodoptera litura* was reared on natural food (castor leaves, *Ricinus communis*) and on a several semi-synthetic diets using quasi mass rearing techniques. The effect of the different diets and rearing regimes on *S. litura* growth, development, reproductive competence and adult behaviour was measured. *Spodoptera litura* reared from a modified chickpea-based diet provided the greatest growth index and index of adequacy. These studies were conducted as a prerequisite for the evaluation of F_1 sterility technique.

1. INTRODUCTION

Spodoptera litura (Lepidoptera: Noctuidae), the common cutworm, is an economically serious and polyphagous pest in India. This pest attacks a wide range of food plants belonging to diverse botanical origins (112 cultivated food plants belonging to 44 families all over the world; 60 plants known from India) [1–4]. A multifaceted approach is required for the control of this pest because it has developed resistance against a range of insecticides and because of limitations in other control strategies when applied as a single tactic [5, 6]. The sterile insect technique (SIT), including F_1 sterility, can be used for Lepidoptera (group to which *S. litura* belongs). In a preliminary study, the effect of substerilizing doses of gamma radiation on the growth, development and reproductive behaviour of *S. litura* in F_1 progeny of treated moths suggested this pest might be managed by the F_1 sterility technique [7]. As a pre-requisite to in-depth evaluations of the reproductive performance and behaviour of *S. litura* in response to two substerilizing doses (100 Gy and 130 Gy), we developed quasi mass rearing techniques and evaluated several semi-synthetic diets.

2. MATERIALS AND METHODS

Quasi mass rearing technology was evaluated using the natural food (castor leaves) and semi-synthetic diets for the ability to produce high quality *Spodoptera litura* required for radiation biology experiments. Environmental conditions in the insectary were 26.8±1°C, 75±5% R.H. and 12L:12D photoperiod.

2.1. Rearing on natural food

The eggs laid by mated females were incubated at high relative humidity (about 80%) and maintained in containers with castor leaves (*Ricinus communis*) to provide the 1^{st} instar neonates immediate access to food. First instars were placed in groups of 100 each in a 500 ml container. From the 4^{th} instar onwards, larvae were reared in groups of 12–15 in 1 litre containers on castor leaves. Larvae were allowed to pupate in moist, loose soil. To avoid any mechanical injury, the pupae were sexed on the 3^{rd} or 4^{th} day after the sclerotization and hardening of pupal integument. Adult moths eclosed in 7–8 days. Moths, generally 10–12 pairs, were held for mating and oviposition in cages (20 x 20 x 20 cm) with 15–20% honey solution as food. Castor leaf was provided as an ovipositional substrate. After 8–10 generations adult moths were collected from agricultural fields and mixed with the laboratory colony so that vigour could be maintained and genetic deterioration caused by extended laboratory culture could be avoided.

2.2. Rearing on semi-synthetic diet

In order to develop a suitable semi-synthetic diet for mass rearing, various combinations of ingredients were evaluated for optimal growth and development of this moth. Recipes were modified from a variety of different diets used for different species of *Spodoptera* [8–11]. The proposed semi-synthetic diet consisted mainly of a ground dry seed source (chickpea, wheat, wheat germ or soybean) mixed with yeast and synthetic additives in an agar base. A chickpea-based (CpN), in which chickpea was used as a main carbohydrate complement, was reasonably satisfactory in preliminary experiments. Therefore, two more chickpea-based, semi-synthetic diets (with little modification) were prepared for evaluation: CpCs (chickpea based semi-synthetic diet with castor leaf powder) and CpSn (chickpea-based, semi-synthetic diet with sinigrin) (Table 1).

Agar was added to water and autoclaved. All ingredients of parts B and C were mixed thoroughly and added to the dissolved agar. Finally, the antibiotic and vitamin mix (part D)

Table 1. Constituents of the semi-synthetic diet proposed for rearing *Spodoptera litura.*

Ingredients	Amount
PART A	
Agar	25.00 g
Deionized water	750.00 ml
PART B	
Casein	44.00 g
Ground chickpea seeds	93.50 g
Wesson's salts	12.50 g
Cholesterol	1.25 g
Brewer's yeast	19.00 g
Methyl-p-hydroxybenzoate	1.25 g
Sugar	39.00 g
Sorbic acid	2.00 g
Deionized water	400.00 ml
4 M KOH solution	6.25 ml
PART C	
Corn oil	2.50 ml
Linseed oil	2.50 ml
Formaldehyde 10% solution	5.50 ml
Sinigrin (1%)	3.53 ml
PART D	
Antibiotic and vitamin mixture[1]	7.50 g
Choline chloride	1.25 g

[1] Composition: chloramphenicol (2 g), streptomycin (4 g), tetracycline (36 g), ascorbic acid (80 g), vitamin E (Evion, 0.2 g; Merck Co.), vitamin mixture (2 g; Roche Co.).

was added when the mixture cooled to about 70°C. When the diet cooled completely it was covered and stored at 4°C. Neonates were placed in a plastic chamber (8 cm diam x 8 cm) containing a strip of diet. About 100 larvae were placed in each chamber and allowed to feed and grow in a gregarious manner. After 4–5 days, 3rd instars were placed individually with diet in glass specimen tubes (2.5 cm diam. x 10 cm) or plastic containers (6 x 6 x 6 cm). Fresh diet was replaced after 72–96 h. Larvae pupated inside the diet. Pupae were collected after 48–72 and were allowed to eclose in mating/oviposition cages.

2.3. Handling techniques to control microbial contamination

Various protocols were adopted to prevent microbial infection during the rearing of *S. litura*. Examples of these protocols included:

(i) formaldehyde fumigation to disinfect the insectary before introduction of insects,

(ii) washing glassware and plastic containers with detergent, 5% formalin and oven drying at about 70–80°C,

(iii) washing castor leaves with water and 0.001% $KMnO_4$,

(iv) surface sterilization of eggs for 3–5 seconds with 0.2% sodium hypochlorite or 2% formalin,

(v) surface sterilization of pupae for 10 s with 1% sodium hypochlorite or 4–5% formalin, and

(vi) adding diet to insect containers under aseptic conditions in a laminar flow hood.

2.4. Diet suitability for insect maintenance

Suitability of the various diets was determined using an index incorporating growth, development, fecundity (indicated in terms of female pupal weight) and survival into one empirical factor, similar to the index described by Raulston [12]. The suitability of the diets was also evaluated in terms of reproductive behaviour of the reared insects. Experiments on mating success were conducted in cages (each cage having 10–15 pairs, comprising one replicate). The mating success of moths was assessed by dissection of females immediately after the death. The presence of a spermatophore in the bursa copulatrix indicated that the female had mated; the number of spermatophores indicated the number of matings.

3. RESULTS

Growth indices revealed that the chickpea-based diets were better than the other semi-synthetic diets. Of the three types of chickpea diets evaluated, the CpSn diet (Table 1) was more suitable for insect growth. Insects that fed on CpSn diet had a growth index of 2.61 and an index of adequacy of 1.54. These values were similar to those calculated for insects reared on castor leaves (Table 2). The index of adequacy for larvae that developed on the Soybean-based diet was about 20% lower than that for larvae that developed on the CpSn diet.

Table 2. Growth and development of *Spodoptera litura* on different diets.

Nature of Food	% Pupation[1]	Larval Period	Female Pupal Weight (g)	Developmental Period (days)	% Adult Emergence[1]	Growth Index[2]	Sex Ratio M:F	Index of Adequacy[3]
Castor leaf	88.4a	16.2±0.7a	0.339±0.007a	27.9±0.3a	83.6±3.3a	2.99a	1:0.98	1.748a
Wheat germ diet	57.3c	17.5±0.8ab	0.320±0.012ab	1.4±0.9bc	52.3±2.6d	1.66c	1:1.01	0.955d
Wheat diet	71.8b	18.1±0.5b	0.313±0.010b	31.9±0.7c	64.3±2.4c	2.01bc	1:1.10	1.109cd
Soybean diet	76.7b	17.1±0.4a	0.310±0.008b	29.5±0.8b	67.8±3.3bc	2.29bc	1:1.02	1.233c
Chickpea diet CpCs[4]	78.4b	16.8±0.5a	0.314±0.002b	29.8±0.7b	69.5±2.9b	2.33b	1:0.95	1.304bc
Chickpea diet CpN[5]	79.1b	16.8±0.4a	0.344±0.008a	29.7±0.8b	71.8±2.5b	2.41b	1:0.94	1.470b
Chickpea diet CpSn[6]	81.4ab	16.5±0.4a	0.339±0.009a	28.6±0.6ab	74.9±2.9ab	2.61ab	1:0.96	1.540ab

[1] Observed in groups of 25 larvae = 1 replicate (analyzed with ANOVA; data transformed using arcsine square root).
[2] Growth index = % adult formation / developmental period.
[3] Index of adequacy = (female pupal weight / larval period) x % adult formation.
[4] Chickpea based diet + yeast + synthetic constituents + castor leaf powder (3.5 g/litre).
[5] Chickpea based diet + yeast + synthetic constituents.
[6] Chickpea based diet + yeast + synthetic constituents + sinigrin.

Means ± SE followed by the same letter in a column are not significantly different at $P < 0.05$ (calculated using ANOVA followed by LSD post test); n = 10.

Table 3. Reproductive and mating parameters of *Spodoptera litura* reared on different diets.

Nature of Food	Preoviposition Period (d)	Oviposition Period (d)	Eggs per Female	Mating Frequency	Mating Success[1] (%)	Fertility[1] (%)	Longevity (days)	
							Male	Female
Castor leaf	1.72±0.08a	7.62±0.31a	2088±49a	1.7±0.1a	94.1±1.9a	88.9±1.4a	10.3±0.4a	9.3±0.3a
Chickpea diet (CpSn)	1.69±0.09a	7.33±0.24a	2155±74a	1.7±0.2a	89.6±2.4ab	78.6±3.1b	9.8±0.5ab	9.5±0.2a
Soybean diet	1.75±0.09a	7.25±0.31a	1750±105b	1.6±0.2a	87.5±2.9ab	72.5±3.5b	9.5±0.6ab	9.0±0.3a
Wheat diet	1.85±0.08a	5.65±0.35b	985±125c	1.5±0.3a	86.6±4.3ab	53.4±4.2c	7.8±0.9b	7.3±0.6b
Wheat germ diet	1.92±0.12a	5.35±0.59b	746±172c	1.4±0.6a	77.0±6.9b	50.3±3.5c	7.1±0.7c	6.9±0.5b

[1] For statistical analysis by ANOVA, the percentage data were transformed using arcsine square root.
Means ± SE followed by the same letter in a column are not significantly different at $P < 0.05$ (calculated using ANOVA followed by LSD post test); n = 10.

Because the adult's behavioural competence is a crucial parameter in deciding the success of the F_1 sterility technique, assessment of adult behaviour was also examined on the semi-synthetic diets. Average longevity of mated males and females was not significantly different for castor leaves, the CpSn diet and the soybean diet, however, longevity decreased on the wheat germ and wheat-based diets. Mean oviposition was highest for females that developed on the CpSn diet (Table 3). On soybean diet, the mean number of eggs laid per mated female was 1750. Oviposition was reduced by 54.2% on wheat-based diet and by 65.3% on wheat germ diet compared to that on CpSn diet. Egg viability was greatest on castor leaves (88.9%), followed by on CpSn diet (78.6%), soybean diet (72.5%), wheat-based diet (53.4%) and wheat germ diet (50.3%). Insects reared on CpSn diet exhibited better fertility in comparison with the insects reared on other semi-synthetic diets (Table 3). Among all the semi-synthetic diets evaluated, CpSn diet was found to be most suitable for *Spodoptera litura* rearing. This diet showed an increased growth index due to the incorporation of sinigrin as a phagostimulant. This addition caused enhanced survival and higher fecundity. Therefore, the improved chickpea based diet (CpSn) along with the natural food were selected as the diets to use for rearing *S. litura* for further studies.

4. DISCUSSION

The operation of a mass rearing facility is a basic pre-requisite for conducting evaluations on sterile insect technique (SIT) and F_1 sterility principle. Castor leaves were used to culture *S. litura* given that growth and reproduction of the insects reared on this host was comparable with that of wild insects. In addition, the quality of insects could be maintained for a long time (R. K. Seth, unpublished). Moreover, the use of castor leaves economical compared with the cost of semi-synthetic diet. Therefore, besides developing semi-synthetic diet, a culture of *S. litura* also was maintained on castor leaves for evaluation of F_1 sterility.

The main carbohydrate component in the different semi-synthetic diet was supplied as ground, dry seeds (i.e., chickpea, wheat, wheat germ, or soybean). Satisfactory growth of *Spodoptera* species has been reported from soybean-based [9,10] and wheat germ-based diets [11]. However, in this study the best index of adequacy for growth of *S. litura* was recorded when insects were reared on the chickpea-based diet. The diet's major component was carbohydrates, of which sucrose provided 6.6% of the total. Microbial inhibitors (formaldehyde, methyl-p-hydroxybenzoate, sorbic acid, and antibiotic mix) comprised about 1.14% of the diet. Brewer's yeast was added as a source of vitamin B complex. Although brewer's yeast contains choline, the amount has been reported to be insufficient [13]. Therefore, multivitamin tablets (Roche Co.) and choline chloride were added. Vitamin C has been shown to be an essential requirement for a limited number of herbivores [14]. Our diet contained 0.33% of L-ascorbic acid and provided satisfactory growth of *S. litura.* These results agree with the findings reported by Levinson and Navon [9]. Vitamin E was added to improve adult survival and reproduction.

Most insects require sterols in their diet. As such, we added cholesterol to the diets tested. In addition, a dietary requirement for polyethenoid fatty acids has been demonstrated for several lepidopterans [15]. Unsuccessful moulting, eclosion and reproduction are common symptoms of linoleic or linolenic acid deficiency. David et al. [11], working with *Spodoptera exempta,* showed that diets containing corn and linseed oil yielded more adults than diets containing cholesterol. As such, corn oil and linseed oil were added to the chickpea diet. A sinigrin solution (0.0025%) was added as a phagostimulant which resulted in better growth and increased the index of adequacy for this diet.

In our experiments the fecundity of *S. litura* was slightly enhanced in moths reared on chickpea diet (up to 2,155 eggs per mated female) compared to moths reared on castor leaves. Similarly, Lukefahr and Martin [16], Tamhankar et al. [17] and Tamhankar and Dongre [18] reported that synthetic diet-reared lepidopterans had better fecundity than those reared on natural food. We conducted an assessment of the quality of the reared insects. This included calculation of the growth index, and evaluation of adult behaviour and mating competence of moths. We found that pupal characteristics were important and useful in assessing the quality and vigour of *S. litura* [19]. Threshold values for adult survival, sperm transfer and mating competitiveness also should be established for assessing the use of F_1 sterility as a control strategy.

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REFERENCES

[1] LEFROY, H.M., The tobacco caterpillar, *Prodenia littoralis*, Mem. Dept. Agric. India Entomol. Ser. 2 (1908): 79–93.

[2] MOUSSA, M.A., M.A. ZAHER AND F. KOTBY, Abundance of cotton leafworm, *Prodenia litura* (F.) in relation to host plants. I. Host plants and their effect on biology, Bull. Soc. Entomol. Egypte. 44 (1960): 241–251.

[3] THOBBI, V.V., Growth potential of *Prodenia litura* in relation to certain food plants in Surat, Indian J. Entomol. 23 (1961): 262–264.

[4] CHARI, M.S. AND N.G. PATEL, Cotton leafworm *Spodoptera litura* (Fabr.): its biology and integrated control measures, Cotton Development 13 (1983): 7–8.

[5] ARMES, N.J., J.A. WIGHTMAN, D.R. JADHAV AND G.V. RANGA RAO, Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India, Pestic. Sci. 50 (1997): 240–248.

[6] RAMAKRISHNAN, N., V.S. SAXENA AND S. DHINGRA, Insecticide resistance in the population of *Spodoptera litura* (Fb.) in Andhra Pradesh, Pesticides 18 (1984): 23–27.

[7] SETH, R. K. AND S.S. SEHGAL, "Partial sterilizing radiation dose-effect on the F_1 progeny of *Spodoptera litura* (Fabr.): growth, bioenergetics and reproductive competence", In: Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques, (Kitto, Kelleher and Ramesh, eds.) Vienna, 19–23 October 1992. International Atomic Energy Agency, Vienna. (1993): 427–440.

[8] MOORE, I. AND A. NAVON, An artificial medium for rearing *Prodenia litura* F. and two other noctuids, Entomophaga 9 (1964): 181–185.

[9] LEVINSON, H.Z. AND A. NAVON, Ascorbic acid and unsaturated fatty acids in the nutrition of the Egyptian cotton leafworm, *Prodenia litura*, J. Insect Physiol. 15 (1969): 591–595.

[10] BURTON, R.L. AND W.D. PERKINS, WSB, a new laboratory diet for the corn earworm and the fall armyworm, J. Econ. Entomol. 65 (1972): 385–386.

[11] DAVID, W.A., L.S. ELLABY AND G. TAYLOR, Rearing *Spodoptera exempta* on semi-synthetic diets and on growing maize, Entomol. Exp. Appl. 18 (1975): 226–237.

[12] RAULSTON, J.R., B-vitamin supplements required for soy flour-wheat germ diet used in rearing tobacco budworm, Ann. Entomol. Soc. Amer. 68 (1975): 387–388.

[13] BECK, S.D., J.H. LILLY AND J.F. STAUFFER, Nutrition of the European corn borer, *Pyrausta nubialis* (Hbn.). I. Development of a satisfactory purified diet for larval growth, Ann. Entomol. Soc. Amer. 42 (1949): 483–496.

[14] DADD, R.H., D.L. KRIEGER AND T.E. MITTLER, Studies on the artificial feeding of the aphid *Myzus persicae* (Sulzer) - IV. Requirements for water-soluble vitamins and ascorbic acid, J. Insect Physiol. 13 (1967): 249–272.

[15] SCHMIDT, G.H., Polyensauren in Insektlipiden und ihre Bedeutung für die Metamorphose, Fette Seifen Anstr. Mittel. 66 (1964): 573–579.

[16] LUKEFAHR, M.J. AND D.F. MARTIN, The effects of various larval and adult diets on the fecundity and longevity of the bollworm, tobacco budworm and cotton leaf worm, J. Econ. Entomol. 57 (1964): 233–235.

[17] TAMHANKAR, A.J., K. SHANTARAM, M.R. HARWALKAR AND G.W. RAHALKAR, Development of an artificial diet for the laboratory rearing of the spotted bollworm, *Earias vitella* (Fabricius) (Lepidoptera: Noctuidae), Insect Sci. Appl. 13 (1992): 241–243.

[18] TAMHANKAR, A.J. AND T.K. DONGRE, Improved artificial diets for laboratory rearing of the spiny bollworm, *Earias insulana* (Biosduval) (Lepidoptera: Noctuidae), Trop. Pest Management 38 (1992): 85–88.

[19] SETH, R.K., V.P. SHARMA, D.K. RAO, AND J.J. KAUR, Pupae as a physiological and morphogenetic indicator for assessing F_1 sterility technique for management of *Spodoptera litura* (Fabricius), J. Nuclear Agric. Biol. 26 (1997): 247–253.