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Artificial diet formulation and its efficacy evaluation on development and reproduction of *Spodoptera litura* F. a polyphagous pest

KEYWORDS: *Spodoptera litura*, artificial diet, mass rearing

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ABSTRACT *The tobacco cutworm Spodoptera litura Fabricius (Lepidoptera: Noctuidae) is a serious polyphagous pest distributed throughout the tropical and subtropical world causing damage to more than 150 species of host plants. A suitable artificial diet is desirable for producing uniform insects for research purpose. In pursuit, a diet was formulated and assessed against a similar diet formulated for Helicoverpa armigera (Shobha et al., 2009) and castor leaves a natural diet for rearing the larvae of Spodoptera litura in laboratory. The biological parameters based on mass rearing of eleven continuous generations showed higher pupation (89.3±1.84) and survival (70.4±1.21) of larvae on new modified diet formulation, compared to that of Helicoverpa armigera diet (81.6±1.61 and 48.6±1.43) and castor leaves (78.1±1.18 and 45.3±0.06). The average number of eggs laid by insects reared on this diet was 3546.4±10.21, against 508.3±15.21 and 2618.2±17.31 those reared on castor leaves and Helicoverpa armigera diet respectively.*

INTRODUCTION

Mass rearing of *Spodoptera litura* is normally reared on castor leaves (*Ricinus communis*) in the laboratory a laborious job as it demands supply of fresh leaves throughout the feeding stage of the insect sometimes needs change of leaves twice daily; besides imparting infections to the larvae resulting in poor harvest of any of the stages. Though, successful establishment of colonies involve monitoring of photoperiod (Hashmat and Khan, 2009), temperature (Zhu *et al.*, 2000), humidity (Patel *et al.*, 1986) and sanitation; cannibalistic nature of the larvae (Jason *et al.*, 1999) limits the success of mass rearing, as group rearing of larvae is impossible. Individual rearing on castor leaves is not feasible as it requires large supply of leaves and larger space. This limitation, one can, overcomes by rearing on artificial diet. Though the insect is reared on artificial diet (Gupta *et al.*, 2005) by a number of investigators satisfactory diet formulations for continuous rearing of this moth are still eluding the researchers. After three to four generations fresh stock culture is required to reestablish the colony.

In the absence of a well defined artificial diet for a long term rearing and to handle the cannibalistic larvae to achieve maximum harvest of larvae/moths the current investigation was undertaken to develop a feasible technique for mass rearing.

MATERIAL AND METHODS

Parental stock

The success of the rearing program depends upon obtaining a disease free and a genetically efficient and uniform parental stock. The starter colony was established from pupae (National Accession No. NBAII-MP-NOC-02) collected from National Bureau of Agriculturally Important Insects (NBAII), Bangalore.

Rearing

Rearing was carried out on surface sterilized castor leaves till completion of one life cycle. The second cycle was continued on castor leaves, artificial diet developed for *Helicoverpa armigera* (Shobha *et al.*, 2009) and a composition formulated for *S. litura* based on *H. armigera* diet at 25±2 °C and 70±5% RH with a photoperiod of 12L: 12D.

Adults soon after emergence were paired and transferred into a new cage. After successful mating the female was provided with a suitable substratum for egg laying and the eggs were transferred to a fresh container every day to avoid the emerged larvae feeding on freshly laid eggs. The adults were provided with 10% honey as food. Larvae emerged from the eggs were transferred to Petri-plates containing the test diets (Table 1.) as well as castor leaves.

***H. armigera* Diet (Shobha *et al.*, 2009)**			**Modified Diet for *Spodoptera litura***	
Ingredients	**Quantity**	**Batch**	**Ingredients**	**Quantity**
Chickpea flour	90 g	I	Chickpea flour	90 g
Milk Powder	10 g	I		
Sorbic acid	1 g	I	Sorbic acid	1 g
Ascorbic acid	3 g	I	Ascorbic acid	3 g
Sterile water	400 ml	I	Sterile water	400 ml
Agar-agar	12 g	II	Agar-agar	12.75 g
Sterile water	400 ml	II	Sterile water	400 ml
Bavistin	2 g	III	Bavistin	2 g
Formaldehyde-40%	1 ml	III	Formaldehyde-10%	2 ml

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Absolute alcohol	10 ml	IV	Absolute alcohol	10 ml
Methyl p-hydroxy benzoate	2 g		Methyl p-hydroxy benzoate	2 g
			Streptomycin sulphate	0.25 g
Yeast (Brewer's)	30 g	V	Yeast (Brewer's)	10 g
Vitamin Syrup	2 ml		Multi Vitamin (Becadexamin)	2 capsules
			Vitamin E (Evion)	2 capsules

Table 1. Composition and preparation of artificial diet

The ingredients of Batch-I were mixed thoroughly in a blender with lukewarm 400ml distilled water. Agar-agar (Batch-II) dissolved in 400ml lukewarm distilled water was boiled and cooled to 50 °C. The molten agar was transferred to the blender and mixed thoroughly for two minutes. Ingredients of Batch-III were added to the blender and mixed for two minutes. Once the diet temperature was less than 50 °C the Batch-IV ingredients are added and mixed well. Finally Batch-V ingredients were added and whirl mixed. The diet was transferred to Petri-plates and individual vials while it was still hot and allowed to cool before transferring the larvae to diet.

Early instars larvae were reared in Petri-plates while later instar i.e. third instars onwards in individual vials containing about 5 ml diets. Larvae burrowed in to the diet on entering pre-pupal stage. The vials were inverted to safeguard the larvae which will otherwise get buried in the diet as the diet caves in. The pupae collected were disinfected with 0.2% hypochlorite solution and thoroughly rinsed in distilled water and maintained separately after sexing. At each stage of development the duration taken to advance to the next stage was recorded besides the mortality rate.

Diet evaluation

The indices of larval growth and total development were calculated based on % pupation and % survival respectively using the formulae (Gupta *et al.*, 2005) mentioned below to evaluate the diet influence on growth and development.

$$\text{Larval Growth Index} = \frac{\%\ \text{Pupation}}{\text{Larval Period (days)}}$$

$$\text{Total Developmental Index} = \frac{\%\ \text{Survival}}{\text{Total Developmental Period (days)}}$$

Male and female moths were allowed to mate for three hours. After mating the females were transferred to a separate chamber and a sheet of paper was provided as substratum to lay eggs. The egg sheets were replaced every day to record the rate of egg laying as well as total number of eggs laid.

Results

The biological attributes of *Spodoptera litura* reared on the castor leaves, artificial diet developed for *Helicoverpa armigera* (Shobha *et al.*, 2009) and composition formulated for *S. litura* over eleven successive generations are given in Table 2.

Table 2. Growth & Survival parameters (Mean ± SD*) under different diet formulations

Parameters	Natural diet Castor leaves	*Helicoverpa armigera* Diet (Shobha et al., 2009)	*Spodoptera litura* Modified Diet
1st instars	2.27 ± 0.23	2.35 ± 0.06	2.26 ± 0.13
2nd instars	2.33 ± 0.09	2.18 ± 0.02	2.73 ± 0.05
3rd instars	2.15 ± 0.07	3.32 ± 0.12	3.55 ± 0.18
4th instars	2.71 ± 0.13	3.16 ± 1.04	3.32 ± 0.13
5th instars	3.35 ± 0.15	2.66 ± 0.07	4.59 ± 0.06
6th instars	2.45 ± 0.27	3.11 ± 0.03	5.33 ± 0.34
Pre-pupal period	2.24 ± 0.12	3.52 ± 0.02	2.02 ± 0.07
Larval period (Days)	17.50 ± 1.06	18.3 ± 1.36	23.8 ± 0.96
Pupal period (Days)	10.7 ± 0.71	11.3 ± 0.98	12.3 ± 0.36
Pupation (%)	78.1 ± 1.18	81.6 ± 1.61	89.3 ± 1.84
Survival (%)	45.3 ± 0.06	48.6 ± 1.43	70.4 ± 1.21
Larval Growth Index	4.46	4.45	3.75
Total Developmental Index	1.60	1.64	1.95
Fecundity (eggs/ female) **	508.3 ± 15.21	±17.31	3546.4 ± 10.21

*Means of eleven generations and three replicates per generation
** Means of ten females per generation

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Larval and pupal period
The total larval period as well as pupal period was found to be longer on modified diet (23.8 ± 0.96, 12.3 ± 0.36) compared to castor leaves (17.50 ± 1.06, 10.7 ± 0.71) and *Helicoverpa armigera* diet (18.3 ± 1.36, 11.3 ± 0.98).

Pre-pupation
The pre-pupal stage lasted for 2.02 ± 0.07 days in modified diet compared to that of castor leaves (2.24 ± 0.12) and *Helicoverpa armigera* diet (3.52 ± 0.02) fed larvae.

Pupation
Number of pupae harvested was slightly better on modified diet compared to other two diets. The maximum percentage of pupation was observed in the larvae fed with the modified diet (89.3±1.84) compared to that of castor leaves (78.1±1.18) and *Helicoverpa armigera* diet (81.6±1.61) fed larvae.

Survival rate
The survival rate was 1.5 times and 1.4 times more on the *Spodoptera litura* modified diet compared to that of castor leaves and *Helicoverpa armigera* diet respectively.

Fecundity
The fecundity (3546.4±10.21) was seven times greater compared to those fed on castor leaves (508.3±15.21) and nearly one and half times greater than those fed on *Helicoverpa armigera* diet (2618.2±17.31).

Growth and Development indices
The larval growth index and total developmental index are inversely proportional . The larval growth index was 4.46, 4.45 and 3.75 respectively in castor leaves, *Helicoverpa armigera* diet and *Spodoptera litura* modified diet fed larvae with least in the latter. Conversely, the developmental index was higher in *Spodoptera litura* modified diet fed larvae which was 1.95 against 1.60 and 1.64 in castor leaves and *Helicoverpa armigera* diet fed larvae respectively.

Discussion
Numerous diets for Lepidoptera larval rearing have been already modified/ simplified (Shorey and Hale, 1965; Wakil *et al.,* 2011; Bing-Chun *et al.,* 2011). However, there is always a scope to improve the diets as well as rearing techniques in any laboratory which can be adopted by other researchers with little or no modifications. The present study has evaluated efficacy of different diets on rearing of larvae of *Spodoptera litura* a polyphagous pest.

Spodoptera litura larvae exhibits cannibalism whenever they are group reared. Rearing on an artificial diet provides an opportunity to rear these insects in small vials singly to avoid cannibalism which is a limitation in case of castor leaves which are normally used for rearing in the laboratory. The cannibalistic larva of *Spodoptera litura* was either bigger in size than its prey or more active and it had completed the respective instar faster than the control larvae fed only with artificial diet.

Addition of streptomycin sulphate and vitamin E to the artificial diet of *Helicoverpa armigera* (Shobha *et al.,* 2009) and removal of milk powder from the diet in order to prepare a modified diet lead to an increase in survival rate and fecundity of *Spodoptera litura* by nearly two and one and a half times respectively compared to original *Helicoverpa armigera* diet. Streptomycin sulphate and vitamin E generally protects the organisms from infections (Srinivasan *et al.,* 2000) and the shelf life of diet which is in semi solid form might have improved. Hence, an increased survival rate of *S. litura*. Rueda *et al.,* (2010) have demonstrated that milk powder based diet resulted in lower larval life duration in *L. seicata*. Further, enhanced fecundity could be attributed to increase in the larval period which might have offered an opportunity to feed more as later instar larvae are normally voracious feeders. The female fecundity was higher in the insects, the larvae of which were fed on modified diet than those fed even on castor leaves a natural diet which is also evident from the total developmental index which was higher (1.95) in modified diet compare to that of in castor leaves (1.60) and *H. armigera* diet (1.64) respectively.

The enhanced parameters such as pupation percentage (89.3±1.84) and survival percentage (70.4±1.21) in addition to enhanced total developmental indices are suggestive of suitability of the modified artificial diet for rearing of *Spodoptera litura* larvae. The larval growth index and total developmental index are inversely proportional (Gupta *et al.,* 2005) and enhanced development index is an evidence of suitability of the diet as the index in other two diets are low.

Inbreeding depression is another problem encountered in laboratory rearing that generally occurs after the fourth/fifth generation in laboratory reared colonies of any insect culture (Gupta *et al.,* 1998). However, in the present study it was observed only after the eighth generation where pupation and survival decreased up to 5% which further supports the suitability of the diet for mass rearing of *Spodoptera litura*.

Conclusion
Mass rearing of *Spodoptera litura* under laboratory conditions on castor leaves, a general practice is not only laborious but also inflicts infections to the larvae. The artificial diet of *Helicoverpa armigera* (Shobha *et al.,* 2009) with small modifications yielded very good results in terms of increased pupation percentage, survival rate and fecundity. Because of all these the modified diet is ideal for mass rearing of *Spodoptera litura*.

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