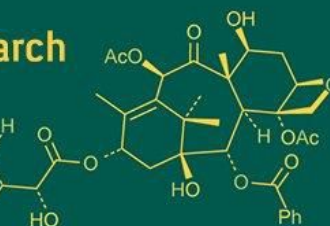
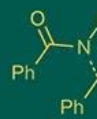


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Studies on the biology of *Spodoptera litura* (Lepidoptera: Noctuidae) on artificial diet under laboratory conditions

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Abstract

The development of a viable mass-production technique for the generalist herbivore *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), using modified artificial diets, is crucial for conducting experiments to study its biology and propose control methods. This study aims to investigate the developmental biology of *S. litura* fed on artificial diet. The mean developmental period of the first, second, third, fourth, fifth larval instar on artificial diet was 2.57 ± 0.51 , 3.50 ± 0.57 , 4.55 ± 0.68 , 5.51 ± 0.71 , 5.51 ± 0.71 days, respectively. The mean total life period of male and female insect was 38.50 ± 0.88 and 41.70 ± 0.56 days, respectively. Observations on the life cycle parameters, including pupal duration (8.00 ± 0.71 days), and adult longevity (8.00 ± 1.00 days, female, and 7.10 ± 0.88 days, male), were recorded. The current results indicate that *S. litura* can be effectively cultured on artificial diets under controlled laboratory conditions.

Keywords: Artificial diet, biology, *S. litura*, instar, larva

Introduction

India is a major producer of cereals, pulses, oilseeds and vegetables with over varieties of different crops grown under varied agro-climatic conditions. Key crops include Cotton, groundnut, soybean, tomato, greengram, onion, chickpea, brinjal, tobacco, cabbage, cauliflower, okra, and peas. Crop yield loss owing to a variety of biotic and abiotic variables is a major problem for the worldwide agriculture sector, and insect pests are a major contributor to this drop. Insects can live on a wide range of plants, including ornamental plants, weeds, and trees in forests. Additionally, it can contaminate packaged food items stored in facilities, leading to substantial food loss and deterioration of quality. According to Dhaliwal *et al.* (2010), insect pests are classified as minor pests when the damage falls between 5% and 10% and as important pests when the damage exceeds 10%. *Spodoptera litura* (Fabricius), is also known as the tobacco caterpillar, beet armyworm, smaller armyworm, little mottled willow, cutworm and pigweed caterpillar belonging to the family Noctuidae in the order Lepidoptera (Qin *et al.*, 2020) [12]. Native to Asia, this species is found in tropical and temperate regions of Asia, Australia, Africa, the Middle East, Europe, and the Pacific Islands (Singh *et al.*, 2024) [15]. The pest can also found in India, Pakistan, Bangladesh, Sri Lanka, South East Asia, China, Korea, Japan, the Philippines, Indonesia, Australia, the Pacific Islands, Hawaii, and Fiji (Cameron *et al.*, 1993) [3]. It causes serious damage to the nature and also acts as crucial feeder on a number of agricultural and horticultural crops. Next to *Helicoverpa armigera*, the tobacco caterpillar, *S. litura*, is regarded as one of the biggest challenges to modern intensive agriculture and shifting cropping patterns globally. It is an economically significant polyphagous pest in India. It feeds on nearly, 112-150 kinds of plants. This insect pest skeletonizes leaves in the early stages and causes intensive defoliation in later stages, which reduces the ability of affected plants to photosynthesize. The young insect pest larvae/caterpillars eat the complete leaves, buds, flowers and even contaminate the fruits in the infected fields. Caterpillars burrow themselves in the soil up to several centimeters and pupate without the cocoon.

Rigid sclerotized sections, such as the insect head, grow stepwise in all arthropods with the aid of ecdysis, which is easily analyzed by comparing the size of the body structure in successive instars. The breadth of the head capsule was used to identify the instar stage in numerous research on the growth of young insects, particularly in lepidoptera. Therefore, measuring the head capsule's diameter offers fundamental data for the creation of morphometric studies that are helpful for controlling pests (Tanwar *et al.*, 2010) [16]. The age of different lepidopteran pests was frequently ascertained by measuring the head capsule width of various larval instars. The number of larval instars and other information concerning with insect biology help in the development of pest forecasting models based crop phenology, or in the refinement of existing models (Nisha Pradeepa *et al.*, 2017) [11]. Control of insect pests might be achieved by means of a diversity of tactics. Host plant resistance, biological, chemical and cultural methods, are involved in this category. Biological control through the release of sterile insect is a vital part of integrated pest management (IPM) strategies, and does not contaminate the air, water and landscape. A constant supply of experimental insects is as well important for conducting field and laboratory experimentations (Siddiqui *et al.*, 2023) [14]. There are currently many artificial diets available for raising *S. litura* (Gupta *et al.*, 2005; Hashmi *et al.*, 2023) [4, 5]. Although there have been some successes in raising this insect on a synthetic diet, the influence of both fitness and reproductive potential has resulted in a longer developmental period and a reduced fecundity rate. The artificial diets used for raising *S. litura* to date have led to decreased fecundity, longer developmental periods, and worse survival rates despite a number of issues with the semi-synthetic formulation. The current study assesses the advancements, adjustments, and continuous standardization of artificial diets to increase *S. litura* rearing efficiency with

the goal of minimizing detrimental effects on the insect's biological characteristics over subsequent cohorts.

Material and Methods

The experiments were conducted at BLDEA's, Commerce, B.H.S Arts and T.G.P Science College, Jamkhandi, Bagalkot, Karnataka, India.

Insect culture

Egg clusters of *S. litura* were collected from the cotton and tobacco farmer fields to start a nucleus colony. Neonates were reared on cotton leaves, in an environmentally controlled growth chamber (25±2 °C, 60-65% Relative humidity and photoperiod of 12 hours light and 12 hours dark). Cotton leaves were washed thoroughly with running water and treated with 0.02% formaldehyde solution, to avoid microbial infection, before feeding to the larvae. Sterilized moist sandy soil was provided to the fifth instar larvae for pupation. Three-day-old pupae were treated with 0.02% formaldehyde solution to protect them from infection. Inside the cage five % honey solution was provided as food for the adults by dipping of absorbent sponge in it. Thus, a continuous culture was maintained on cotton leaves for growth and development studies at initiation stage to obtain good number of insects.

Diets composition and preparation

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 larvae. Recipes were modified from a variety of different diets used for different species of *Spodoptera* (Moore *et al.*, 1964; Gupta *et al.*, 2005) [9, 4]. 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. The ingredients of the artificial diet (1000 ml) for *S. litura* are listed in Table 1.

Table 1: Composition of artificial diet for rearing of *Spodoptera litura* larvae

Groups	Ingredients	Quantity(g/ml)
Fraction-A	Wheat germ	40.00
	Chickpea flour	55.00
Fraction-B	Sorbic acid	1.5
	Ascorbic acid	5.5
	Methyl-p-hydroxybenzoate	4.0
	Formaldehyde solution (4%)	25
	Streptomycin sulphate	0.10
	Vitamin mixture	5.00
Fraction-C	Yeast	34.00
	Agar	21.00

The ingredients of fraction 'A' were mixed thoroughly in a blender with 500ml of deionized distilled water. Agar-agar and yeast powder (fraction 'C') was weighed separately, dissolved in 500ml of deionized distilled water and boiled in a hot air oven for three minutes. After boiling the mixture was cooled to room temperature for 2 minutes and the mixture blended thoroughly for about 1min at high speed for uniform mixture. The ingredients of fraction 'B' was then added to Fraction-A and the mixture stirred well using glass rod. After uniform mixture of Fraction-A&B, Fraction-C mixed with them and boiled the mixture in hot air oven for 2 minutes. The prepared diet was poured into sterilized plastic dropping bottle. The diet was refrigerated until use after

solidifying at room temperature (25-27 °C). The diet was removed from the fridge and conditioned to room temperature for 2-3h before use. The semi-solid diet should be placing in small plastic vial (4×3 cm) using dropping bottle. Approximately 4 ml diet were put in each plastic vial.

Rearing procedure

Rearing of *S. litura* was completed under environmentally controlled conditions in the laboratory. About 25 neonates larvae were reared on 7 ml diet per plastic vial. four days later, these larvae were transferred into individual rearing plastic vial until pupation to avoid cannibalism. The diet should be regularly changed at every three days to avoid

microbial contamination. Pupation occurred inside pupal cells formed with the artificial diet. The pupae were collected and disinfected with 0.02% formaldehyde solution, air-dried and kept in glass jars until adult emergence. After emergence of adults, the adults were transferred to the oviposition jar (25×17 cm). The mouth of each jar was covered with a sterilized white muslin cloth and kept tight. Inside the cage five % honey solution was provided as food for the adults by dipping of absorbent sponge in it. The observations were recorded on incubation period, different larval instar duration, pupal, adult, Pre-oviposition, oviposition, post oviposition and total life span.

Results and Discussion

Table 1: Duration of various life stages of *Spodoptera litura* under laboratory condition

Life stages	Duration in day(s)			Sample size (n = 25)
	Minimum	Maximum	Mean ± SD	
	Eggs			
Hatching (%)	92 (%)			25
Incubation period	2.0	3.0	2.78±0.39	
Maggot period				
I instar	2.0	3.0	2.57±0.51	23
II instar	3.0	4.0	3.50±0.57	23
III instar	3.0	6.0	4.55±0.68	23
IV instar	4.0	6.0	5.51±0.71	23
V instar	4.0	6.0	5.51±0.71	23
Total larval period	16.0	25.0	21.64±3.18	23
Pre-pupal period	0.75	1.0	0.87±0.26	23
Pupal period	7.0	9.0	8.00±0.71	23
Pre-oviposition period	1.0	3.0	1.92±0.49	13
Oviposition period	2.0	3.0	2.57±0.51	13
Post-oviposition period	1.0	3.0	1.92±0.49	13
Fecundity (Eggs/female adult)	0.0	1855.20	925.6±2.92	13
Adult period				
Male	6.0	8.0	7.10±0.88	10
Female	6.0	9.0	8.00±1.00	13
Total life span				
Male	31.0	46.0	38.50±0.88	10
Female	32.75	50.0	41.70±0.56	13
Sex ratio (Male: Female)	1: 1.30			
Note: SD = Standard Deviation				

The observations recorded on the developmental duration of different life stages of *Spodoptera litura* are summarized in Table-2.

Description and Duration of Egg Stage

An investigation on the egg stage of *S. litura* was carried out using a stereo binocular microscope to examine its morphological features. Tiny buff-colored hairs that matched the hairs on the gravid female's abdomen tip coated the grouped eggs. The eggs were set up in groupings with two to three distinct levels each. The round, pale white eggs had their scales removed before they hatched, exposing the first instar's embryonic head capsule. Number of egg mass laid by single female of *S. litura* during its life span as known as fecundity. female can laid approximately 1855.20

eggs. The observed reproductive output (1855 eggs per female) exceeded previously reported values for *S. litura* under standard diets (774 and 452 eggs per female), likely reflecting enhanced nutrient quality, controlled rearing conditions, and optimized experimental design (Zhang *et al.*, 2016) [17].

Incubation period

The time between laying an egg and the larvae emerging from it was referred to as the egg incubation period. According to Table-2, the average incubation period for eggs laid by female *S. litura* on an artificial diet was 2.78±0.39 days. The minimum and maximum incubation times that were noted were two and three days, respectively. Jadhav *et al.* (2024) [6] observed an egg period of 4.53±0.01 days to 4.80±0.01 days which was more or less similar to our findings.

Description and Duration of Larval Period

In order to determine the larval instars, the individual maggot was observed daily for the shedding off exuviae by the larvae. The moulting was confirmed by the presence of casted-off exuviae. During the experiment, it was revealed that larvae undergoes through five instars and form to the prepupal stage.

1st instar: The neonate larvae were pale green in colour with dark black head having distinctly visible black hairs on the body and tiny black spot on first abdominal segment and which later became yellowish green in colour. The body tapered toward the back and had a cylindrical shape. First instar larval period lasted for about 2-3 days on artificial diet with an average of 2.57±0.51 days. Newly hatched slow moving pale green larvae are cylindrical in shape with a wide head size and tapering abdomen towards caudal region (Latha *et al.*, 2014).

2nd instar: The size and appearance of the second instar larva differed significantly from the first instar. In comparison to the first instar, the larva expanded greater in length. The noticeable triangle mark on the head changed from black to pale brown. The body was bright green in color. However, the second instar larvae at a later stage of development started dispersing to feed on their own after moulting. The second instar larval stage was raised on an artificial diet for 3.50±0.57 days. However, *S. litura* minimum and maximum second larval instar periods were observed to be 3 and 4 days, respectively.

3rd instar: The third instar larvae changed their body colour to dark green with two dorsal black spots on the first abdominal segment and dark crescent shaped spots on the sides of the subsequent abdominal segment. Whitish color inverted 'Y' shape ecdysial line seen on head and completion of third instar confirmed by head capsule and exuviae. The larval duration ranged from 3 to 6 days with an average of 4.55±0.68 days.

4th instar: The larvae's color changed from green to dark blue-green on the dorsal side and pale greenish yellow on the ventral side during the fourth instar. The color of the three lines of dorsal bands altered; the two lateral bands turned yellow, and the middle band turned vivid orange.

Compared to the previous three instars, the fourth instar larval stages was slightly longer, measuring 5.51 ± 0.71 days.

5th instar: Following the fourth molt, the larvae grow larger and take on a dark brownish hue. Black spots are particularly noticeable on the first and eighth abdominal segments. 5.51 ± 0.71 days was the longer fifth instar development time. However, it was found that the minimum and maximum second larval instar periods of *S. litura* were 4 and 6 days, respectively noted during the experiment.

Total larval period

The data presented in Table-2 indicated that the larval stage of *S. litura* passed through five instars and was completed between 16 to 25 days with a mean of 21.64 ± 3.18 days. Additionally, Yadav *et al.* (2014) [2] noted that *S. litura* entire larval lifespan on castor and okra was 17.33 and 17.03 days, respectively. Therefore, the current findings are closely aligned with these workers as well. *S. litura* raised on a modified diet had a total larval period of 13.08 days, which is significantly shorter than the 17.2 and 15.08 days reported by Saljoqi *et al.* (2015) [1] on artificial diets based on beans and grams, respectively.

Pre-pupal and Pupal Period

The pre-pupal phase was described as the time between the end of larval feeding and the beginning of the pupal stage. The fifth-instar larva was in the pre-pupal stage and stopped feeding during the experiment. The mature larvae coiled into a C shape before pupation, and the prepupal stage lasted an average of roughly 0.75-1.00 days. Pupal period lasted for about 7-8 days on artificial diet with an average of 8.00 ± 0.71 days. Pupae appeared pale yellowish initially, later became dark reddish brown in colour. In support of present result, Kandagal and Khetagoudar (2013) [7] reported that the male and female pupal period ranging from 8-9 and 9-12 days, respectively.

Pre-oviposition period

Pre-oviposition period of *S. litura* raised on artificial diet was on an average of 1.92 ± 0.49 days with a minimum pre-oviposition period was 1 day while maximum pre-oviposition period was 3 days.

Oviposition period

The data from Table-2 stated that oviposition period of *S. litura* was observed from 2 to 3 days with an average of 2.57 ± 0.51 .

Post-oviposition period

The data (Table-2) depicted that *S. litura* raised on artificial diet had an average 1.92 ± 0.49 days post-oviposition period with a minimum of 1 days and a maximum of 3 days.

Adult period

The imago of *S. litura* can emerge by creating a tiny slit along the pupa's middorsal line. Shortly after emerging, it was observed that the wings were folded and did not completely enclose the abdomen. After some time, the moth started to move, and its wings spread out to their typical length. The adult moth was brown with a complex pattern of creamy coloured crisscrossing markings on the forewings. The hind wings were silvery white in colour. In contrast to females, males had a conspicuous white stripe on their forewings, and adult males were somewhat more colorful.

Brightly colored scales coated the thorax. The data on adult longevity (days) are depicted in the Table-2 and indicate that the female adult period was varied from 6 to 9 days with an average of 8.00 ± 1.00 days. The male adult period was varied from 6 to 8 days with an average of 7.10 ± 0.88 days. The observed sex ratio was 1: 1.30 (male: female). In his research on the biology of *S. litura* on a variety of crops, including castor, okra, tapioca, sweet potato, mulberry, groundnut, cowpea, and taro, Narvekar (2018) [10] discovered that the minimum and maximum adult longevity were 5.33 and 7.67 days, respectively.

Total life cycle

The total life cycle of the respective sex of *S. litura* from eggs to adult data is summarized in Table-2. It was indicated that male and female from egg to emergence of adult flies under laboratory condition ranged from 31 to 46 days and 32.75 to 50 days, respectively. While, male and female adult flies on an average of 38.50 ± 0.88 and 41.70 ± 0.56 days, respectively. These results are consistent with other research, such as that done by Ahmad-ur-Rahman *et al.* (2015), who found that *S. litura* raised on a chickpea-based diet had a total developmental time of 29.6 days as opposed to 31.9 days on a bean-based diet. Beans and chickpeas are members of the Fabaceae family of legumes, which are high in protein, fiber, carbs, and important minerals.



Fig 1: Life cycle along with life stages of *Spodoptera litura*

Conclusion

Food security and agricultural productivity are significantly impacted by the spread of pesticide resistance in *Spodoptera* populations. In order to develop and apply integrated pest management techniques that can effectively control the populations of this insect while preventing the emergence and spread of chemical resistance, farmers, researchers, and policymakers must collaborate. In summary, the current work effectively created artificial diets that are affordable and simple to use for the best possible laboratory rearing of *S. litura*. Large-scale mass rearing on any natural diet, however, is a very time-consuming procedure with a high danger of infection. Therefore, an attempt was made in the current study to combine typical artificial diet elements with chickpea and wheat flour. The effectiveness of artificial diets was assayed in terms of palatability and time taken for completion of various life stages of *S. litura*.

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