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Development of castor based oligidic diet for tobacco caterpillar, *Spodoptera litura* (Fabricius) and its comparative study with other artificial and natural diets

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Abstract

The present research was aimed to study the development of castor based oligidic diet for tobacco caterpillar, *Spodoptera litura* (Fabricius) and its comparative study with other artificial and natural diets. Study was carried out at Department of Agricultural Entomology, Kerala Agricultural University during 2015-16. The results revealed that populations reared on castor based oligidic diet showed a significant reduction in total larval period (13.08 days) and total life period (32.66 days) with high development indices and survival per cent (73.26) when compared to populations raised on natural diets.

Keywords: *Spodoptera litura*, artificial diet, natural diet, mass rearing, tobacco caterpillar

1. Introduction

S. litura is a polyphagous insect pest with wide host range causing enormous losses to many commercial crops in India, China, Pakistan and Bangladesh [1-4]. It is primarily a defoliator but also feeds on buds, flowers, legume pods and cotton bolls. The larvae initially scrap the leaf tissue gregariously and feed in clusters and quickly skeletonize the leaves [5]. The late instars defoliate the plants completely causing heavy yield losses. In order to develop sustainable pest management programs, it is highly essential to mass rear the insect to meet the requirement of various bioassay studies. Also, it is important to rear the insects to study their life history, behaviour, feeding habits and their susceptibility and resistance to chemical and biological pesticides. A number of studies have been conducted on the biological parameters of *S. litura* on different artificial diets in the Asian countries under different environmental conditions [3, 4, 6]. Total larval duration 17.8 days, pre-pupal duration 2.8 days, pupal duration 8.5 days and total immature duration 31.2 days was reported in *S. litura* reared on gram based artificial diet [7]. Whereas, diet based on groundnut, red gram and winged bean based flour recorded 17.2 days total larval duration, pre-pupal duration 2.7 days, pupal duration 8.3 days and 30.8 days total immature duration of *S. litura* [8]. The present study aimed to develop castor based oligidic diet, to investigate and compare the life cycle of *S. litura* reared on artificial and natural diets tested.

2. Materials and Methods

Present study was carried out during 2015- 2016 in the Department of Agricultural Entomology, College of Agriculture, Vellayani, Kerala Agricultural University. Initial populations of *S. litura* were collected from the field and used for the mass rearing study.

2.1 Laboratory rearing of tobacco caterpillar, *S. litura* in different diets

To maintain population of *S. litura* in the laboratory, sufficient for bioassay studies, an appropriate laboratory rearing technique was standardized by suitably modifying the artificial diet. The culture was also maintained in fresh leaves of castor, cowpea and amaranthus.

2.1.1 Artificial diet

The egg mass and larva of *S. litura* collected from the infested vegetable fields of various experimental locations were used for mass rearing on artificial diet. Initially, the diet recipe given by Saljoqi *et al.* [9] (2015) was followed with few modifications (Diet 1). An up gradation for this diet has been made by adding dried castor leaf powder (diet-2) to increase

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the similarity in composition to natural diet and to increase the palatability for *S. litura*. Ingredients include chickpea flour (200g), yeast powder (20g), ascorbic acid (3.5g), methyl-p-hydroxybenzoate (2g), sorbic acid (1g), formaldehyde solution (1.5ml), agar (10g) and 500ml distilled water. Modified diet contained 50 g of dried castor leaf powder, 150 g chickpea flour and added multivitamin capsules while keeping the other ingredients constant. All the dry and wet ingredients of the diet were weighed/measured carefully, and kept in separate containers. Half of the total water was taken in a steel vessel, brought to boil and agar was added. The total quantity of gram flour was added to the boiling agar. Remaining water was poured and stirred continuously to reduce the clump formation. Then, all the dry and wet ingredients were added to the mixture and thoroughly homogenized using blender. The prepared diets were then poured into the sterilized plastic boxes (Plate 1a), and allowed for solidification. Diet was left over for whole night and used next day for feeding to *S. litura*. The diet was cut into small pieces of size 2 x 2 cm (Plate 1b) and introduced to the plastic rearing troughs. The *S. litura* larvae were introduced to the diet piece in the trough. The same procedure was used to rear the larvae using diet-2 also (Plate-2). Based on good palatability and reduced duration of life stages, the best diet was selected for further rearing of *S. litura*.

2.1.2 Mass rearing of *S. litura* on natural diet

Certain natural hosts of *S. litura* like amaranthus, cowpea and castor were selected for the rearing based on its host preference. The egg and first instar larvae of *S. litura* collected from the infested vegetable fields were released on to fresh leaves of castor, cowpea and amaranthus kept in cylindrical polyvinyl containers of 9 cm height and 11 cm diameter. The open end of the containers were closed with muslin cloth for providing aeration and secured tightly with a rubber band to prevent escape of larvae. Dried leaves were removed periodically by using forceps and larvae present on the dried leaves were transferred carefully onto fresh leaves by using camel hair brush. The leaves were replaced with fresh ones every day to ensure their suitability for the larvae. All these containers with larvae were kept in a rectangular iron rat proof cage (4 x 2 feet) to make then rat proof. Containers and cage were cleaned every day to maintain sanitation.

The larvae pupated periodically on different dates were collected by using a camel hair brush and kept in polyvinyl cylindrical jars of 20 cm height and 8cm diameter with multi layered tissue paper bed at the bottom for adult emergence and mating. Cotton buds soaked in ten per cent honey solution were provided as food source to the adult moths (Plate 3). Pieces of muslin cloth were placed inside to facilitate oviposition. Fresh and tender leaves were introduced into the containers for neonates to feed.

2.2 Comparison between natural diet and artificial diet

The data on time taken to complete different life stages of *S. litura* reared on artificial and natural diet were compared and documented. Larval growth index and total developmental index were calculated based on per cent pupation and per cent survival respectively using the formulae^[10] mentioned below to evaluate the effect of diet on growth and development of *S. litura*.

Larval growth index = % Pupation / Larval Period (days).

Total developmental index = % Survival / Total Developmental Period (days).

2.3 Statistical analysis

Design followed was completely randomized design (factorial). Pooled data obtained from three generations was subjected data analysis by using OP Stats software.

3. Results

The data documented on the biological parameters, including both immature and mature adult stage of *S. litura* fed on the tested artificial and natural diets, up to 3 generations and average mean data regarding the overall 3 generations have been presented.

3.1 Laboratory rearing of tobacco caterpillar, *S. litura* in different diets

The results of the mass rearing of *S. litura* on artificial and natural diets was presented below.

Mass rearing on artificial and natural diets

The results of the present study on duration of life stages of *S. litura* are given in Table 3 and Mean number of days taken by the first instar larva was 3.16 days on cowpea leaves and it was significantly on par with *S. litura* raised on amaranthus with duration of 2.66 days. However, duration of 1st instar larvae taken on castor leaf was 2.00 days and was significantly different with other two natural diets tested and on par with diet-2 which registered duration of 1.91 days. The larva raised on artificial diet-1 showed a duration of 1.75 days which was significantly varied with all other diets tested except artificial diet-2. Second instar larvae showed duration of 3.91, 3.34 and 2.34 days on amaranthus, cowpea and castor leaves respectively and are significantly different. Whereas, larvae reared on artificial diet-1 showed duration of 2.83 days and it on par with cowpea (3.34) and castor (2.24). However, diet-2 showed a reduced duration of 1.91 days and found to be effective when compared to other diets.

Third instar larvae reared on cowpea leaf registered duration of 4.66 days and is found to be significantly different. Larval duration of 3.25, 3.08 and 2.83 were documented with amaranthus, castor and diet-2 and were statistically on par with each other. Larva reared on artificial diet-1 showed duration of 2.58 days and was on par with diet-2. Larval duration of 4th instar *S. litura* was significantly higher on amaranthus and cowpea leaves with 3.25 and 3.5 days respectively. Whereas, larvae raised on castor, artificial diets-1 and diet-2 showed duration of 2.58, 2.50 and 2.33 respectively and were statistically on par.

Mean number of days taken for 5th instar *S. litura* was 3.00, 2.91 and 2.66 days respectively on diet-2, cowpea and diet-1 and were on par with each other. Whereas, larva raised on amaranthus leaf showed a duration of 2.58 days which was statistically on par with diet-1 (2.66) and castor (2.08). Amaranthus registered highest larval duration for 6th instar *S. litura* with 3.50 days and was on par with cowpea (3.33 days). Duration registered on castor leaf was 2.33 days and was on par with diet-1 (2.16 days). Lowest duration was documented for diet-2 (1.66) and is significantly different with other diets tested.

Total mean larval duration was found to be highest in larvae raised on cowpea leaf (20.83) followed by amaranthus (18.33) and were significantly different. Whereas, duration documented for diet-1 and castor leaf raised larvae are 14.50 and 14.08 respectively and were statistically on par. Lowest mean larval duration was recorded for diet-2 with 13.08 days and was statistically significant.

Highest pre-pupal duration was recorded under cowpea leaf with 3.08 days followed by amaranthus which was found to

be 2.66 days and is on par with cowpea (3.08) and castor (2.33). Whereas, diet-1 and diet-2 documented pre-pupal durations of 1.58 and 1.16 days respectively and were statistically different.

Pupal duration was highest in larva raised on cowpea with 7.66 days followed by amaranthus with 7.16 days and diet-1 with 6.66 days. Pupal duration of *S. litura* raised on amaranthus was significantly on par with both cowpea and diet-1. Whereas, pupal duration was recorded with diet-2 was 6.41 days and is statistically equal with pupal duration on castor (5.91 days).

Highest adult period of 6.33 days was recorded in *S. litura* raised on diet-2 followed by castor with 6.16 days and diet-1 with 5.83 days and were statistically on par with diet-2. Adult period recorded on cowpea was 5.50 days and was on par with diet-1 (5.83) and amaranthus (5.00).

Incubation period was found to be higher in *S. litura* raised on cowpea (4.16) followed by amaranthus with 4.00 days and diet-1 with 3.50 days and is statistically equal with amaranthus. Incubation period recorded with diet-2 and castor was 3.25 days and 3.16 days respectively and was statistically equal.

Total life cycle period was highest for *S. litura* raised on cowpea with 37.58 days. Amaranthus and castor recorded next highest duration of 34.91 and days 34.16 and were statistically on par. Total life cycle period of 33.58 and 32.66 was recorded for *S. litura* raised on diet-1 and diet-2 and were statistically on par.

Growth and Development indices

The larval growth index was 6.84 for diet-2 followed by 5.80, 4.40, 3.43 and 5.80 respectively for *S. litura* raised on diet-1, amaranthus, cowpea and castor leaves. The developmental index was also higher in diet-2 (3.54) followed by castor (3.21), diet-1 (2.96), amaranthus (1.85) and cowpea (1.76).

4. Discussion

The development of artificial diets, pioneered by Vanderzant *et al.* [11] (1962) facilitated the continuous production of insects. Since then, several species of dipterans, lepidopterous and coleopterans have been successfully reared under controlled laboratory conditions. In the present study, in order to guarantee a continuous and adequate supply of larvae, an effort was made to standardize a viable rearing technique. Initially, the artificial diet (Diet-1) was prepared based on the procedure described by Saljoqi *et al.* 2015 [9] (Table 1). Further, diet-2 was standardized by slightly modifying the procedure and composition of diet-1 to increase its palatability and to make its composition near to natural diet (Table 2). Total larval period documented for *S. litura* reared on modified diet was 13.08 days and is comparatively lower with 17.2 days and 15.08 days reported by Saljoqi *et al.* [9] (2015) on bean based and gram based artificial diets. Present study is in coordination with Sorour *et al.*, 2011 [12] who reported a total larval duration of *S. littoralis* (13.78) days on bean based artificial diet. Total developmental index reported by Gupta *et al.* (2005) [10] and Diwakara and Manjulakumari, 2015 [6] were 2.84 and 1.95 which were much lesser than the present castor based oligidic diet where total developmental index recorded was 3.54. Castor is one of the most favourite

food of *S. litura* whose composition is very essential especially for skeleton build-up process of its early instars. However, large scale mass rearing on any natural diet is very laborious process with high risk of infections. Hence, in the present study an attempt was made by mixing dried castor leaf powder with traditional artificial diet ingredients. The effectiveness of artificial diets was assayed in terms of palatability and time taken for completion of various life stages of *S. litura*. Rearing of larvae of *S. litura* in artificial diet was successful and the number of days taken to complete the total larval period is lower in larvae raised in diet-2 followed by diet-1. However, total life cycle was significantly lesser in both the diets tested than populations raised in natural diet viz., amaranthus, cowpea and castor (Fig. 1). Survival percentage and total growth index were also highest in *S. litura* raised on diet-2 followed by larvae raised on castor and diet-1.

In conclusion, the present study showed the effect of modified castor based diet in mass rearing of *S. litura* both in terms of palatability and short life period. This indicated necessity of oligidic diets in order to increase the palatability as well as to improve the survival per cent of economically important insect in mass rearing programs. Hence, it is necessary to continue further research on standardization of an effective and viable rearing techniques for the mass rearing of *S. litura* for continuous supply of larva required for bioassay studies.

Table 1: Ingredients of artificial diet (diet-1) for rearing *S. litura*

Sl. no	Composition	Quantity
	Fraction-A	
1	Water	500 ml
2	Yeast	20 g
3	Ascorbic acid	3.50 g
4	Chickpea flour	200 g
5	Methyl-p-hydroxybenzoate	2 g
6	Vitamin E	0.16 g
7	Multivitamin capsules	1 no.
8	Sorbic acid	1 g
	Fraction B	
9	Agar- agar	10 g
10	Water	80 ml
11	Formaldehyde	1.5 ml

Table 2: Ingredients of modified oligidic diet for rearing *S. litura*

Sl. no	Composition	Quantity
	Fraction-A	
1	Water	500 ml
2	Yeast	20 g
3	Ascorbic acid	3.50 g
4	Dried castor leaf powder	50 g
5	Chickpea flour	150 g
6	Methyl-p-hydroxybenzoate	2 g
7	Vitamin E	0.16 g
8	Multivitamin capsules	1 no.
9	Sorbic acid	1 g
	Fraction B	
10	Agar- agar	10 g
11	Water	80 ml
12	Formaldehyde	1.5 ml

Table 3: Growth and survival parameters (Mean ± SD) of *S. litura* (F) reared on different diets (Means of three generations data).

Life stages	Mean number of days taken in different diets					CD
	Artificial diet	Artificial diet with castor leaf powder	Amaranthus leaf	Cowpea leaf	Castor leaf	
First instar	1.75± 0.62 ^c	1.91± 0.67 ^{bc}	2.66± 0.67 ^{ab}	3.167± 0.49 ^a	2.00± 0.51 ^b	0.904
Second instar	2.83± 0.58 ^{bc}	1.91± 0.83 ^c	3.91± 0.67 ^a	3.33± 0.45 ^b	2.33± 0.49 ^c	0.536
Third instar	2.58± 0.67 ^{bc}	2.83± 0.83 ^b	3.25± 0.74 ^b	4.66± 0.49 ^a	3.08± 0.79 ^b	0.642
Fourth instar	2.50± 0.52 ^b	2.33± 0.65 ^b	3.25± 0.45 ^a	3.50 ± 0.52 ^a	2.58± 0.67 ^b	0.507
Fifth instar	2.66± 0.65 ^a	3.00± 0.43 ^a	2.58± 0.51 ^{ab}	2.91± 0.79 ^a	2.08± 0.67 ^b	0.553
Sixth instar	2.16± 0.83 ^b	1.66± 0.49 ^c	3.50± 0.52 ^a	3.33± 0.65 ^a	2.33± 0.49 ^b	0.543
Total larval period*	14.50± 2.11 ^c	13.08± 1.31 ^d	18.33± 0.89 ^b	20.83± 1.90 ^a	14.08± 1.31 ^a	0.98
Pre-pupa	1.58± 0.51 ^c	1.16± 0.72 ^d	2.66± 0.49 ^{ab}	3.08± 0.29 ^a	2.33± 0.49 ^b	0.452
Pupa	6.66± 0.65 ^b	6.41± 0.67 ^{bc}	7.16± 0.83 ^{ab}	7.66± 0.49 ^a	5.91± 0.90 ^c	0.633
Pupation (%)	84.12± b	89.47± a	74.23± c	71.56± c	81.67± b	3.56
Survival (%)	67.34± a	73.26± a	52.34± b	55.78± b	71.87± a	7.77
Larval Growth Index	5.80	6.84	4.04	3.43	5.80	-
Total Developmental Index	2.96	3.54	1.85	1.76	3.21	-
Adult	5.83± 0.94 ^{ab}	6.33± 0.89 ^a	5.00± 0.95 ^{bc}	5.50± 0.80 ^{bc}	6.16± 0.58 ^{ab}	0.712
Egg	3.50± 0.67 ^{ab}	3.25± 0.45 ^b	4.00± 0.74 ^a	4.16± 0.83 ^a	3.16± 0.72 ^b	0.593
Total life cycle**	33.58± 2.91 ^c	32.66± 2.02 ^c	33.91± 1.19 ^c	37.58± 1.96 ^a	34.16± 1.51 ^b	1.972

Means followed by identical letters are not significantly different for comparisons between treatments within each row (P <0.05).

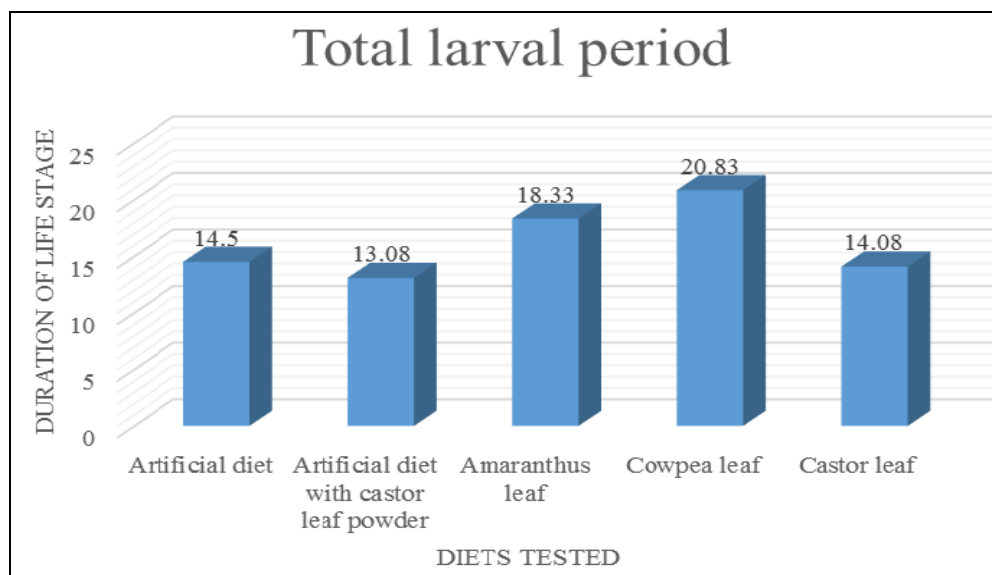


Fig 1: Total larval duration taken on different diets tested

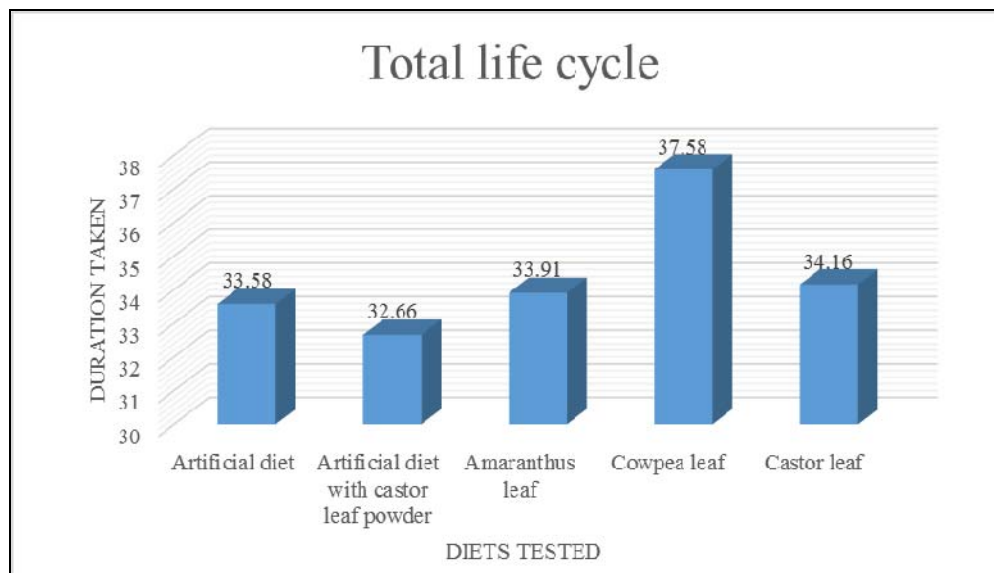


Fig 2: Total life duration taken on different diets tested



Plate 1a. Diet-1



Plate 1b. Diet-2



Plate 2: *S. litura* larvae feeding on diet



Plate 3: Adults feeding on honey syrup

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