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Effects Different Biomaterials on Biological and Histopathological Aspects of Cotton Leafworm, *Spodoptera littoralis* (Boisd)

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ABSTRACT

The cotton leafworm. *Spodoptera littoralis* (Boisd) was reared on two artificial diets named, kidney bean and broad bean incomparable with feeding on castor bean leave under laboratory conditions i.e. 27 ± 1 °C and 70 ± 5 % R.H. The biological aspects were obtained through two generations. The two artificial diets kidney bean and broad bean were more preferred to the insect as compared to the fresh host plant (castor bean leaves). The results revealed that the mean weight of larval instars, when reared on artificial diets, was higher than that recorded when feeding on castor bean leaves and also egg deposited by females. Moreover, the duration of generation was affected by the rearing on artificial diets (31 and 33 days), respectively. On the other hand, the generation that was reared on castor bean leaves was not affected (37 days). The larval duration was higher (21days) when feeding on castor bean than the two other diets Kidney bean and broad bean which recorded (18 and 16 days), respectively, On the other hand, the adult longevity females were (7, 9 and 6 days) when feeding on Kidney bean, broad bean and castor bean leaves, respectively. It is concluded that brood bean diet is the best to conduct out the different bioassays for the cotton leafworm,

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* occasionally becomes a serious pest on cotton in Egypt 25 years ago. It was reported to cause damage in the cotton field in Egypt Abdel-Raheem, *et al.,* (2016, 2020). Female moths lay most of their egg masses the infection rate could reach 119 048 egg-mass/ha leaves, buds, flowers and cotton (Temerak 2002; Ahmed *et al.,* 2015a), the first three larval instars feed mainly on the lower surface of the leaves, whereas later instars feed on both surfaces. The larvae feed mainly in the dark, although this behavior pattern may be less noticeable in early instars so, *S.littoralis* (Boisduval), the cotton leafworm, is considered the most serious cotton pests (Hatem *et al.,* 2009). Information on development on different host crops is given by (Dimetry, 1970). Studies in Egypt indicate that there are seven overlapping generations of *S. littoralis* when feeding on cotton and that there are three peak infestation periods Abdel-Raheem, (2019).

The present study was undertaken to find a suitable laboratory method for rearing *S. littoralis* on artificial and natural diets, aiming to obtain information on its nutritional requirements as well as its biology to help for its control. Furthermore, laboratory-reared insects are useful for insects of uniform size and age may be produced in relatively great numbers under controlled conditions.

MATERIALS AND METHODS

Original of Stock and Maintenance of Culture:

The initial culture individuals collected as newly hatched larvae from cotton fields at Kafr El-Shekh Governorate were reared in the laboratory for several generations. This strain was reared on caster leaves under constant temperature (27 ± 1 °C) and 70 ± 5 % R.H. The hatched larvae were transferred to glass jars (2-liter capacity) covered with muslin cloth secured with rubber bands larvae were provided daily with fresh Castor leaves, *Rieinus communis*. The number of larvae per jar was gradually grown as the larvae develop reaching for the 6th instar. Each 20 pre-pupae were allowed to pupate in glass jars containing a 2-inch layer of dry sawdust, the resulting pupae were then placed on filter paper discs in uncovered petri- dishes, which were kept 1 cubic foot wire screen cages. The resulting moths were fed on 20% sugar solution, and allowed to lay their eggs on fresh *Neriumoleader* leaves as a physical surface for moths mating, ovipositor and resting egg- masses were collected every2 days and transferred to petri- dishes for another generation.

Artificial Diets:

Diets used in the present investigation were principally based on Shorrry and Hale (1965). However, several modifications were added to these diets in order to make them more suitable for larval feeding. It was our interest to develop artificial diets principally based on ingredients available in local markets. There are two artificial diets, the first diet was kidney beans *phassealus vulgaris* while the second broad bean, *viciafaba*.

Diet Preparation:

Diet was prepared as follows: Eash of diet ingredient except with agar and amount of water for dissolving it was blended with the amount of water required for blending. Agar was separately dissolved in the remaining water at 100 °C**.** The agar solution was cooled to a loss of 70 °C and mixed with the other blended ingredients. The stock media were prepared by pouring the diet in a deep glass container and kept in the refrigerator until needed.

Rearing Larvae on Artificial Diet:

The newly hatched larvae were fed on two tested diets and caster leaves (60 larvae) in each treatment were transferred single with a hairbrush and then put in 1-litermuslin-covered mason Jars and plastic ice cube until pupation stage. After pupation couples of males and females were placed in jars and supplied with 20% of sugar solution and *Nerium oleander* leaves to depose their egg, this egg was collected from *Nerium oleander* leaves and cut muslin.

Histopathological Studies:

Samples of larvae *S. littoralis* were fixed in 10% neutral formalin, the sample was dehydrated in graded ethanol to xylene and embedded in paraffin. Sections were cut at a thickness of 4-5 Mm and stained using Haematoxylin/eosin for larval tissue according to Bancroft and Stevens, (1996). The stained sections were observed and photographed using an optical microscope.

Statistical Analysis:

All experiments were in 3-5 replicates. The values were shown as means ± standard deviations. Data were subjected to analysis of variance (ANOVA), and Duncan's multiple range tests to differentiate between the means at $P< 0.05$ (SAS Institute, 1988).

RESULTS AND DISCUSSION

Determination of Reproductive Biology of *Spodoptera littoralis* on Artificial Diets and Natural Food:

A newly hatched larvae were used for biological studies, the effect of various diets was evaluated by comparing results obtained also calculated larval duration, pupation percentage, pupation period, pupation weight, percentage of adults emergence and number of eggs.

Yeast: supplies the insect with vitamin B group Wiggles worth (1972) stated that vitamin deficiencies in the diet may cause generally a delayed effect on the insect growth. George *et al.,* (1960) used brewers' yeast for rearing *A. ipslon* larvae.

Ascorbic acid: supplies the insect with vitamin C that important for normal developmental stages and egg production Vanderzant*et al.,* (1962). Methylparaben and Sorbic acid: both have no nutritional value they act as microbial inhibitors to prevent diet contamination. Formaldehyde (40%): Although using protective methods for Resistance (polyhydros is a virus). The artificial media of Sorey and Hale (1965) after being modified rearing *S. littoralis*(modification as in material and method) modified broad bean and kidney bean diet were held in comparison with castor bean leaves revealed that the development and survival rate of larvae not only as well as better than caster bean leaves (Tables 2 & 3). Successful rearing of the insect was continued for 8 generations on the artificial diet without any deterioration in the survival rate or larval weights.

Table 1: Component of artificial diets.

Diets component (gm.)	Kidney bean	Broad bean
Kidney bean	500.0 g	-----------------
Broad bean	---------------	500.0 g
Ascorbic acid	4.5 g	4.5 g
Sorbic acid	7.0 g	7.0g
M. paraben	15.0 g	15.0 g
Formaldehyde (40%)	10.0 ml	10.0 ml
Yeast	15.0 g	15.0 g
Water	4.0 Liter	4.0 Liter
Agar	30.0 g	30.0 g

Table 2: Effect of different diets on the larval period of the cotton leafworm.

Evaluation criteria	Kidney bean	Broad bean	Castor leaves	L. S. D.
1st	2.00	3.00	4.00	1.63
2nd	2.00	3.00	3.00	1.63
3rd	3.00	2.00	2.00	1.15
4th	4.00	2.00	3.00	2.30
5th	3.00	2.00	4.00	2.57
6th	4.00	4.00	5.00	1.99
Total instar larvae	18.00	16.00	21.00	4.89
Pupal period (days)	6.00	8.00	10.00	1.99
Longevity/ Female (days)	7.00	9.00	6.00	1.99
Duration (days)	31.00	33.00	37.00	----------

Table (2) indicated larval period of cotton leafworm we note that the total larval period in the kidney bean diet was 18 while in the Broad bean diet was 16 compared with those feed on castor leaves it was 21days. Also, when we compared Longevity/ Female it recorded 7.00, 9.00 and 6.00 days in Kidney bean diet, Broad bean diet and Castor leaves, respectively.

On the other hand, when we calculate the duration time of generation it recorded 31.00, 33.00 and 37.00 days in the kidney bean diet, Broad bean diet and Castor leaves, respectively.

From the previous results, we reported that the kidney bean diet was the best and most fever able diet because it gives moderate larval period, least longevity of female and moderate time of generation compared with Broad bean diet and Castor leaves,

Data in table (3) indicated that the mean weight of last instar larvae on artificial diets (0.186 & 0.202) days in kidney bean and broad bean was significantly higher than on castor bean leaves 0.125. This was confirmed by the higher parameters of pupal weights on the diets than on castor bean leaves.

Egg deposited by females ensuring from artificial diets laid slightly more egg (1042 and 1165) eggs in broad bean and kidney bean than those ensuring from castor bean leaves (890) eggs.

Table 3: Comparison between the two artificial diets and normal food on biological aspects of the cotton leafworm

Diets	Mean of larval weight	% Pupation	Mean of pupal weight	% Moth emergency	Number of Egg
Kidney bean	0.186	84.06	0.28	94.30	1165
Broad bean	0.202	80.05	0.25	83.85	1042
Castor leaves	0.125	71.92	0.280	80.62	890
L. S. D.	0.03	4.37	0.04	5.44	83.13

As shown in Table (4) the sex ratio of female to male pupae was approximately 1:1 in all studied generations of *S. littoralis* on the experimented diets. Sex ratio of male to female were 1 : 0.63 , 1 : 0.57 , 1 : 0.67 for pupae developed on Kidney bean diet ; 1 : 0.60 , 1 : 0.61 , 1 : 0.58 on Brood bean diet and 1 : 0.60 , 1: 0.58 , 1 : 0.66 on Castor oil leaves for the 3^{RD}, 5^{th} , 8^{th} generations , respectively .

These results can be concluded based on successive generations that feeding on the diet of broad bean was the least impact on the biological development and vitality of cotton leafworm compared to feeding on a diet of kidney bean and so is with the feeding on castor oil leaves.

El-Guindy *et al.* (1979) recorded nearly similar results with *S. littoralis* using an artificial diet of Shorey and Hall (1965) and castor oil leaves as a natural diet. Dimetry (1970) reared *S. littorals* on the same artificial diet of Shory and Hall (1965) using horse beans instead of pinto beans. The results revealed no significant differences in the pupal durations and weight by rearing on this diet and castor oil leaves the fecundity and longevity were similar in both cases. Cabello *et al.* (1984), reared *Spodoptera littoralis* (Boisd.) for one generation on eight diets based on four meals (made from dried alfalfa leaves, corn kernels, broad beans, or soybeans) offered with and without a vitamin-aminoacid supplement. The length of development, percentage of pupae and adult longevity changed according to the basic meal used in the diet. The weight of pupae and adult fecundity were affected by both: the kind of meal and the presence or absence of the vitamin-aminoacid additive. Diets based on soybean meal reduced the percentage of pupae, adult longevity and fecundity.

Table 4: Life span ratio of the cotton leafworm, *Spodoptera littoralis*pupae reared on different diets.

Diet	Generation	No. of pupae	Pupal sex		Sex ratio	
			Female	Male	Female	Male
Kidney bean diet	**3**	35	22	13	0.63	0.37
	5	47	27	20	0.57	0.43
	8	43	29	14	0.67	0.33
Broad bean diet	**3**	42	25	17	0.60	0.40
	5	57	35	22	0.61	0.39
	8	69	40	29	0.58	0.42
Castor oil leaves	**3**	33	20	11	0.60	0.40
	5	41	25	18	0.58	0.42
	8	40	27	14	0.66	0.34

Sex ratio of femalepupale = no. of female pupae/total no. of pupae x100

Histological Effects:

The structure of the midgut in larvae is documented by Chapman, (1988) as seen in figure (1). The midgut is lined with an epithelial layer, which rests on a basement membrane while is composed of a single layer in addition to columnar cells containing a large coarse nucleus which occupies a middle position within the cell and bears a brush in figure (2). Goblet cells or calyx- shaped are seen between the columnar cells, each of these cells has a large ampulla opening by the narrow neck on the inner surface.

While in figures (3 & 4) we found that within the midgut lumen, there is a thin membrane surrounding food mass, a musculosa surrounds the epithelial layer, composed an inner and out layer of longitudinal muscle. His with agree Lofty, Dalia *et al.*, (2016) which reported that midgut containing different cell (i) A majority of columnar cells, (ii) Goblet cells are seen between columnar cells. (iii) Regenerative cells are small in size and are round or elongated.

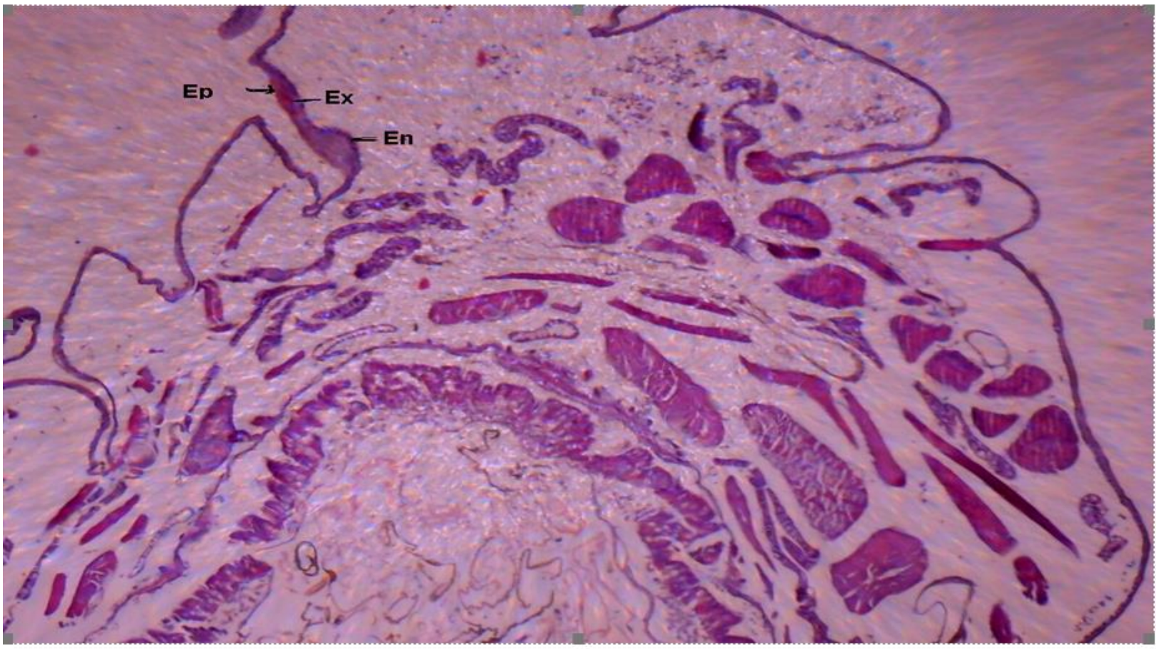

Fig. 1: Cross-section in the cuticle of untreated 4th instar larvae (control) of *S.littoralis* (HE 200X) showing normal fiber layer and the cellular layer.
Where: **Ep**: Epicuticle **Ex**: Exocuticle **En**: Endocuticle

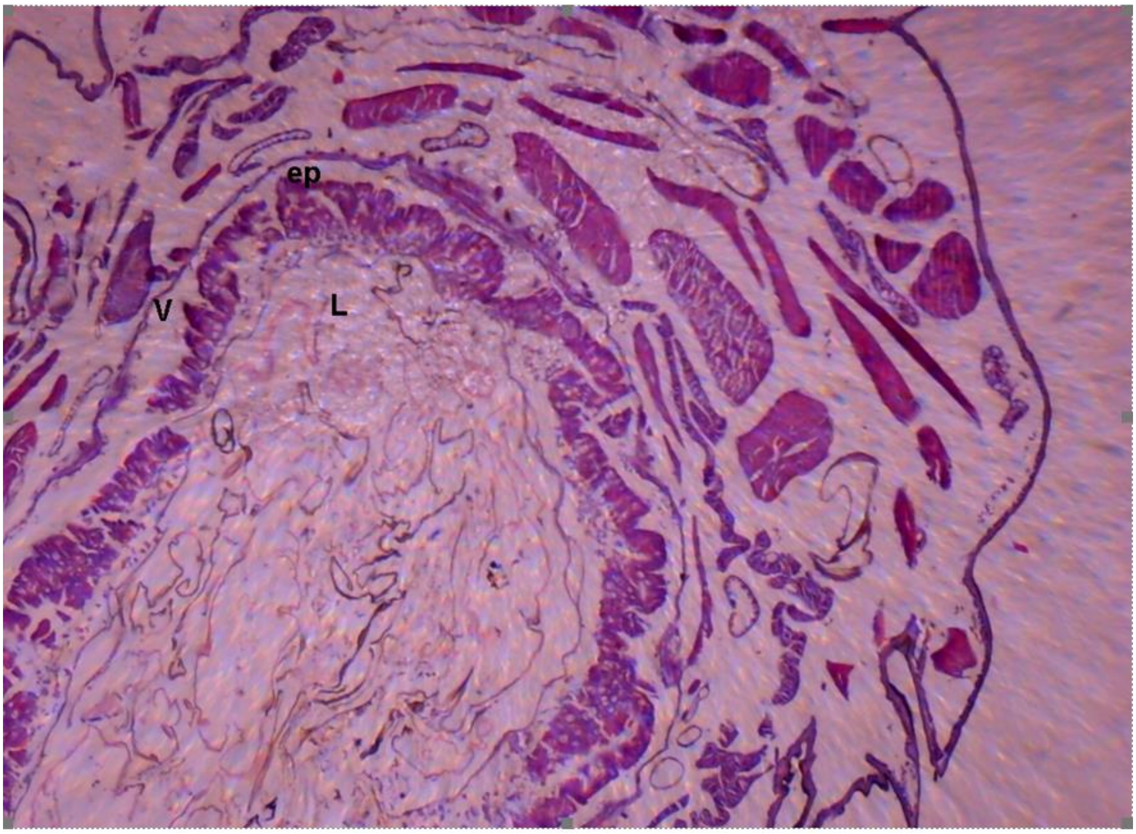

Fig. 2: Cross-section in the midgut of untreated 4th instar larvae (control) of *S. littoralis* (HE 400X) showing normal columnar layer with brush border, goblet cells, circular and longitudinal muscles.

Where: **L**: longitudinal muscle **ep** :epithalial cell,**V**:vacuole

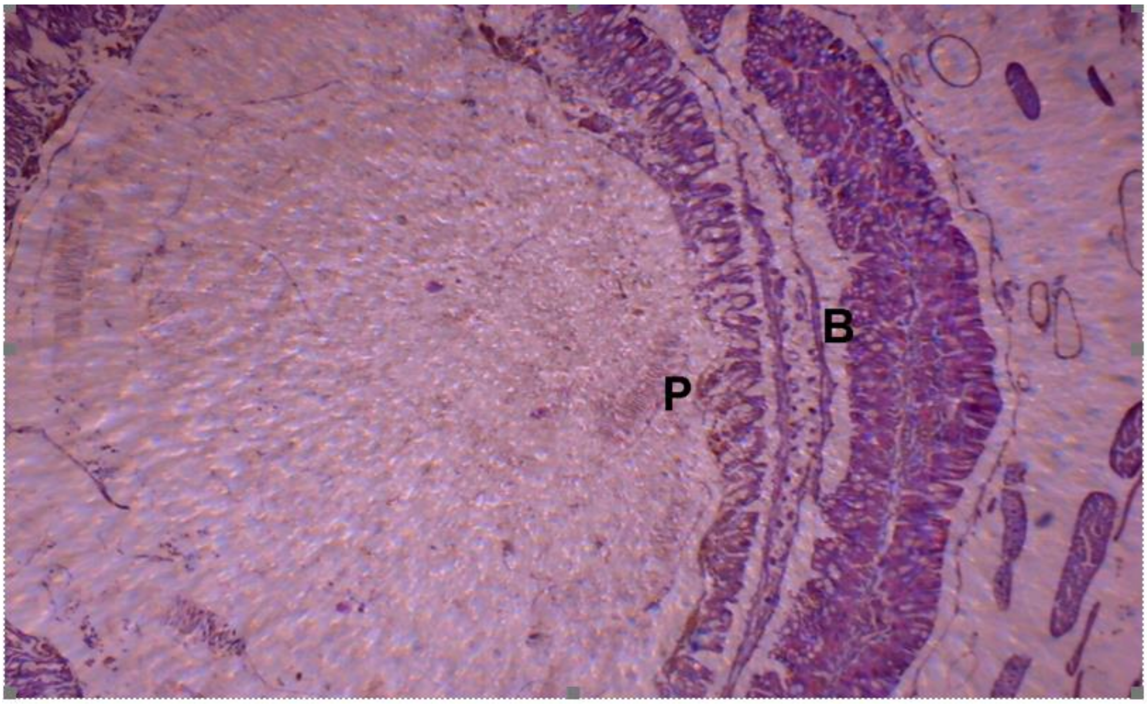

Fig. 3: Cross-section in the 4th instar larvae of *S. littoralis* living on artificial (Kidney bean) diet (HE 200X) showing the midgut with vacuoles in the columnar cells, cells are separated from the basement membrane, necrosis of muscles cells**.**

Where: **B**: basmentmembrane , **p**:peritrophicmembrane

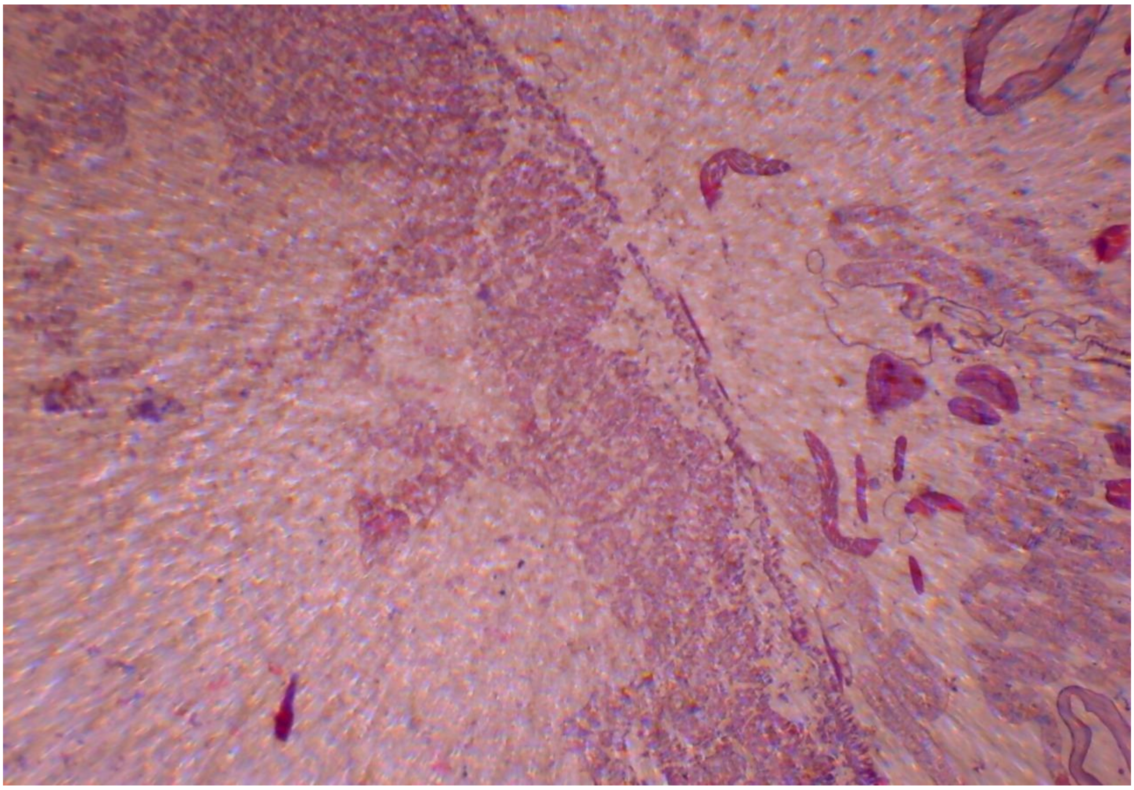

Fig. 4: Cross-section in the larvae of *S. littoralis* living on artificial (Kidney bean) diet (HE 200X) showing the midgut with necrosis of muscles cells &destroyed peritrophic matrix**.**

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