J. Plant Prot. and Path., Mansoura Univ., Vol. 6 (11): 1527 - 1535, 2015

INFLUENCE OF LARVAL FOOD ON NUMBER OF SPERMATOPHORES, FECUNDITY AND SOME BIOCHEMICAL PARAMETERS OF THE SPINY BOLLWORM, *Earias insulana* (BOISD.)

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ABSTRACT

Experiments were carried out to study the effect of larval food types (cotton bolls, okra pods and artificial diet) on number of spermatophores, fecundity and total soluble protein, lipid and carbohydrate contents of the spiny bollworm, *Earias insulana* (Boisd.). The obtained results showed that the larvae reared on artificial diet gave the highest weight, while, those collected from cotton bolls gave the lowest weight. The lowest pupal mortality percentage and the highest adult emergence percentages, number of eggs/female and hatchability percentages recorded for the larvae obtained from artificial diet followed by those collected from okra pods and cotton bolls. Pupal duration, pre & post- oviposition periods and male longevity were differed insignificantly as affected by feeding larvae on okra pods, cotton bolls and artificial diet. The newly emerged female moths from larvae collected from cotton bolls were recorded the lowest mean number of the eggs/ovariol and spermatophores, while, those emergence from larvae obtained from artificial diet was recorded the highest one. The highest value of total soluble protein, lipid and carbohydrate contents were recorded in larvae obtained from artificial diet followed by those collected from okra pods and cotton bolls.

Keywords: *Earias insulana*, larval food, anatomy, biological, physiological aspects

INTRODUCTION

The spiny bollworm, *Earias insulana* (Boisd.), is one of the most dangerous pests attacking cotton and okra in Egypt (Nada *et al.*, 2010). The quality and quantity of food consumed by the pest can affect its entire biology, rate of growth, development, reproduction, and history (Bavaresco *et al.*, 2004). The preferred hosts have a great influence on oviposition, fecundity, development period and longevity of adults of *Earias vittella* (Fab.) and *E.insulana* (Khan and Rao, 1960). Khalifa *et al.* (1981) found that the females of *Pectinophora gossypiella* (Saund.) fed as larvae on okra pods laid the highest number of eggs followed by those fed on cotton bolls and kenaf fruits. The fecundity and longevity of *Helicoverpa armigerea* (Hubner)were affected by fed during larval stage on different host plants (Shanower *et al.*, 1997 and Kulkarni and Gawande, 1999). The developmental periods and fecundity of *H. armigerea* different stages when fed as larvae on different host plants (Amer and EL-Sayed, 2014). Tamhankar *et al.* (1993) found in the

E. *vittella* a positive correalation exists between mating frequency and fecundity. The proteins, lipids and carbohydrates are known to influence growth and development in insects (Beck, 1950; House, 1963 and Soo Hoo and Fraenkel, 1966). Sundararaj and David (1987) observed decrease in body weight, fecundity, percent of larvae becoming adults, adult longevity of both males and females and prolongation of larval period of *E. vittella* when reared on *A. indium* compared with okra and cotton which was due to less amount of reducing sugars, protein and free amino acids but high amount of non reducing sugars. Also, decreased the total lipid caused decreased on the mean number of eggs and hatchability percentage for adult. The reduction in total lipid and protein caused reduction in the fecundity and fertility of *E. insulana* adult female (Kunkle and Nordin, 1985 and Kandil, 2013). There was significant inhibition in the level of both total soluble protein and total lipids in *Spodoptera littoralis* larvae which fed on all the tested varieties of cotton compared to that found in larvae fed on castor bean leaves (Khedr and Al-Shannaf, 2015).

There, fore the present work aims to determine the effect of larval food, cotton bolls, okra pods and artificial diet on number of spermatophores, fecundity and total soluble protein, lipid and carbohydrate contents of the spiny bollworm, *E. insulana*.

MATERIALS AND METHODS

The experiments were carried out to study the effect of larval food, cotton bolls, okra pods and artificial diet on number of spermatophores, fecundity and total soluble protein, lipid and carbohydrate contents of the spiny bollworm, *E. insulana*.

The spiny bollworm strains:

Field strain:

One hundred full grown larvae of *E. insulana* were collected from green cotton bolls and another one hundred was collected from okra pods from okra (*Abelmoschus esculantus* L.) intercropped with cotton field (*Gossypium barbadence* L.) At Bahr Albaqar Sharkia Governorat (the cotton crop occupied in area of 7875 m² and okra crop about 525 m² planted on the border of the cotton area). The experimental area was sprayed according to the recommendation Ministry of Agriculture program. It transferred to Bollworm Research Department, Plant Protection Research Institute, Sharkia Branch. It fed separately on cotton seeds or okra pods (collected from same previous area) in glass tubes (2.5 × 7 cm) and placed under laboratory conditions until pupation. The food was daily renewed till pupation.

Laboratory strain:

One hundred full grown larvae of *E. insulana* was obtained from a culture maintained for many generation at Bollworms Research Department, Plant Protection Research Institute, Sharkia branch on artificial diet have been described by Rashad and Ammar (1985), Rashad *et al*. (1993) and Amer *et al.* (2010).

Effect of larval food on some biological aspects of the spiny bollworm:

Full grown larvae of the spiny bollworm collected from cotton bolls, okra pods and artificial diet were weighted. Pupae transferred separately to clean glass tubes and placed in an incubator at 26 ± 1°**C** and 70 ± 5 % RH until adult emergence. Pupal weight and pupal duration were recorded. The emerged moths were sexed and caged to eggs laying (five Paris / cage) and replicated three times for each treatment. Moths were provided with 10 % honey solution. The cages were inspected daily until moth death. Pre-oviposition, oviposition and post-oviposition periods, female & male longevity, number of deposited eggs/female and hatchability percentages were recorded.

Effect of larval food on total soluble protein, lipid and carbohydrate contents of the spiny bollworm:

Preparation of samples:

The full grown larvae of the spiny bollworm were picked up from each host plant and artificial diet (one g /each treatment). The larvae were homogenized in distilled water with jacket of crushed ice for 3 minutes. Centrifugation was done at 35000 r.p.m for 10 minutes using a refrigerated centrifuge (5 ºC). The samples were kept in a deep freezer (-20 ºC) until required.

Total soluble protein:

Colorimetric determination of total soluble protein in the supernatant of the homogenated of larvae of spiny bollworm was carried out as described by Bradford (1976), and expressed as mg protein/g. b.wt.

Total carbohydrates:

Total carbohydrates were determined according to the method of Miller, (1959).

Total lipids:

Total lipids were estimated according to the method of Zollner and kirsch (1962), and expressed as mg lipid/g b.wt.

Effect of larval food on the number of eggs in the ovary and spermatophores of the spiny bollworm female:

Five newly emerged females from each treatment were dissected under stereomicroscope; the ovary was separated and the numbers of eggs in two ovariols one from left and the other from right were counted. After ovipostion period the five females from each treatment were dissected and the number of eggs and spermatophors were count.

Statistical analysis:

The obtained data were subjected to statistical analysis according to Little and Hills (1975).

RESULTS AND DISCUSSION

Larval stage:

Data presented in Table (1) showed that highly significant differences between larval weigh of *E. insulana* collected from cotton bolls & okra pods and those obtained from artificial diet. The larval obtained from artificial diet

gave the highest weight (0.082 g) while, those collected from cotton bolls gave the lowest weight (0.063 g).

Pupal stage:

Data arranged in Table (1) indecated that in-significant differences between pupal duration. The duration of *E. insulana* pupae was shorter, when the larvae obtained from artificial diet (9.67 days) and prolonged to 10.0 and 10.76 days for the larval collected from cotton bolls and okra pods, respectively. Highly significant differences were found between pupal weights. The averages of pupal weights were 0.057, 0.051, and 0.060 g, respectively. The pupal mortality percentages resulted from the larvae fed on artificial diet were significantly lower than those fed on okra pods and cotton bolls and recorded 5.0 ,11.0 and 14.0 % , respectively.

Adult emergence:

Data are given in Table (2) showed that highly significant differences in the adult emergence percentage from pupal resulted from the larval collected from okra pods & cotton bolls and artificial diet. The percentages of adult emergence were 89.0, 86.0 and 95.0 %, respectively

Table (1): Some biological aspects of immature stages of the spiny bollworm reared on cotton bolls, okra pods and artificial diet.

Foods	Larval weight (g)	Pupal duration (days)	Pupal weight (g)	Pupal mortality %
Okra pods	0.073 b	10.76	0.057 b	11.0b
Cotton bolls	0.063 c	10.0	0.051 c	14.0a
Artificial diet	0.082 a	9.67	0.06 a	5.0c
P	**	NS	**	**
$LSD_{0.05}$	0.002		0.002	1.99

Adult longevity:

Data in Table (2) cleared that the oviposition periods of *E. insulana* females resulted from larvae collected from okra pods , cotton bolls and artificial diet were 11.0, 9.0 and 13.0 days, respectively. The oviposition periods of *E. insulana* females were significant affected as larval food but the pre- oviposition and post-oviposition periods were non-significantly affected and lasted (3.0, 4.0 and 2.0 days) and (2.0, 2.0 and 3.0 days), respectively. The male longevity of *E. insulana* was 15.0, 13.0 and 16.0 days, respectively. In significant differences were recorded between male longevity resulted from collected okra pods & cotton bolls and artificial diet.

Table (2): Adult longevity and reproductive potential of spiny bollworm reared on cotton bolls, okra pods and artificial diet .

Foods	Adult emergence %	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Female longevity (days)	Male longevity (days)	No. of eggs /female	Hatchability %
Okra pods	89.0	3.0	11.0 b	2.0	16.0 b	15.0	79.0 b	77.0 c
Cotton bolls	86.0	4.0	9.0 c	2.0	15.0 B	13.0	52.0 c	88.0 b
Artificial diet	95.0	2.0	13.0 a	3.0	18.0 A	16.0	165.0 a	95.0 a
P	*	Ns	**	Ns	**	NS	**	*
LSD 0.05	6.21		1.99		1.76		7.56	10.38

Eggs laying and hatchability percentage:

Data in Table (2) indecated that the average numbers of laid eggs / female resulted from the larval collected from okra buds and cotton bolls were decreased significantly than those obtained from artificial diet which recorded 79.0, 52.0 and 165.0 eggs, respectively. Hatchability percentages were differed significantly as affected by larvae food. The hatchability percentages were 77.0, 88.0 and 95.0 %, respectively. The preferred hosts have a great influence on oviposition, fecundity, development period and longevity of adults of *E. vittella* and *E. insulana* (Khan and Rao, 1960). Khalifa *et al.* (1981) found that females of *P. gossypiella* fed as larvae on okra pods laid the highest number of eggs followed by those fed on cotton bolls and kenaf fruits. The fecundity and longevity of *H .armigerea* was affected by fed during larval stage on different host plants (Shanower *et al.*, 1997 and Kulkarni and Gawande, 1999). The developmental periods and fecundity of *H .armigerea* different stages when fed as larvae on different host plants (Amer and EL-Sayed, 2014).

Effect of larval food on the number of eggs in the ovary and spermatophores of the spiny bollworm female:

Data preseneted in Table (3) and illustrated in Fig. (1) showed that the spiny bollworm female moths emergence from larvae collected from cotton bolls were recorded the significantly lowest in the mean number of eggs/ovariol (17.30 eggs/ovariol), while, those emergence from larvae obtained from artificial diet was recorded the significantly highest one (26.45 eggs/ovariol). After eggs deposition stopped (post-oviposition period); average numbers of eggs found were 8.86, 4.66 and 4.74 eggs/ovariol. The average numbers of spermatophores/ female were 1.00, 0.80 and 1.60 spermatophores/female, respectively. Khalifa *et al.* (1981) found that feeding of *P.gossypiella* on okra pods caused an increase in the number of egg rudiments in the ovarioles as compared with those fed on cotton bolls and kenaf fruits. Tamhankar *et al.* (1993) found in the *E. vittella* a positive correlation exists between mating frequency and fecundity.

a

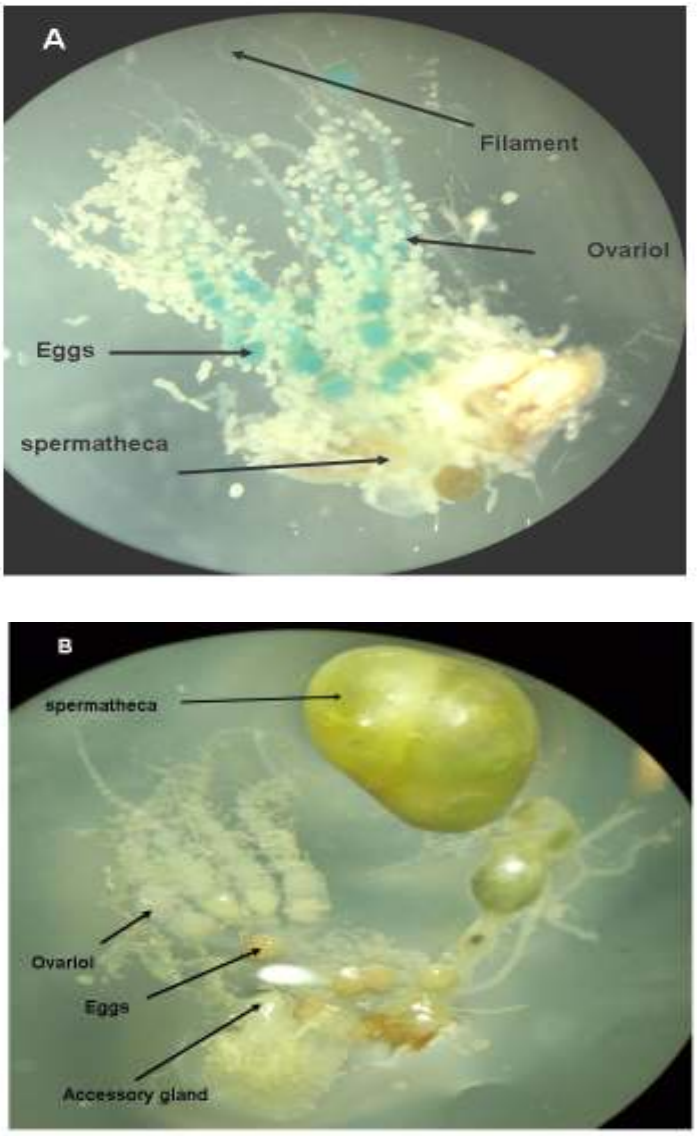

Fig. (1): A; *Earias insulana* ovary after emergence, B; ovary after post ovipostion period.

Table (3): Effect of larval food on t he number of eggs in the ovary and spermatophores of the spiny bollworm female.

Food	No. of eggs after emergence/ovariol	No. of eggs after post ovipostion/ovariol	No. of spermatophor/female
Okra pods	19.56b	8.86b	1.00b
Cotton bolls	17.30c	9.66a	0.80b
Artificial diet	26.45a	4.78c	1.60a
P	0.014*	0.011*	0.008**
LSD 0.05	5.41	2.82	0.41

Effect of larval food on total soluble protein, lipid and carbohydrate contents of the spiny bollworm:

The changes in larval total soluble protein, lipid and carbohydrate contents are given in Table (4). The highest value of total soluble protein content was obtained in the case larvae collected from artificial diet (83.30 mg protein /g BW) followed by those collected from okra pods (72.85 mg protein /g b.wt.), while, the lowest value was obtained in the case of larvae collected from cotton bolls (51.00 mg protein /g b.wt.). There was significant decreased in the level of total lipid of *E. insulana* larvae which fed on cotton bolls (111.42 mg lipid /g b.wt.) and okra pods (244.28 mg lipid /g b.wt.) Compared to that found in larvae fed on artificial diet (281.85mg lipid /g b.wt.). The total carbohydrate was significant affected by fed during larval stage on okra pods & cotton bolls and artificial diet. The total carbohydrate were 48.0, 90.0, 90.0 mg carbohydrate /g b.wt. for the larvae fed on okra pods, cotton bolls and artificial diet, respectively. Sundararaj and David (1987) observed decrease in body weight, fecundity, percent of larvae becoming adults, adult longevity of both males and females and prolongation of larval period of *E. vittella* when reared on *A. indium* compared with okra and cotton which was due to less amount of reducing sugars, protein and free amino acids but high amount of non reducing sugars. Also, decreased the total lipid caused decreased on the mean number of eggs and hatchability percentage for adult. The reduction in total lipid and protein caused reduction in the fecundity and fertility of *E. insulana* adult female (Kunkle and Nordin, 1985 and Kandil, 2013). There was significant inhibition in the level of both total soluble protein and total lipids in *Spodoptera littoralis* larvae which fed on all the tested varieties of cotton compared to that found in larvae fed on castor bean leaves (Khedr and Al-Shannaf, 2015).

Table(4):Effect of larval food on total soluble protein, lipid and carbohydrate contents of the spiny bollworm:

Foods	**Total soluble protein** mg protein /g b.wt.	**Carbohydrates content** mg carbohydrates/g b.wt.	**Lipids content** mg lipids /g b.wt.
Okra pods	72.85	48.00	244.28
Cotton bolls	51.00	90.00	111.42
Artificial diet	83.30	90.00	281.85
P	**	**	**
$LSD_{0.05}$	4.75	5.41	7.38

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تأثير تغذية اليرقات على عدد مرات التزاوج والكفاءة التناسلية وبعض القياسات الكيميائية لدودة اللوز الشوكية

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أجريت هذة التجارب لدراسة تأثير تغذية اليرقات على لوز القطن وقرون الباميا و البيئة الصناعية على عدد مرات التزاوج و الكفاءة التناسلية و محتوى البروتين الكلى والدهون والكربوهيدرات لدودة اللوز الشوكيه. وأوضحت النتائج أن اليرقات المغذاة على بيئة صناعية أعطت أعلى وزن يرقات بينما المغذاة على لوز القطن أعطت أقل وزن. و سجلت أقل نسبة موت فى العذارى وأعلى نسبة خروج فراشات وعدد بيض موضوع ونسبة فقس فى حالة تغذية اليرقات على بيئة صناعية تلاها المرباة على قرون الباميا ولوز القطن. كما وجد اختلاف غير معنوى فى فترة طول عمر العذراء وفترة قبل وبعد وضع البيض وطول عمر ذكور الفراشات نتيجة التغذية على الباميا والقطن والبيئة الصناعية .على الجانب الآخر سجلت الفراشات الخارجة من اليرقات المجموعة من لوز القطن أقل متوسط عدد بيض لكل فرع مبيض وكذلك عدد مرات التزاوج بينما سجلت الفراشات الخارجة من اليرقات المرباة على البيئة الصناعية أعلى متوسط. أيضا سجلت أعلى قيم للمحتوى الكلى للبروتين الذائب والدهون والكربوهيدرات لليرقات المغذاة على بيئة صناعية تلاها المجموعة من قرون الباميا ولوز القطن.