J. Plant Protection and Pathology, Mansoura Univ., Vol. 1 (5): 299 - 306, 2010

IMPROVED TECHNIQUES FOR LABORATORY REARING OF THE SPINY BOLLWORM, *Earias insulana* (BOISDUVAL) (LEPIDOPTERA: NOCTUIDAE)

Amer, A.E.A.; A.A.A. El-Sayed and S.A.A. Raslan
Plant Protection Research Institute, ARC, Dokki, Giza Egypt

ABSTRACT

Modifications were made in rearing technique of the spiny bollworm, *Earias insulana* (Boisduval), to obtain perfect methods to increase their reproductive ability. Changing the larval artificial diet of *E. insulana* after seven days with the fresh one of the same diet or natural okra fruits, caused non significant short in the larval and pupal duration, and significant increasing the larval and pupal weight, adult emergency, fecundity and hatchability percentages in comparison with larvae continuously reared on artificial diet without change the diet until pupation.

The use of honey solution either alone or mixture with yeast or vitamin E instead of sugar solution alone or mixture with yeast or vitamin E, in feeding spiny bollworm moths increased the quantity and viability of egg laid per female. The number of eggs per female were 146, 154, 149, 156, 166, 161 eggs, for sugar, sugar + yeast, sugar + vitamin E, honey, honey + yeast and honey + vitamin E solutions, respectively. The numbers of egg per female in presence of cotton boll or okra fruit were 183 and 178 eggs, respectively, which was higher than that obtained in absent of host plant (156 eggs per female).

INTRODUCTION

Spiny bollworm, *Earias insulana* (Boisd.) considers one of the most important insect pests attacking cotton and okra plants in Egypt and Mediterranean countries, causing a greet loss in the yield, Person and Maxwell-Darling (1958) and Dhawan and Sidhu (1984). Laboratory rearing of insect is an ongoing process, and more efficient methods are continually being adopted. Sometimes, very minor changes in techniques can have dramatic results, both positive and negative Patana (1977). Several studies have been conducted to develop artificial diet of spiny bollworm, *Earias insulana*, for improving its reproductive potential, Klein, *et al* (1981), Rashad and Ammar (1985) and Tamhankar and Dongre (1992).

The aim of this study is to do modification in rearing technique of the spiny bollworm, *E. insulana*, to obtain perfect methods to increase their reproductive ability.

MATERIALS AND METHODS

This work was carried out in Bollworms Research Department, Plant Protection Research Institute, Sharkia branch, to study the influence of larval and adult diet techniques and stimulation of host plants on development and reproductive ability of spiny bollworm *Earias insulana*. The experiments were conducted under constant conditions of 26±1 °C and 75±10% r.h.

Effect of different feeding techniques for spiny bollworm larvae on some biological aspects:

The full grown larvae of field strain of spiny bollworm *E. insulana* collected from infested cotton bolls in Bahr Elbakar region – Sharkia Governorate, and reared in the laboratory for six generations. The neonate larvae were transferred into glass tubes (2.5x7cm) containing about 4g artificial diet according to Rashad & Ammar (1985). Diet medium consists of 2133 g soaked kidney beans, 320.0 g medical dried yeast, 20.0 g ascorbic acid, 10.0 g methyl-p- hydroxy benzoate, 10.0 g sorbic acid, 10 ml formaldehyde 40% and 128.0 g agar to which 6400 ml distilled water was added. The different larval diet techniques used in this study were:

A- Neonate larvae continuously reared on the previous artificial diet without changing the diet until their pupation (4 replicates x 75 larvae).

B- Neonate larvae fed on the previous artificial diet for seven days and then transferred into another clean glass tubes containing fresh one of the same diet until their pupation (4 replicates x 75 larvae).

C- Neonate larvae fed on the previous artificial diet for seven days and then transferred to another clean glass tubes containing natural food (okra fruit) which changed daily with another one until their pupation,(4 replicates x 75 larvae).

The glass tubes were incubated and examined daily until their pupation, then the pupae were transferred individually to clean glass tubes until moths emergence. Moths resulted from pupae of all treatments were transferred into glass gars (0.5 kg). Each gar was containing three pairs of males and females. Five replicates were used for each treatment. The gars covered with muslin as a media for egg laying and a piece of cotton socked in 10% honey was offered to moths as a source for feeding. The durations, weights, mortality percentages of larval and pupal stages, moths emergency, pre-oviposition, oviposition, post-oviposition periods, numbers of egg per female and hatchability percentages were recorded.

Effect of adult diets on the reproductive ability of spiny bollworm:

This experiment aimed to study the influence of different moths diets on reproductive ability of spiny bollworm *E. insulana*. The emerged male and female moths of spiny bollworm resulted from larvae which reared on artificial diet for seven days and then transferred to another fresh one of the same diet were transferred into glass gars (0.5 kg). Each gar was containing three pairs of males and females. Five replicates were used for each treatment. The gars covered with muslin for stimulating the egg laying and a piece of cotton socked in each diet solution was offered to moths for feeding. The moths diets were as follows:-

a- 10% sugar solution only.
b- 10% sugar solution + yeast (10 g yeast +100 ml sugar solution)
c- 10% sugar solution + vitamin E (5 g vitamin E +100 ml sugar solution)
d- 10% honey solution only.
e- 10% honey solution + yeast (10 g yest+100ml honey solution)
f- 10% honey solution + vitamin E (5 g vitamin E+100 ml. honey solution)

The different treatments are examined daily until all moths dead. Pre-oviposition, oviposition, post-oviposition periods, numbers of egg per female and hatchability percentages were recorded.

Influence of host plants on the reproductive ability of spiny bollworm moths:

This experiment was carried to study the effect of natural cotton bolls and okra fruits on the reproductive ability of spiny bollworm moths. The emerged male and female moths of spiny bollworm which resulted from larvae which reared on artificial diet for seven days and then transferred to another fresh one of the same diet were transferred into glass gars (0.5 kg). Each gar was containing three pairs of males and females. Five replicates were used for each treatment. The gars covered with muslin as a media for egg laying and a piece of cotton socked in 10% honey solution was offered to moths for feeding. The treatments classified as follows:-

a- The gars were contain one cotton boll covered with muslin cloth.
b- The gars were contain one okra fruit covered with muslin cloth.
c- The gars without any host plants.

The experiment was examined daily until all moths dead. Pre-oviposition period, oviposition period, post-oviposition period, numbers of egg per female and hatchability percentages were recorded.

The relation between larval diets of spiny bollworm and the durations, weights, mortality percentages of larval and pupal stages, moths emergency, pre-oviposition, oviposition, post-oviposition periods, numbers of egg per female and hatchability percentages, also the relation between adult diets or host plants and pre-oviposition, oviposition, post-oviposition periods and numbers of egg per female and hatchability percentages were performed using one way ANOVA. The means were separated using Duncan's Multiple Rang Test at 0.05 or 0.001 probability level using Costat program (1990).

RESULTS AND DISCUSSIONS

Effect of different feeding techniques for spiny bollworm larvae on some biological aspects:

The effects of the different larval diet techniques on some biological aspects of larval and pupal stages are presented in Table (1). Larval durations recorded 14.60, 13.0 and 13.2 days for A, B and C diet techniques, respectively, without any significant differences between them. Diet techniques B and C, resulted in mean larval weight of 0.079 and 0.076 gm, which increased highly significant than diet technique A, where recorded only 0.067gm. The higher larval mortality percent recorded with diet technique A (12%), while technique B, resulted in least one (6%). In the case of diet technique C recorded (9% larval mortality). The three techniques were significantly differed. Pupal durations of spiny bollworm recorded 10.2, 9.5 and 9.5 days for A, B and C diet techniques, respectively, without any significant differences between them. Diet techniques B and C, resulted in mean pupal weight of 0.058 and 0.056 gm, which increased highly significant than technique A, where recorded only 0.045 gm. The highest pupal mortality

percent recorded with diet technique A (10%), while diet technique B, resulted in least one (4%). Diet technique C recorded (6% pupal mortality). The three techniques were significantly differed. When spiny bollworm larvae reared on diet technique C and B, significantly increased the emergency percentages than A. The three diet techniques recorded, 90, 96 and 94% emergency, for A, B and C diet technique, respectively.

Table (1): Effect of different feeding techniques for spiny bollworm larvae on some biological aspects

Larval diets	Larval duration (days)	Larval weight (gram)	% Larval mortality	Pupal duration (days)	Pupal weight (gram)	% Pupal mortality	% Emergency
A	14.62	0.067b	12.0a	10.2	0.045b	10.0a	90.0b
B	13.0	0.079a	6.0c	9.5	0.058a	4.0c	96.0a
C	13.2	0.076a	9.0b	9.5	0.056a	6.0b	94.0a
P	NS	***	***	NS	***	***	*
LSD 0.05	---	0.003	1.997	---	0.003	1.997	3.995

A- Neonate larvae continuously reared on artificial diet (Rashad & Ammar (1985)) without change the diet until their pupation.

B- Neonate larvae fed on the previous artificial diet for seven days and then transferred into another clean glass tubes contain fresh one of the same diet until their pupation.

C- Neonate larvae fed on the previous artificial diet for seven days and then transferred to another clean glass tubes containing natural food (okra fruit) which changed daily with another one until their pupation.

The effects of the different larval diet techniques on reproductive ability of spiny bollworm moths are presented in Table (2). Diet techniques B and C, significantly shorted the pre-oviposition period than A. The three diet technique recorded, 2.8, 2.2 and 2.0 days as pre-oviposition period, for A, B and C, respectively. Diet techniques B and C, recorded 14.0 days as oviposition period, which were significantly longer than diet technique A, where recorded 11.6 days. Diet techniques B and C, recorded 3 days as post-oviposition period, which were significantly shorter than A, which recorded 3.6 days. The numbers of eggs laid by female moths fed as a larvae on diet techniques of B and C were 156 and 149 eggs per female, respectively, which were highly significant than that obtained with moths of diet technique A, which recorded 90 eggs per female. Diet techniques B and C, significantly increased the hatchability percentages than diet technique A. The three diet techniques recorded, 83, 91 and 95% hatchability percentages, for A, B and C, diet techniques respectively.

Generally, Change the larval diet after seven days with fresh one of the same diet or natural okra fruit, caused non significant difference in larval and pupal durations, and significantly increased the larval and pupal weight, adult emergency, egg numbers per female and hatchability percentages for spiny bollworm compared with larvae continuously reared on artificial diet without change the diet until pupation.

These results are in agree with that obtained with Adkisson (1961), who found that quality of larval diet is an important factor in determining the reproductive capacity of *Pectinophora gossypiella* (Saund.).

Table (2): Effect of different feeding technique for spiny bollworm larvae on reproductive ability

Larval diets	Pre-oviposition period	Oviposition period	Post-oviposition period	Egg numbers / female	% Hatchability
A	2.8a	11.6b	3.6a	90.0b	83.0b
B	2.2b	14.0a	3.0b	156.0a	91.0a
C	2.0b	14.0a	3.0b	149.0a	95.0a
P	**	*	*	***	**
LSD 0.05	0.326	1.997	0.399	7.32	5.993

A- Neonate larvae continuously reared on artificial diet (Rashad & Ammar (1985)) without change the diet until their pupation.

B- Neonate larvae fed on the previous artificial diet for seven days and then transferred into another clean glass tubes contain fresh one of the same diet until their pupation.

C- Neonate larvae fed on the previous artificial diet for seven days and then transferred to another clean glass tubes containing natural food (okra fruit) which changed daily with another one until their pupation.

Effect of adult diets on the reproductive ability of spiny bollworm:

The effects of six different diet solutions for feeding moths of spiny bollworm on reproductive ability are presented in Table (3). There were significant differences between the treatments in the effects on pre-oviposition periods of spiny bollworm moths, where they last 2.4 days, for (10% sugar solution), and 1.8 days for (yest+10% honey solution). Data show that no significant differences between the treatments in the effects on oviposition periods of spiny bollworm moths, where they last 13.8 days, for (10% sugar solution), and 14.4 days for (yest+10%honey solution). Their were slight significant differences between the treatments in the effects on post-oviposition periods of spiny bollworm moths, where they last 3.4 days, for (10% sugar solution) and (vitamin E+10% sugar solution), and 2.8 days for (yest+10%honey solution).

Obviously, from Table (3), The use of honey solution either alone or mixture with yeast or vitamin E instead of sugar solution alone or mixture with yeast or vitamin E, in feeding spiny bollworm moths increased the quantity and viability of egg laid per female. The numbers of eggs per female, were 146, 154, 149, 156, 166 161 eggs, for sugar, sugar + yeast, sugar + vitamin E, honey, honey + yeast and honey + vitamin E solutions, respectively. E. The numbers of eggs per female were 146, 154,149, 156, 166 and 161 eggs, for sugar, sugar+ yeast, sugar+ vitamin E, honey, honey+ yeast and honey+ vitamin E solutions, respectively. The effect of the different moth foods on deposited eggs hatchability percentages were the same trend as in the effect on number of eggs per female, the honey solution and its mixtures with yeast or vitamin E resulted in better hatchability percentages than sugar solution and its mixture. The hatchability percentages were 88, 92, 93, 91, 93 and95%, for sugar, sugar+ yeast, sugar+ vitamin E, honey, honey+ yeast and honey+ vitamin E solutions, respectively.

Table (3): Effect of adult diet on the reproductive ability of spiny bollworm

Adult diet	Pre-oviposition period	Oviposition period	Post-oviposition period	Egg numbers / female	% Hatchability
10% sugar solution	2.4a	13.8	3.4a	146.0e	88.0b
10% sugar solution +yeast	2.2ab	14.2	3.2ab	154.0cd	92.0ab
10% sugar solution +vitamin E	2.0bc	14.0	3.4a	149.0de	93.0a
10% honey solution	2.2ab	14.0	3.0bc	156.0bc	91.0ab
10% honey solution +yeast	1.8c	14.4	2.8c	166.0a	93.0a
10% honey solution +vitamin E	2.0bc	14.2	3.0bc	161.0ab	95.0a
P	*	NS	*	*	*
LSD 0.05	0.355	--	0.355	5.944	4.043

Influence of host plants on the reproductive ability of spiny bollworm moths:

The effects of use host plant as stimulation for reproductive ability were recorded in Table (4). The presence of natural cotton bolls and okra fruit recorded 1.8 days pre-oviposition period, significantly shorter than resulted with host plant absent treatment, which recorded 2.2 days. The treatments recorded oviposition periods of 14.0, 14.6 and 14.4 days, for host plant absence, cotton bolls and okra fruit presence, respectively, without any different significant. The treatments recorded post-oviposition periods of 3.0, 2.6 and 2.4 days, for host plant absence, cotton bolls and okra fruit presence, respectively, without any different significant. The numbers of eggs laid by females moths in presence of cotton boll or okra were 183 and 178 eggs per female, respectively, which were highly significant than that obtained with moths in absent of host plant, which recorded 156 eggs per female. In the presence of cotton boll and okra fruits, no significantly increased in the hatchability percentages than that recorded with moths in absent of host plant. The treatments recorded Hatchability percentages of 91, 95 and 94% for host plat absence, cotton bolls and okra fruit presence, respectively.

The results are in agree with Tamhankar (1994), who mentioned that presence of okra fruit increased numbers of eggs and hatchability percentage of spiny bollworm, *Earias insulana.* El-Sayed *et al*. (2008), found that the presence of cotton bracts increased oviposition period, fecundity and fertility of pink bollworm moths.

Table (4): Influence of host plants on the reproductive ability of spiny bollworm moths:

Presence of host plants	Preoviposition period	Oviposition period	Postoviposition period	Egg numbers / female	% Hatchability
host plants absent	2.2a	14.0	3.0	156.0b	91.0
Cotton bolls	1.8b	14.6	2.6	183.0a	95.0
Okra fruits	1.8b	14.4	2.4	178.0a	94.0
P	*	NS	NS	***	NS
LSD 0.05	0.399	---	---	5.41	---

REFERENCES

Adkisson, P. L. (1961): Fecundity and longevity of the adult female pink bollworm reared on synthetic diets. J. Econ. Ent., 54: 1224-1227.

Costat, S. (1990). Microcomputer program analysis, version4-20 CoHort Software, Berkly CA, USA.

Dhawan, A.K. and A.S. Sidhu (1984): Incidence and relative abundance of different species of spotted bollworms on okra at Ludhiana, Punjab. J. Res. Punjab-Agric. Univ., 21(4): 533-542.

El-Sayed A.A.A., A.E.A. Amer and M.G. Ragab (2008): Pink bollworm moths in relation to diapause, heat units and host plant. Bul. Ent. Soc. Egypt, 85: 109-119.

Klein M., A. Naavon, M. Andreadou and S. Keren (1981): Development of an artificial diet for mass rearing of the spiny bollworm, *Earias insulana*. Phytoparasitica, 9 (2): 145-148.

Patana R. (1977): Rearing selected western cotton insects in the laboratory. U.S. Department of Agriculture, Agricultural Research Service, ARS W-51, 8 pp.

Person, E.O and O.B.E. Maxwell-Darling (1958): The insect pests of cotton in tropical Africa, Empire Cotton Growing Corporation and Commonwealth institute of Entomology, London.

Rashad, A.M. and E.D. Ammar (1985): Mass rearing of the spiny bollworm, *Earias insulana* (Boisd.) on semi artificial diet. Bull. Soc. Entomol. Egypt, 65:239-244.

Tamhankar J. and T. K. Dongre (1992): Improved artificial diets for laboratory rearing of the spiny bollworm,*Earias insulana* (Boisduoval) (Lepidoptera: Noctuidae). International Journal of Pest Management, 38 (1):85-88.

Tamhankar A. J. (1994): Host influence on mating behavior and spermatophore reception correlated with reproductive output and longevity of female *Earias insulana* (Boisduoval) (Lepidoptera : Noctuidae). Journal of Insect Behavior, 8 (4): 499-511.

تحسين طرق التربية المعملية لدودة اللوز الشوكية

عادل السيد علي عامر، علي احمد أحمد السيد و سامي ابو الفتوح عبد الواحد رسلان
معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - الجيزة

تم أجراء تعديلات في طريقة تربية دودة اللوز الشوكية فى المعمل للوصول الى طرق أفضل تؤدى الى زيادة كفاءتها التناسلية. وجد أن تغيير البيئة الصناعية التى تتغذى عليها اليرقات بعد 7 أيام بنفس البيئة حديثة التجهيز، أو عوائل طبيعية (لوز القطن أو ثمار الباميا) أدت الى قصر معنوي فى طول عمر اليرقات والعذارى، وزيادة معنوية فى وزن اليرقات والعذارى، نسبة خروج الفراشات، عدد البيض الموضوع للانثى وكذلك نسبة فقس البيض مقارنة باليرقات التى تم تغذيتها على البيئة الصناعية بأستمرارية بدون تغيير حتى التعذر.

أستخدام محلول عسل النحل لوحده أو بأضافة الخميرة أو فيتامين ه اليه فى تغذية فراشات دودة اللوز الشوكية أدى الى زيادة عدد البيض الموضوع للانثى وكذلك نسبة فقس البيض مقارنة بالفراشات التى تم تغذيتها على محلول سكرى لوحده أو بأضافة الخميرة أو فيتامين ه اليه حيث كان عدد البيض الموضوع للأنثى 146، 154، 149، 156، 166 و 161 بيضة لأناث الفراشات المغذاة على محلول سكرى، محلول سكرى+خميرة، محلول سكرى+ فيتامين ه ، محلول عسل النحل ، محلول عسل النحل +خميرة، محلول عسل النحل + فيتامين ه، على الترتيب.

عدد البيض الموضوع بواسطة أناث فراشات دودة اللوز الشوكية أزداد معنويا فى وجود العائل النباتى سواء لوز القطن الأخضر أو ثمار الباميا (183 و 178 بيضة) مقارنة بعدد البيض الموضوع بواسطة أناث فراشات دودة اللوز الشوكية فى عدم وجود العائل النباتى (156 بيضة).

قام بتحكيم البحث

أ.د / سمير صالح إبراهيم عوض الله — كلية الزراعة – جامعة المنصورة
أ.د / محمد أحمد محمد ندا — مركز البحوث الزراعية