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ECONOMIC ARTIFICIAL DIETS FOR REARING SPINY BOLLWORM, *Earias insulana* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The present investigation was conducted to evaluate the use of four artificial diets that don't contain agar and less expensive for mass rearing the spiny bollworm as compared to the agar – based artificial diet. These four diets A, B, C and D contained dried active yeast, ascorbic acid, sorbic acid, methyl parahydroxy benzoate, starch, liquid milk, vitamin mixture and formaldehyde 34 - 38%, except diet A not containing starch. In addition to the essential ingredient in diet A is kidney beans and wheat grated, diet B is kidney beans and soybeans flower , diet C is kidney beans and diet D is kidney beans and yellow lentils .The obtained results showed that the mean larval and pupal durations were affected significantly by diets A & B and non-significantly by diets C & D as compared with control diet .The longest larval and pupal periods were on control diet and shortest were on diet A .Weight of larvae and pupae reared on diets A, B, C and D were significantly higher than those of the larvae and pupae reared on control diet .The larval fed on the diet A gave the highest larval and pupal weights, while those fed on control diet gave the lowest weights .The highest larval and pupal mortality percentages were on control diet followed by diets C, B, D and A. The lowest pupation and emergence percentages were on control diet and the highest were on diet A .The highest number of deposited eggs and hatchability percentage were observed on diet A followed by diets B, D and C and the lowest were on control diet .The growth index and fitness index were highest on diet A and the lowest were on control diet. Moreover, the total cost of ingredients reduced by 59.11. 43.88 , 43.75 and 45.24% per 1000 g. diet of diets A, B, C and D, respectively as compared to the agar – based control diet.

INTRODUCTION

Spiny bollworm , *Earias insulana* (Boisd.) is considered one of the most harmful insect pests which attack cotton , maize and okra plants in Egypt and causes a great loss in the yield , Amer (2004) and Nada *et al.* (2010) . It is reared in Egypt on artificial diet to study its life history, behavior, susceptibility and resistance to chemical and biological pesticides. Rearing it on artificial diets is an expensive – process. Also, all artificial diets used in rearing of bollworms in Egypt contain agar which is very expensive, Abd El-hafez *et al.* (1982), Rashad and Ammar (1985), Rashad *et al.* (1993) and Ahmed (2001) . So ,considerable effort has been invested in Egypt and the world in the development of low cost effective artificial diets based on the use of cheaper ingredients and testing potential agar substitutes Patana (1969), Klein *et al.* (1981), Abbasi *et al.* (2007) and Sorur *et al.* (2011) .

The aim of this work is to evaluate the use of four artificial diets that don't contain agar and less expensive for mass rearing the spiny bollworm as compared to the agar – based artificial diet (Ahmed, 2001).

MATERIALS AND METHODS

The present study was carried out in Bollworms Research Department, Plant Protection Research Institute, Sharkia branch to evaluate four artificial diets that don't contain agar and less expensive for mass rearing the spiny bollworm, *E. insulana* as compared to the agar - based artificial diet (Ahmed, 2001). The experiment was performed at constant temperature 26 ± 1 °c and 70-75% R.H. .

The composition of the four diets evaluated in this work is detailed in Table (1 and 2).

Table (1): Component of the control and tested diets for rearing the spiny bollworm.

Ingredients	Control diet	Diet A	Diet B	Diet C	Diet D
Kidney beans (g)	215.00	250.00	250.00	275.00	215.00
Wheat grated (g)	-	125.00	-	-	-
Soybeans flour (g)	-	-	50.00	-	-
Yellow lentils (g)	-	-	-	-	85.00
Dry active yeast (g)	32.00	49.00	49.00	49.00	49.00
Ascorbic acid (g)	3.00	3.00	3.00	3.00	3.00
Sorbic acid (g)	1.25	1.75	1.75	1.50	1.50
Methyl parahydroxy benzoate (g)	1.25	1.75	1.500	1.50	1.50
Agar agar (g)	11.25	-	-	-	-
Starch (g)	-	-	5.00	5.00	5.00
Liquid milk (ml)	-	100.00	175.0	90.00	100.00
A mixture of vitamins(ml)	-	8.00	8.00	8.00	8.00
Formaldehyde 34-38%(ml)	3.00	2.50	2.50	2.50	2.50
Distilled water (ml)	650	-	-	-	-

Control diet (Ahmed, 2001) was modified from those developed by Rashad & Ammar (1985) and Rashad *et al.*, (1993).

- **Soybeans flower, Glycine max .**
- **kidney beans, *Phaseolus vulgaris*.**
- **.Yellow lentils, Lens culinaris.**
- **. wheat grated , *Triticum aestivum* L .**

Table (2): Ingredients of vitamins mixture (Grand Vit with Iron Syrup), Produced by Sigma Pharmaceutical industries for Sina Pharm.

Each 5ml contains

Ingredients	Quantity
Vitamin A	1200 I.U
Vitamin D3	100 I.U
Vitamin B1	1 mg
Vitamin B2	1 mg
Vitamin B6	0.5 mg
Vitamin C	50 mg
Vitamin E	1 mg
Nicotinamide	5 mg
Panthenol	2 mg
Calcium gluconate	25 mg
Calcium phospholactate	25 mg
Ferrous gluconate	43.2 mg

Diet preparation:

To preparing the tested diets A, B and C. The boiled water is added to kidney beans or kidney beans + wheat grated and put over heat for seventy minutes , lifted and left for twenty minutes to be cooled and clarifying water from them. They are blended with milk in a electric blender and put in the refrigerator for 24 hours. After that adding the other components to them and thoroughly blended and put in the refrigerator for 24 hours before being used. On the other hand, to preparing the diet D, the water is added to yellow lentils and put over heat for seven minutes, lifted and clarifying water from them. Also, kidney beans was cooked (as previously). They are blended with milk in a electric blender and put in the refrigerator for 24 hours. After that adding the other components to them and thoroughly blended and put in the refrigerator for 24 hours before being used.

Insect:

The newly hatched larvae of the spiny bollworm used in this study was obtained from a culture maintained for many generations 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) .

Nine generations of insect were reared on each tested diets before beginning recording the experiment observations.

Diet evaluation:

Newly hatched larvae of the spiny bollworm were transferred individually to glass tubes (2×7.5 cm) containing about 4 - 5 g artificial diet (Table 1), each tube were plugged tightly with absorbent cotton and placed in an incubator at the same previous conditions . Four replicates of 25 larvae were used for each tested diet. Larvae were examined daily until pupation to record larval duration, larval mortality percentage and larval weight. Pupae were transferred to clean glass tubes and examined daily until moth emergence to record pupation percentage, pupal weight, pupal duration and pupal mortality percentage. When adults emerged, moths were sexed and caged to eggs laying (five pairs / cage) and replicated four times for each diet. Moths were provided with 10 % honey solution. The cages were inspected daily until moth death. The pre-oviposition, oviposition and post-oviposition periods, average number of eggs / female, hatchability percentage and longevity of adult females and males were calculated. The larval and pupal growth index, immature growth index, standardize growth index and fitness index of the spiny bollworm reared on each tested diet were calculated using the following equation (Pretorius, 1976; Itoyama *et al.*, 1999):

$$\text{Larval growth index} = \frac{\text{Pupation (\%)}}{\text{Larval period (days)}}$$

$$\text{Pupal growth index} = \frac{\text{Emergence (\%)}}{\text{Pupal period (days)}}$$

$$\text{Standardized growth index} = \frac{\text{Pupal weight}}{\text{Larval period (days)}}$$

$$\text{Immature growth index} = \frac{\text{Emergence (\%)}}{\text{Immature stages (Larval period + pupal period (days))}}$$

$$\text{Fitness index} = \frac{\text{Pupation (\%)} \times \text{pupal weight}}{\text{Larval period + pupal period}}$$

Statistical analysis:

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

RESULTS AND DISCUSSION

Larval stage : The results of the effect of tested diets on development time of larval stage of the spiny bollworm are shown in Table (3).The mean larval durations were affected significantly by diets A & B and non-significantly by diets C & D as compared with control diet . The longest larval duration (15.0days) was on control diet and shortest (13.5 days) on diet A, whereas 14.0 days on diet B and 14.5 days on diet D, significantly differences were found between larval weight fed on tested diets and control diet. The larval fed on the diet A gave the highest larval weight (0.100 g) while those fed on control diet gave the lowest weight (0.070 g) in other diets , larval weight were observed as 0.088 , 0.083 and 0.086 g on diets B , C and D , respectively . Highly significant differences were found between the mortality percentages for tested diets and control diet. The mortality percentages of larval stage were 5.0, 6.0, 7.0, 5.0 and 13.0 % for diets A, B, C, D and control diet, respectively.

Pupal stage : Data presented in Table (3) showed that the pupal duration was shorter when the larvae were fed on diets A & B (9.5 days) followed by diet D (10.0 days) and diet C (10.5 days). While, pupal duration was prolonged to 11.0 days on control diet. The mean pupal durations were affected significantly by diets A & B and non-significantly by diets C & D as compared with control diet. Pupal weight was 0.072 g on diet A that was highest among all diets. The lowest weight per pupae (0.052 g) was recorded on control diet. Weight of pupae on diets B, C and D were 0.066, 0.059 and 0.068 g., respectively. Tested diets have highly significant effect on the pupation percentage as compared with control diet. The pupation percentages were 87.0, 95.0, 94.0, 93.0 and 95.0 % on control diet, diets A, B, C and D, respectively. Significant differences were found between pupal mortality percentages resulted from larval fed on tested diets and control diet. The highest percentage of pupal mortality (13.79 %) was observed on control diet. The lowest percentage of pupal mortality (7.37 %) was obtained on diet A, whereas diets B, C and D showed intermediate effect on percentage of pupal mortality.

Adult emergence: Data given in Table (4) showed highly significant differences in the adult emergence as larval stage fed on tested diets and control diet. The percentages of adult emergence were 92.63 , 91.49 , 90.32 , 91.58 and 86.21 % for diets A, B, C, D and control diet , respectively .

Adult longevity: The oviposition period of the spiny bollworm female moths developed from larvae reared on tested diets and control diet are summarized in Table (4). The oviposition period was significantly different among the tested diets and control diet but the pre-oviposition and post-oviposition periods were affected non - significantly by the tested diets. The longest oviposition period was obtained on diet A (14.0 days) and shortest was observed on control diet (10.0 days). Also, resulted showed significant differences were recorded between male longevity fed as larval on tested diet and control diet. The male longevity of the spiny bollworm moths were 16.0 , 15.5, 15.0, 15.5 and 14.0 days on diets A , B , C , D and control diet , respectively .

Eggs laying: The number of eggs per female of the spiny bollworm are given in Table (4). There was significant differences in the effect of tested diets and control diet as larval fed on the number of eggs per female. The highest number of eggs per female was 190.0 eggs on diet A , followed by 175.0 , 170.0 and 145.0 eggs on diets B , D and C , respectively and the lowest number was 115.0 eggs on control diet. Hatchability percentages were differed significantly as affected by larvae, fed on tested diets and control diet , The hatchability percentages were 94.0 , 92.0 , 90.0 , 92.0 and 87.0 % for diets A , B , C , D and control diet , respectively. Abbasi *et al.* (2007) showed that *Helicoverpa armigera* when fed the tapioca-based artificial diet during larval stage, larval and pupal development period, percent pupation, pupal weight, emergence rate of male and female, longevity, fecundity and hatching were non – significantly different than that of the control agar - based artificial diet. Sorour *et al.* (2011) showed that the a higher larval weight, pupal weight, adult emergence and fertility of *Spodoptera littoralis* when fed the semi synthetic diets based on combination of starch agar during larval stage as compared to the control agar - based diet.

Table (3): Immature stages of the spiny bollworm reared on control and tested diets.

Artificial diets	Larval duration (days)	Larval weight (g)	Pupation %	Larval mortality %	Pupal duration (days)	Pupal weight(g)	Pupal mortality %
Control diet	15.00 a	0.070 d	87.00 c	13.00 a	11.00 a	0.052b	13.79 a
Diet A	13.50 c	0.100 a	95.00 a	5.00 c	9. 50 b	0.072a	7.37 c
Diet B	14.00 bc	0.088 b	94.00 ab	6.00 bc	9.50 b	0.066a	8.51 bc
Diet C	14.50 ab	0.082 c	93.00 b	7.00 b	10.50ab	0.059a	9.68 b
Diet D	14.50 ab	0.086 bc	95.00 a	5.00 c	10.00 ab	0.068a	8.42 bc
P	**	***	***	***	*	**	***
$LSD_{0.05}$	0.615	0.004	1.651	1.651	1.029	0.009	1.326

Table (4): Adult longevity and reproductive potential of the spiny bollworm reared on control and tested diets

Artificial diets	Adult emergence %	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Female longevity (days)	Male longevity (days)	No. of eggs /female	Hatchability %
Control diet	86.21 c	2.00	10.00 b	3.00	15.00 b	14.00 b	115. 00 d	87.00 c
Diet A	92.63 a	2.00	14.00 a	2.00	18.00 a	16.00 a	190.00 a	94.00 a
Diet B	91.49 ab	2.00	13.00 a	2.50	17.50 a	15.50 a	175.00 b	92.00 ab
Diet C	90.32 b	2.00	13.00 a	2.00	17.00 a	15.00ab	145.00 c	90.00 b
Diet D	91.58 b	2.00	13.50 a	2.50	18.00 a	15.50a	170.00 b	92.00 ab
P	***	NS	***	NS	***	*	***	***
$LSD_{0.05}$	1.489		1.167		1.51	1.215	6.534	2.01

Growth and fitness index: Data obtained in Table (5) showed that the highest growth index of larval, pupal and immature stages of the spiny bollworm were 7.04, 9.75 and 4.03 on diet A, respectively. The lowest one was 5.80, 7.84 and 3.32 on control diet, respectively. While , on diets B , C and D were 6.71, 9.63 and 3.89 ; 6.41 , 8.60 and 3.61 and 6.55 , 9.16 and 3.74, respectively . The growth index emphasizes the importance of both survival rate and developmental time in measuring of food quality Setamou *et al.* (1999). Higher survival rates and shorter development times yield higher values of growth index. The highest fitness index was 0.297 on diet A while the lowest was 0.174 on control diet. The fitness index was 0.264, 0.219 and 0.264 on diets B, C and D, respectively.

Cost of rearing the spiny bollworm on artificial diets: Data in Table (6) showed that the total cost of ingredients reduced by 59.11 , 43.88 , 43.75 and 45.24 % per 1000g of diets A, B, C and D, respectively as compared to the control diet. Abassi *et al.* (2007) showed that the cost to rear on tapioca - based approached 2.13 times than the cost of rearing on the agar - based artificial diet. Sorour *et al.* (2011) showed that the modified by substituting the agar amounts partially with starch reduced the cost of ingredient by 45.6 and 33.3 % per one litter of diets A and B , respectively .

Table (5): Growth index of the spiny bollworm reared on control and tested diets.

Artificial diets	Larval growth index	Pupal growth index	Immature growth index	Standardized insect growth index	Fitness index
Control diet	5.8	7.84	3.32	0.0035	0.174
Diet A	7.04	9.75	4.03	0.0053	0.297
Diet B	6.71	9.63	3.89	0.0047	0.264
Diet C	6.41	8.60	3.61	0.0041	0.219
Diet D	6.55	9.16	3.74	0.0047	0.264

Table (6): Cost comparison of the composition of the control and tested diets used for rearing the spiny bollworm larvae.

Ingredients	Control diet		Diet A		Diet B		Diet C		Diet D	
	Quantity	Cost (pound)	Quantity	Cost (pound)	Quantity	Cost (pound)	Quantity	Cost (pound)	Quantity	Cost (pound)
Kidney beans (g)	215.0	2.58	250.0	3.00	250.5	3.00	275.0	3.30	215.0	2.58
Wheat grated (g)	-	-	125.0	0.63	-	-	-	-	-	-
Soybeans flour (g)	-	-	-	-	50.0	0.60	-	-	-	-
Yellow lentils (g)	-	-	-	-	-	-	-	-	85.0	0.85
Dry active yeast (g)	32.0	0.64	49.0	0.98	49.0	0.98	49.0	0.98	49.0	0.98
Ascorbic acid(g)	3.0	0.54	3.0	0.54	3.0	0.54	3.0	0.54	3.0	0.54
Sorbic acid (g)	1.25	0.19	1.75	0.27	1.75	0.27	1.5	0.23	1.5	0.23
Methyl parahydroxy benzoate (g)	1.25	0.16	1.75	0.22	1.50	0.20	1.5	0.20	1.5	0.20
Agar agar(g)	11.25	6.19	-	-	-	-	-	-	-	-
Starch(g)	-	-	-	-	5.00	0.05	5.00	0.05	5.0	0.05
Liquid milk (ml)	-	-	100	0.60	175.0	1.05	90.0	0.54	100.0	0.60
A mixture of vitamins(ml)	-	-	8.0	0.51	8.0	0.51	8.0	0.51	8.0	0.51
Formaldehyde 3438%(ml)	3.0	0.04	2.5	0.03	2.5	0.03	2.5	0.03	2.5	0.03
Distilled water	650.0	-	-	-	-	-	-	-	-	-
Total weight of productive diet (g) and cost for each diet	670	10.34	1075	6.78	835	7.23	735	6.38	768	6.49
Cost of 1000 g diet	15.43		6.31		8.66		8.68		8.45	
Total cost compared with control (%)			59.11		43.88		43.75		45.24	

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بينات صناعيه اقتصاديه لتربية دودة اللوز الشوكيه

عادل السيد علي عامر

معهد بحوث وقاية النباتات الدقي- جيزه -مصر

أجريت هذه الدراسه لتقييم استخدام أربع بينات صناعيه لا تحتوي علي أجار ورخيصة الثمن لتربية دودة اللوز الشوكيه بالمقارنه ببينه صناعيه محتويه علي أجار . هذه البينات الأربعه أ ، ب ، ج . د تحتوي علي خميرة جافه نشطه ، أسكوربيك أسيد ، سوربيك أسيد ، ميثيل باراهيدروكسي بنزوات ، نشا ، لبن سائل ، مخلوط من الفيتامينات وفورمالدهيد ٣٤- ٣٨ % عدا البينه أ لا تحتوي علي نشا . بالاضافه الي ان المادة الاساسيه في البينه أ الفاصوليا والقمح المبشور وفي البينه ب الفاصوليا ودقيق فول الصويا وفي البينه ج الفاصوليا وفي البينه د الفاصوليا والعدس الاصفر . أوضحت النتائج أن متوسط فترة طورى اليرقه والعذراء تأثرت معنويا بواسطه البينات أ ، ب وغير معنويا بواسطه البينات ج ، د بالمقارنه بالبينه الصناعيه المحتويه علي أجار . وأطول فتره لطوري اليرقه والعذراء كانت علي البينه الصناعيه المحتويه علي أجار واقلها علي البينه أ . ارتفع وزن اليرقات والعذاري التي ربيت علي البينات أ ، ب ، ج ، د معنويا عن تلك اليرقات والعذاري التي ربيت علي البينه الصناعيه المحتويه علي أجار . اليرقات التي تغذت علي البينه أ أعطت أعلي وزن يرقات وعذاري بينما التي ربيت علي بينه صناعيه محتويه علي أجار اعطت أقل وزن . أعلي نسب سوت لليرقات والعذاري كانت علي البينه المحتويه علي أجار تلاها البينات ج ، ب ، د ، أ . أقل نسب تعذير وخروج فراشات كانت علي البينه المحتويه علي أجار وأعلاها كانت على البينه أ. أعلي تعداد بيض وضع ونسب فقس لوحظ علي البينه أ تلاها البينات ب ، د، ج واقلها كانت علي البينه المحتويه علي أجار .أعلي مؤشرات نمو وملائمه كانت علي البينه أ واقلها علي البينه الصناعيه المحتويه علي أجار .بالإضافه إلي أن اجمالي تكلفة المواد اللازمه لإنتاج ١٠٠٠ جرام بينه إنخفضت الي٥٩,١١، ٤٣,٨٨، ٤٣,٧٥و ٤٥,٢٤ %للبينات أ ، ب ، ج ، د علي الترتيب بالمقارنه بالبينه الصناعيه المحتويه علي أجار .