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Original Research Article

Successful Laboratory Mass Production of the American Bollworm *Helicoverpa armigera* (Hubn.) on Artificial Diet

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Abstract

The ever increasing demand for a large numbers of laboratory reared insects has necessitated the development of efficient and economic methods of production. The American bollworm or Tomato fruitworm *Helicoverpa armigera* (Hubn.) (Lepidoptera: Noctuidae) has long been recorded as a pest of many crop in many countries of the world, like Tomato, Maize, Zucchini, Green paper, Gourd, Muskmelon, Okra, Cassia, Potato, Bean, Chickpea, Sunflower, Red beet, Tobacco, Cowpea, Turnip. It also attacks the cultivated cotton plants. *H. armigera* can be successfully reared on artificial diet for the continuous maintenance of laboratory colonies and to facilitate investigations into different studies. The artificial diet composed of dry powdered chickpea, brewer's yeast, agar, ascorbic acid and mould inhibitor. The addition of vitamin B_{12} and riboflavin to the diet significantly increased the percentage of survivor larvae, moths fecundity, larval weight, fertility and moths fecundity, while the addition of sesame oil as a feeding stimulant significantly increased the pupal weight, percentage of deformed pupae and adults, these as a result of the fat tissues accumulation in the pupae during the feeding period of larvae . The pupae was passing through a period of diapauses from December to February, where the pupal period lasted about 100 day during this period for *H. armigera* of Saudi Arabia strain.

Keywords: *Helicoverpa (Heliothes) armigera*, successful artificial diet, rearing technique

INTRODUCTION

The American bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) is a major pest of cotton, tomato, okra, chickpea and some other crops and vegetables in Saudi Arabia (AL-Dhafer and Sharaby, 2001). This species has a wide range of host plants, including both cultivated crops and wild plants causing extensive loss to major legume, fiber, cereal, oilseed and vegetable crops (Krishnareddy and Hanur, 2015). Laboratory rearing using synthetic diet is a better option for knowing its biology under controlled conditions. Successful rearing becomes the first priority to study its life history and behavior using artificial diet. Many researches were done to rear the insect under laboratory condition using artificial diet (Castani and Zapata, 2005). Till today many techniques are available for rearing *H. armigera* (Abbasi *et al*., 2007), and still efforts were continued towards improvement for increase in potential rearing. Nutritive value of various diets may vary based

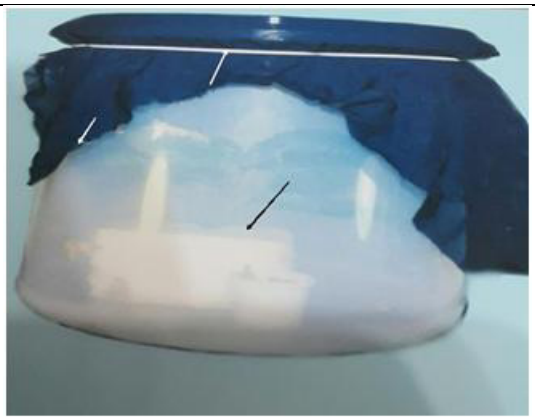

Fig 1. Plastic jar 1 liter capacity for mating the adult ♀and ♂, its mouth covered with black cloth as a site for eggs deposition (white arrows) fastened with a rubber band. A cotton swab moistened with honey solution inside the jar (black arrow).

Fig 2. Upper is Dorsal view and the lower is Ventral view of H. armigera adult

Fig 3. Egg stage with white yellow color deposited on black cloth.

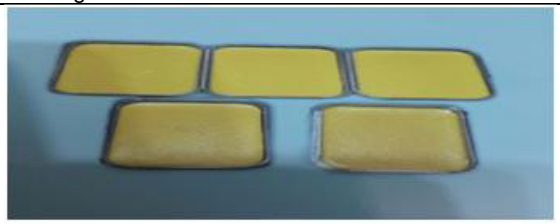

Fig 4. Artificial diet powered in plastic containers.

Fig 5. Newly hatched larvae on artificial diet.

Fig 6. Second larval instar on artificial diet.

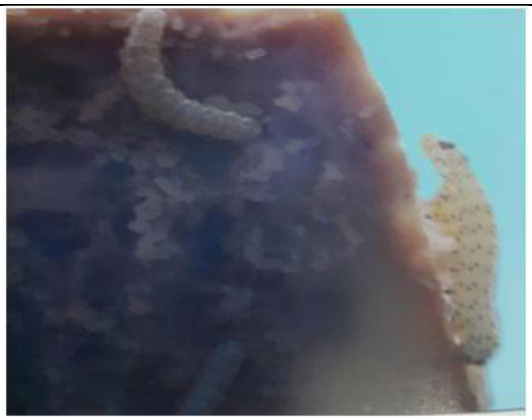

Fig 7. Third larval instar on artificial diet.

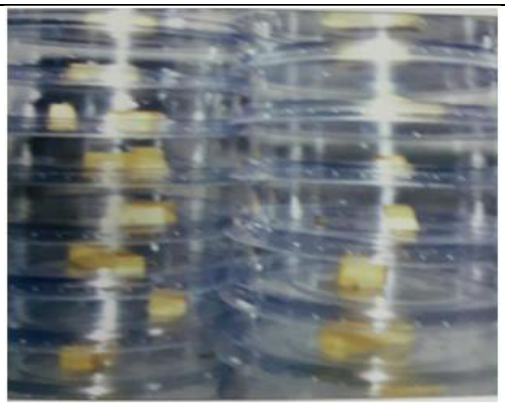

Fig 8. Larvae separated individually, one larva / Petri dish with piece of artificial diet.

Fig 9. Last larval instar trial to pupate in the diet

Fig 10. Pupa inside its cocoon in the diet

Fig 11. Newly formed pupa each in one container with dry and not contaminated diet.

Fig 12A. The resulting normal mature pupae

Fig 12B. Deformed pupa full of fats (white arrow) and shrinkage in their integument

upon its composition available in the diet, the uptake of nutrients by insects depend upon its feeding with artificial diet. Laboratory mass production of the insect on artificial diet can also be used for the study of insect pathogens, plant resistance factors, effects of insecticides and many other scientific researches on the insect. The essential ingredients for insect development of artificial diets include proteins, carbohydrates, fats, cholesterol, glucose, vitamins, minerals and preservatives (Sieber and Rembold, 1983). The present study was undertaken to evaluate two kinds of artificial diets as better diet in terms of growth and feeding of larvae and increase in egg laying of *H. armigera* under laboratory condition. All diet ingredients are locally and easily available in low cost.

MATERIALS AND METHODS

Insect collection and rearing on the artificial diets

This study was conducted at the laboratory of zoology department, faculty of science for girls, Dammam, KSA. *H. armigera* moths were collected from the field in KSA by the net from around the light traps that fastened near the green houses of tomato plant, Okra and Clover, that belonging to the project of reclaim agriculture at AL- Qatif and AL-Hasa governorate at eastern rejoin, larvae also were collected at different stages from the Maize and Lettuce in October and March. The insects that collected were transferred to the laboratory for mass production. Adults were introduced separately in gars 1 liter capacity (Fig. 1) one old ♂ with one newly ♀ in each jar for success mating(Colvin *et al.,* 1994), and a cotton swab soaked in 10% honey solution as a food source for adults after emergence, jars covered with a piece of black cotton cloth as a site for egg deposition .The eggs were gathered (Fig. 3) then confined with a piece of artificial diet in Petri dishes 9cm (Fig. 8), newly hatched larvae were moved towards the diet and accumulated on it (Fig. 5) for feeding till reaching 3rd larval instar (Fig. 7) then separated 1 larva for each cup with diet (for cannibalism habit) till larva attain the pupation stage

Table 1. Constituents of the artificial diet for mass production of *H. armigera* (Hubn.)

Constituents	Quantity	
	Diet 1	Diet 2
Flour of chickpea, *Cicer arietinum*	100 gm.	105 gm.
Agar	10 gm.	17 gm.
Brewers yeast	16 gm.	16 gm.
Ascorbic acid	2 gm.	2 gm.
Benzoic acid	2 gm.	2 gm.
Tetracycline hydrochloride capsule (Julphar)	250 ml.	250 ml.
Benexol		
B12 (F.Hoffmann-La Roch Ltd, Basel)	500 µg.	500 µg.
Riboflavin	-----	1 gm.
Casen	-----	2 gm.
Folic acid		
(Vifolin) (APM Co. Ltd, Sult- Jordan)	-------	15 gm.
Sorbic acid 20% in ethanol 95%	1.5 ml.	1.5 ml.
1% Hcl	1 ml.	0.5 ml.
Formaldehyde 40%	1 ml.	0.5 ml.
Sesame oil	2 ml.	-------

(Fig. 11) All laboratory experiments conducted for rearing was kept at 27±2Cº, 65±5% RH. When the diet was eaten or consumed another piece was added, diet was not spoiled during the feeding by the larvae. A field collected larva was separated from different host plants and was placed in artificial diet for rearing. The diet was changed regularly with three day intervals till completion 3rd instar then separate as mentioned before. The process of pupation occurred by forming a cocoon within the diet itself (Fig. 9 and 10) during rearing. Initial stage of pupae was light green in color (Fig. 11) and turns dark brownish upon completion (Fig.12A). Males and females Pupae were in appearance and were transferred to the mating containers as previously mentioned. After eclosion period of pupae, the adults were released into the mating cage. The eggs laid was clearly observed on the black cloth (Fig. 3) and upon egg hatching, neonate larva was collected using fine camel hairbrush and was released into fresh diet for feeding. The stock culture was maintained regularly. All biological aspects were recorded daily at the two tested diets (larva duration, larval weight after 12 day from feeding, % pupation, pupa duration, pupal weight after 2 day from pupation, % of deformed pupae, % emerged moths, sex ratio, % deformed adults, moths fecundity and fertility finally incubation period.

Artificial diet constituents and preparation

Table 1 consist of the two diets that were used in this study as described by Salama and Sharaby (1980) with some modification in their constituents. The components of the two diets are illustrated in Table 1. Diets were prepared as follows: All of each diet ingredients except of agar were well dissolved in 700ml. of distilled water. Agar was kept separately in 300ml. of distilled water then boiled to 100°C. It was cooled to 50°C then blended with the other 700ml. (contained ingredients). The prepared diets each bowered before cooling in a thin layer of 5cm. height plastic containers and kept in a refrigerator until used. Data was statistically analyzed following t test to differentiate between means.

RESULTS AND DISCUSSION

Biological aspects of the *H. helicoverpa* maintained on artificial diet under laboratory condition

Table 2 shows the different biological parameters of *H. armigera* obtained from rearing on the two diets 1 and 2. Data indicated no obvious significant differences between means of the larval weight, moths longevity for males and females, females fecundity, incubation period for eggs and sex ratio. There were significant differences of $p< 0.05$ between means of larval and pupal duration, pupal weight. They were respectively 16.38 day, 15.10 day and 439mg. on diet 2 comparing with 18 day, 21.45 day and 347mg. respectively on diet 2. Diet 2 cleared increase in percentage of both alive larvae with 13%, egg fertility by 32.65%, 13% of moths emergence and recorded13% of pupation than diet 1. There was decrease in pupal duration 13.09% in comparison with 11.49% for diet 2. We could conclude that increase of pupal weight resulting from larvae reared on diet 1 as the increase of fat bodies accumulated in the larvae and pupae Fig 12B. Because

Table 2. Different biological aspects of H.armigera (Hubn.) maintained on artificial diet

Biological aspects in days	Artificial diet					
	Diet 1			Diet 2		
	min	max	mean±SE	min	max	mean±SE
Larval duration	14	20	16.38± 0.14	14	30	18± 0.44 **
Larval weight in mg.	170	660	316.5± 24.8	210	490	360± 13.30
% pupation	-	-	85	-	-	98
% of pupal deformation	-	-	14.11	-	-	1.02
Pupal weight /mg.	280	540	439± 24.90	300	400	347±5.49 **
Pupal duration	13	16	15.10± 0.16	13	29	21.45±1.06 **
% of moths emergence	-	-	83	-	-	96
% of deformed moths	-	-	12.94	-	-	1.45
Six ratio ♂	-	-	37.64	-	-	51.04
♀	-	-	62.35	-	-	48.95
Moth longevity ♂	17	25	21± 1.39	12	29	22.86± 1.70
♀	18	31	25.30± 1.29	17	31	24.20± 1.19
Egg production/ female	149	1324	651.50± 128	700	1700	939.50± 109.44 *
Incubation period	2	4	3.96± 0.03	2	4	3.96± 0.03
% 0f egg hatchability	-	-	59.68	-	-	92.33

*Significant **highly significant at p> 0.05

diet 1 contained Sesame oil that lack of Riboflavin and vitamin B12 showed increased in the percentage of pupal and moths deformation. Riboflavin and vitamin B12 have an impact on the hormonal system of the insect, they caused increased percentage of the alive larvae and moths fecundity on diet 1 with decrease percentage of deformed pupae and moths. The previous data agreed with that recorded by Krishnareddy and Hanur (2015) that the artificial diet contained protein-X during through egg laying. Miller and Silhacer (1995) mentioned that adding 5% Riboflavin to the *H. zea* the diet increased the growth of the larvae and pupae of *H. armigera* and increased moths fecundity. Results obtained on diet 2 agreed with Salama and Sharaby (1980). They succeeded in rearing *H, armigera* on diet contained dry kidney bean, larval duration was 1702 day and pupal weight 303mg., fecundity 580egg/female and 88% egg fertility. The variation in *H.helicoverpa* development on different diet related to variation of the diet components and their present percentages. Kinds of food have great effect on the development and its rate, larva; duration and pre-pupal stage greatly affected by the agroclimatic condition. The insect fertility have been dependent on the kind of laval diet (Tripathy and Singh 1989). Larvae reared on host plants takes 6 stages on cotton, seven on tobacco and 8 on tomato plants (Ramos and Marallo-Rejesus 1980). We could conclude that diet 2 was more suitable for laboratory mass production of *H. armigera* under the constant condition (27±3ºC and RH 65±5%) and under normal day light. Larvae that consumed the two diets passing through five instars and pupae entered diapauses for 100dayfrom December to February, these agreed with mentioned by (Hmimina *et al* 1993). Females emerged before males with 3-4 day for this reason old

emerged females should confined with newly emerged male for success mating and good fertility and fecundity.

CONCLUSION

Hlicoverpa. armigera can be successfully reared on artificial diet for the continuous maintenance of laboratory colonies and to facilitate investigations into different studies. The artificial diet composed of dry powdered chickpea, brewer's yeast, agar, ascorbic acid and mould inhibitor. All diet ingredients are locally easily available in low cost. The addition of vitamin B_{12} and riboflavin to the diet significantly increased the percentage of survivor larvae, moths fecundity, larval weight, fertility and moths fecundity. Diet 1 contained Sesame oil and lack of Riboflavin and vitamin B12, it showed increased in the percentage of pupal and moths deformation. Riboflavin and vitamin B12 caused increased percentage of the alive larvae and moths fecundity on diet 1 with decrease percentage of deformed pupae and moths. We could concluded that diet 2 was more suitable for laboratory mass production of *H. armigera* under the constant condition (27±3ºC and RH 65±5%) and under normal day light.

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