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Modified Artificial Diet for Mass Rearing of Chickpea Pod Borer, *Helicoverpa* (*Heliothis*) *armigera* (Hubn)

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Abstract
This paper reports on a comparison of a modified diet with other diets and procedures used in mass rearing of *Helicoverpa armigera*. The results on the biology of *H. armigera* reared on the modified diet for up to six consecutive generations indicate that the pupal recovery percentage ranged form 71.2 ± 9.59 to 83.7 ± 5.98; the adult energence percentage varied from 59.6 ± 3.15 to 78.4 ± 3.29.The maximum egg yield/female was recorded to be 273.3 ± 13.6 in the first generation. The average cost to produce one pupa of this insect was calculated to be Rs. 0.60, which in equivalent ot half the cost of production per pupa using other diet formulas.

Key words: Modified diet; Helicoverpa *(Heliothis) armigera*; rearing; oviposition, techniques; larvae

Introduction
The ever-increacing demand for a large number of laboratory reared insects has necessitated the development of efficient and economical methods of production. The shifting emphasis in insect control, using biological entities such as natural enemies and insect pathogens (bacteria, viruses and fungi), has increased the demand for constantly reliable sources of such insects. More over, the rearing of phytophagous insects on artificial media, rather than on their host plants, is advantageous in a variety of investigations. Laboratory-reared larvae can be used for the study of insect pathogens, plant resistance factors, and effects of insecticides and radiation of fecundity and growth, as well as for the study of insect life cycles.

Beck *et al*. (1949), reported the first seccessful aseptic rearing of a phytophagous lepidopteran, the European corn borer, *Ostrinia mubilalis*, on a meridic diet. Ishii and Urushibara (1954) were able to rear the rice stem borer, Chilo suppersalis, on a modification of the diet reported by Beck *et al*. (1949). Vanderzant and Reiser (1956) were the first to formulate a meridic diet for the pink bollworm, *Pectinophora gossypiella*, without a plant adjuvant. Later Adkisson *et al*. (1960), developed a satisfactory artificial medium supplemented with wheat germ for rearing pink bollworm. The wheat germ medium has been used to rear several species of plant-feeding lepidopterans, including the bollworm *Helicoverpa* (*Helipothis*) *zea* (Vanderzant *et al*., 1962); the cabbage looper, *Trichoplusia ni* (Getzin, 1962); the white large butterfly, *Pieris brassicae* (David and Gardiner, 1976); and the coding moth, *Carpocapsa pomonella* (Rock *et al*., 1964).

Since, these studies were performed, both diets and rearing techniques have been modified/simplified (Ignoffo, 1965; Shorey and Hale, 1965; Burton and Perkins, 1972; Dulmage *et al*., 1976, Ahmed, 1983; Ahmed *et al*., 1985; Kulkarni and Amonkr, 1988; Navon *et al*., 1990).

The chickpea pod borer, *Heliothis armigera*, is an important, polyphagous insect pest that inflicts economic losses to chickpea, maize, tomato, tobacco, sunflower, cotton, vegetable, and fodder crops (Ahmed *et al*., 1986, 1989). In morthern Pakistan, *H. armigera* caused 90 percent pod damage in unprotected fields (Ahmed *et al*., 1986, 1990; Anonymous, 1987).

To facilitate research on *H. armigera*, efforts were focused on developing an economical diet formula and rearing techniques. The present study and its results elaborate upon the development of a diet (with all ingredients locally avilable), tools, and techniques applied successfully for maintenance of healthy insect cultures of *H. armigera* to conduct bioassays of *Bacillus thuringiensis* (an insects pathogen currently being used in many biological insecticides for control of lepidoterous pests). The artificial diet formula and producers reported herein represent further simplification/modification of the artificial diet and rearing techniques reported by the author (Ahmed, 1983).

Materials and Methods
At the start, 1050 larvae (different instars) of *H. armigera* were collected from chickpea fields and reared on artificial diet. The rearing room temperature ranges from 20 to 29°C, relative humidity varied from 60 to 80 percent, and a 15.9 light and dark cycle was maintained.

Ingredients for the preparation of diet: The ingredients and quantities used in preparation of a 4.251 batch of the diet are as follows:

Ingredients	Quantity
Agar	25.0 g
Bean powder (*Vigna unguiculata*)	600.0 g
Ascorbic acid	7.0 g
Sorbic acid	3.0 g
Vitamin E	0.2 g
Dried active yeast	20.0 g
Mehtyle-p-hydroxyhenzoate	10.0 g
Vitamin mixture	5.0 ml
Formaldehyde (10%)	6.0 ml
Tap water	3500.0 ml

The following vitamin mixture preparation in 200 ml sterile distilled water was used:

Ingredients	Quantity
Calcium pantothante	4.8 g
Nicotine acidamid	2.4 g
Rboflavin	1.2 g
Folic acid	1.2 g
Thiamine hydrochloride	0.6 g
Pyridoxine hydrochlorid	0.6 g
Biotin	0.048 g
Vitamin B_{12}	0.0024 g

The dry ingredients of the diet wer weighed carefully and kept in separate containers. The wet ingredients were measured and also kept in separate containers. The entire quantity of agar was suspended in a 51 capacity container and brought to a boil. The total quantity of bean powder (*Vigna unguiculata*) was added to the boiled agar and mixed, which resulted in cooling of the mixture to nearly 80°C. Then all the dry and wet ingredients were added to this mixture and the entire mass was mixed. The vitamin mixture, including vitamin E, was added last, and the mass was again mixed thoroughly. The prepared diet was the poured into the desired number of sterilized glass capsule vials (5-6 ml diet/capsule vial) with the help of a squeeze bottle and allowed to cool and harden.

Rearing procedures: Glass capsule vial technique for individual larval development: A standard-sized glass capsule vial (2.5 cm in diameter & 5.5 cm high) was used in this technique. The sterilized capsule vial containing diet was infested with a newly hatched first instar larva with the help of a sterilized camel hair brush. After the vial was infested with instar larva, its mouth was tightly closed with a sterilized cotton wool plug, which provided an exchange of air and did not allow the diet to dry out before the developing lrva pupated. A number of sets, each comprising 25 to 50 vials (one larva/vial) were used for mass larval development.

Petri plate technique for group larval development: This insect develops cannibalism from the late third to fifth instars. Technique were developed that allows rearing *H. armigera* in groups of 90 to 100 larvae per petri plate (17.0 x 3.0 cm), with a thin layer of diet at the bottom and in the cover of the plate. After infestation of the petri plate with about 110 first instar larvae, sterilized two ply tissue paper was folded and placed in the petri plate to soak up excessive diet moisture and allow the developing larvae to move into the tissue paper folds, therby reducing chances of possible larval cannibalism. This enabled aseptic rearing upto the early third instar larvae for use specifically in second or third instar larval bioassays. This rearing technique significantly reduced the cost of development of third instar larvae as compared with the individual rearing technique (one larva/container).

Adult emergence: Plastic jars (11.5 cm in diameter and 10 cm high) were used for adult emergence. The larvae that pupated in the, capsule vials were taken out and placed on a circular pieccof blotting paper in the plastic jar (10-15 pupae/jar). The mouth of the jar was closed with muslin cloth held tightly in position by rubber bands. After emergence, the adults were collected individually into capsule vials (one adult/vial). Care was taken not to disturb the adults to reduce the chances of their escape from the emergence jar. The emerged adults were checked and removed daily.

Mating-oviposition cage: A 30 cm cubic box made of acrylic sheet was fabricated into an oviposition cage. Four slits, each 25.0 cm long X 6.0 mm wide, were made at equal spacing on three sides of the cage. The slits were closed with cotton wool strips for oviposition. On two opposition sides of the cage, slits 6.0 cm wide x 30.0 cm long were left open. These wide slits were covered with muslin cloth to allow air to pass through the cage. The adults were also introduced into the cage ,through these wide slits. Two medium-sized petri plates containing cotton wool pads soaked in 10% sucrose solution were also placed in the cage to provide food for the ovipositing adults.

The desired number of pairs (20-30) of *H. armigera* were released in the cage through one of the slits. The eggs deposited on cotton wool strips were removed daily, checked, and kept in polyethylene bags for incubation and hatching.

Egg incubatio: The eggs were incubated at optimum temperature in the laboratory. Hatched larvae were transferred into capsule vials containing diet. Egg sterilization was not considered necessary as no incidence of bacterial or viral disease was observed. However, a, low level of fungal contamination (off-born contamination) was found after approximately 12 days of larval feeding in the vials.

For evaluation of significance, a range test (DMRT) was done using the MSTAT-C computer programme (Version 1.42, developed by Michigan State University, USA).

Results and Discussion

Table 1 shows a comparison of the diet formulae developed by Ahmed (1983), Ahmed *et al*. (1985), Kulkarni and Amonkar (1988) and Navon *et al*. (1990) for rearing the chickpea pod borer, *Helicoverpa (Heliothis) zea,* and tobacco budworm, *Heliothis virescens*); and Shorey, (1963) (for rearing the cabbage looper, *Tricholusia ni*). The modified diet developed in the present work, which supported six generations of the insect, is similar in composition to the diet of Ahmed (1983), except for the addition of vitamin E, an increase in tap water (3500.0 ml), and a reduction of about 30.0 to 45.0 percent in the quantities of all the ingredients. Vitamin E was incorporated in the diet to event a possible reduction in reprodictive vigor and egg viability of the insects. The diet reported by Ahmed *et al*. (1985), supported six successive generations, was similar in composition to the diet developed by Ahmed

Table 1: Artificial diet formula presently developed by the authors and its comparison with previously published artificial diet formulae used for rearing Helicoverpa (*Heliothis species* and *Trichoplusia nil*)

Ingredients[A]	Present Formula	Ahmed[1] (1993)	Ahmed[1] *et al*. (1985)	Shorey and[2] Hale (1965)	Shorey[3] (1963)	Kulkarni[1] and Amonkar (1988)	Navon[1] *et al*. (1990)
Agar (g)	25.0	50.0	50.0	128.0	4.0	19.0	74.0
Bean powder (g)	600.0	400.0	400.0	2133.0*	100.0	136.0**	1200.0***
Ascorbic acid (g)	7.0	11.0	3.0	32.0	1.0	2.9	16.0
Sorbic acid (g)	3.0	3.0	-	10.0	-	-	5.0
Vitamin E (g)	0.2	-	-	-	-	-	-
Dried active yeast (g)	20.0	20.0	20.0	320.0	10.0	42.0	160.0
Methyl-para-oxybenzoate(g)	10.0	7.0	7.0	20.0	1.0	2.7	16.0
Vitamin mixture (ml)	5.0	4.0	-	-	-	-	-
Formaldehyde (ml)	6.0 (10.0%)	6.0 (10.0%)	6.0 (10.0%)	20.0 (40.0%)	-	10.8 (10.0%)	20.0 (37.0%)
Tap water(ml)	350.0	2000.0	2000.0	6400.0	200.0	825.0	3000.0
Cholestrol (g)	-	-	-	-	-	-	3.0
Chloramphanical (g)	-	-	-	-	-	-	6.0
Alfalfa meal (ml)	-	-	-	-	-	-	200.0
Quantity of diet (approx.)	4250.0	2500.0	2500.0	8500.0	300.0	1000.0	4250.0
No. Of generation raised	6.0	23.0	6.0	-	12.0	-	-

*Soaked lima bean **Bengal gram powder ** * Phaseolus vulgaris presoaked in water for 24 hr. 1 For rearing *Helicoverpa* (*Heliothis*) *armigera*, 2 For rearing *Heliothis zea* and *H. virescens*, 3 For rearing *Trichoplusia ni*.
[A]All the ingredients were that of analar grade except bean powder, yeat and formaldehyde

Table 2: Mean ± SE of larval development of *H. armigera* from field collected larvae to 6th laboratory generations

Generation	Larvae placed on diet	Average pupal** Recovery (%) ± SE	Average larval** period (days) ± SE	Temperature range(°C)
Field	1050*	71.2 ± 9.56a	-	24-29
First	900	83.7 ± 5.98a	22.9 ± 4.04	17-29
Second	500	75.3 ± 8.85a	26.3 ± 3.50	16-26
Third	706	76.9 ± 11.51a	26.2 ± 1.55	18-26
Fourth	723	79.0 ± 11.51a	26.2 ± 4.72	22-30
Fifth	757	74.5 ± 5.34a,	20.2 ± 2.54	23-35
Sixth	330	79.4 ± 4.97a	23.2 ± 0.18	23-33

A category which shares common letters is non-significantly different at $p < 0.05$. *Larvae collected from chickpea field were in different instars. **Average of 4 batches

Table 3: Mean ± SE pupal development and emergence of *H. armigera* from field to 6th laboratory generation

Generation	Number of pupa recovered	Adult emergence* (%) ± SE	Average pupal period* (Days) ± SE	Temperature range (°C)
Field	759	73.2 ± 5.88ab	13.60 ± 0.26	24-30
First	742	78.4 ± 7.07a	17.1 ± 2.34	16-29
Second	372	65.3 ± 2.05bc	19.5 ± 0.69	18-26
Third	557	59.6 ± 3.15c	20.2 ± 1.82	18.29
Fourth	603	78.4 ± 3.29a	15.5 ± 0.45	22-31
Fifth	561	76.0 ± 7.05a	13.3 ± 1.38	23-35
Sixth	264	78.2 ± 5.38a	14.2 ± 0.53	23-31

A category which shares common letters is non-significantly different at $p < 0.05$. *Average of 4 batches

Table 4: Mean ± SE of oviposition per female of *H. armigera* from field to 6th laboratory generation

Generation	Number of adults recovered	Number of adult pairs released in cage	Average No. Of eggs* /female ± SE	Temperature range 1°C)
Field	547	1-10	202.2 ± 35.4b	21-28
First	589	130	273.3 ± 13.6a	16-27
Second	243	115	102.9 ± 13.3c	18-26
Third	326	144	153.9 ± 17.1b	18-29
Fourth	476	155	158.9 ± 8.8b	22-30
Fifth	424	88	188.3 ± 40.8b	23-35
Sixth	208	67	166.1 ± 35.3b	24.32

A category which shares common letters is non-significantly different at $p < 0.05$. *Average of 4 batches

(1983) (likewise supported 23 successive generations of *H. armigera*), except that sorbic acid and vitmins were omitted, and the quantity of ascorbic acid was decreased to 3.0 g for a 2.51 batch of diet.

The diet formula developed by Shorey and Hale (1965) also contained the same ingredients, in different quantities and with complete omission of vitamins as compared with the present formula. Shorey (1963), also worked out a simple diet consisting of only six ingredients on which he successfully raised twelve generation of cabbage looper, *Trichoplusia ni.* The present diet modification contained all the ingredients reported by Shorey (1963). Kulkarni and Amonkar (1988) also used similar diet ingredients, though in different quantities and without sorbic acid and vitamins; they used Bengal gram powder as the basic ingredient instead of bean powder. The diet formula used by Navon *et al*. (1990) for brearing *H. armigera* consisted of 11 ingredients, of which 8 were common to the present modification.

The modified diet developed here in is compartively in expensive, and one batch (4.251) can be used to rear about 700 larvae (6.0 ml diet/larva). The average cost to produce each pupa, including labor charges, was calculated to be Rs. 0.60, which is equivalent to 1.8 cents. Twenty eight years ago, Shorey and Hale (1965) reported that approximate cost to produce on *H. zea/H. virescens* pupa was 0.9 cent. In Pakistan, analar grade chemicals (many of the diet ingredients) are imported items. Thus their cost is high (e.g., one tin [454.0 g] of Difco Agar-Agar cost Rs. 3300 which is equivalent to $100).

Helicoverpa armigera larval development and pupal recovery data from field to sixth laboratory generations (Table 2) showed no significant difference among the pupal recovery percentage in all the generations. The maximum pupal recovery (83.7%) was noted in the first generation. Average pupal period ranges were from 20.2 days (minimum in the fifth generation) to 26.3 days (maximum in the second generation) at temperature ranges of 23-35 and 16-26°C, respectively. Similar results were observed by Burton and Perkins (1972) and Dang *et al*. (1970), who ahieved 86.2 percent pupation of *H. zea* reared on bean diet and 75.0% pupation of *C. zonellus* reared on Kabuli gram diet, respectively. Ahmed (1983) and Ahmed *et al.* (1985) also reported *H. armigera* pupal recovery ranging from 61.6 percent (in the second generation) to 97.3 percent (in the second generation respectively.

The data in Table 3 illustrate results on pupal period and adult emergence. The maximum adult emergence (78.4%) noted in first generation, was significantly different from second and third generation. The cause of the high pupal mortality in the third generation is unknown. The pupal period varied from 13.3 to 20.2 days, according to the temperature range. Ahmed (1983), observed the maximum adult emergence of *H. armigera* (92.8%) in the fourth generation and the minimum (50.2%) in the twenty-third generation. High adult emergence rates of 96.8 percent for *C. zonellus* (reared on bean diet) and 90.0 percent for *H. zea* (reared on bean diet) also reported by Dang *et al.* (1970) and Burton and Perkins (1972), respectively. Ahmed *et al.* (1985) reported low adult emergence of *H. armigera* (44.23 to 70.97% in different generations).

The maximum number of eggs laid/female (Table 4) was 273.2, in the second generation. The inimum number of eggs/female (102) was recorded in the third generation. The reason for such significant variation in oviposition per female is unknbwn. Similar findings (145 to 326 eggs/female of *H. armigera)* were reported by Ahmed *et al.* (1985). Burton and Perkins (1972) obtained 1901 eggs/mated female of *H. zea* reared on WSB diet.

The modified artificial diet reported herein proved to be successful for mass rearing of *H. armigera* and supported development of this insect for at least six labortory generations. Moreover, the techniques applied for maintaining healthy cultures of the insects satisfied experimental requirements (bioassays), The present modified diet was also found economical in comparison with other diets. Hence, this diet is recommended for rearing of *H.armigera* for long periods.

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