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A SEMI-SYNTHETIC CHICKPEA FLOUR BASED DIET FOR LONG-TERM MAINTENANCE OF LABORATORY CULTURE OF *HELICOVERPA ARMIGERA*

G CHITTI BABU,*** HC SHARMA,* T MADHUMATI,** G RAGHAVAIAH,** KVM KRISHNA MURTHY** AND VS RAO**

** International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad-502,324, Andhra Pradesh,*

*[*]Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad 500 030, Andhra Pradesh Corresponding author: *h.sharma@cgiar.org*

ABSTRACT

Artificial rearing of insects in the laboratory is a pre-requisite for undertaking studies on insecticide/ biopesticide bioassays, and evaluation of germplasm, segregating breeding material, mapping populations, and transgenic plants for resistance to insects. For successful rearing of insects in the laboratory, there is need for standardizing a semi-synthetic diet that supports survival and development of the insect for several generations. We tested different semi-'synthetic diets for rearing the legume pod borer, *Helicoverpa armigera* for two generations under laboratory conditions. All the semi-synthetic diets tested supported the growth and development of *H. armigera* up to adult emergence, but there were significant differences in survival and development and fecundity on different diets. The highest larval survival was observed in the wheatgerm based diet in first (97.5%) and second generation (93.5%), followed by chickpea based modified diet (93.75% survival in both the generations). The lowest larval weights (270.7 and 283.7 mg) were recorded in the tapioca granules diet in 1st and 2nd generation, respectively. The adult emergence ranged from 68.7 to 83.3%, and 60.4 to 81.2% in the 1st and 2nd generation, respectively. Least fecundity was observed in the tapioca granules based diet (422 and 603 eggs per female) in both the generations, but the differences were not significant. Complete or partial replacement of agar-agar with tapioca granules was not suitable for use in artificial diets. The results indicated that modified chickpea flour based diet was quite appropriate for rearing *H. armigera* under laboratory conditions.

Key words: Insect rearing, Artificial diets, Tapioca granules, *Helicoverpa armigera,* Survival and development, Fecundity.

The cotton boll worm/legume pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the most devastating crop pests worldwide (Fitt, 1989; Sigsgaard *et al*. 2002; Sharma, 2005). It is a polyphagous pest, and attacks more than 181 cultivated and uncultivated species of plants (Manjunath *et al.* 1985). The extent of losses have been estimated to be over US$ 2 billion in the semi-arid tropics, despite application of insecticides costing over $500 million annually (Sharma, 2005). Rearing of phytophagous insects such as *H. armigera* on artificial diets rather than on their host plant is advantageous for a variety of investigations. It is important that the larvae reared under laboratory conditions have characteristics similar in viability, vigor and behavior to the wild populations, and show corresponding resistance to pathogenic viruses, bacteria and fungi (Griffith and Haskell, 1988).

Artificial rearing of insects in the laboratory is a pre-requisite for undertaking studies on insecticide/ biopesticide bioassays (Surekha Devi *et al.* 2011), and evaluation of germplasm, segregating breeding material (Sharma *et al*. 2005a,b), mapping populations (Clement *et al*. 2010), and transgenic plants for resistance to insects, and studying mechanisms and inheritance of resistance to insects (Sreelatha *et al*. 2007; Narayanamma *et al*. 2007; Sharma *et al*. 2008). Several research workers have successfully reared *H. armigera* on artificial diets under laboratory conditions. Ahmad (1983) evaluated an artificial diet (except with sorbic acid and vitamins), which supported 23 successive generations of *H. armigera*. This diet was further modified with the addition of vitamin E (which prevents a possible reduction in vigor and egg viability), and supported six successive generations of *H. armigera* (Ahmad *et al.* 1998). Gupta *et al.* (2004) standardized an artificial diet that supported the growth and development of *H. armigera* for 10 generations, while Armes *et al.* (1992) standardized the chickpea flour based artificial diet for rearing of this insect under laboratory conditions. This diet has been used at ICRISAT for the past 20 years, but we often experienced pathogen infection, and reduced fecundity of the insect after 5 - 6 generations. The rate of survival and development of *H. armigera* larvae has been found to differ significantly depending upon diet ingredients such as chickpea, soybean and maize (Singh and Rembold 1992). Seven artificial diets having chickpea, mung bean, soybean, wheat, maize, cottonseed, or water chestnut flour have been evaluated for rearing *H. armigera,* and the chickpea flour based diet produced healthy larvae and pupae that gained the maximum weight (Hamed and Nadeem 2008). The *H. armigera* larvae fed on tapioca-based artificial diet (instead of agar-agar as the binding agent) did not differ significantly from the agar-agar based artificial diet in terms of larval and pupal developmental periods, %age pupation and adult emergence, pupal weight, longevity, fecundity and egg hatching (Abbasi *et al.* 2007).

Although several diets have been developed that support optimum survival and development of *H. armigera*, there is a continuing problem of loss of viability and vigour in the laboratory culture, in addition to build of viral, fungal and bacterial infections, resulting in collapse of the insect cultures quite frequently. Therefore, we evaluated different artificial diets for successful rearing *H. armigera* under laboratory conditions. In addition, we also evaluated tapioca granules, which are cheap and easily available in the market, as a complete or partial substitute for agar-agar in the artificial diet.

MATERIAL AND METHODS

The experiments were conducted in the insect rearing laboratory at the International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Different semi-synthetic diets used in these experiments are listed in the Table 1. Based on a number of experiments, we also modified the chickpea flour based artificial diet (Chitti Babu, 2013), whereby, we used 1.5 g sorbic acid instead of 1.0 g, 3.0 g of methyl-p-hydroxy benzoate instead of 1.5 g, 4.7 g ascorbic acid instead of 1.67 g, 1.0 ml multivitamin mixture instead of 3.0 ml, 48.0 g yeast instead of 16.0 g, and 48.0 g of agar-agar instead of 16.0 g. In addition, 0.2 g streptomycin sulphate, 10.0 ml formaldehyde, and 400 mg capsule of vitamin E were also included in the modified diet.

Table 1. Ingredients of the artificial diets used for rearing *H. armigera* under laboratory conditions

Ingredients	Wheatgerm diet	Chickpea * flour diet	Tapioca granules diet	Tapioca granules agar-agar diet	Chickpea flour modified diet
Chickpea flour	100 g	100 g	100 g	100g	100 g
Wheat germ	13 g	-	-	-	-
Sorbic acid	1.2 g	1.0 g	1.0 g	1.0 g	1.5 g
Methyl p-hydroxy benzoate	2 g	1.5 g	1.5 g	1.5 g	3 g
Ascorbic acid	6 g	1.67 g	1.67 g	1.67 g	4.7 g
Casein	15 g	-	-	-	-
Streptomycin sulphate	0.2 g	-	-	-	0.2 g
Cholesterol	0.6 g	-	-	-	-
Multivitamin solution (ABDEC drops)	1.5 ml	3.0	3.0	3.0	1.0 ml
Multivitamin capsules	2	2	2	2	-
Formaldehyde 1%	1 ml	-	-	-	10 ml
Vitamin E (400 mg)	1	-	-	-	1
Yeast 24.5 g	16 g	16 g	16 g	48 g	
Agar-agar	15 g	5.8	-	3.0	14 g
Tapioca granules	—	-	40	30	
Water	825 ml	400 ml	400 ml	400	700 ml

*Chickpea flour based diet used at ICRISAT.

After preparing the diets, the semi-solid diet was dispensed into the sterilized multi-cell well plates (Chitti babu. 2013). After solidification of the diet (in 30 – 45 min), 12 neonate larvae of *H. armigera* were released individually in cell wells by using a camel hair brush. There were four replications for each treatment in a completely randomized design. The larvae were reared in the cell wells until pupation. The pupae were collected carefully, and placed in a glass jar with moist vermiculite at the bottom for adult emergence. Observations were recorded on larval and pupal survival, larval and pupal development periods, and fecundity. The fecundity was observed by keeping a pair of adults in an oviposition cage (30 x 30 x 30 cm), and the numbers of eggs laid were counted daily, until the females died.

STATISTICAL ANALYSIS

The results were subjected analysis of variance using GENSTAT Version 10.1. The significance of differences between the treatments was judged by F-test, while the treatment means were compared by least significant difference (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

There were significant differences in survival and development of *H. armigera* on different artificial diets. The larval survival ranged from 81.2 to 97.5%, and 72.0 to 93.5% in the first and second generations, respectively. The highest larval survival was observed in the wheatgerm based diet in both the generations (97.5 and 93.5%), followed by chickpea flour (93.75% in both the generations) modified diet (Table 2). Lower larval survival was observed in insects reared on the *Tapioca* granules based diet in both the generations (81.2 and 72.0 %). The larval survival decreased in second generation in the *Tapioca* granules diet. %age larval mortality was greater in *Tapioca* granules based diet as compared to the other diets tested. The larval period lasted for 12.8 days in insects reared on the modified chickpea flour diet as compared to 14.2 days on the *Tapioca* granules based diet in the first generation. Similar trend was observed in the second generation (Table 3). The lowest larval weights (270.7 and 283.7 mg) were recorded in insects reared on *Tapioca* granules agar-agar diet. The larval weights were highest (357.3 mg) in insects reared on wheatgerm based diet in first generation, and modified chickpea diet (402.8 mg) in the second generation (Table 3).

There were significant differences in %age pupation on different diets, which ranged from 77.0 to 91.7% and 64.5 to 87.5% in the first and second generations, respectively. The highest pupation (91.7%) was recorded in larvae reared on modified chickpea flour based diet, followed by those reared on the wheatgerm diet (89.5%) in the first generation. In the second generation, highest pupal recovery was observed in larvae reared on wheatgerm diet (87.5%), followed by modified chickpea flour diet (83.33%) (Table 2). Pupal period was longer (9.8 days) in insects reared on the *Tapioca* granules - agar-agar mixed diet and *Tapioca granules* diet (9.6 days) than in insects reared on modified chickpea flour diet (8.8 days). In the second generation, longer pupal period (10.5 days) was observed in *Tapioca* granules and agar-agar mixed

Table 2. Survival and development of *H. armigera* on different artificial diets under laboratory conditions

Diets	Generation I			Generation II		
	Larval survival (%)	Pupation (%)	Adult emergence (%)	Larval survival (%)	Pupation (%)	Adult emergence (%)
Wheat germ diet	97.92	89.58	83.33	93.75	87.50	79.17
Chickpea flour diet	91.67	85.42	79.17	89.58	81.25	75.00
Tapioca granules + agar-gar diet	81.25	77.08	68.75	77.08	66.67	62.50
Tapioca granules diet	85.42	79.17	72.92	72.92	64.58	60.42
Modified chickpea flour diet	93.75	91.67	83.33	93.75	83.33	81.25
Fp (4,15)	0.01	0.05	0.06	0.002	0.002	<0.01
VR	5.46	3.10	2.91	6.93	6.94	10.82
SE ±	0.34	0.43	0.46	0.37	0.38	0.25
LSD (P 0.05)	1.03	1.30	NS	1.10	1.15	0.75

NS = Nonsignificant. VR = Variance ratio.

Table 3. Duration of post-embryonic development of *H. armigera* on different artificial diets

Diets	Duration (days)				Weight (mg)			
	Generation I		Generation II		Generation I		Generation II	
	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae
Wheat germ diet	13.0	9.0	13.2	9.3	357.34	235.92	397.37	263.5
Chickpea flour diet	13.3	9.2	13.5	9.4	334.28	235.73	394.09	263.8
Tapioca granules + agar –agar diet	14.1	9.8	14.3	10.5	270.70	208.68	283.74	209.5
Tapioca granules diet	14.2	9.6	14.5	10.2	285.51	212.30	292.01	209.8
Modified chickpea flour diet	12.8	8.8	13.1	9.2	341.29	247.06	402.85	264.9
Fp (4,15)	<0.001	<0.001	<0.001	<0.001	<0.001	0.060	<0.001	<0.001
VR	26.64	21.38	28.29	15.22	12.80	2.25	32.60	10.90
SE ±	0.12	0.10	0.12	0.16	10.40	1106.00	10.50	9.00
LSD (P 0.05)	0.37	0.29	0.37	0.47	31.50	NS	31.80	27.10

NS = Nonsignificant. VR = Variance ratio.

diet. Adult emergence ranged from of 68.7 to 83.3%, and 60.4 to 81.2% in the first and second generations, respectively. The highest adult emergence was observed in insects reared on modified chickpea flour diet, followed by those reared on the wheatgerm diet. The fecundity decreased in insects reared on *Tapioca* granules – agar-agar diet, but not in insects reared on the other diets tested.

The numbers of eggs laid per female (fecundity) was greater in insects reared on wheatgerm diet (595 eggs and 665 eggs per female in 1st and 2nd generation, respectively) and the modified chickpea flour diet (594 and 680 eggs) (Fig. 1) than in sects reared on *Tapioca* granules – agar-agar based diets (422, and 603 eggs per female in 1st and 2nd generation, respectively). However, the differences in fecundity between the diets tested were not significant.

Brewer and King (1979) successfully reared *Heliothis* spp. on artificial diets based on soybean flour and wheat germ, while Hamed and Nadeem (2008) showed that chickpea flour based diet produced healthy larvae and pupae that gained maximum weight and completed development in 14.5 and 11 days, respectively. The present studies indicated that modified chickpea flour based diet supported better survival and development of *H. armigera,* and produced healthy larvae and pupae with a maximum weight of 340 – 400 mg and 240 – 260 mg, respectively. The larval and pupal periods were shorter in insects reared on chickpea flour based diets than on the other diets tested. The survival and development of *H. armigera* on wheatgerm based diet was also

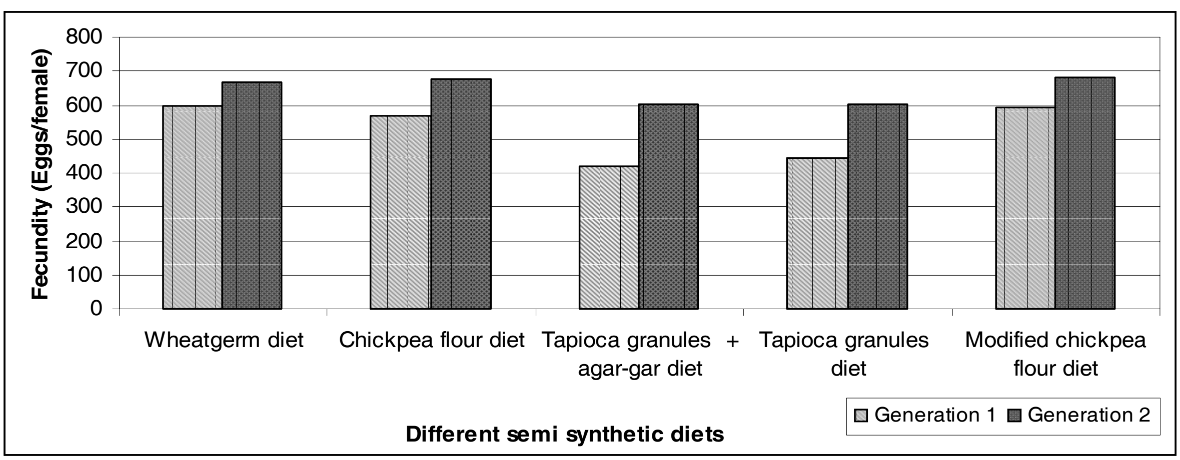

Fig 1. Fecundity of *Helicoverpa armigera* on different artificial diets under laboratory conditions.

similar to the modified chickpea flour diet. Ahmad *et al.* (1998) reported 71.2 to 83.7% pupation and 59.6 to 78.4% adult emergence, while in the present studies, we recorded 81 - 83% adult emergence on the modified chickpea flour diet. Singh and Rembold (1992) suggested that even though the nutritive value of soybean diet was high, food consumption by the larvae was better on chickpea flour based diet as compared to soybean and wheatgerm based diets. We recorded 594 - 680 eggs per female on modified chickpea flour based diet as compared to 326.6 eggs recorded earlier by Ahmed *et al.* (1998). Complete or partial replacement of agar-agar with *Tapioca* granules was not suitable for rearing *H. armigera* in artificial diets. The modified chickpea flour based can be used for rearing *H. armigera* under laboratory conditions for undertaking studies on insecticide/biopesticide bioassays, and evaluation of germplasm, segregating breeding material, mapping populations, and transgenic plants for resistance to this insect.

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