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Enhanced synthetic diet for rearing *H. armigera* under laboratory conditions

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Abstract
This manuscript majorly reports the modification in synthetic diet using Protein-X towards improvement in rearing procedure for *H.armigera*. Development of numerous synthetic diets in laboratory for rearing *H.armigera* has been given high priority by using a mixture of basic ingredients like chickpea, wheat flour and tapioca. Egg fecundity is being commonly studied and various formulations for mass rearing have been endeavored. Protein- X is used for enhancement in egg production along with this chickpea diet and observations in egg mass proved noteworthy. The results on maximum egg yield reported better in comparison with regular diet. This artificial diet along with Protein-X is suitable to overcome the drawbacks during egg laying and is helpful in obtaining appropriate successive generations.

Keywords: Artificial diets, Protein-X, *H.armigera*, egg fecundity.

1. Introduction
The tomato fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is one of the most serious insect pests in several countries like China, Australia, and India. 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. Amongst many agricultural pests *H. armigera* is widely distributed throughout the world by menacing due to its polyphagus feeding (a typical of Nocutidae), increased adult mobility, obscured larval stages, resistant to pesticides and strong diapauses. In polyphagus insects, the availability of different host plants plays an important role in triggering population increase and outbreaks. Mobility and facultative diapause manages with adjusting on spatial and temporal distributions of host plants. In India almost all major states like Andhra Pradesh, Madhya Pradesh, Karnataka, Assam, Punjab, Bihar, West Bengal, Haryana and Gujarat is reported to have considerable damage by *H armigera* wherein the loss estimates vary from year to year and from crop to crop which is mostly depended on the pest population density. Cotton being highly affected with 80% loss [1] followed by 72% in chickpea and in tomato the loss is upto 40% [2]. Current methodologies applied towards appropriate control of *H. armigera* need to be redefined and knowing its biology is more helpful in understanding this key pest. Laboratory rearing using synthetic diets is a better option for knowing its biology under controlled conditions. Successful rearing becomes the first priority to study its life history and nourishing behavior using synthetic diets. Many researchers attempted to rear *H. armigera* under laboratory conditions using synthetic diets [3] by attaining positive results with petite challenges [4]. Maximum number of insect population was not obtained through these synthetic diets [5] and hence requirement of large populations can be overwhelmed with appropriate mass rearing techniques. An attempt to mass rear *H. armigera* was initiated due to screening of large population by tomato plants by challenge inoculation. The bulk requirement was aimed to screen transgenic tomato plants having BtCry2A gene developed in this lab [6]. Nutrition requirement becomes much crucial for insect nourishing across many generations. In general vitamins play a major role in egg fecundity and in nature it is captivated by several means through available essential nutrients. At laboratory conditions, vitamins must be provided at optimal concentrations for effective egg laying and its emergence. Till today many techniques are available for rearing *H. armigera* [7] and still efforts were made towards improvement for increase in potential rearing. Commercially available Protein-X (Wockhardt Nutrition) is rich in essential aminoacids, minerals and vitamins that helps in systemic growth of an organism. Nutritive value of various diets may vary based upon its composition available in the diet, but sufficient uptake of nutrients by insects depends upon its feeding with synthetic diet. Rearing of *H. armigera* using different food substrates was quite successful with having slender

tribulations like moderate feeding behavior, inadequate egg laying and forced or early pupation. The chickpea diet has been clearly verified as better artificial diet in terms of growth and feeding of larvae [8] and hence this chickpea ingredient diet was selected for this experiment. The present study was carried out to assess the protein – X efficiency in chickpea synthetic diet with respect to increase in egg laying by *H.armigera* under laboratory conditions.

2. Materials and Methods

This study was performed at Biopesticide laboratory, Division of Biotechnology, Indian Institute of Horticultural Research, Bangalore during October 2013 to January 2014 to evaluate the effect of Protein-X on egg fecundity of *H. armigera*. To facilitate a pure culture of *H. armigera* huge number of larva were collected regularly from different plant crops and were reared using modified semi synthetic diet till larva attain the pupation stage. Manual collection of different stage larva from the plants of tomato, chickpea and pigeon pea at various locations in and around Bangalore (Latitude: $13^{0}58^{1}$ N Longitude: 78^{0}) was done regularly till the standardization of culture in the laboratory. All laboratory experiments conducted for rearing was kept at 25±2 ^{0}C, 65±5% RH. Field collected larvae was separated from different host plants and was placed in artificial diet for *in vitro* rearing. Precautionary measures were followed throughout rearing by elimination of parasites and diseased larva. *H. armigera* larvae were reared individually on 6 well trays/ containers with artificial diet (Fig 1). These six well containers were covered with brown paper to provide dark conditions for larva. *Helicoverpa armigera* rearing in the laboratory was done on modified semi-synthetic diet [8] with slight modifications. The diet was changed regularly with three days interval till completion of all larval stages or until larva attained pupation. The process of pupation occurred by forming a cocoon within the diet itself during rearing. Initial stages of pupae was light green in color and turns dark brownish upon completion with black head formation. After complete pupation the pupae were separated based on the size and abdominal appearance into males and females and was transferred to glass containers (20 cm × 10 cm) covered with brown paper on all sides of glass containers to provide dark condition necessary for pupation and adult emergence. Healthy pupae were selected and separated individually as pairs till eclosion (emergence from pupal case). After eclosion period the adults were released into cages (32 cm×32 cm×32 cm) from the glass containers containing pupae which were placed on sand enclosed with water in plastic trays to maintain humidity. Temperature was maintained between 25±5 °C during pupation. A cotton swab prepared was dipped in honey mixed with multivitamin capsule (Becadexamin Capsule by GlaxoSmithkline Pharmaceuticals Ltd.,) and Protein-X was placed inside the cage on the sixth day after pupation as a food source for adults after emergence. The adults emerge inside the glass container was allowed for mating. The cages was covered with white cloth inside essential for egg laying. These cages was kept in the basin containing sand filled with water to maintain moisture and humidity required for egg laying. The eggs was laid in two to three intervals by female moth on the cloth provided at the top or sides of cloth covered on cages (Fig 2). The eggs laid was clearly noticed on white cotton cloth and observed under a stereo microscope (Fig 3) for its fertility and upon egg hatching neonate larva was collected using fine camel hairbrush and was released into fresh diet for feeding. Routinely used apparatus like forceps, containers, brushes, plastic trays, glass containers and cotton cloth for rearing was sterilized frequently with absolute alcohol or decont 0.1% (commercially available cleaning agent). The stock culture was maintained regularly at the Biopesticide laboratory, Division of Biotechnology, Indian Institute of Horticultural Research, Bangalore for phenotypic screening of transgenic tomato plants. The maximum and minimum temperature and relative humidity ranged was 27 ºC and 22 ºC and 65-70 percent respectively. The composition of the diet (Table 1) was mixed thoroughly with boiling and cooled to room temperature before pouring to multiwell diet plates. The artificial diets without protein-X and with protein-X were compared and results were tabulated. A one way ANOVA was performed to compare the effect of Protein-X on *H.armigera* egg fecundity in the artificial diet at laboratory conditions.

Table 1: Synthetic Diet composition

Composition of *H. armigera* larval diet (1 litre)	
Chickpea	100 gm
Yeast extract	30 gm
Sucrose	25 gm
Wesson's salt mixture	5 gm
Methyl para hydroxy benzoate	5 gm
Ascorbic acid	10 gm
Sorbic acid	6 gm
Streptomycin sulphate	100 mg
Choline chloride (5%)	10 ml
Formaldehyde	2 ml
Multivitamin capsules	03
Protein-X	2 gm
Autoclaved double distilled water	1000 ml

3. Results and Discussion

In this study, the escalation during egg setting in *H.armigera* with the addition of a commercially available multivitamin ingredient Protein-X was attempted. The current synthetic diets available to rear *H. armigera* are majorly used to study its biology on different diet compositions due to its polyphagus nature. Addition of several ingredients like wheat flour, maize flour [8] and tapioca based diet [7] are readily established for laboratory rearing of *helicoverpa armigera*. The egg laying and larval emergence is an important factor in the insect lifecycle by which the survival percentage of this insect pest can be determined. Earlier studies on egg fecundity [9] mostly focused on several food substrates and suggested that maximum number of eggs can be laid, but still an efforts were made to increase the egg fecundity using this diet. The control diet used regularly without protein-X and along with Protein-X selected was taken as treatments along with five replications. There was a significant effect of Protein –X on *H. armigera* egg fecundity at the $P<0.05$ level [$F=4.224$, $p= 0.029$] in contrast with routinely used synthetic diet. The results (Table 2) suggest that addition of Protein –X in reality has a substantial consequence on enhancement in egg fecundity, on an average around 9.6% increase was seen with egg laying capacity in adults. In particular the insertion of this commercial product Protein-X plays a significant role in the perfection of rearing *H. armigera*. The optimization in addition of Protein-X levels has to be performed in combination with other synthetic diets for obtaining superior results in egg fecundity and continuous rearing across generations of this lepidopteron pest. This study provides knowledge to researchers regarding the importance of essential ingredients like vitamins, essential nutrients and minerals

during rearing. The influence of Protein – X in combination with other diets including tapioca, moong bean and wheat flour on successful rearing of this pest is under investigation.

4. Conclusion

In conclusion, the optimization of this rearing procedure deliver an efficient methodology to understand egg laying details and towards optimization of mass rearing in *H.armigera*. Hence, this Protein-X along with common diet is a better option for efficient egg production during rearing of *H. armigera* under laboratory conditions.

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Table 2: One way ANOVA performed for Egg fecundity in *H. armigera*.

Sl. No	ANOVA	SS	df	MS	F	P(<0.05)
1	Between	16,897.90	4	4,224.47	4.224	0.029
2	Within	10,000.09	10	1,000.01		
3	Total	26,897.99	14			

Fig 1: Laboratory rearing using Synthetic diet

Fig 2: Eggs laid by *Helicoverpa armigera*

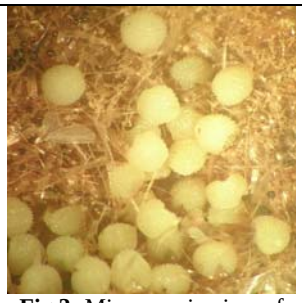

Fig 3: Microscopic view of fertile eggs

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