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Substrate preference for eggs laying pattern and Modification of larval rearing unit of *Helicoverpa armigera* (Hubner)

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Abstract
The fertile egg production was observed from day of 5th to day 11th having two peaks on the 5th and on the 10th day of the egg laying period. The overall mean fertile egg production per female was 1173.25 and sterile egg production per female was 298.50. The maximum sterile eggs were laid on day 13th with 46.5 numbers. Eggs were found on different substrates such as cotton cloth cover, hung vertically cotton cloth, cotton swabs and inner layer of oviposition cage. There were 54 per cent survival in tooth-picks and 46 per cent in without tooth picks which was statistically significant. Male and female pupal weight was 0.378gms and 0.368gms in tooth-picks trays while it was 0.357gms and 0.361gms in without tooth picks.

Keywords: *Helicoverpa armigera*, fecundity, substrate preference, modification of larval rearing unit

1. Introduction
Helicoverpa armigera (Hub.) is a polyphagous pest. It causes serious damage to pulses, fibers and other crops in India and many tropical parts of the world. This insect acquired resistance to majority insecticides and has generated research interest across the world. Therefore, large number of insects are required for laboratory experiment. Large numbers of *Helicorvepa* is also used to produce most favoured microbial agent, nuclear polyhedrosis virus (NPV) which is favoured management tool for the management of *Helicoverpa armigera* on many crops. Nuclear polyhedrosis virus of strain *Helicoverpa* is most promising control agent of *Helicoverpa*. In India, the incidence of a NPV in a laboratory culture of *H. armigera* was first reported by [6]. The virus is produced *in vivo*. Of the many factors influencing the rearing of the *Helicoverpa* thus far, substrate preference for eggs laying and the diet with physical facilities for rearing methodology has received little attention. There are certain factors which reduce the prospects of commercial success of *Helicoverpa* rearing. During rearing the *H. armigera*, there were production of both sterile and fertile eggs have been observed. Cannibalism and disease incidences are two major bottlenecks during larval rearing of *H. armige*ra. A multicellular larval rearing unit was developed for mass rearing of *H. armigera* larvae at the Project Directorate of Biological Control, Bangalore [2]. Further studies indicated that the larvae of *H. armigera* preferred thinner layer of diet surface in its initial stages of development.
The present investigations were taken up to find out the suitable egg laying substrate as well as the impact on the development of *Helicoverpa armigera* on thin a layer of diet. So the objectives of the experiments were: (1) to study the oviposition pattern of *Helicoverpa armigera* on different oviposition substrates and (2) in order to provide thin layer of diet surface for the young larvae, the provision was made by providing toothpicks dipped in diet medium in the cells of the multicellular tray.

2. Materials and Methods
Plastic containers (20x19cm, Fig.1) were selected for the experiment after conducting initial preliminary trials using containers of different sizes. A moist foam piece of 14cm diameter was kept at the bottom of the container. This foam sheet was covered with a moist cotton cloth piece to maximize the humidity and for the counting of eggs as described by [3]. The emergence container was covered with a cotton piece from which another long cotton cloth piece (15x10cm) was hung vertically (Fig.1).

Two small plastic lids with 10 per cent sucrose solution and water swabs were placed in the container. In this mating cum oviposition unit (Fig.2), a pair (male and female) of moths of the same age was released. The fertile eggs and the yellowing colour of the sterile eggs [1]. The eggs laid on substrates provided (like vertically hung cloth, cover cloth, bottom cloth, swabs and container wall) were counted using lens under a table lamp. Egg counts were recorded from day 1st till mortality of moths. The data obtained from 10 units were subjected to ANOVA to determine the best ovipositional substrate. Commercially available toothpicks (Trade Mark Popular Mfd. by Delhi Trading Syndicate (India) were used for developing thin layer diet experiment. The initial trials were conducted to optimize the method of sterilization to avoid mold formation on the toothpicks when in the diet filled cells. It was standardized that 0.5 per cent solution of NaOCl was perfected for wet sterilization of the toothpicks. The toothpicks were dipped for an hour in 0.5 per cent solution of NaOCl. They were taken out and spread individually on a white clean paper sheet for shade drying. After 24 hrs, these were utilized in the experiment. The toothpicks were dipped in the freshly prepared diet (semisynthetic diet, standardized by) [5] and subsequently taken out individually. A thin layer of diet which was formed on the toothpicks was allowed to dry up to 10 minutes. One set of diet was prepared and poured into the plain bottom tray of the multicellular unit and the cellular tray was placed over it. Through each opening of the cell, the diet adhered toothpicks were inserted into each cell (which acted as a substrate for the growing larvae and provided an ideal diet surface for larval feeding). The experiment was set up in multicellular trays (Fig. 3&4), one set contained toothpicks and one without (a control). The data on pupal weight and survival were recorded. The data obtained were subjected to ANOVA to check on the effectiveness of utilizing toothpicks for larval rearing.

3. Results and Discussion

3.1 Age specific fecundity

It was found that the moth started egg laying on the day after pairing and it continued to lay eggs for 15 days till one day before mortality. Generally it was observed that more sterile eggs were laid during the first two days (26.50-31.50) and last three days (39.00-46.50).However, there were instances of sterile egg production even during mid-oviposition period (31.75 eggs on the 8th day). Maximum sterile eggs were laid on the day (13). The maximum number of fertile eggs was laid on the 10th day (172.50) while minimum number was on the 15th day (9). Fertile egg production was maximum from day 5 to day (11). The overall mean fertile egg production per female was 1173.25 and sterile egg production per female was 298.50. Armes *et al*., 1992 [1] found 80 to 200 fertile eggs in an established laboratory culture and they reported 8-14 days as the peak oviposition period of field culture in the laboratory. However, in pure laboratory culture, the peak of oviposition was reported as 4-7 days. Review of literature reveals that estimates of fecundity are highly variable and probably reflect differences in the way the adults are maintained in the laboratory.

3.2 Ovipositional substrate preference

It was found from the statistically analyses data, that ovipositing moths had a maximum preference for the bottom cloth where 56.80 per cent eggs were laid and there were no significant difference between the egg laying on the other substrates (2.6 to 17.3%) However, least preference was for the swabs. Mitchell *et al*., 1988 [4] reported that moths oviposited more readily on moist cloth than the dry cloth. Our results also reflect similar observations wherein the egg laying were maximum on the bottom cloth which was highly moist as it was covering the moist foam. The container cover, vertically hung cloth and container wall were dry substrates and hence egg laying, probably because of the lesser surface area. By identifying the most suitable substrate for egg laying by *H. armigera*, it is possible to utilize such substrate to improve the cultures of *H. armigera*.

As indicated in Table 1, significant increase in survival was observed in the trays with toothpicks (54%) in comparison to control (46%). It was found that the mean pupal weight of male pupae was 0.378g and 0.357g and female pupae was 0.368g and 0.361g, respectively, in the trays provided with toothpicks and those without toothpicks. Pupal weights in the treatment trays were higher in comparison to control. But the differences were statistically insignificant. Significant difference was also not observed with respect of developmental time which was 19.6 days in treatment and 20.7 days in control.

Table 1: Biological Parameters of *H. armigera* larvae reared in the Modified Rearing Unit

Treatment	Mean survival (%)	Mean pupal weight (g)		Mean larval developmental period (days)	
		Male	Female	Range	Mean
Modified unit	54.00 (47.31)	0.378	0.368	20-70	19.61
Control unit	46.00 (42.64)	0.357	0.361	17-21	17.21
CD (P≤0.05)	3.28*	NS	NS	NS	NS
CD(≤0.01)	4.55*	NS	NS	NS	NS

* Showing significance

Figures in parentheses are angular transformed values.

C.D at P ≤0.05=12.41 & C.D. at P ≤ 0.01= 16.41

Fig 1: Emergencies

Fig 2: Oviposition cages

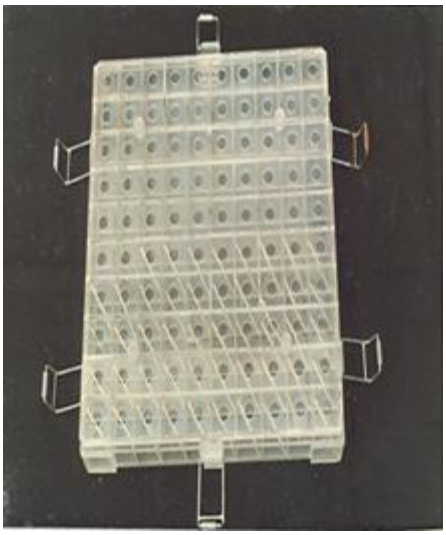

Fig 3: Modified Multi-cellular tray with tooth picks

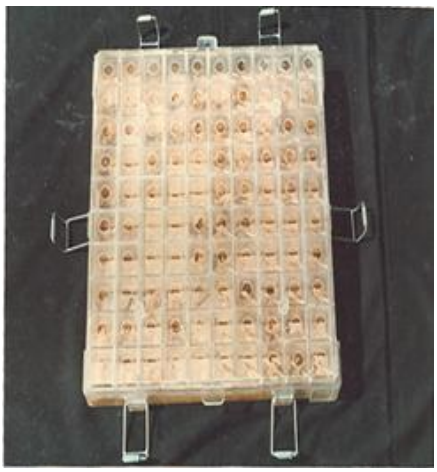

Fig 4: Helicoverpa larvae feeding in the cells of modified multi-cellular tray

4. Conclusion

It was concluded that the most preferred substrate for eggs laying (56%) was bottom cloth. In the toothpicks rearing cells, significantly better survival (54%) were found. So the use of suitable eggs laying substrate and provision of toothpicks in the rearing cells could be best suitable to improve the culture of *Helicoverpa armigera*.

Here, toothpicks were provided as a physical facility for the developing larvae in the multicellular rearing unit. It was observed that the larvae were generally found resting on the toothpicks. The toothpicks also provided a thinner layer of diet surface, which the neonate larvae preferred. For full grown larvae, the toothpicks provided a point (where the tooth-picks are in contact with the diet surface) for boring into the diet, just prior to pupation. In addition to the above facts, the toothpicks eased the process of larval introduction into the diet cells in comparison to control where larvae are transferred directly to the normal diet surface. The toothpicks in the rearing unit provided a physical support and also the thin diet surface preferred by the neonate larvae. This leads to increased pupal production. Thus this modification could be a useful tool for improving the rearing units of *H. armigera*.

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6. References

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