

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Survival and Development of *Chilo partellus* (Swinehoe) (Lepidoptera: Pyralidae) Green Gram Based Diet in Laboratory Conditions.

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ABSTRACT

Chilo partellus (Swinehoe) (Lepidoptera: Pyralidae) is a detrimental pest infecting maize a major staple food crop in India. In order to get rid of such negative impact on productivity insect resistant cultivars may be a better alternative. Artificial field screening programs with effective mass rearing technologies were in practice. Hence present investigation was conducted In vitro conditions to examine the performance of *Chilo partellus* fed with an artificial diet standardized using green gram. Respectively the growth rate was significantly increased. Development of pupa was observed between 8-10 days, Life span of moth in 7-9 days while hatching of eggs was recorded as of 4-6 days with 70-75 per cent in the first generation. Results revealed a significant increase in the growth of *Chilo partellus* when compared to that of previous literature at a low of cost.

Keywords: *Chilo partellus*, lepidopteron, artificial diet, sorghum, survival, development.

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INTRODUCTION

Maize is a principal cereal crop cultivated worldwide and its production remains more than 960MT during 2014-15. Maize is the third most important cereal crop in India after rice and wheat. It is grown throughout the year in India. It is an important staple food crop in many countries and used in animal feed along with industrial applications. It is predominantly a *kharif* crop with 85 per cent of area under cultivation. It accounts for ~9 per cent of total food grain production in the country. Maize is generally affected by different pests with respect to environmental conditions.

Chilo partellus was one among major stem borer diseases seen in cereal crops; like maize, sorghum, paddy and sugarcane [14] all over the world. Most insect pests are not monophagous [5]; they can survive on a wide range of host plants. Maize stem borers can cause severe damage at different stages in the development of cereal crops from seedling to maturity. When infestation is severe, a physiological disruption occurs in plant growth, panicle emergence and grain formation resulting in reduction of kernel number and mass [1].

The spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), is far by the most serious pest causing yield losses from 26.7–80.4% in different agro climatic regions of the country. There is no permanent solution to control this pest except chemical control. However, bio control agents such as parasitoids, predators and pathogens suppressed the population outgrowth of *Chilo partellus* but their activity was not enough to reduce the pest populations below the economic damage level. Effective mass-rearing technology on artificial diet is being used at several institutions [10]. In India, many of the researchers prepared methyl para bean instead of linoleic acid for *Chilo partellus* development *in vitro* conditions [11]. The diet optimization was very low in laboratory conditions. Researchers have improved the quality of diet by changing its ingredients [10]. In our research the diet was optimized with green gram (*Phaseolus vulgaris* L.), which reduced the developmental time and increased the number of larvae per generation.

In nature an insect locates a host plant through a sequence of behavioral and biological responses. among the Six main categories in insect behavioral and physiological responses of insects are considered to be important during insect establishment in plants: (i) Orientation and settling; (ii) feeding (iii) metabolism of ingested food (iv) growth (v) survival and fecundity and (vi) oviposition⁸. In the present study standardization of artificial diet during the growth of *Chilo partellus* larvae, behavioral responses and larval development of *Chilo partellus* in determining of resistance or susceptibility of different maize lines for pest studies in the field. We provide statistical data containing tables for a possible explanation for the apparent by *Chilo partellus*.

MATERIALS AND METHODS

Collection of larvae

The *Chilo partellus* was collected from infested maize fields, located on Prakasam district (AP), India. These larvae were arranged according to their size and age, and transferred into plastic containers provided with an artificial diet.

Research and development of rearing techniques

The first artificial diet used to rear *Chilo partellus* in laboratory, researchers used different chemicals like casein, glucose, salt mixture, yeast, choline chloride, cholesterol, cellulose, leaf factor, agar, methyl para bean and water [12,3] reared *Chilo partellus* on wheat gram based diet which was earlier used by keaster and harrendorf (1965) for rearing *Zeadiatraea grandiosella*. The major breakthrough in mass rearing of *Chilo partellus* came with the use of kabuli gram based diet [5]. The diet had fewer and more rapidly available ingredients. Most of the diets employed in India were addition or deletion of ingredients in diet, list of ingredients used in standardizing diet were shown in Table 1

Table 1: List of various ingredients employed by researchers for rearing *Chilo partellus*- In vitro

| S.No | Ingredients | Dang et al., (1970) | Lakshmi narayana and Soto (1971) | Moorty (1973) | Siddiqui and Chateerji (1972) | Siddiqui et al., (1977) | Sharma and Sarup (1978) | Seshu Reddy and Davies (1979) | Taneja and Leuschner (1985) | Balaji et al., 2013 |
|------|-----------------------------------|---------------------|----------------------------------|---------------|-------------------------------|-------------------------|-------------------------|-------------------------------|-----------------------------|---------------------|
| 1 | Agar (g) | 51 | 25 | 20 | 5.1 | 5.1 | 6 | 51 | 40.8 | 12.6 |
| 2 | Ascorbic acid (g) | 13 | 4.3 | 4 | 1.3 | 1.3 | 1.5 | 13 | 10.4 | 2.5 |
| 3 | Formoldehyde (ml) | 8 | 2.7 | 2.6 | 1 | 1 | 1 | 10 | 3.2 | 2 |
| 4 | Methyl para hydroxy benzoate (g) | 8 | 2.7 | 2.6 | 0.8 | 0.8 | 0.9 | 8 | 64 | 2.0 |
| 5 | sorbic acid (g) | 4 | 1.5 | 1 | 0.4 | 0.4 | 0.5 | 5 | 4 | 1.3 |
| 6 | yeast (g) | 40 | 13.3 | 13.2 | 4 | 4 | 5 | 40 | 32 | 22.7 |
| 7 | water (ml) | 3120 | 1000 | 1000 | 380 | 380 | 390 | 4500 | 3600 | 400 |
| 8 | sorghum leaf powder (g) | - | 40 | 40 | - | - | - | 200 | 160 | - |
| 9 | sucrose (g) | - | 60 | 20 | - | - | - | - | - | 35.3 |
| 10 | Vitamin Fortification mixture (g) | - | 5 | 1 | - | - | - | - | - | - |
| 11 | Vitamin E (g) | - | - | - | 0.1 | 0.1 | 0.1 | 5.2 | 4.6 | 2.1 |
| 12 | Wheat flour (g) | - | - | - | 20 | 20 | 20 | - | - | - |
| 13 | pulses kabuli gram (g) | 420 | 100 | 100 | - | - | - | - | 438.4 | - |
| 14 | Rajmah | - | - | - | 74.8 | - | - | 548 | - | - |
| 15 | Green gram/ Dew gram cow pea | - | - | - | - | 75 | - | - | - | - |
| 16 | Lentil | - | - | - | - | - | 75 | - | - | - |
| 17 | Casein | - | - | - | - | - | - | - | - | 25.2 |
| 18 | Maize powder | - | - | - | - | - | - | - | - | 22.7 |

Procedure for preparation of artificial diet

Bean powder act as a natural effect of diet, casein and yeast were rich of protein source, vitamin E in pupa formation, methyl para hydroxy benzoate in development of pupa to moth, sucrose gives to the carbon source, Ascorbic acid and formaldehyde are preservatives and agar is solidifying agent for diet. This is the cheapest and best diet for *Chilo partellus* compare to the previous diets. Diet was prepared in 3 fractions (A, B, and C), first add fraction A components were heated and mixed on magnetic stirrer than add fraction B finally cool the diet and add fraction C and mix the diet, distribute in to the diet boxes.

Laboratory Bio Assay

Larvae raring from eggs

The sterilized dark spotted egg masses were kept in to the 7.8cm length and 6.7cm width of plastic jars filled with 20ml of the artificial diet. The flasks were closed with cotton wool and kept in an incubator at 26°C and 60±10% RH. When the egg hatching was started the cotton wool stopper was removed.

Larvae

The matured larvae were transferred into plastic jars containing 20ml of the artificial diet shown in figure: 1. These plastic jars were transferred into the rearing chamber, maintained at a temperature of 26°C and 60±10% RH, with a photoperiod of 16 hr light and 8 hr darkness (L: D 16:8) for *Chilo partellus* until emergence of pupation and adult formation. Fresh diet was replaced as and when required.



Figure 1: *Chilo partellus* larvae were reared on an artificial diet

Collection of pupa

Pupae were carefully transferred into sterile glass jars. Each jar is maintained with one male and one female separately until they get changed into adult moths.

Collection of moths

Adult moths (male and female) were carefully transferred into smooth-walled glass jars facilitating the moths to lay eggs. A ball of cotton soaked in distilled water was introduced into cages as a source of nourishment.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 15). Statistical significance between treatments was evaluated at the 5% probability level. General linear model (GLM) ANOVA was used for further analysis of data. Values were expressed as means ± S.E.M.

RESULTS AND DISCUSSION

Larvae rearing from eggs

Chilo partellus completes its life cycle in the following five stages: eggs, early instar larvae, late instar larvae, pupae, and moths. The *Chilo partellus* larvae appear in purplish-pink color on the dorsal side and white color on the ventral side. They have a black or dark brown head and appear off-white or gray, with rows of black dots on the body. Adult larvae remain stout and smooth with 15-25 mm in size; male larvae were observed to be 1.44 ± 0.03 cm and female larvae in 1.70 ± 0.05 cm in length. Mortality rate of larvae reared in controlled conditions usually remains less in number. Artificial diet described by Koul et al., (1997) and Balaji et al., (2013) showed 60% larval survival rate, but our results show a 90% survival rate, which is promising at a low cost. *Chilo partellus* larval stage gets completed in 4-5 weeks on artificial diet. The young larva consumes artificial diet until the formation of pupa. The fresh diet was replenished in the containers as and when required, until pupation.

Pupa

The pupae were cylindrical in shape and dark brown in colour (figure: 2), average weight was $200 \pm 1.52\text{mg}$. Pupa period takes 8-10 days. The size of female pupa was slightly larger than that of the male pupa. Female pupa was larger ($1.6 \pm 0.05\text{cm}$) than that of male pupa ($1.21 \pm 0.02\text{cm}$).

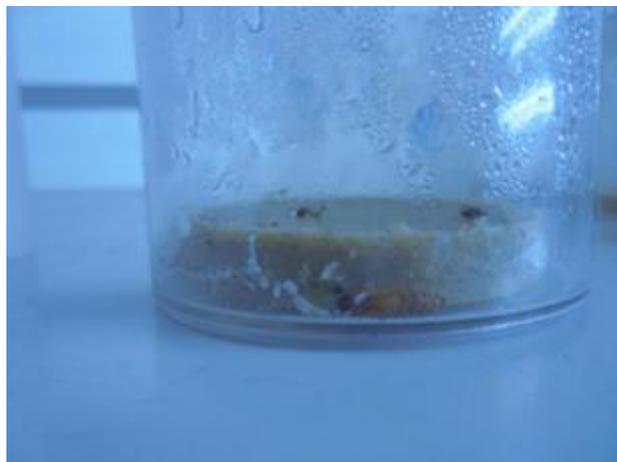


Figure 2: Pupa formations after feeding an artificial diet in laboratory condition

Moth

The moths appear in pale brown in color (figure:3), the male has pale brown forewings with dark brown scale forming a line on the tips of the wings, the hind wings were pale yellow the female was much lighter than the male. The male moth was much darker and appears olive brown with less distinct lines on the wings. The average length of male moth has $1.36 \pm 0.05\text{cm}$ and female moth length was $1.70 \pm 0.03\text{cm}$. The adult male lived for 3 to 7 days and and female for 3-8 days with a mean of 6.30 ± 0.85 and 5.10 ± 0.69 days, respectively. *Chilo partellus* mean fecundity was recorded to be 547 eggs, which are reasonably more than 434 as reported [2].



Figure 3: *Chilo partellus* moths a) ventral side b) dorsal side

Production of *Chilo partellus* in mass culture is very difficult procedure because there is no slandered diet, many scientists' proposed different diets with less reproducibility. In our research above cited composition (table 2) with different chemical composition and green gram powder showed good results with reproducibility. The *Chilo partellus* larvae life span was previously reported 50-60 days [19]. In our results it showed 47 – 51 days. To compare with previous data the life span of *chilo partellus* was decreased by one week.

Table 2: Composition of Ingredients employed in standardizing artificial diet

| S.No | Ingredients | Quantity |
|-------------------|-----------------------------|----------|
| Fraction A | | |
| 1 | D. H ₂ O | 100 ml |
| 2 | Bean powder | 13.6gms |
| 3 | Casein | 1gms |
| 4 | Carboxyl methyl cellulose | 0.25gms |
| 5 | Methyl-p- hydroxyl benzoate | 0.25gms |
| 6 | Yeast | 2.8gms |
| 7 | Sucrose | 4.37gms |
| 8 | Ascorbic acid | 0.16gms |
| 9 | Vitamin E | 0.31gms |
| Fraction B | | |
| 10 | Agar | 1.5gms |
| Fraction C | | |
| 11 | Formaldehyde | 0.25ml |

CONCLUSION

The above cited results and discussion indicated that rearing conditions of *Chilo partellus* In vitro, many scientists employed different diets in different research centers but they did not get satisfactory results at lowest cost. In our research the diet was standardized by using different chemicals along with bean powder for low cost. In this diet the culture growth was apparently fast. Conditions on artificial diet, the affecting factors of laboratory conditions, diet maintenance, larval survival, and growth parameters were observed. The diet infestation has been a major problem in an artificial diet, because larvae of the *Chilo partellus* had to be reared singly in plastic container. However, various aspects of these techniques have been modified to suit our conditions, and also to make the rearing process more efficient. Fecundity and life history of the artificially reared *Chilo partellus* were similar those of the wild colonies. Cheaper alternatives for the expensive items in the artificial diet must be sought. The time taken to rear adequate *Chilo partellus* was reported to be a function of time.

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