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Biology studies on screening of suitable diet for fall armyworm, *Spodoptera frugiperda* (J. E. Smith)

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Abstract
The present investigations on the effects of different foods on the biological parameters of *Spodoptera frugiperda* (J. E. Smith) were studied in laboratory. The highest larval and pupal weights were observed on D2 (Armes diet) and it was significantly superior over other two diets. The larval and pupal periods were found significantly lowest on D2 (Armes diet) followed by D3 (Cowpea + Corn diet) and both the artificial diets were found significantly superior on D1 (maize leaves). Significantly highest fecundity and egg hatchability of *S. frugiperda* were recorded on D1 (maize leaves). In this respect treatment D2 was found superior over D3. Significantly highest male longevity was recorded on D1 (maize leaves). It was followed by D2 (Armes diet) and both these treatments were found statistically superior over D3. The effects of various diets on female longevity, however found statistically non significant.

Keywords: Biology, screening, *Spodoptera frugiperda*, artificial diet, natural diet

Introduction
Maize (*Zea mays* L.) belongs to family Poaceae is one of the most important cereal crop of the world and contributes to food security in most of the developing countries. In India maize is emerging as third most important crop after rice and wheat. Its importance lies in the fact that it is not only used as human food and animal feed but at the same time it is also widely used in corn starch industry, corn oil production and as baby corn in different recipes (Singh, 2014) [13]. It contains various major phytochemicals such as carotenoids, phenolic compounds and phyto sterols which are useful in the prevention of some chronic diseases. It is believed to have potential anti HIV activity due to the presence of *Galanthus nivalis* agglutinin (GNA) lectin or GNA maize (Shah *et al.*, 2016) [14]. The production of maize in 2017-18 was 20,118 MT in *Kharif* and 8,634 MT in *Rabi* with a total production of 28,753 MT in India. After the entry of fall armyworm, the production is reduced to 19,410 MT in *Kharif* and 8300 MT in *Rabi* with a total of 27,720 MT in 2018-19 (Anonymous, 2019b) [5]. Amongst several insect pests damaging this crop, the one fall armyworm, *Spodoptera frugiperda* (J. E. Smith) is an occasional but sometimes serious pest observed on this crop. This pest commonly referred to as FAW is a native of America. However, it was first formally reported in West Africa in January, 2016 and has spread to several countries across Africa except a few countries in North Africa (Padhee and Prasanna, 2019) [12]. In India, the pest has been reported for the first time in India in Karnataka in July 2018 and subsequently in a few other states such as Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra and Odisha. Based on results of surveys conducted between 9-18 July 2018 that recorded more than 70 % prevalence of the FAW in a maize field in Chikkaballapur, Karnataka (Annonymus, 2018b) [3].
In India, *Rabi* maize was sown in around 15.56 lakh ha as of 22nd February, 2019 which was lower than 17.28 lakh ha during corresponding period last year. All India *Rabi* maize production is estimated by at 5.67 MMT for the year 2018-19 (Anonymous, 2019a) [4]. The fall armyworm damage has been the highest in maize while several other crops such as sorghum, sugarcane, millets, vegetables and cotton (Padhee and Prasanna, 2019) [12] are also vulnerable to the attack. Maize crop is subjected to attack by over 130 insect pests during different growth stages of crop. The FAW moth populations are capable of migrating very fast (almost 100 km per night and nearly 500 km before laying eggs) and thus, can invade new areas quickly (Johnson, 1987) [6]. The pest completes its life cycle in about 30-45 days (depending on weather conditions). In cooler temperatures the life cycle may extend up to 60-90 days. The female moth lays on an average about 1500 eggs attaching them to the foliage. The egg stage lasts for only 2 to 3 days in warmer weather. The FAW in general has six larval instars (stages) before it goes for pupation.

The entire larval stage lasts for 14 to 30 days depending on the weather conditions especially temperature and humidity (Padhee and Prasanna, 2019) [12]. Keeping in view the above facts, the study on "Biology studies on screening of suitable diet for *S. frugiperda* (J. E. Smith)" was carried out under Laboratory condition. In the present studies different diets were screened against this pest, which may be used to mass rearing of this pest in near future.

Materials and Methods

The materials used and methodology followed in the present investigations are described under respective subheads.

Materials Used

Test insect used: The first instar larvae of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) were used for biology studies.

Plastic and glass wares used: 1. Glass petriplates (50 ml), 2. Glass beljars (15 cm diameter and 25 cm height) for adult mating and oviposition, 3. Measuring cylinder, 4. Glass beakers (250 ml), 5. Plastic jars (16 cm diameter and 18 cm height) as incubation chamber, 6. Conical flasks (250 ml), 7. Plastic containers (25 cm diameter and 10 cm height) with rubber band for larval rearing, 8. Plastic boxes (10 cm height X 16 cm width X 25 cm length), 9. Glass test tubes (25 ml) for relative toxicity studies.

Chemicals used: 1. Sodium hypochlorite solution (0.2 %) in water for disinfection of tools and table tops, 2. Formaldehyde solution (2 %) for disinfection of insect rearing room, 3. Alcohol (70 %) solution in water for disinfection of scissors, brushes and washing of hands, etc.

Equipment's used: 1. Hot air oven (Lab Hosp. Make), 2. Refrigerator (Make Godrej, 170 Lit.), 3. Stereozoom Microscope (Make Nilcon), 4. Digital balance (Contach make, 500 g capacity) 5. Accupippettee (Cap. 1 to 1000 µl), 6. BOD incubator (Make - Sanco), 7. Air conditioner (Make Hitachi), etc.

Other material and accessories used: 1. Glass marking pens, 2. Sterile distilled water, 3. Muslin cloth, 4. Forceps, 5. Cotton thread, 6. Hand lens (10 x), 7. Absorbent cotton, 8. Needle, 9. Scissor, 10. Tissue paper, 11. Paper towel, 12. Black paper sheets, 13. Honey (Dabur), 14. Camel hair brush, 15. Fresh maize leaves as larval food, etc.

Methods adopted

Growing of food plants for fall armyworm, *S. frugiperda* (J. E. Smith): Sowing of maize (local variety) in isolation was under taken in the field of Department of Agricultural Entomology by following recommended tillage practices. In order to ensure continuous and fresh availability of food throughout the experimental period, staggered sowing of maize seeds was followed. Since the crop is served as food source for laboratory reared population of *S. frugiperda,* every precaution was taken to keep the plot free from insecticide contamination and possible drifts from neighboring plots. The fresh tender leaves of maize from this plot were used for feeding *S. frugiperda* larvae in the laboratory.

Mass rearing of *S. frugiperda* in laboratory: For mass rearing of fall armyworm, *S. frugiperda* method described by Sisay *et al.* (2019) [16] was followed with slight modifications with respect to rearing materials and food. Initially, FAW starter colony was collected from an unsprayed maize field in the university campus and brought to the laboratory. In order to avoid cannibalism the larvae were placed individually in ventilated plastic jars and fed with fresh tender maize leaves from the food plot grown in the field. The full grown larvae were transferred to a plastic jar one third filled with soil and were allowed to undergo pupation. The pupae were collected and placed in a moistened petri dish in an oviposition cage. Sterile cotton soaked in a honey based adult diet solution was placed in a petri dish inside the oviposition cage as a food source for the emerging adults. The walls of the cages were lined with wax paper as an oviposition substrate. A photoperiod of 12L:12D was maintained for oviposition. After 2-3 days, old egg batches were collected from the oviposition cages and placed in sterile plastic jars. Eggs were monitored daily for hatching. The neonates were used for conducting biology studies on different foods, while the third instar larvae from this laboratory culture were used for bioassay experiments. The rearing was performed at ambient temperature and RH conditions in laboratory.

Biology studies on screening of suitable diet for *S. frugiperda* (J. E. Smith): Use of a suitable diet for test insect is a prerequisite for toxicological studies. In order to know most suitable diet for growth and development of *S. frugiperda* the effects of different foods on the biological parameters of *S. frugiperda* were studied in laboratory. The studies were conducted using maize leaves (natural diet) an artificial diet prescribed by Armes *et al.* (1992) [2] and a new cowpea + corn floor based diet. The ingredients used for manufacturing of two artificial diets are listed in Table 1. For preparation of the artificial diets procedure prescribed by Armes *et al.* (1992) [2] was followed. The fresh diets were stored in refrigerator between 8º to 12ºC temperature and used periodically for feeding the larvae during the experiment. A standard larval rearing method was followed as described earlier under 'Mass rearing of *S. frugiperda* in laboratory. The neonates of *S. frugiperda* were reared on three foods in separate containers and periodically fed with sufficient quantity of food until pupation. The adults on each food were paired and allowed to mate in cages. They were provided with honey based diet to ensure proper nourishment and oviposition. Observations on various biological parameters like larval period, larval weight, pupal weight, pupal period, adult fecundity, egg hatchability and adult longevity were recorded on various foods. The data were analyzed under Completely Randomized Design (CRD) to draw the inferences.

Results and Discussion

Use of a suitable diet for test insect is a prerequisite for toxicological studies. In order to know most suitable diet for growth and development of *S. frugiperda* studies were conducted in laboratory to know on different foods to know their effects on biological parameters of *S. frugiperda.* The results are presented in following tables and discussed in light of available literature.

Effect of different diets on larvo-pupal growth and development of *S. frugiperda* (J. E. Smith): The results from Table 3 revealed that the highest larval weight (435.63 mg) and pupal weight (271.15 mg) were observed on D2 and

it was significantly superior over other two diets. However, larval and pupal weights on D2 were significantly superior over D1 indicating more feeding by *S. frugiperda* on this diet than on the natural diet i.e. maize leaves. The larval period (14.38 days) and pupal period (7.28 days) were found significantly lowest on D2 followed by D3 and both the artificial diets were found significantly superior on D1 (i.e. maize leaves). The enhanced phagostimulation and body weight gain by *S. frugiperda* larvae on artificial diet was evidenced from the earlier studies reported by Pantoja *et al*. (1987) [11], Ali *et al*. (1990) [1] and Pinto *et al*. (2019) [10]. Kulkarni and Gawande (1999) [7] recorded higher growth index values in *Helicoverpa armigera* (Hub.) on natural foods compared to laboratory prepared artificial diets. These findings support the outcome of the present studies. Ali *et al*. (1990) [1] observed that the rate of larval and pupal development in *S. frugiperda* is influenced by temperature ranges in addition to the type of diet provided in rearing. The differences in the larvo-pupal developments in *S. frugiperda* in the present investigations may be attributed to this fact.

Effect of different diets on fecundity and egg hatchability of *S. frugiperda* (J. E. Smith): The results presented in Table 3 revealed that the significantly highest fecundity (1084.87 eggs / female) and egg hatchability (96.55 %) of *S. frugiperda* were recorded on D1 (maize leaves). In this respect treatment D2 was found superior over D3. Superiority of natural food in enhancing the fecundity and egg hatchability of insects was evidenced from the earlier woks of Pantoja *et al*. (1987) [11], Kulkarni *et al*. (2004) [8] and Lekha *et al*. (2020) [9] supporting the findings in the present studies. The high fecundity of females in the present study in laboratory is supported by the findings of Sharanabasappa *et al.* (2018) [15] who found that females of *S. frugiperda* lay more number of eggs in captivity as compared to open field conditions. Pinto *et al*. (2019) [10] observed no oviposition in females of *S. frugiperda* developed from larvae fed on artificial diet (D2) even though larval and pupal development on this diet was normal. This signifies that imbalanced nourishment in immature stages of insect is a major cause for infertility problems of adults. Pantoja *et al*. (1987) [11] reported slow growth of fall armyworms, *S. frugiperda* on rice foliage as compared to artificial diet producing lighter adult females. However, this difference did not extend to reproductive parameters and adult longevity. These findings support the observations recorded on adult longevities in the present studies.

Effect of different diets on male and female longevity of *S. frugiperda* (J. E. Smith): The results presented in Table 3 revealed that the significantly highest male longevity (8.16 days) was recorded on D1 (maize leaves). It was followed by D2 (7.74 days) and both these treatments were found statistically superior over D3. The effects of various diets on female longevity, however found statistically non significant. Lekha *et al*. (2020) [9] recorded variation in adult longevity of *S. frugiperda* developed as larvae on various diets. However, in contradictory to the findings of present investigation the variation observed were considerable and found in the range of 4.50 - 8.00 days for males and between 7.00 - 10.33 days for females. The female longevity is generally co-related with fecundity. However, this general perception found deviating in the present studies. Pinto *et al*. (2019) [10] concluded that there was no difference among the diets in relation to the fertility life table parameters of *S. frugiperda* reared under controlled conditions which supports the findings in the present studies.

Comparative biology of *Spodoptera frugiperda* (J. E. Smith) on different diets: The results of the studies on comparative biology of *S. frugiperda* on different diets (Table 3) revealed that maximum larval and pupal weights, minimum larval and pupal periods were recorded on D2 (diet prescribed by Armes *et al*. 1992) [2], While all the fertility related parameters like maximum fecundity, egg hatchability, male longevity and female longevity were observed on D1 (maize leaves). It is evidenced that the laboratory prepared modified (Cowpea + maize) diet (D3) was quite supporting the growth and development of *S. frugiperda.* However, for mass rearing of *S. frugiperda* generations in laboratory use of natural diet i.e. maize leaves may be more useful.

Table 1: Composition of artificial diet ingradients used for *S. frugiperda* (J. E. Smith)

Sr. No.	Ingredients	Quantity used for preparation of 1 L diet	
		Artificial diet by Armes *et al.* (1992) [2]	Modified artificial diet
1.	Chickpea flour	300 g	60 g
2.	Cowpea flour	--	120.0 g
3.	Corn flour	--	120.0 g
4.	Yeast	48 g	72.0 g
5.	Sorbic acid	1.5 g	2.4 g
6.	Methyl paraben	--	4.4 g
7.	Ascorbic acid	4.7 g	7.3 g
8.	Becadexamin multivitamin multimineral capsule	--	200 mg
9.	Vitamin mixture (Table 2)	10 ml	--
10.	Formaldehyde	--	6.0 ml
11.	Linseed oil (refined)	12 ml	--
12.	Aureomycin (as water soluble powder containing 5.5% chlortetracycline hydrochloride)	0.4 g a.i.	--
13.	Methyl-4-hydroxybenzoate (Nipagin M)	3.0 g	--
14.	90-95% ethanol	3.0 ml	--
15.	Agar agar	17.25 g	20.0 g
16.	Distilled water	400 ml	400 ml
17.	Distilled water (for yeast/agar)	700 ml	400 ml

Table 2: Vitamin mixture for artificial diet (Armes *et al.*, 1992) [2]

Sr. No.	Ingredients	Quantity
1	Nicotinic acid (Niacin)	1.53 g
2	Calcium pantothenate (pantothenic acid calcium salt) B_3	1.53 g
3	Riboflavin B_2	0.76 g
4	Thiamine hydrochloride (aneurine hydrochloride) B_1	0.38 g
5	Pyridoxine hydrochloride B_6	0.38 g
6	Folic acid BC	0.38 g
7	D-biotin H	0.31 g

Table 3: Comparative biology of *S. frugiperda* (J. E. Smith) on different diets

Diet used	Biological parameters							
	Larval weight (mg)	Larval period (days)	Pupal weight (mg)	Pupal period (days)	Fecundity (eggs/ female)	Egg hatching (%)	Male longevity (days)	Female longevity (days)
D1 - Maize leaves (Natural diet)	355.78	15.57	200.10	8.43	1084.87	96.55	8.16	10.52
D2 - Artificial diet by Armes *et al.* (1992) [2]	435.63	14.38	271.15	7.28	1072.13	95.42	7.74	10.30
D3 - Modified artificial Diet	393.9	14.92	229.42	7.87	1009.29	93.52	7.16	10.43
'F' Test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Non- Sig.
SE m (±)	2.82	0.12	5.45	0.07	3.26	0.54	0.1	0.18
C. D. (5%)	8.19	0.34	15.81	0.19	9.46	1.56	0.28	0.51
C. V. (%)	2.26	2.5	7.38	2.66	0.98	1.79	4.0	5.32

Conclusions

The results of the studies on comparative biology of *S. frugiperda* on different diets revealed that maximum larval and pupal weights, minimum larval and pupal periods were recorded on D2 (diet prescribed by Armes *et al*. 1992) [2]. While, all the fertility related parameters like maximum fecundity, egg hatchability, male longevity and female longevity were observed on D1 (maize leaves). It is evidenced that the laboratory prepared modified (cowpea + maize) diet (D3) was quite supporting the growth and development of *S. frugiperda*. However, for mass rearing of *S. frugiperda* generations in laboratory use of natural diet i.e. maize leaves may be more useful. Based on the results of the present investigations it can be concluded that artificial diets D2 (Chickpea flour based proposed by Armes *et al.* 1992) [2] and D3 (Cowpea + Corn flour based) can be used for larval feeding of *S. frugiperda* in laboratory. For sustainability in growth and fertility *S. frugiperda* should be reared on the natural food (maize leaves).

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