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Research Article

Laboratory Rearing of Herbivore Larva of *Spodoptera Frugiperda* (*Order: Lepidoptera*) to Assess Yield Loss of Economically Important Crops

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Abstract

Lepidopteran larvae are one of the most destructive pests in agricultural ecosystems. To assess the yield loss caused by lepidopteran larvae, *Spodoptera frugiperda* was reared in the laboratory and daily food consumption, total feed intake, pupal weight and percentage of pupal emergence were calculated. The larvae of S. frugiperda were provided with maize and brinjal as a diet. Per day food consumption was calculated by subtracting remaining food and evaporative loss from total provided food. The entire experiment was conducted at 25°C ± 5°C temperature, 60 ± 5 relative humidity and 16: 8 light and dark period. Larvae of S. frugiperda consumed 16.1 ± 2.50 g of maize and 8.85 ± 1.73g of brinjal. 0.61 ± 0.01g was maximum pupal weight of S. frugiperda on maize while on brinjal pupal weight was 0.32 ±0.03g. Food consumption by each instar was also calculated. The 6th instar stage was most damage causing stage. The rate of adult emergence was highest in maize (89%). Adults of S. frugiperda attained longevity of 14 days on maize and 9 days on brinjal. This life table study could be valuable in assessing the damage caused by the disastrous lepidopteran pest. The results could help to formulate pest management strategies for sustainable crop production.

Keywords: Yield loss, food consumption, pupal weight, herbivorous larva, life table analysis.

1. Introduction

Massive crop cultivation not only fulfills human food requirements but also provides favorable habitats for plant eating insects. But in this manner, there are more chances for plants to get infested by pests. Phytophagous insects cause loss equivalent to one-fifth per annum crop production worldwide [1]. Due to climate change and global trade many insect pests have emerged that are major threats to crop production both qualitatively and quantitatively [2, 3]. Among these pest species of insects many species of Order Lepidoptera are key pests of agroecosystem that require immediate control to protect crops from significant loss [4].

Lepidoptera, refers to caterpillars, which are one of the most damage-causing insects of agriculture [5]. Lepidopterans manifest a permutated role in cropland because they are beneficial as they help in pollination and meanwhile, they are serious pests of crop. Their larvae feed on leaves because they have chewing mouth parts and, at adult age they bore into stems of plants. Due to this unique feature they are economically important pests of agriculture, fruits, and agro-forestry [6].

Out of 1.4 million species 53% are insect's species on earth and Lepidoptera is the 2nd largest order of insects that comprises butterflies, moths, and skippers [7, 8]. This order is further classifying into 46 super families and 126 families. Approximately 180,000 insect's species have been identified from order Lepidoptera [9, 10]. Out of these more than 28,000

species are butterflies and 80% of these butterflies are distributed in tropical areas of the world. In Pakistan there are more than 5000 species of insects of which 400 species are butterflies and moths [11, 12].

Caterpillars and butterflies relied on specific plants for pollens, nectar, and foliage. Regions with high plant varieties and vegetation diversity accommodate large numbers of butterflies and moths' communities. So, the diversity of lepidopterans greatly depends upon the diversity of plants [13].

Areas with high plantation density ensure the presence of butterflies. These are host specific and depend upon specific plants for sucking nectar, pollen, or foliage. As different crops are being cultivated throughout the year, so this continual availability of auxiliary host plants is a food source for the development and growth of lepidopterans. Different host plants are being eaten by polyphytophagous insects during successive generations and in this way, they become resistant to applied chemicals [13].

The feeding habits of early instars and late instars of lepidopterans are markedly different. Consumption of food, metabolic rate, growth rate and utilization efficiency of early instars are higher than instars of later stages [14]. Feeding behavior greatly depends upon types of mouthparts. Larvae of Lepidoptera possess chewing type of mouth parts and they can eat almost every part of a plant [15].

Spodoptera frugiperda is also a herbivorous lepidopteran pest that is cosmopolitan in distribution [16, 17, 18]. Its larval stages damage the economically important crops from June to August. Larvae feed on leaves and stems of more than 100 plant species, including maize, millet, sugarcane, wheat, chickpea, soybean, rice, sorghum and many vegetables [19].

S. frugiperda commonly known as fall armyworm (FAW), causes substantial yield loss to important food crops, including maize, rice, sorghum and vegetables. The larvae consume the stems, reproductive parts of plants and leaves and have damaged more than 350 species of plants [20, 21]. It has been estimated that in sub-Saharan Africa this pest has caused a loss of over $31 billion in sugarcane, sorghum and maize crops [21]. The first outbreak of this pest originated in 2016 in Africa, and later invaded different territories worldwide, such as parts of East, West and South Asia, Oceania, southern Australia and Europe [22].

A most common method to control these phytophagous pests is the application of pesticides. However the abusive and massive application of pesticides causes many troublesome issues like killing the non-targeting insect species, human health issues, environmental pollution and resurgence and resistance of pest species [23]. To combat these issues, a comprehensive knowledge of feeding behavior, ecological requirements and life cycle analysis proved to be the chief pillar in framing in an eco-friendly pest control strategies. [24]. The present study focused on determining the feeding behavior and life cycle of *S. frugiperda* for designing efficient pest control strategies.

2. Methodology

2.1. Collection and handling of Spodoptera frugiperda

Eggs and 1st instar stage of *S. frugiperda* were collected from croplands, including brinjal, maize and fodder crops of District Sialkot. In the field eggs of Lepidoptera were identified by shape, coloration and position on leaves [25]. Some eggs and 1st instar larvae along with their host plant leaves were brought in the Zoological research laboratory of Government College Women University Sialkot in a well aeriated container and were separated in insect rearing box. The insect rearing boxes were made up of transparent plastic having two moveable lids for opening the boxes. Rearing boxes were equipped with two windows covered with very small sized net for proper aeriation. The size of rearing cage was 15× 15 × 9 cm.

2.2. Determination of feeding efficacy of *S. frugiperda*

In single cage 4 larvae were kept with three replicates. Total of 12 larvae were reared for each experiment. Larvae were provided with pre weighed fresh Brinjal (Solanum melongena) and maize (Zea mays L.) leaves. In another box same pre

weighed diet was kept without larvae to calculate evaporative loss. After 24 hours boxes were cleaned, excreta of larvae was removed and fresh pre weighed food was provided to larvae. The same practice was repeated until larvae became pupae and stopped feeding. Food consumption by larvae was calculated by subtracting evaporation loss and weight of remaining food from initial diet method described by [26]. After somedays, 8 days in case of maize and 11 days in case of brinjal, when pupae hatched into adults, adults were shifted to separate boxes relatively of smaller measurement. They were provided with 20% honey solution and adult lifespan was observed. The entire experiment was conducted at 25°C ± 5°C temperature, 60 ± 5 relative humidity and 16: 8 light and dark period.

2.3. Statistical analysis

As development of adult and pupa is directly dependent upon per day food consumption and total food consumption by larva. So, the significance between per day food consumption and total food consumption was analyzed by one-way Analysis of Variance (ANOVA) by keeping the level of significance at 0.05. ANOVA was applied by using SPSS version 21.0.

3. Results

3.1. Life Table of Spodoptera frugiperda

Total food consumption: Larvae of Spodoptera frugiperda were collected from maize. In Laboratory conditions it was given maize as it was its host diet and brinjal was to check the damage extent on these crops. Table 1 indicates the total consumption and per day food consumption on these two diets by *S. frugiperda*.

Table 1. Life table and feeding efficiency of Spodoptera frugiperda on two selected host plants (maize and brinjal).

Days	Maize (grams)	Brinjal (grams)
1	0.23 ± 0.03	0.17 ± 0.35
2	0.48 ± 0.15	0.39 ± 0.20
3	0.59 ± 0.04	0.61 ± 0.03
4	0.84 ± 0.5	0.71 ± 0.06
5	1.18 ± 0.5	0.94 ± 0.20
6	1.49 ± 0.4	1.05 ± 0.04
7	1.74 ± 0.07	1. 37 ± 0.21
8	1.79 ± 0.03	1.55 ± 0.05
9	1.82 ± 0.20	1.01 ± 0.06
10	1.91 ± 0.40	0.84 ± 0.07
11	1.95 ± 0.03	0.12 ± 0.46
12	1.23 ± 0.07	_
13	0.51 ± 0.06	_
14	0.34 ± 0.02	_
Total food consumption (grams)	16.1 ± 2.50	8.85 ± 1.73
Per day consumption (grams)	1.15 ± 0.17	0.80 ± 0.15
Pupal weight (grams)	0.61 ± 0.01	0.32 ±0.03
% of adult hatching	89	67
Adult life duration (days)	14	9

Note: mean ± SD: mean value of all three replicates and their standard deviation to show dispersion of values from their mean value

Table 2. One-way Analysis of Variance (ANOVA) applied on per day food consumption and total food consumption to analyze the level of significance.

		Sum of Squares	df	Mean Square	F	Sig.
Per day food consumption	Between Groups	1.924	1	1.924	5.621	.025
	Within Groups	8.899	26	.342		
	Total	10.823	27			
Total food consumption	Between Groups	367.938	1	367.938	7.38E+31	0.000
	Within Groups	.000	26	.000		
	Total	367.938	27			

Note: df: Degree of freedom, F: ratio of the mean square for the between groups divided by the mean square within groups, sig.: significant value

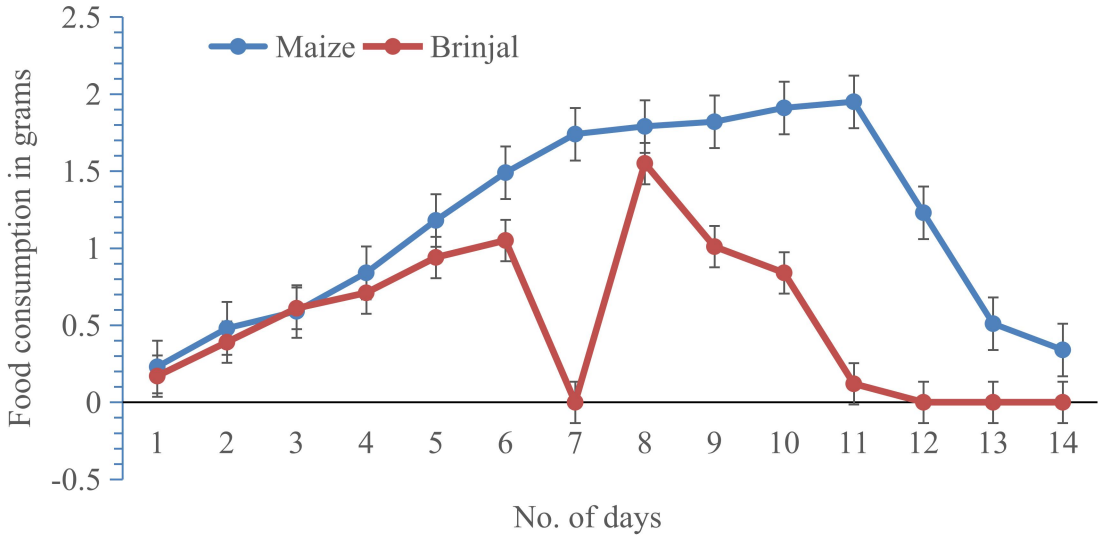

Figure 1. Food consumption (grams) by *S. frugiperda* larvae on Maize and Brinjal.

From 1st instar to 6th instar larval duration on maize was 14 days and while on brinjal it was 11 days. Maximum food consumption was recorded on maize i.e., 16.1 ± 2.50 and on brinjal total food that was consumed in 11 days was 8.85 ± 1.73. Statistical analysis of the results also exhibited significant results p-value= 0.000 indicating that larvae preferred maize crop (Table 2).

Per day consumption: Per day food consumption by *S. frugiperda* was calculated by taking an average of total food consumption. 1.15 ± 0.17 g food was per day consumed by larvae on maize and on brinjal per day consumption was 0.80 ± 0.15 (Figure 1). Significant results were found (p-value = 0.025) when analyzed by ANOVA (Table 2).

Maximum food consumption: On maize maximum food was consumed on 11th day and on brinjal maximum food was consumed on the 8th day. On 11th day maximum food consumption was 1.95 ± 0.03 on maize while on 8th day maximum food was consumed at 1.55 ± 0.05g on brinjal (Table 1).

Pupal weight: Pupal weight was associated with food consumption. The larvae that consumed more food have maximum pupal weight. As maximum food was consumed on maize, the highest pupal weight was also recorded on maize as well. On maize, pupal weight was 0.61 ± 0.01, and on brinjal, pupal weight was 0.32 ± 0.03 (Fig 2).

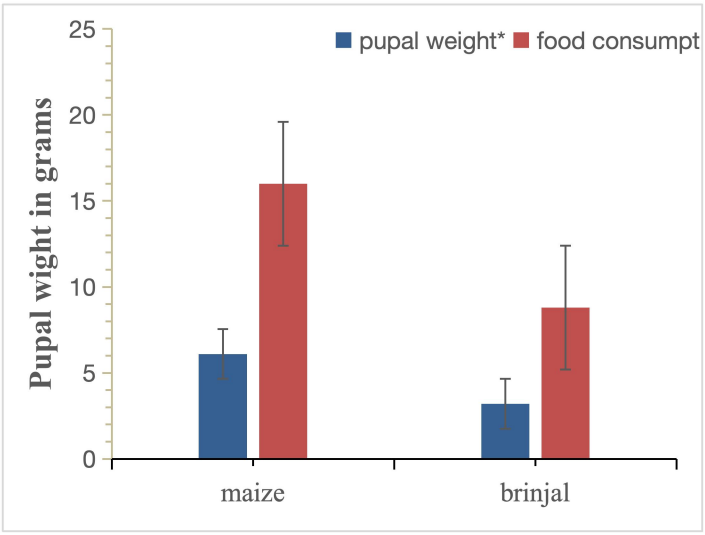

Figure 2. pupal weight and total food consumption of *S. frugiperda*. *pupal weight has been multiplied by 10 to make it visible on graph.

Table 3. Larval Instars duration and diet utility of *S. frugiperda* on maize and brinjal as host plants.

	Maize		Brinjal	
Instars	Duration (days)	Food consumption (g)	Duration (days)	Food consumption (g)
1st instar	2.00-3.00	0.71 ± 0.18	2.00 - 3.00	0.56 ± 0.55
2nd instar	2.00-2.50	1.43 ± 0.54	1.0 - 2.00	0.61 ± 0.03
3rd instar	2.00	2.67 ± 0.90	2.00 - 3.00	1.65 ± 0.26
4th instar	2.00-3.00	3.53 ± 0.10	2.00 - 2.50	2.42 ± 0.25
5th instar	1.50-2.50	3.73 ± 0.60	1.0 - 1.50	1.55 ± 0.05
6th instar	4.00-5.00	4.03 ± 0.18	3.00 - 4.00	1.97 ± 0.59
Pupal duration	8.00±1.00	-	11.00 ± 2.00	-

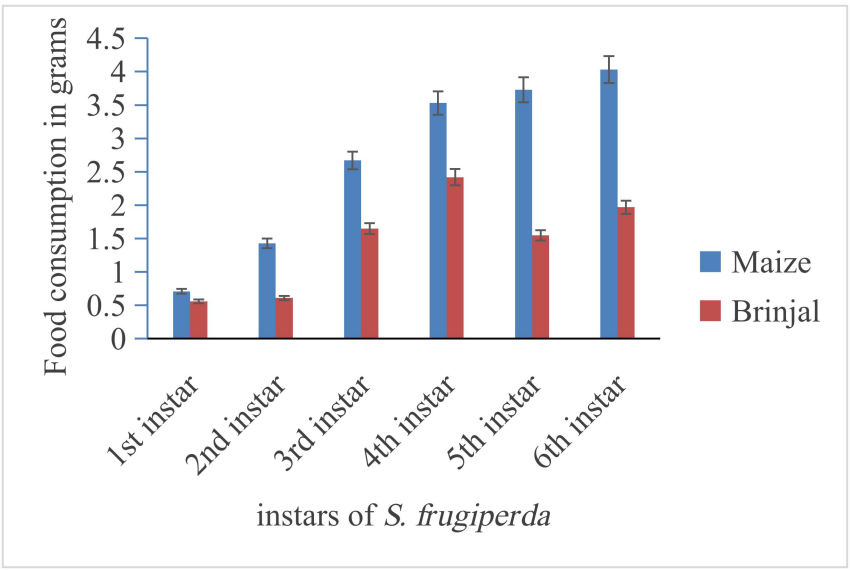

Figure 3. per instar food consumption on maize and brinjal by *S. frugiperda*

Percentage of adult hatching: Table 1 showed that the percentage of adult emergence was also correlated to food consumption and pupal weight. The percentage of pupal hatching was 89 on maize and 67 on brinjal.

Adult life span: The larvae that fed most food also show more life duration of their adults. As maize was consumed more as compared to brinjal, the life duration of maize-fed adults was also higher than that of adults whose larvae were fed on brinjal. 14 day life span was observed for maize feeding, and 9 days of adult life duration was on brinjal (table 1).

3.2. Determination of per instar duration and food consumption on two selected host plants

Larval duration: Larvae of *S. frugiperda* have 6 instar stages. The duration of each instar on both host plants has been listed in Table 3 During molting between instars, change in body length, breadth and color was observed. The longest instar stage was 6th instar, which lasted for 4.00 – 5.00 days on maize and 3.00 - 4.00 days on Brinjal.

Food consumption by each instar stage: From 1st instar to 6th instar food consumption in grams has been indicated in figure 3. During 1st and 2nd instar stages larvae did not feed as much. From 3rd to 5th instar stages food consumption gradually increases.

Pupal duration: During the end of 6th instar larvae stop feeding gradually and become pupa at the end of 6th instar. The pupal period lasts for 8.00 ± 1.00 days in case of maize feeding and 11.00 ± 2.00 days on Brinjal (Table 3).

3.3. Life stages of *S. frugiperda*

Spodoptera frugiperda is an economically important pest that causes crop loss in many countries. Figure 4 shows different life stages of *S. frugiperda* from egg masses to 4th instar. Eggs of *S. Frugiperda* were rounded white and can be observed in bunches or clusters on the upper side of leaf. After few days mesh like structure formed around eggs and egg masses stuck to the leaves. 1st instars of *S. frugiperda* were minute blackish gray in color. From single cluster of eggs hundreds of larvae were hatched figure 4 (a-c).

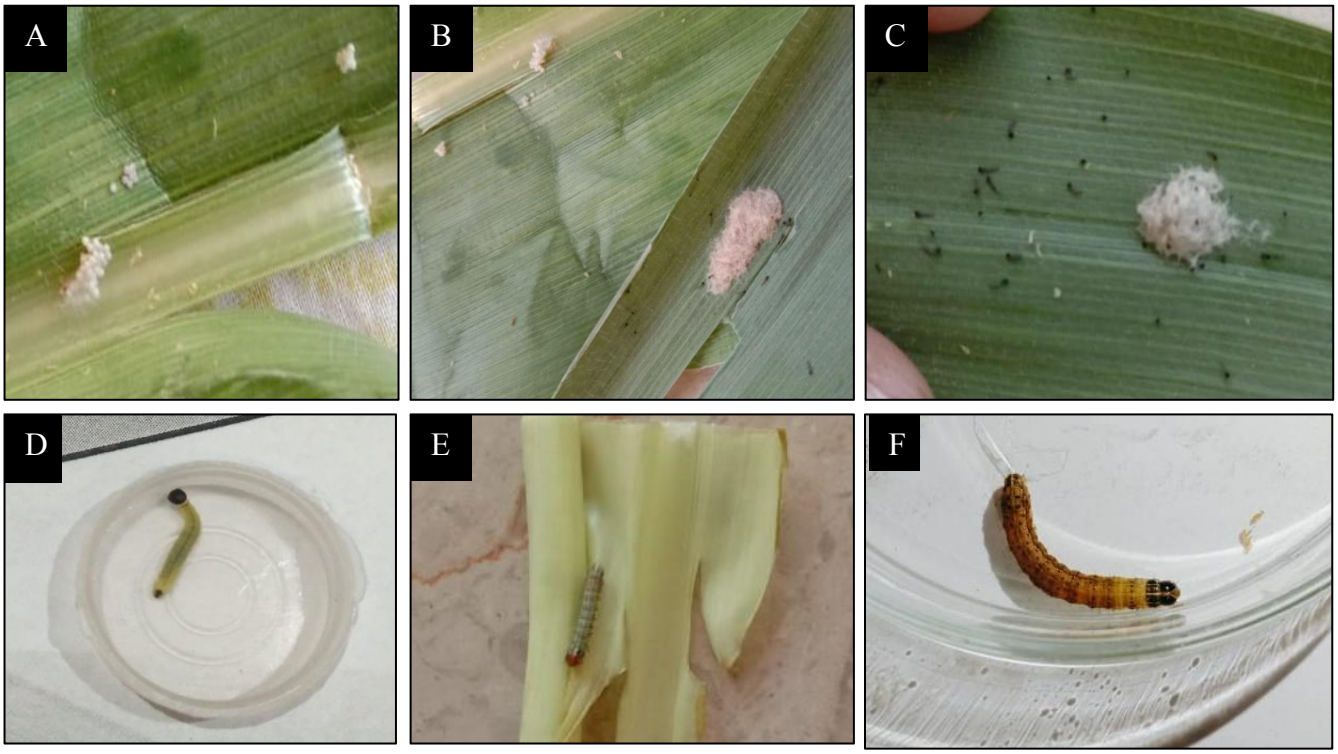

Figure 4. Photographs of egg masses and different larval stages of *S. frugiperda* captured during laboratory trials. (a, b): Egg masses of *S. frugiperda* collected from maize crop. (c): 1st instars emerging from egg masses. (d): 2nd instar stage. (e): 3rd instar feeding on maize. (f): 4th instar stage.

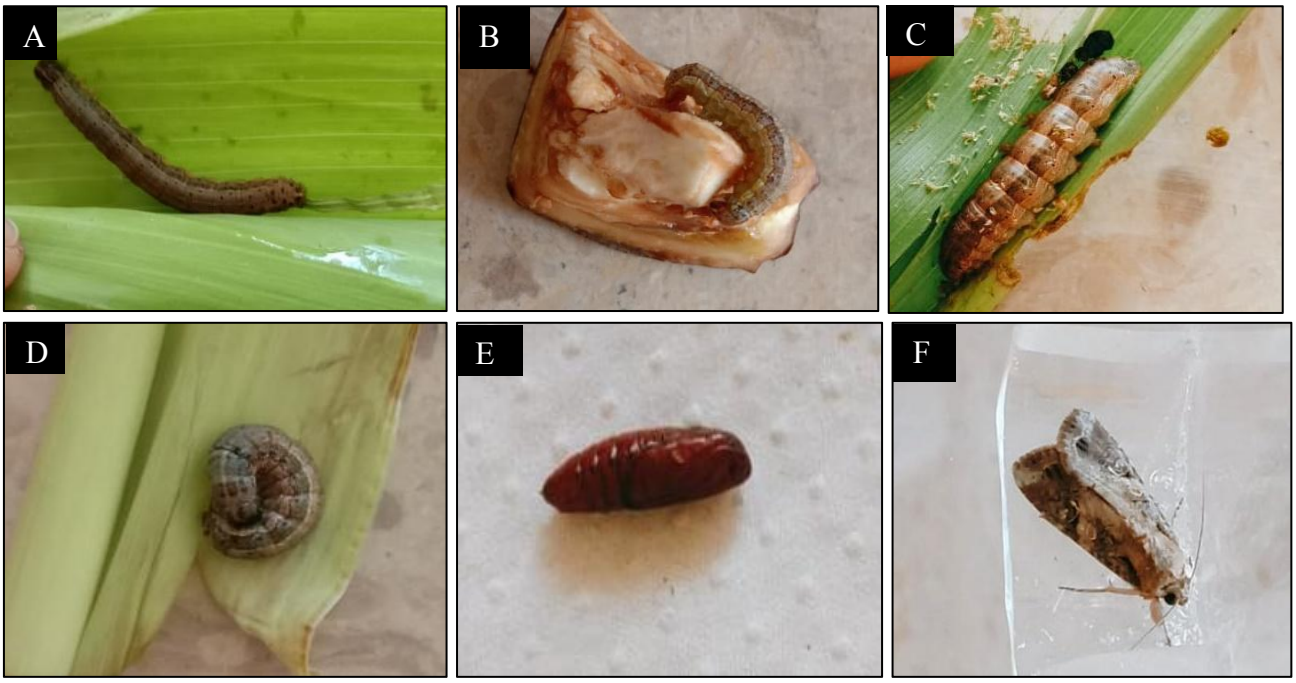

Figure 5. Photographs of egg masses and different larval stages of *S. frugiperda* captured during laboratory trials.(g): 4th instar feeding on maize. (A): 5th istar feeding on brinal. (B): 6th instar. (C): pre pupal stage. (D): pupa of *S. frugiperda*. (F): adult of *S. frugiperda*.

Figure 4 (D) indicates the 2nd instar stage which was identified by rounded black head and yellowish green slender body. During 3rd instar stage body color transitioned from yellowish green to greenish brown color and the head turned redfigure 4 (E). Body length increased during 4th instar stage. During this stage feeding efficiency also increased (Figure 4F & 5A)

Figure 5 (A) 5th instar was brown gray in color and yellow strip was visible on lateral sides of larvae. During 6th instar body length did not increase as much, but larva gained weight and became stout figure 4 (C). At the end of 6th instar feeding efficiency was lower and body length started decreasing figure 5 (D).

Figure 5 (E) showing pupa of *S. frugiperda*. It was dark brown in color. Moth in figure 5 (F) is the adult of *S. frugiperda*. Moth is blackish brown in color and have pattern on forewing. Hind wing is whitish and has black lining.

4. Discussion

S. frugiperda is an important insect pest of many crops including, maize, sugarcane, tomato, fodder, wheat, rice, sorghum, cotton and many other vegetables [27]. *S. frugiperda* exhibits poly phytophagous behavior and feeds on more than 60 cultivated crops reported by [28, 29, 30]. The present study was conducted to estimate the damage extent caused by *S. frugiperda* on selected crops. Maize and brinjal were selected as diet. Maize was the most damaged crop by *S. frugiperda* as reported by Keute et al. [31]. In Pakistan, maize crop is heavily infested by lepidopteran pests as compared to any other cereal crop. About 20-30% yield loss in maize is caused by maize stem borers annually [32, 33, 34]. Six larval stages were recorded when fed under laboratory conditions. Each larval stage was disastrous for crops, but 5th instar larvae are the most damaging and cause reduction of crop yield. As growth proceeds larval stages need more food that's why from 3rd to 5th instar stage food consumption was higher. The severity, damage and diversity of larvae were correlated with each other. Severe damage occurs when more than one larva co-exists in a

single plant. Larvae were hugely spread among plants, all stages, from egg masses to 6th instar, were observed in the fields. Moreover, larvae also move from one plant to another plant to feed [35].

Larval growth varies on the plants and leaves that are given as diet. The larvae of *S. frugiperda* bore into the host plant stem or shoots and then take nutrition. Because in the inner region of stem water content and other nutrition are present abundantly as compared to older and fully grown regions so larvae prefer to bore into stem and take diet from inner plant materials. Kursar and Coley [36] also reported that those larvae that feed on younger leaves were grown faster than those that were fed on older leaves. Pupal weight and pupal hatching depend upon the food consumed by larvae. Those larvae that consume more food have more pupal weight and their chance of hatching are also greater. In present studies as larvae feed more on maize crop as compared to brinjal therefore pupal duration was short on maize than brinjal. But pupal weight also varies when same type of larvae fed on different foods as reported by Naseri et al., [37].

Silva et al., [38] Developmental period, body mass and pupal weight and survival rate of larvae are the key parameters to estimate the damages caused by lepidopteran pests. Short feeding period, fast larval growth, high larval and pupal weight, greater number of pupal emergence and low death rate suggest that larvae have high survival rates and is a serious pest of crop land ecosystem. In the present study as larvae of *S. frugiperda* fulfills the above-mentioned criteria for maize crop because its foraging efficiency and pupal weight were higher on maize as compared to brinjal. *S. frugiperda* can be considered as serious economic pest of maize as reported by Prowell et al., [39]. In maize certain volatile compounds including linalool are attractive for *S. frugiperda* larva. And hence maize crop proved to be more preferred food source for larvae [40]. On mid to late growth stages of maize damage from the infestation by *S. frugiperda* can be 15-73% and yield loss can be more than this if not controlled timely. Depending upon the larval stage instars can be found on maize leaf, stem or in the whorl [40, 41]. As damage caused by herbivorous larvae can be assessed by feeding efficacy of larvae on host plants, future research should focus on developing eco-friendly methods to control these destructive larvae.

5. Conclusion

This study analyzed that larval stages of *S. frugiperda* are one of the most damage causing pests of economically important crops. Understanding their feeding efficacy, larva-host plant association and larval developmental stages could provide beneficial insights for designing effective and eco-friendly pest control measures.

Data availability statement

The data supporting the results of this study can be obtained from the corresponding author upon request.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Ethical approval

Not applicable (N/A)

Authors contribution

Ayesha Hafeez conducted the research, collected and analyzed the data and wrote the manuscript. Sadia Maalik investigated, visualized and supervised the work. Sajida Mushtaq and Moazama Batool helped in formal analysis and conceptualization. Naheed Bano and Nazia Ehsan edited and reviewed the manuscript.

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