Research Article

Development of Protein Based Artificial Diet for Mass Rearing of *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)

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Abstract | Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) is one of the significant insect pests of agricultural crops due to its polyphagous nature. The plants on which it feeds possess variable chemical and physical properties which may influence its biology particularly for growth and reproduction. This study evaluated the growth and fecundity of *S. frugiperda* on artificial diets. The FAW larvae were reared on two distinct composed artificial diets (bean D1 and chickpea flour D2) and one natural diet D3 (fresh and healthy maize leaves). In both artificial diets, only the flours were different, while the rest of the ingredients remained the same. Sixty first instar larvae were introduced in a Petri dish to observe larval duration, larval weight, and adult fecundity. Each larva was offered 0.5 mg of diet in a plastic cup and replaced after every 24 hrs. A prominent effect of different diets on larval development was noticed. The survival percentage was 100% on D3. The difference ($P<0.05$) was much noticeable in early larval stages from L1 to L3. The overall larval duration was maximum 18.2±0.23 days on D1. The maximum larval and pupal weight were 491.1±19.41 mg and 261± 10.19 mg on Diet 2. In last, the highest oviposition period was 5.7±0.10 days found on D2. The maximum number of egg masses 23.11± 2.5 with the highest fecundity of 1758.61± 245.9 eggs was noted on D3. The impact of larval and pupal body weight on adult's oviposition of *S. frugiperda* was correlated (larva r^2=-0.84 & pupa r^2=-0.79) that showed a negative with non-significant difference ($P>0.05$). Thus, based on the findings in the present study, it is concluded that D2 (chickpea flour) was much appropriate for FAW rearing as it produced short larval developmental period, maximum body weight with efficient oviposition. Further it is suggested; more future research should be conducted in order to know the impact of artificial diet on FAW during lab condition.

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Introduction

Maize (*Zea mays* L.) is the third most vital cereal crop and staple food of many countries. In Pakistan during 2017-2018, maize was cultivated on 1.65 m ha having 6.43 million tones production annually (FAOSTAT, 2021). It is known as the Queen of cereals due to its extremely productive impending and cheaper cost in comparison to other grown grains. It is a cash crop as it plays a significant role in the agricultural economy as a human food, animal feed and organic matter for industrial purposes (Jaliya *et al*., 2008). Its widespread utilization as an animal feed is quite common due to higher proportion of vitamins, carbohydrates, proteins, fiber and riboflavin that contain reasonably good amounts of calories (Abdulrahaman and Kolawole, 2008; Kumar and Jhariya, 2013).

The crop is attacked by 140 different insect species with their different levels of damage. However, 12 species are the most serious pests of maize causing damage from sowing to the harvesting and in the storage conditions (Siddiqui and Marwaha, 1993). In insect pests, the fall armyworm (FAW) *Spodoptera frugiperda* J.E. Smith, 1797 (Noctuidae: Lepidoptera) is a major insect pest of many annual crops and causes severe losses including corn, soybean, cotton and vegetables (Bueno *et al*., 2011; Tahir *et al*., 2022). It is an invasive species and highly migratory polyphagous in nature, voracious feeder, higher fecundity and devoid of diapauses. FAW feeds on over 100 different plant species but the most economically damaging is to maize in different regions of the world (FAO, 2018). In 2016, FAW was the first detected from African nations (Georgen *et al*., 2016) and later it expanded to Bangladesh, India, Thailand, China, Sri Lanka, and Myanmar (FAO, 2019a, b) causing the significant damage. However, its invasion was successfully reported in Pakistan (Sindh) in 2019 (Bhatti *et al*., 2020; Gilal *et al*., 2020). Because of its economic importance to agriculture, many studies are being conducted on different aspects, but little is known about the factors affecting their larval development and fecundity on artificial diets which may influence their growth and development under laboratory conditions. As its development on 15 grasses and sedges have already been found which showed the host diet significantly affected the larval duration, pupal weight and fecundity (Pencoe and Martin, 1981). Besides, artificial diets do not only provide a better condition to rear insects, but also provide a depth information about nutritional requirements of different insects and behavioral responses during their mass rearing which are essential for the development of Integrated Pest Management programs (IPM) (Panizzi and Parra, 2009). Thus, all this information is quite imperative to introduce any effective control measures against pests (Zapata *et al*., 2005). Unfortunately, information about proportions of nutrients for larval diets are still insufficient though this may essentially impact on biology of insects by preventing their development and later reproductively. However, few studies had been previously evaluated on impact of insect diets or food consumption indices (Panizzi and Parra, 2009). These indices are essential and helpful to examine the effect of food on insects' growth and development and later reproduction depending upon food that has been ingested and absorbed by insects. Mostly insects consume about 70-75% of their intake in order to maintain their different life stages and in such scenario if taken up of non-balanced or low-quality diet may increases the chances insect's metabolic stress which further lead to suppression of insect immune system and more importantly affect the insect fertility and fecundity (Woods *et al*., 2019). The insects which are polyphagous in nature like different known species of *Spodoptera* prefer to visit physically different host plants for foraging because of their variable nutrients which directly influence the growth and development of their mobile immature stages (Sarate *et al*., 2012). For the most insects, the balance portion of carbohydrate and protein are the key nutritional factors for their progress because with their proper and balance intake better composition of difference hormones and enzymes takes place for insect (Bae and Sicher, 2004; Sarate *et al*., 2012). Though, some achievements have been achieved to culture successful insect generations and other lepidopteran on various artificial diet based on protein and carbohydrate (Truzi *et al*., 2021). Chickpeas and bean flour are cheaper and good source of making artificial insect diet as these both have sufficient amount of protein and carbohydrate (Masood *et al*., 2014), further their possible preservation made easiness to rear insects throughout the year when main host plants normally absent due to off seasons (Nair *et al*., 2019); however in most cases the fitness loss particularly in early stages of insect and less productiveness of adults resulted which further leads to longer developmental period of larvae or immature stages and lower reproductive rate (less number of eggs laid by female) (Coudron *et al*., 2002). Several studies on ovipositional behavior of *S. exigua* (Azidah and Sofian-Azirun, 2006) have been recorded but there is still a lack of published literature on oviposition behavior of the FAW with influence of artificial food. These studies explored that the basic materials used in insect diets are soybean flour, wheat germ, and yeast powder (Pinto *et al*., 2019; Wang *et al*., 2019; Su *et al*., 2019; Lekha *et al*., 2020). No doubt, development of effective tactics for managing insects requires a deep knowledge of the biological interactions of the insect and its host plants. Keeping in view the importance of nutrition on insect population growth, we designed to evaluate the impact of protein based artificial diet on developmental and reproductive parameters of *S. frugiperda* and compared with natural diet under laboratory conditions.

Materials and Methods

Place of work

The experiment was carried out in the Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tando Jam. The larvae of FAW were collected from the maize field surrounding Tando Jam and later reared at the Department of Entomology, FCPT, SAU, Tando Jam on natural diet at 27±2 °C with 60-70 % (RH). The experiment was laid out in a Completely Randomized Design (CRD) with three treatments and three replications to determine the effect of artificial diet on larval development, larval duration, larval weight, and larval mortality of *S. frugiperda*. The larvae were reared on three different diets (two artificially prepared and one natural). For the 2nd diet, the bean flour was replaced by the Chickpea flour in a similar quantity while the rest of the ingredients remained the same. However, in the control treatment, the maize leaves were offered to the experimental larvae. The experimental larvae were taken from F2 generation after successful rearing of F1 generation on artificial diets.

Diet preparation

Two artificial diets were prepared based on bean flour and chickpea flour. Both grains were purchased from the local market and kept in a microwave hot oven for 3-5 min at 65-70 °C to insure the killing of any possible pathogen. Later, the grains were milled to produce powder or flour. The powder ingredients except methyl-p-hydroxybenzoate were mixed well under a fume hood in a sterile jar. The distilled water was boiled at 100 °C and left it to cool up to 60°C. After cooling, water was mixed with the pre-prepared ingredients through a blender for 2-3 min. Later, methyl-p-hydroxybenzoate was added (that was previously dissolved in 20 ml in absolute ethanol) with the mixture in the blender and again all these were blended for more 1-3 min. Agar powder was weighed in a separate sterile container and then added distilled cold water in a separate container. Stirred periodically while boiling and then cooled at 60°C for proper mixing ingredients and blended for further 3 min. Finally, add 40% formaldehyde to the mixed ingredients in the blender and then mix for 3 min at room temperature. All the ingredients used in making a diet are listed in Table 1 and preparation mentioned in Figures 1, 2, 3.

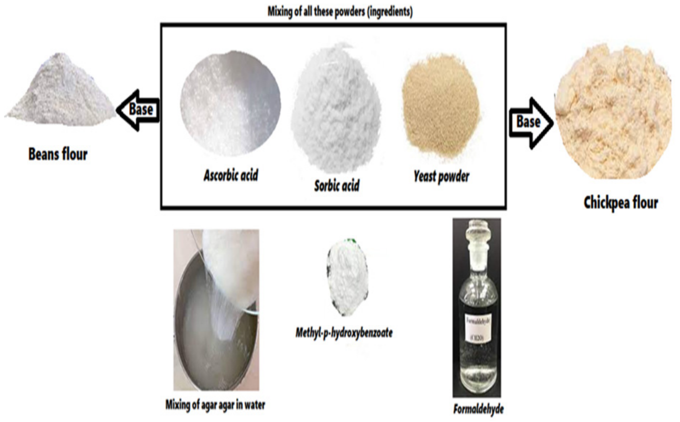

Figure 1: *Ingredients used for artificial diets stepwise based on bean flour and chickpea flour.*

Figure 2: *Preparation of artificial insect diet.*

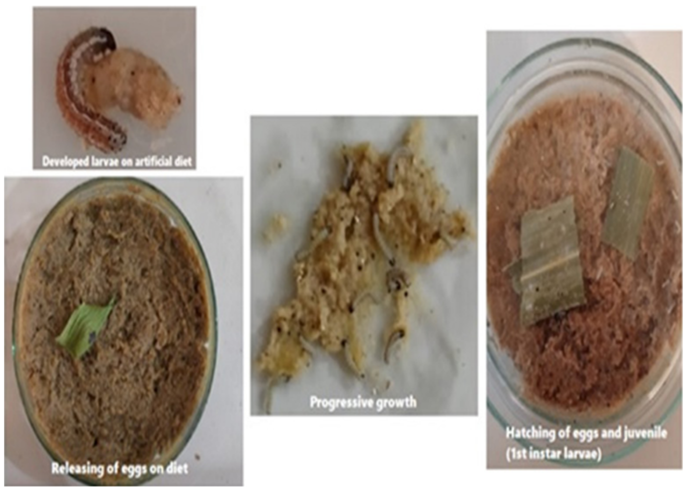

Figure 3: *Development of Spodoptera frugiperda on artificial diet.*

Table 1: *The ingredients of artificial diets (D1 and D2) and D3 consisted of maize leaves.*

Bean flour based (D1)	Chickpea flour based (D2)	Control (D3)
Flour bean (500g)	Chickpea flour (500g)	Fresh maize leaves
Powder yeast (52g)	Yeast powder (52g),	
Ascorbic acid (5.3g)	Ascorbic acid (5.3g),	
Formaldehyde solution (5 cc)	Formaldehyde solution (5 cc)	
Sorbic acid (1.7g)	Sorbic acid (1.7g)	
Methyl-p-hydroxybenzoate (5.3g)	Methyl-p-hydroxybenzoate (5.3g)	
Agar Agar (30 g)	Agar Agar (30g)	
Distilled water (500 ml)	Distilled water (500 ml)	

Table 2: *The larval developmental period and survival percentage of Spodoptera frugiperda on different diets.*

Developmental stages (Larval instar 1-6)	Natural diet		Diet 1		Diet 2	
	Developmental period (days)	Survival (%)	Developmental period (days)	Survival (%)	Developmental period (days)	Survival (%)
L1	1.2±0.01a	91.3	2.1±0.02b	62.2	2.2±0.02b	65.5
L2	3.1±0.02a	93.6	3.6±0.03b	71.4	3.2±0.03b	75.1
L3	3.1±0.01a	95.1	3.6±0.01b	75.6	3.2±0.02b	72.1
L4	3.6±0.02a	100	3.4±0.02a	89.1	3.3±0.04a	88.8
L5	3.1±0.02a	100	3.2±0.03a	90.00	3.2±0.04a	97.1
L6	2.8±0.02a	100	2.9±0.04a	91.00	2.8±0.03a	97.4
Total larval period (days)	16.51±0.35A	96.66	18.2±0.23B	79.88	17.8±0.19B	82.66

Means followed by different letters in column are significantly different (P ≤ 0.05)

Observation to be recorded

Each treatment consisted of sixty (60) 1st instar larvae; these were kept separately from 3rd instar in a small jelly cup to avoid cannibalism and further divided into three different groups/replications (each replication consisted of 20 larvae) for data analysis. In larval development, larval duration, larval weight, larval mortality on different diets were observed from 1st larval instar to last larval instar (L1-L6). Each larva was offered 0.5 mg quantity of diet in plastic cups and replaced at every 24 hours. In control, fresh maize leaves were offered after proper washing with distilled water. In reproductive performance, the adult fecundity was recorded after taking three pairs (each pair was considered as replication) from each treatment. Newly emerged adults were collected and released in each transparent box cage at a sex ratio of 1:1 after proper identification of either sex based on morphological characters and fed with 20% Honey solution through a cotton ball in a small plastic cage (3.5×1.3 cm^2). The upper side of the cage was covered with muslin cloth. The maize plants were placed inside cage for egg laying. Leaves were observed on daily basis for egg collection.

Statistical analysis

The collected data were analyzed through ANOVA (Analysis of variance) using Statistix 8.1 computer software package and the difference among the treatment means was compared by Least Significant Difference (LSD) at ($P<0.05$).

Results and Discussion

The results of the proposed study indicated the prominent effect of different diets on the larval development of *S. frugiperda* (Table 2). No variation ($P > 0.05$) from L4 to L6 was found when these larvae were reared on two artificial diets in comparison to a natural diet.

Meanwhile, the survival percentage for these instars was 100% on a natural diet in comparison to Diet 1 (89.1-91%) and on Diet 2 (88.8-97.1%). The statistical difference ($P<0.05$) was much noticeable in the early larval stage from L1 to L3. The developmental period was the lowest 1.2±0.01 days in L1 on a natural diet with 91.3% survival, 2.2±0.02 days on Diet 2 with 65.5 % survival and 2.1±0.02 days with 62.2 % survival on D1, respectively. The trend was similar with no statistical variation (p>0.05) in both artificial diets on L2 and L3 but varied (P<0.05) in comparison to natural diets. These findings pointed out the much effect of food on survival rate rather than developmental period. However, the vulnerability at early stage of FAW larvae was natural thus noticed higher mortality but fitness of early larval on natural diets was remarkable. The overall larval duration was maximum 18.2±0.23 days on Diet 1 composed of chickpea flour with 79.88 % survival followed by 17.8±0.19 days on Diet 2 composed of bean flour with 82.66 % survival. The minimum 16.51±0.35 days with the highest 96.66 % survival was recorded on a natural diet that is maize leaves. To confirm the further effect of different diets, the larval body and pupal masses were weighed. A significant difference (P<0.05) in larval weight was found when FAW larvae prior reared on a variety of diets and subsequently noticed in pupal masses. The maximum larval weight of 491.1±19.41 mg was recorded on Diet 2 followed by 487±25.5 mg on Diet 1; whereas the minimum larval weight of 443±12.4 mg on the natural diet was recorded. Similarly, the variation among pupal weight was also observed (P<0.05) in which the maximum pupal weight of 261± 10.19 mg on Diet 2, 255± 9.51 mg on Diet 1 and the minimum 234.5± 8.1 mg was recorded on natural diet, respectively (Figure 4). Furthermore, these findings suggested no variation in larval body weight and pupal masses weight was recorded when statistically compared on both artificial diets (P>0.05) but the variation was prominent when comparing both results with natural diets.

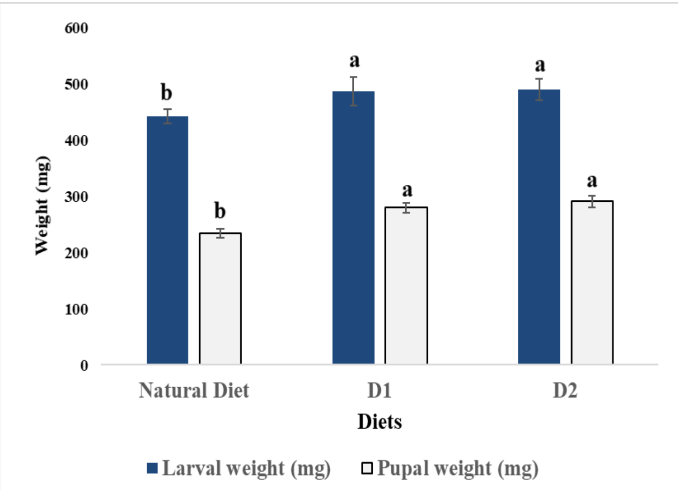

Figure 4: *Effect of different diets on larval and pupal weight of Spodoptera frugiperda.*
Means followed by different letters in bar diagrams are significantly different (P ≤ 0.05).

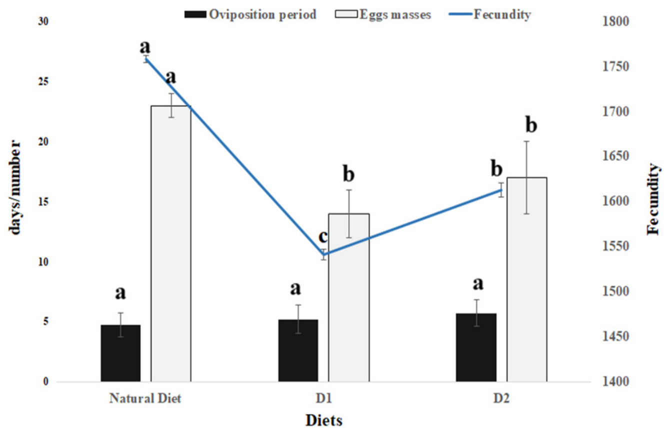

Figure 5: *Effect of different diets on reproductive performance of Spodoptera frugiperda.*
Means followed by different letters in bar diagrams are significantly different (P ≤ 0.05).

In the last, *S. frugiperda* adult's fecundity was recorded in terms of influence by larval diets (Figure 5). The three parameters including egg masses, oviposition period and fecundity were recorded. The egg masses are the bunch of eggs laid by females, oviposition period is the time spent by female for eggs laying and fecundity is the rate of eggs laid by female was observed respectively. A significant difference (P<0.05) related to the reproductive performance of *S. frugiperda* was recorded except oviposition period (P>0.05). The highest oviposition period was 5.7±0.10 days found at D2 with no significant difference of 5.2± 0.12 days on D1.

The reasonable lowest period of 4.7± 0.19 days for oviposition by female was shown on the natural diet. Though, oviposition period was found highest on both artificial diets but the maximum eggs masses of 23.11± 2.5 with the highest fecundity of 1758.61± 245.9 eggs was noted on Natural Diet as compared to D1 (14.21±3.1 eggs masses with fecundity of 1541.31±353.61) and D2 (17.11±5.71 eggs masses with fecundity of 1612.71 ±389.7 eggs). These findings suggested that it (fecundity) was the only and essential parameter that found higher on Natural diet as compared to both artificial diets; but fitness of *S. frugiperda* on artificial diets in comparison to natural diet (maize) for laboratory rearing was also incredible for future further studies. Furthermore, the larval and pupal body weight was also correlated with fecundity and represented through a regression line. It showed that both larval (r^2=-0.847) and pupal (r^2=-0.799) body weight was negatively correlated (P>0.05) with fecundity and showed non-significant difference (Figures 6 and 7).

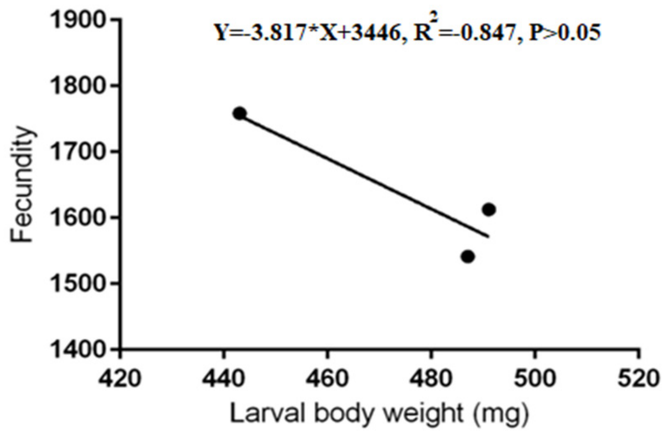

Figure 6: *Regression line showing relationship between larval body weight and fecundity on three different diets.*

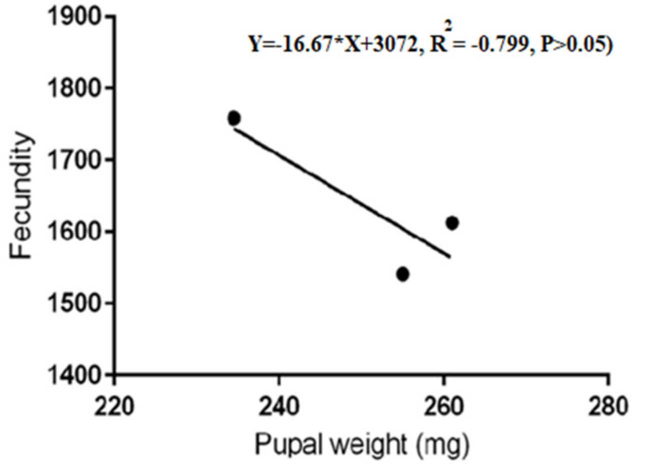

Figure 7: *Regression line showing relationship between pupal body weight and fecundity on three different diets.*

Being a threatening global agricultural insect pest, the mass rearing cost of FAWs should be lesser but effective. Basically, an artificial diet is an advantageous in comparison to natural diets because natural diet relates to seasonal availability and is quite laborious to be arrange (Pinto *et al*., 2019). Artificial diets do not provide only known quality but also confers continuous mass rearing of different insects in laboratory throughout the year for various experiments (Cohen, 2015). The growth and development insect is greatly related with the quality of diet (Silva *et al*., 2020) and in this contest the consumption of distinct plant species may elicit variation in survival and life history of insect (Guo *et al*., 2021) as host plant quality is an essential indicator for life and insect fecundity (Hong *et al*., 2019).

In the present study, we strived to develop a cheaper but high-performance protein based artificial diets from chickpea and bean flours for culturing of *S. frugiperda* first time in Sindh, Pakistan. The observed biological parameters of insect indicated a prominent effect of artificial diets as compared to the natural diet most particularly on the fitness of young juvenile larval instars. However, the overall growth, development and body weight were not much different when rearing of FAW larvae on both chickpea flour and beans flour diets; possibly due to similar amount of nutritional contents but in comparison to natural diets (maize leaves) the outcome was varied and such phenomenon is natural and well understood due to the fitness of a polyphagous insect on maize leaves and on artificial diets. Once the FAW larvae were adjusted (fitness) on artificial larvae then very few deaths were recorded and it occurred particularly after 2nd instar larvae. Previously, it has also been documented well in most lepidopterans insect pest including *Helicoverpa armigera* (Hübner, 1808) and *Plodia interpunctella* (Hübner, 1813), the studies showed that less-protein diets brought the changes in larval mortality and body weight, overall developmental period, pupation rate and adult emergence ratio (Borzoui *et al*., 2018; Truzi *et al*., 2019). Further, the quantity of food utilized by insect and its nutritional attributes specifically protein may influence on larval phase duration (Pinto *et al*., 2019). Thus, both protein based artificial diets and natural food offered to *S. furgiperda* certainly were discrete in proportion of protein and other nutrients which resulted differently in this experiment. Further, change in diet or environment always produce a higher mortality in early stage of insect until or unless they become used to of it. Merkx-Jacques *et al*. (2008) reported that mortality were high with sufficient developmental delay on the high-carbohydrate and low-protein diets particularly in early larval instars which displaying the potential detrimental effects of excess carbohydrates and the requirement for protein survival and growth of *S. exigua*. Similarly, Ashouri *et al*. (2023) observed in their experiments on artificial diet that the early larvae were unable to moult and consume little amount of food and few failed for their transition to pupa on a diet containing corn flour instead of beans, demonstrating the importance of bean flour as protein-rich which were varied in proportion. In similar pattern, we also found a little delay but not significantly higher in larval development on both artificial diets in comparison to natural diet but they finally survived well and produced almost similar results in larval duration of natural diet. The food quality and nutritional value of different host plants and artificial diets are basic matter of concern for the fitness of insects (Atrchian *et al*., 2016).

Similar observations of Zhang *et al*. (2014) and Silva *et al*. (2020) on larvae reared on tobacco and Chinese cabbage in comparison to reared on man-made diets displayed a varied developmental times.

Moreover, the larval and pupal body weight were higher on artificial diets but both these correlated negatively with fecundity so it is not always true that higher masses of immature always helpful to produce higher fecundity. Host plant preferences during the larval and other developmental stages of insects can lead to both behavioral isolation and phenotypic plasticity (Etges and Oliveira, 2014). In most Lepidopteron species, developmental period delay (Silva *et al*., 2020), mortality higher and fecundity lower on artificial given diets in comparison to natural diets specifically in early generation of insects due to their adoptability on newly developed diets due to higher ingestion which uplift the body weight but poor digestion. Artificial diets are not sometime naturally balanced in nutrition and also get pathogenic activity more easily and frequently that may affect the insect health. On the other hand, artificial diets contain a variety of nutrients like phosphorus, lipids, minerals and more importantly proteins which play an important role in insect development (Cambron *et al*., 2019) and their deficiency could adversely affect insect developmental time (Khanamani *et al*., 2017) and body weight. Generally, man-made insect diets are more properly balanced for insect development in comparison to development of insect on natural single host plant (Zhang *et al*., 2014; Silva *et al*., 2020) until or unless its adoption on it. Tuzi *et al*. (2021) reported the larval weight of *S. frugiperda* using nutritional indices (i.e., RCR and RGR) were higher in the diet with double protein (D_3). This showed that the larvae required a larger amount of food with protein to meet their nutritional needs, which led to greater weight gain in comparison to natural diet. In addition, the variation on growth and development of insect is physiological influenced. As the natural and artificial diets are varied in pH and moisture content. According to Cohen's (2015) the most insects required a slightly acidic pH range in their diet and which often varied in natural plants and dependent of soil, weather and other agronomic application. Further, he added that most plant phytophagous insects require high water content in their diets and in laboratory condition, sometime procedure of giving natural host plant leaves often and quickly dry and loss the water content (evaporate) which make difficulty for insects to sustain their life process. In natural host plants (food), water is preserved in plant cell wall and cellular constituents whereas in man-made diets carrageenan gel or agar is used to bind water (Woods *et al*., 2019), thus when the diet moisture evaporated greatly then larvae failed to access enough nutrients and produce poor development which may consequence in maximum mortality. However, the natural diet proved the insects to get more fecundity instead of lower weight of immature stages (larvae and pupae particularly in case of lepidopteron insects).

Therefore, based on the present study and in the light of literature, it is obvious that larval and adult diets or their preference to host plants is associated with their nutritional requirements which make them more attractive to them. Information from the present study displayed in terms of FAW ecology in which it showed how its female's decision affects survival of its offspring and their reproductive fitness. The most important findings of this study were the impact of natural diet on body masses of both larva and pupa of *S. frugiperda*. The early maturity of larval instars on natural diets rather than artificial diets showed the fitness of natural host plants. The moth laid a number of egg clusters, but there were less in number of eggs on artificial diets than natural diets. However, it is quite a fact that artificial diet can be as beneficial as natural but friendly availability and fitness of FAW, or artificial larvae is a good achievement of this study. Thus, it is to be said that artificial diet for the fall armyworm is very essential to conduct research on the physiology, toxicology, biology, behaviour and mass rearing of natural enemies on it. We do not need to forget that *S. frugiperda* is a polyphagous insect in nature and its ability to feed on a variety of plant species also suggest developing a variety of artificial diets need to be developed in future for the success of indoor rearing (Wang *et al*., 2019).

Conclusions and Recommendations

We successfully developed an inexpensive but effective protein based artificial diet from chickpea flour for mass culturing of FAW which is the notorious an invasive insect pests of many economical crops. Based on the results of observed biological parameters of insects, the rearing performance on the prepared diet was consistent with the natural diets except in the initial stage of young larvae. Thus, this work can promote the mass rearing of FAWs for further studies in order to devise a proper control management based environmental friendly application of pesticide or its synchronization with any bio-control agent.

Acknowledgement

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Novelty Statement

FAW and other Lepidoptera insect pests can easily be reared in lab without live plants on these developed artificial diets which support and facilitate various experiments on this deadly and other harmful insects in vivo

Author's Contribution

All research ideas and lab facilities were provided by Agha M. Ahmed and Fahad N. Khoso. Touseef A. did laboratory work, M. Ibrahim K. performed and designed statistical analysis. Alizachi A. A. and Tehniyat N. S helped in write up, results interpretation and proof reading of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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