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Biological Evaluation of *Spodoptera Frugiperda* (Lepidoptera: Noctuidae) Reared on Various Artificial Diets Compared to the Main Host

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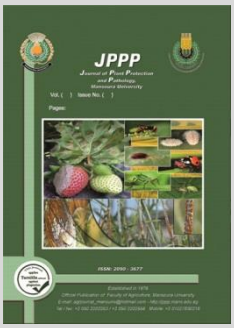

ABSTRACT

Spodoptera frugiperda is a destructive lepidopteran pest that seriously affects many crops. To obtain experimental individuals for use in IPM experiments., it is crucial to improve the rearing technique for *S. frugiperda*. Fundamental and applied entomology problems are resolved by advances in artificial diet rearing techniques. In this work., we studied the development of *Spodoptera frugiperda* on different artificial diets compared with the main food source(corn). Three diets were modified: artificial diet composed of beans (D1)., artificial diet based on corn flour (D2)., artificial diet based on wheat bran (D3) and a diet of castor leaves (D4) compared with a diet of green corn as the main host. The biological characteristics that were analyzed were the lifespan and duration of the larvae and pupae., the weight of male and female pupae., the sex ratio., fertility., and the longevity of the adult. Diet 3 showed the longest larval duration period while diet 5 showed the shortest larval duration period. Diet 4 recorded the longest pupal duration., while diet 2 recorded the shortest pupal duration period. No significant difference in the number of hatched eggs between larvae fed on artificial diets and those fed on castor bean leaves as compared with control (green corn).

Keywords: *Spodoptera frugiperda.*, artificial diet., mass rearing., biology., total life cycle

INTRODUCTION

The tropical and subtropical Americas serve as the native habitat of the polyphagous fall armyworm. Prior to 2016., the FAW's distribution was confined to its native range. However., in 2016., it was reported to have caused significant harm to maize crops in Africa (Goergen *et al.*, 2016). The pest has rapidly proliferated across much of sub-Saharan Africa since its introduction in 2016. The species causes severe damage to several crops (Nagoshi 2009., Bueno *et al.*, 2011). *S.frugiperda* entered Egypt in 2019 then spread in all governorates. Due to the major threat of this insect to maize., understanding its biology and morphology is crucial for developing effective management strategies. The lack of its favorite food in apart of the year necessitated the search for alternative diets to sustain research and control measures (Silva and Parra 2013).

To facilitate year-round research., alternative food source was identified to supplement the study of *S. frugiperda* during periods when its preferred host., maize., is unavailable. One of the alternative food sources was artificial diet which used for other lepidopterous insects.

Many studies on artificial diets have increased rapidly in recent years (wang *et al.*, 2019., Li *et al.*, 2019., Su *et al.*, 2019., Lekha *et al.*, 2020). The basic components used in FAW diets contain., Soybean flour., wheat germ., and yeast powder (Li *et al.*, 2019., Su *et al.*, 2019., Lekha *et al.*, 2020). According to Panizi and Parra (2012) and Rajisha et al., (2022) the findings of all studies have improved our understanding of the biology of insect and the use of integrated pest management techniques like biological control and host plant resistance. Three artificial and two natural diets were use as follows: artificial diet composed of beans (D1)., artificial diet based on corn flour (D2)., artificial diet with wheat bran (D3) and a diet of castor leaves (D4) compared with a diet of green corn as the main food source. We investigated the impact of various diets on development., survival., reproduction., sex ratio., fecundity., and adult longevity., using a diet consisting of maize leaves as the control.

This work was done to examine morphological characters *S. frugiperda.* Also., to study the possibility of rearing *S. frugiperda* on other diets at a time when the main source of food is limited.

MATERIALS AND METHODS

Larvae collection and rearing

Larvae of FAW collected from maize fields in Sakha station farm were reared on maize plant under laboratory conditions. Larvae were allowed to feed till pupation. On pupation., the pupae were transferred into clean jars and sexed. The emerged adults were separated and a couple of them were matedin separate mating cages. The adults were fed with 10% sugary solution for proper egg laying. The newly emerged larvae were reared in new jars for the experiment. Larvae were reared individually on different artificial diets., castor leaves and fresh maize leaves which were changed daily.

The rearing and the experiments of *S. frugiperda* were conducted at laboratory of Plant Protection Research Institute at Sakha., Kafr El-Sheikh. The insects were reared under laboratory conditions. conditions.

Modified artificial diet

The synthetic diet that was used in the study is the synthetic diet described by Shorey and Hale., (1965) and modified by Mabrouk *et al.* (1996). This diet is used for rearing *Spodoptera littoralis*. The main diet of *S. littoralis* based on beans (D1)., artificial diet based on corn flour (D2)., and artificial diet based on wheat bran (D3)., a diet of ricinus leaves (D4) compared

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with a diet of green corn as the main food source. The diet compositions are described in Table (1)

Table 1. Constituents of modified synthetic diets.

Constituent	D1	D2	D3
White bean (kidney bean)	150 gm	-	-
Corn flour	-	150gm	-
Wheat Bran	-	-	150 gm
Brewer's yeast	30 gm	30 gm	30 gm
Ascorbic acid	3 gm	3 gm	3 gm
Sorbic acid	3 gm	3 gm	3 gm
Methylparahydroxybenzoate (Nipagin)	3 gm	3 gm	3 gm
Formaldehyde	1ml	1ml	1ml
Agar	15 gm	15 gm	15 gm
Distilled water	500 ml	400 ml	400 ml

Preparation of artificial diet

Three artificial diets., modified from the formulation of Mabrouk *et al.*, (1996)., were prepared using corn flour and wheat bran as primary ingredients. The ingredients were weighed., mixed with sterile water and agar., and blended to form a homogeneous., semi-solid mixture. Anti-microbial compounds were added., and the diets were stored at 10°C to maintain freshness."

Biological parameters

Fifty newly hatched larvae were placed individually in small cubs containing small cubes of diet., replaced with fresh diet when necessary: The biological parameters studied were duration of larva and pupa., pupal weight of male and female., sex ratio., adult longevity and fecundity.

After the emergence of the adults five plastic jars for each treatment containing two couples of *S. frugiperda*., hatched at the same time. Adults were fed with a 10% sugary solution. Adult fecundity and longevity., also were observed.

RESULTS AND DISCUSSION

Results

Morphological identification

Eggs

Eggs were laid in masses. They are white to creamish in color covered with white scales of the abdomen of the female (Fig.1a). The egg color changed from brown to black near hatching (fig.1b&c). Sharanabasappa *et al.*, (2018) reported that the egg masses initially appeared pale green., subsequently changed to a golden yellowish color., and finally turned black prior to hatching.

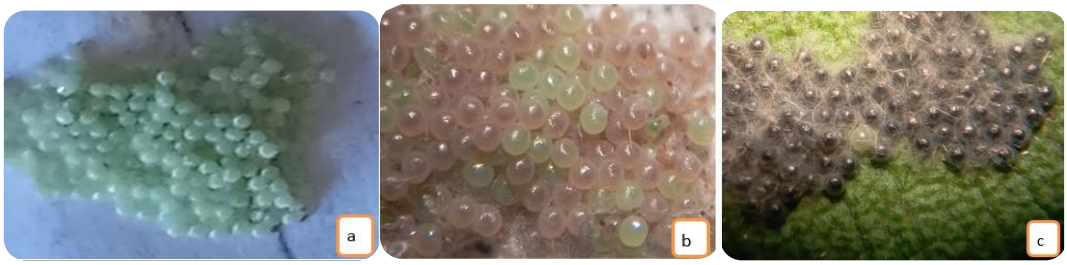

Fig. 1. Different stages of egg mass of Spodoptera frugiperda

Larva

First instar larvae possessed large flattened and circular black head with awhitish hairy body **(**Fig.2 d). They turned greenish brown. in the second instar and brownish color in the third instar. The distinctive features of fourth to sixth instar larvae included a brownish-black coloration., a square pattern of black spots on the eighth abdominal segment., and the inverted 'Y' shape on the head capsule. (fig. 2e).

Pitre and Hogg., 1983., studied the biology of *Spodoptera frugiperda.* larvae and reported that the larval developmental period tends to be about 14 – 30 days. Sharanabasappa *et al.*, 2018 found that the life cycle of the insect was observed to be 32-43 and 34-46 days., respectively.

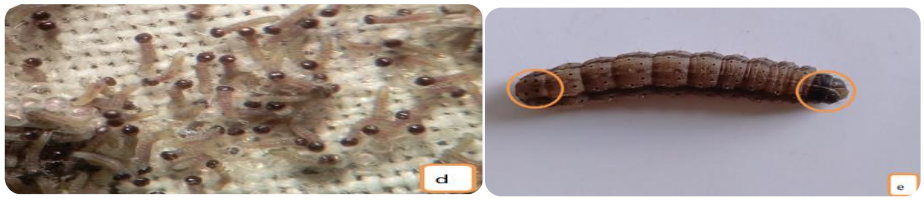

Fig. 2. First instar larva of Spodoptera frugiperda

Pupa

Newly ecdysed pupae of *S. frugiperda.* were white creamy in color., changed to orange-brown., then became dark reddish brown (Fig.3f). The sex of the pupae was determined by examining the morphological differences in their genital organs and by measuring the distance between the genital opening and anal slit., which was significantly shorter in males than in females. (Fig.4 g).

Similarly., Débora et al. (2017) reported that the differentiation between male and female pupa can be done by looking at the genital opening and the distance between it and anal slot.

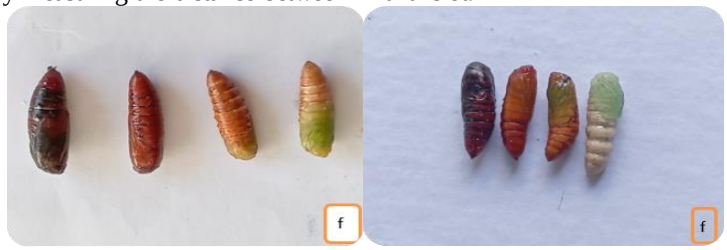

Fig. 3. different stages of pupa

Fig. 4. male and female pupa

Adult

The distinctive features of the male forewing included a gray-brown background., a triangular white apical patch., and a circular spot at the wing's center. Female forewings exhibited a uniform gray-brown coloration., interspersed with fine patches of lighter gray and darker brown. (Fig.5.h). Hind wing is silver-white in color with a narrow dark border in both male and female (Fig.5I). Silva *et al.*, 2021 and Sharanabasappa *et al.*, 2018 reported the same results.

Male forewings are colored with gray and brown., with triangular white patch at the top region and circular spot at the center. The female forewings appeared grayish brown to a fine spotting of gray and brown (Fig.5h). The hind wing appeared as silver-white with a narrow dark. Border in both male and female (Fig.5I). Silva *et al.*, 2021 and Sharanabasappa *et al.*, 2018 reported the same results.

These results were similar to that found by Bhavani *et al.*, (2019)., Manjula *et al.*, (2019) and Malo and Hore (2020). Also., the morphological characters of adult described here. Are similar to those reported by (Deole and Paul (2018)., Oliver and Chapin., 1981).

Fig. 5. Adult moth of Spodoptera frugiperda

Diet preference

The table (2) presents a comprehensive analysis of the different artificial diets on the life cycle of newly. hatched fall armyworm *Spodoptera frugiperda*. There was a significant variation in larval duration across the different diets. Diet 5 exhibited the shortest larval duration. while diet 3 had the longest larval duration (11.6 and 15.3 d respectively). This suggests that the nutritional composition of the. diet has a great influence on larval development rate. On the other hand., diet 2 has the same larval period as the control.

The pupation rate was generally high across all diets., indicating that most larvae successfully. completed their larval stage and entered pupation. However., there were slight differences among the diets., with diet 1 (control) having the highest. Diet 5 showed the lowest pupation rate (82.3) while. diet 2 showed the highest pupation rate (90.3) compared to the control (95.3). Pupal weight was significantly affected by the diet., with diet 2 produced the heaviest pupae. This implies that the nutritional quality of the diet directly influences the size of the resulting adult. Similar results obtained by **Bernardi *et al.*, (2014)**.,

The sex ratio was also influenced by the diet composition. Diet 5 showed the highest. proportion of females (62.50., while diet 3 had the highest proportion of males (54.2). This suggests that the diet may have sex-specific effects on larval development.

Pupal duration was also variable across the diets., with diet 4 having the longest pupal duration (14 ± 0.3) while. d2 recorded the shortest pupal duration (8 ± 0.58). This suggests that the diet may influence the rate of metamorphosis.

Table 2. Impact of the different artificial diet on mean larval duration., pupation rate., Sex ratio., mean pupal weight and mean pupal duration of new hatched larvae of the fall armyworm., *Spodoptera frugiperda* (Smith.).

Different Artificial diet	Mean larval duration (days) ± S. E.	Pupation%	Sex ratio %		Mean pupal weight (gm) ± S. E.	Mean pupal duration (days) ± S. E.
			♀	♂		
Dit 1 (Control)	13.3 ± 0.3 ^{b}	95.3 ^{a}	52.7 ^{d}	47.3 ^{b}	0.162 ± 0.6 ^{b}	10.6 ± 0.6 ^{b}
Dit 2	13 ± 0.58 ^{bc}	90.3 ^{b}	58 ^{b}	42 ^{c}	0.182 ± 0.58 ^{a}	8 ± 0.58 ^{c}
Dit 3	15.3 ± 0.33 ^{a}	88.3 ^{c}	45.8 ^{e}	54.2 ^{a}	0.161 ± 0.6 ^{b}	9.3 ± 0.3 ^{bc}
Dit 4	12.6 ± 0.67 ^{bc}	85 ^{d}	55.6 ^{c}	44.4 ^{b}	0.159 ± 0.6 ^{c}	14 ± 0.3 ^{a}
Dit 5	11.6 ± 0.3 ^{c}	82.3 ^{e}	62.5 ^{a}	37.5 ^{d}	0.153 ±0.3 ^{d}	11.3 ± 0.6 ^{b}
F value	8.15	112.6	191.35	143.15	321.2	12.447
P value	0.0034**	0.0000***	0.0000***	0.0000***	0.0000***	0.0007***

Means with the same letter designation within a column are statistically similar (P < 0.05). Highly significant effect. *** Very Highly significant effect. D1= green corn D2= castor bean D3=beans D4= corn flour D5= wheat bran**

However., in table (3) there were slight differences among the diets., with the control diet (diet 1) having the highest emergence rate. There was a significant variation in adult longevity across the different diets. Females fed on diet 5 had the longest. lifespan., while females fed on diet 3 had the shortest lifespan. For males., diet 5 also showed the

longest lifespan (14)., while diet 3 had the shortest lifespan (9.3). This suggests that the nutritional composition of. the diet has a profound influence on adult lifespan.

Fecundity was significantly affected by the diet. Females fed on diet 2 laid the highest number of eggs (895)., while female fed on diet 5 laid the lowest of eggs (796) as compared with the control diet. Fertility., or the proportion of eggs that hatch., was also influenced by the diet. Females fed on diet 3 had the highest fertility (843.6)., while females fed on diet 4 had. the lowest fertility (773) suggesting. that this diet produced eggs with higher viability.

Table 3. Impact of the different artificial diet on mean adult emergence %., mean adult longevity (♀ and ♂)., fecundity and fertility of new hatched larvae of the fall armyworm., *Spodoptera frugiperda* (Smith.).

Different artificial diet	Adult emergence %	Mean adult longevity (days) ± S. E. ♀	♂	Mean no. of eggs/female ± S.E.	Mean no. hatched eggs/female ± S.E.
Control (Dit 1)	97.3 ^{a}	13.3 ± 0.58 ^{b}	13.6 ± 0.3 ^{ab}	1224 ± 12.4 ^{a}	1104 ± 4.04 ^{a}
Dit 2	94.3 ^{bc}	13 ± 0.28 ^{b}	11.6 ± 0.4 ^{bc}	895 ± 10.7 ^{b}	823.3 ± 3.6 ^{b}
Dit 3	95.3 ^{bc}	9.3 ± 0.57 ^{c}	11 ± 1.0 ^{c}	873.6 ± 12.5 ^{bc}	843.6 ± 4.7 ^{b}
Dit 4	96 ^{ab}	11.3 ± 0.4 ^{b}	13 ± 0.48 ^{ab}	821.3 ± 16.8 ^{bc}	773.3 ± 8.5 ^{b}
Dit 5	94 ^{c}	14 ± 0.57 ^{a}	14.3 ± 1.15 ^{a}	796.3 ± 10.2 ^{c}	747.3 ± 4.9 ^{b}
F value	5.4	10.821	4.0952	47.8463	16.812
P value	0.0140*	0.0012**	0.0321*	0.0000***	0.0002***

Means with the same letter designation within a column are statistically similar (P < 0.05). * Significant effect. **Highly significant effect. * Very Highly significant effect.D1= green corn D2= castor bean D3=beans D4= corn flour D5= wheat bran**

The growth and survival differences between *S. frugiperda* raised on the artificial diets in this study can be attributed to several factors. The performance of insects is known to be greatly impacted by the ratio of protein to carbohydrates in their diet (Roeder & Behmer., 2014). Additionally., insect growth and survival depend on a balanced combination of B-complex vitamins and inorganic salts (Wang *et al.*, 1984). These factors may explain the variation in weight gain and survival observed among *S. frugiperda* individuals fed different diets. Water content and the amount of agar used can also impact diet quality and insect activity (Assemi *et al.*, 2012., Cohen., 2004). Studies by Stipanovic *et al.*, (2008)., Rossi et *al.*, (2012)., and Kojima *et al.*, (2010) have demonstrated that the survival of *S. frugiperda* pre-pupae and pupae fed on castor bean., corn., and cotton leaves is higher than those fed on other diets. This increased survival is linked to the reduced exposure to toxic secondary metabolites such as ricinin., hydroxamic acid., and gossypol., which are present in these plant species. The better outcomes from the artificial diet and corn leaves for the rearing., development and reproduction of *S. frugiperda* is due to their composition., in carbohydrates., amino acids., trace elements., fatty acids., vitamins and water Maldonado and de Polanía (2010)., Behmer., (2009) and chen *et al.*, (2020).

CONCLUSION

In summary., our findings demonstrated that *S. frugiperda* can be raised at a density of one larva per cub on the artificial diet used for *S. littoralis* in order to prevent cannibalism. Also., our findings underscore the critical role of dietary composition in determining the life cycle of *S. frugiperda*.

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تقييم الجوانب البيولوجية لدودة الحشد التي تم تربيتها على أنظمة غذائية صناعية مختلفة مقارنة بالغذاء الرئيسي

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الملخص

دودة الحشد الخريفية، *Spodoptera frugiperda* (J. E. Smith) هي آفة حرشفية الأجنحة غازية وتسبب خسارة اقتصادية فادحة للمحاصيل المختلفة، وخاصة الذرة. يعد تحسين منهجية تربية *S. frugiperda* ضروريًا للحصول على أفراد تجريبيين بكمية ونوعية كافية لاستخدامهم في دراسات الإدارة المتكاملة للآفات. في هذه الدراسة، قمنا بتقييم تطور *Spodoptera frugiperda* على أنظمة غذائية صناعية مختلفة تستخدم لأنواع أخرى من Lepidoptera مقارنة بمصدر الغذاء الرئيسي (الذرة). تم تقييم ثلاثة من الأنظمة الغذائية التالية: نظام غذائي صناعي يعتمد على الفاصوليا (D1)، نظام غذائي صناعي يعتمد على دقيق الذرة (D2)، نظام غذائي صناعي يعتمد على نخالة القمح (D3)، نظام غذائي يعتمد على أوراق الخروع (D4) مقارنة بنظام غذائي يحتوي على الذرة الخضراء كمصدر الغذاء الرئيسي. وكانت العوامل البيولوجية التي تم تقييمها هي فترة وبقاء اليرقات والعذارى؛ وزن العذارى من الذكور والإناث؛ نسبة الجنس والخصوبة وطول عمر الفراشات. تختلف بيانات البقاء والتطور والتكاثر لـ *S. frugiperda* التي نشأت من أفراد تمت تربيتهم على أوراق نبات الخروع عن تلك التي تتغذى على أوراق الذرة أو النظام الغذائي الاصطناعي. لا يوجد فرق كبير في عدد البيض المفقس بين اليرقات التي تتغذى على الأنظمة الغذائية الاصطناعية وتلك التي تتغذى على أوراق نبات الخروع مقارنة بالكنترول (الذرة الاخضر).