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RESEARCH ARTICLE

Comparison of Artificial Diets and Maize Leave on Growth and Development of Fall Armyworm, Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae)

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ABSTRACT

The fall armyworm, Spodoptera frugiperda (J.E.Smith, 1979), is one of the most important pests of maize in Vietnam as well as the greater Americas, causing up to 35% production losses when defoliation occurs near flowering or even complete destruction of plants. The use of artificial diets to mass-reared insect stock under laboratory conditions for Biological control of this pest is necessary. In this study, the comparison was made between four different diets and the control - maize leaves, which was natural food on the growth and development of S. frugiperda larvae and generated information on development time, larval and pupation survival, weight, length of larval, pupa, reproduction (fecundity) and adult longevity. In four artificial diets and maize leaves, The Diet 2 with ingredients inside consisted of white kidney beans (150 g), wheat germ flour (150 g), Methyl p-hydroxyl benzoate (5.0 g), Sucrose (28.0 g), Vitacap (6.0 g), Vitamin 3B (3.5 g), L-cysteine (1.2 g), Chloramphenicol (0.15 g), Ascorbic acid (3.0 g), Baking yeast (60 g) $CuSO4.5H2O$ (0.3 g) $MgSO4.7H2O$ (0.5 g) NaCl (0.5 g), Agar (20 g) and 700 ml distilled water showed the development from larval to pupal was 29.2 days, sex ratio (1:1.09), adult emergence (100 %), survival larval (95.66%), survival pupal (91.33%) and fecundity (1,618.8 eggs) as compared to other diets. Diet 2 can also be used in appropriate quantities to increase the number of female moth population and sustain mass rearing for research biological control.

INTRODUCTION

The Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), native to the Americas, is one of the important invasive pests of various crops in many countries (Goergen et al. 2015, Sharanabasappa et al. 2018; Jam et al., 2018). In Vietnam, it appeared for the first time in April 2019. Since then, FAW has spread rapidly throughout the country, with estimates that over 35,000 hectares of corn have been affected by FAW in 40 provinces (Fao and CABI, 2019; IPPC, 2019; Rashid et al., 2023). FAW damage has resulted in economic loss due to decreased yields and increased costs for insecticides and labor. Although using chemicals is the prevailing method to control this pest, problems such as ecological disequilibrium, pollution, risks during application, and high costs are present. Furthermore, the insecticides kill the fall armyworm's natural enemies, favoring rapid infestation with serious damage to the culture.

The continuous maintenance of laboratory coolly of insects is necessary for biological control and insecticide bioassay research. With the development of artificial diets, the mass rearing of insects has become greatly facilitated. Artificial diets are more convenient than natural because they are easy to handle and can produce many insects that can be reared with minimum time and labor. Using artificial diets will support better studies. Vanderzant et al. (1962) pioneered the study of artificial insect feed. In the following years, scientists discovered a variety of artificial forages for insects belonging to the Diptera, Lepidoptera, and Coleoptera order (Burton, 1967; Burton and Perkins, 1972; Cohen, 2001; Kanval et al., 2024; Gupta *et al*., 2005; Jam et al., 2013). Besides, Shorey and Hale (1965), Trinh Thi Xuan et al. (2016) developed an artificial diet for rearing the larvae of Noctuidae species such as *Spodoptera litura*, *Spodoptera exigua, Spodoptera littoralis*. The investigation aimed to determine and compare the feasibility of mass-rearing on insect larvae on artificial diets. This study tried artificial diets with different ingredients to rearing *S. frugiperda* in the laboratory.

MATERIAL AND METHODS

Biology of Insect

S. frugiperda larvae were collected from maize fields in the Mekong Delta of Vietnam and propagated in the laboratory at the Department of Plant Protection, College of Agriculture, Can Tho University. The larvae were reared on young maize fruits in an environmentally controlled growth chamber (at temperature 25 ±1^{0}C; relative humidity of 75%, photoperiod of 18hrs light/6hrs dark) until pupal stage. The young maize leaves were washed thoroughly with water flow and treated with 0.03% formaldehyde solution to avoid microbial infection before feeding. Pupae were collected and put in paper bags (2 x 5 x 6 cm) until the adult stage. Moths were fed with 10% sucrose and allowed to lay eggs. Newly laid eggs were sterilized with 3% formaldehyde solution for 10 minutes and washed with sterile distilled water. Hatched larvae were development studies.

Composition and preparation of artificial diets

The artificial diets used in this study are modifications of Trinh Thi Xuan et al., 2016; Pinto et al., 2019. The Compositions of the diets are described in Table 1.

Preparation of diet: soak beans with water for 4-5 hours, then grind them in 300 mL of water by a grinder until they become well-kneaded dough (each bean is divided). After that, the ground bean was poured into an autoclave bench and then hardly stirred with wheat germ flour, L-cysteine, baking yeast, Methyl *p*-Hydroxyl benzoate, ascorbic acid, $CuSO_4.5H_2O$, $MgSO_4.7H_2O$, $CaCl_2$, KH_2PO_4 NaCl and 300 mL of water until ensuring that it became a uniform material. Agar (20 g) and Chloramphenicol (0.15 g) were mixed in 100 mL water and boiled for 10 minutes before pouring into the autoclave bench containing the mixture made from the first step. The mixture was then hardly stirred and autoclaved at 110^{0}C for 10 minutes. Next, keep the artificial diet in the refrigerator until used.

Preparation of natural diet, green maize leaves. A local variety of maize, Bap Nu, was planted in plots at the greenhouse on a weekly interval as a source of larval food. Excised leaves were cut into small pieces, 4cm long, disinfected in 0.1% sodium hypochlorite for 20 minutes, and washed under sterile distilled water. Finally, these were blot-dried with sterile tissue paper before use.

Table 1: Composition of the artificial diets for *S. frugiperda*

Diet 1	Diet 2	Diet 3	Diet 4	Natural food
Soya bean (150g) Wheat germ flour (150g) Methyl p-hydroxyl benzoate (5.0g) Sucrose (28.0g) Vitamin 3B**(3.5g) L-cysteine (3.6g) Chloramphenicol (0.15g) Ascorbic acid (3.0g) Baking yeast (60g) $CuSO_4.5H_2O$ (0.3g) $MgSO_4.7H_2O$ (0.5g) NaCl (0.5g) Agar (12g) Distilled water (700ml)	White Kidney Beans, (150g) Wheat germ flour (150g) Methyl p-hydroxyl benzoate (5.0g) Sucrose (28.0g) Vitacap* (capsule) (6.0g) Vitamin 3B** (3.5g) L-cysteine (1.2g) Chloramphenicol (0.15g) Ascorbic acid (3.0g) Baking yeast (60g) $CuSO_4.5H_2O$ (0.3g) $MgSO_4.7H_2O$ (0.5g) NaCl (0.5g) Agar (12g) Distilled water (700ml)	Soya bean (140g) Wheat germ flour (120g) Methyl p-hydroxyl benzoate (4.5g) Vitacap* (capsule) (6.0g) Vitamin 3B** (1.5g) L-cysteine (1.2g) Chloramphenicol (0.15g) Ascorbic acid (3.0g) Baking yeast (72g) $CuSO_4.5H_2O$ (0.3g) $MgSO_4.7H_2O$ (0.5g) NaCl (0.5g) Agar (20g) Distilled water (700ml)	Soya bean (100g) Young Maize fruit (200g) Wheat germ flour (100g) Methyl p-hydroxyl benzoate (5.5g) Sucrose (30.0g) Vitacap* (capsule) (6.0g) L-cysteine (1.0g) Chloramphenicol (0.15g) Ascorbic acid (3.0g) Baking yeast (10g) $CuSO_4.5H_2O$ (0.3g) $MgSO_4.7H_2O$ (0.5g) NaCl (0.5g) Agar (20g) Distilled water (700ml)	Fresh maize leaves

***Vitacap**: vitamin A (Palmitate) BP 5000 IU, vitamin D3 (Cholecalciferol) BP 400 IU, vitamin B1 (Mononitrate) USP 5 mg, vitamin B2 USP 5 mg, vitamin B6 USP 2 mg, vitamin B12 (Cyanocobalamin) TC NSX 5 mcg, nicotinamide USP 45 mg, vitamin C USP 75 mg, D-Panthenol USP 5 mg, folic acid USP 1 mg, vitamin E (dl-alpha-tocopheryl acetate) USP 15 mg, dibasic calcium phosphate USP 70 mg, iron fumarate BP 50 mg, Mangan Sulphate USP 0.01 mg, zinc sulfate USP 50 mg, kali iodide USP 25 mcg, magie Oxide USP 0.5 mg, vanillin, lecithin, soya oil

****Vitamin 3B**: B1, B6, B12

Feeding test

This research was conducted at the Biological Control Laboratory, Department of Plant Protection, College of Agriculture, Can Tho University, Vietnam. The insects were kept under chamber (Sanyo MIR-553) conditions at 25 ±1^{0}C, relative humidity of 75%, and a photoperiod of 18 hours' light/6 hours' dark

For each diet, one hundred newly hatched larvae after 24 hours were selected and placed individually in a cup (3 cm high, 4.5 cm diameter) containing cubes of the diets (2 x 2 x 2 cm), replaced after 1-2 days or when necessary, to observe the larval, pupal, adult periods of FAW. The evaluation followed biological parameters, including larval developmental period, larval survival, pupal period, pupal survival, percentage of deformed pupal, sex ratio of emerged adults, and percentage of deformed adults. Individuals with malformations in the wings, legs, abdomen, or thorax and difficulty in emergence were considered deformed when adults were attached to the pupal exuviate.

Larval growth index = % pupation / Larval period (days)

Pupal growth index = % Adult emergence/ Pupal period (days)

Total developmental index = % Survival / Total developmental period (days)

Data analysis: All the data generated were subjected to statistical analysis (Finney, 1971) using SPSS Statistical Software, and the difference among the treatment means was compared by LSD at $p<0.05$.

RESULTS AND DISCUSSION

Effect of artificial diet and maize leaves on weight and length of *S. frugiperda*

For the larval stage, Table 2 showed that at the 2nd instar stage of FAW larvae, there was no significant difference in larval length between the diets and maize leaves from 7.7±0.9 to 8.9±0.4 mm. The maximum weight in this stage of FAW was 44.5±3.75 mg was recorded on Diet 2, followed by 42.9±2.25 mg on feeding maize leaves, 41.0±1.6 mg on Diet 4, 38.6±3.12 on Diet 3. Similarity on the 3rd, 4th, 5th, and 6th larval stage, the variation among 6th larval stage length was also observed ($P<0.05$) in which maximum 6th larval length of on 31.3±1.6 mm Diet 2 not statistically significant by 30.3±2.68 mm on maize leaves, followed by 29.3±1.0 mm on Diet 4; 27.9±0.95 mm on Diet 3 and 26.4±1.5 mm on Diet 1. The 6th larval weight was no statistically significant difference between Diet 2 (382.8±13.2 mg) and maize leaves (372.0±16.6 mg), but the difference compared with 308.5±13.4 mg on Diet 1 and 333.3±25.5 mg on diet 3 at $p<0.05$. Besides, the length of FAW pupal feed with all diets showed no significant difference, the date reached from 15.1±0.12 mm to 15.6±0.3 mm, and the weight of pupal from Diet 2 was 213.8±10.1 mg similar to Diet 3, Diet 4 and maize leaves (202.0±14.6 mg; 203.7±12.4 mg, respectively).

Table 2*:* Effect of different artificial diets on larval and pupal weight, length of FAW

Parameters	Diet 1		Diet 2		Diet 3		Diet 4		Maize leaves	
	Length (mm)	Weight (mg)	Length (mm)	Weight (mg)	Length (mm)	Weight (mg)	Length (mm)	Weight (mg)	Length (mm)	Weight (mg)
L2	7.7±0.9	35.1±4.45b	8.2±0.7	44.5±3.75a	8.6±1.0	38.6±3.12ab	8.9±0.4	41.0±1.6ab	8.7±0.3	42.9±2.25a
L3	8.7±0.6c	49.7±3.15c	10.8±1.2a	66.7±4.7a	9.2±0.5b	52.5±3.65c	9.8±0.45b	60.5±2.2b	10.3±0.6a	62.0±3.18a
L4	13.1±0.9b	129.3±12.15b	15.9±0.4a	152.4±15.7a	13.4±0.7b	134.4±11.3b	14.9±1.1ab	142.1±9.75ab	15.2±1.0a	148.6±21.7a
L5	17.8±1.2c	217.6±16.3b	20.3±0.9a	244.4±17.5a	18.2±1.2bc	223.6±23.1ab	19.4±0.6ab	233.1±20.4ab	20.1±1.65a	239.2±26.4a
L6	26.4±1.5c	308.5±23.4c	31.3±1.6a	382.8±13.2a	27.9±0.95bc	333.3±25.5bc	29.3±1.0ab	368.4±35.2ab	30.3±2.68a	372.0±36.6a
Pupal	15.2±0.2	186.3±12.3c	15.2±0.2	213.8±10.1a	15.1±0.12	198.7±13.4bc	15.3±0.24	202.0±14.6ab	15.6±0.3	203.7±22.4a

Means for each parameter followed by a common letter are not significantly different for comparison between treatments within each column (Anova, $P<0.05$); L: instar larval 2-6.

Effect of artificial diet and Maize leaves on growth and development of FAW larva.

Besides length and weight, the survival and development for these instar stages of *S. frugiperda* were investigated using four artificial diets and maize leaves (the main diet in natural). The statistical difference $P<0.05$ in the larval development period was highest 19.9±3.2 days in Diet 3 with 80.33% survival, while in maize leaves was 13.2±2.3 days with 96.3% survival and 13.7±1.8 days on Diet 4 with 90.33% survival, 17.8±2.78 days on Diet 2 with 95.66% survival, 18.6±3.45 days with 75.33% survival on Diet 1. This data pointed out how much food affects the development and survival of FAW larvae. This results in the point that these four artificial diets, such as soya bean, white kidney bean, soya bean mixed with young maize fruit with all the ingredients inside, and maize leaves, are sufficient for larval growth and development from the instar larva of FAW.

Table 3 Growth and development of FAW on artificial diets and maize leaves

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Maize leaves
Larval period (Days ± SD)	18.6±3.45b	17.8±2.78b	19.9±3.2c	13.7±1.8a	13.2±2.3a
Pupal period (Days ± SD)	6.3±1.3ab	5.6±0.4a	8.2±2.2c	7.9±1.15b	6.0±0.2ab
Egg period (Days ± SD)	3.6 ±1.2b	3.0±0.95a	4.2±0.8c	3.2±0.68ab	2.8±0.23a
Larval + pupal development period (Days ± SD)	31.7±5.5b	29.2±3.2b	35.9±4.25 c	27.5±3.1ab	24.8±2.5a

Means for each parameter followed by a common letter are not significantly different for comparison between treatments within each column (Anova, P<0.05)

The pupal period of Diet 2, maize leaves, and Diet 1 was not significant, 5.6±0.4 days, 6.0±0.2 days, and 6.3±1.3 days, respectively (p<0.005), while Diet 3 and 4 were longer significant (7.9±1.15 days in Diet 4, 8.2±2.2 days in Diet 2, respectively) than other diets. The egg periods were a statistical difference of p<0.005 between the diets, the lowest 2.8±0.23 days in maize leaves and the highest 4.2±0.8 days in Diet 3. On the other hand, in terms of total development (Larval duration + Pupal period), the four diets were not equally efficient (24.8±2.5 days in maize leaves, 27.5±3.1 days in Diet 4; 29.2±3.2 days in Diet 2, 31.7±5.5 days in Diet 1 and 35.9±4.25 days in Diet 3. Therefore, the growth and development of FAW in the artificial diets of soya beans mixed with young maize fruits (Diet 4) and maize leaves was shorter than observed by Pinto et al. (2019) on their corn-base diet and similar to Marcela et al. (2021) on Insect Mediab^{R} diet. However, the development of FAW in Diet 1 and Diet 2 compared their results to be similar to Giong et al. (2015) and Karthik et al. (2015), who reported a larval period of 17.25 days while Lekha et al. (2020) reported laval periods between 14.0 days to 18.5 days on many kinds of beans such as cowpea, chickpea, soybean.

Table 4. Percentage larval, pupal survival, adult emergence, sex ratio, and growth indes of FAW on artificial diets and maize leaves

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	maize leaves
Larval survival (%)	75.33	95.66	80.33	90.33	96.31
Pupal survival (%)*	69.67	96.33	68.67	72.68	97.67
Sex ratio (♂/♀)	1:1.07	1:1.09	1:1.04	1:1.03	1:1.08
Adult emergence (%)	100.0	100.0	93.12	89.33	97.33
Laval growth index	4.04	5.34	4.06	6.56	6.84
Pupal growth index	11.90	16.67	9.76	11.39	14.52
Total developmental index	4.01	5.75	4.23	5.30	5.88

*: The number of individuals used in this survey was 100 larvae

There was a statistically significant difference between the larvae survival of larvae using artificial diets. The survival of larvae on maize leaves (96.31%) was higher than in other diets. The pupal survival was also significantly higher on maize leaf diets (97.67%) than Diet 2 (96.33%), Diet 4 (72.68%), Diet 1 (69.67%), and Diet 3 (68.67%), respectively. In this case, the sex ratio of all diets in this present study was similar to conventional, from 1:1.08 to 1:1.03. The FAW larval growth index was found on natural maize leaf food (6.84) and the lowest in Diet 1 (4.04). Similarly, the pupal index was higher in Diet 1 (16.67); next is maize leaves (15.42), respectively.

Gupta et al. (2005) reared *S. litura* larvae on a Rajma-based diet. They reported percentage pupation and percentage emergence to be 89.2% and 97.2%, respectively, similar to our studies. The larval (4.31) values and pupal growth index (9.92) were higher because the S. *frugiperda* (6 instars) takes a lesser larval period than the *S. litura* (5 instars).

Table 5 Fecundity of FAW Smith reared on artificial diets and maize fruit

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	maize leaves
Deformation rate (%)	17.03	3.2	12.4	18.1	18.6
Fecundity (egg/female)	1,111.68±189	1,618.8±323	715.09±272	1,140.8±305	1,445±356
Hatch (%)	76.17	93.33	43.4	79.23	89.67
Female (Day)	9.8	11.9	9.3	9.2	12.5
Male (Day)	9.2	10.2	9.1	8.8	11.1
Egg incubator time (Day)	3.6 ±1.2	3.0±0.95	4.2±0.8	3.2±0.68	2.8±0.23

**: The number of individuals used in this survey was 100 larvae*

Other parameters, such as sex ratio, adult emergence percentage, fecundity, and hatching, were recorded. The egg periods in Table 5 were statistically different $p<0.005$ between the diets, the lowest 2.8±0.23 days in maize leaves and the highest 4.2±0.8 days in Diet 3 to 4.2±0.8 days. The average number of Eggs laid by females reared on Diet 2 and maize leaves were 1,618.8±323 and 1,445±356 eggs. Marcela et al. (2021) studied a standardized artificial diet (Insect Mediab^R)-based diet on S. *frugiperda* and recorded a fecundity of 1,471.6 eggs, while Karthik et al. (2022) reported a fecundity On diets D2 (corn-based) and D4 (soybean-based), the fecundity found was 1124 and 526.07 eggs/female, respectively for the same FAW. Murua et al. (2008) showed 955.05 and 519.83 eggs/female for populations reared on maize and soybean, respectively.

In this study, the percentage of eggs hatched was 93.33% at Diet 2; the adult life was also recorded in four diets with honey solution at 3%, and female longevity of four food resources compared to that of male. Feeding with maize leaves, the lives of females and males were 12.5 and 11.1 days, respectively. The females of Diet 1 had 9.8 days life span and of males 9.2 days; Diet 2 had 11.9 days life span and males 10.2 days; Diet 3 had 9.3 days life span and males had 9.1 days, Diet 4 had 9.2 days life span and that of male 8.8 days. The data from this study showed that rearing FAW has been well-developed and used successfully on white kidney beans rather than soya beans.

CONCLUSION

This study concluded that the artificial diet 2 is more conducive to rearing *Spodoptera frugiperda* in a laboratory at a temperature of 25 ±1^{0}C, relative humidity of 75%, and photoperiod of 18hrs light/6hrs dark. This diet supplies the protein resource from white kidney beans for mass rearing FAW, an invasive insect pest on many tropical crops, especially maize or paddy rice. Based on the results, to preserve the biological parameter of FAW, the procedure for mass rearing can be provided; it is possible to create many research studies related to *S. frugiperda* in the laboratory.

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