EFFECT OF REARING CONTANERS ON THE DEVEOPMENT OF FALL ARMYWOM (*Spodoptera frugiperda* Smith) LARVAE FED WITH ARTIFICIAL DIET

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ABSTRACT
Fall armyworm (Spodoptera frugiperda Smith) is an invasive insect that causes a lot of damage to maize in Nigeria. Mass rearing of the insect was carried out in the insect rearing laboratory of the Department of Crop Protection, Faculty of Agriculture, Ahmadu University Zaria, Using different rearing containers. The containers used were plastic cups, plastic vials and plastic dishes which were replicated 10 times and arranged in a Completely Randomized Design. Neonate larvae obtained from the laboratory colony were fed with artificial diet until pupation. The result of the experiment had shown that duration of larval development, larval length and larval weight were significantly (P< 0.05) the highest for the larvae reared in plastic vials, followed by those reared in plastic cups; and the least was recorded for the larvae reared in plastic dishes. Similarly, females of which their larvae were reared in plastic vials had significantly (P< 0.05) the highest fecundity compared to those reared in plastic cups and plastic dishes. Higher fecundity is very vital for mass rearing of fall armyworm larvae. Therefore, plastic vials are the most ideal containers for mass rearing of fall armyworm larvae.

Key words: containers, fall armyworm, larvae, rearing, vials

INTRODUCTION

Fall armyworm (FAW) (*Spodoptera frugiperda* Smith) is an invasive and damaging pest native to tropical and subtropical America. The pest arrived in to Sub-Saharan Africa (SSA) in 2016 at a time when the region is challenged to feed its rapidly growing population, which has now spread throughout the continent, reaching Asia, and therefore, global (Geogen *et al*, 2016; Hruska, 2019; Kassie *et al*.,2020). It has a wide host range. It was reported that the larvae of fall armyworm attack 353 host plant species belonging to 76 plant families with greatest number in family Poaceae (106 texa), Asteraceae and Fabaceae (31 texa each) Mentezaro *et al*., (2018),

How the pest was introduced into Africa from its native habitat in the Americas is unclear. However, such invasive pests as the fall armyworm are known to across continents either through infested commercial grain or through jet streams across oceans. Many fall armyworm moths have been collected in the Gulf of Mexico as far as 250 km from land, indicating the possibility of seasonal trans-Gulf migration between the United States and the tropics. It is particularly hard to control, as the moths are strong flyers, breed at an exponential rate, and the larvae can feed on a wide variety of plant species. In addition, it can quickly develop resistance to pesticides if they are not used judiciously. The larvae burrow into

the growing point of the maize plant and destroy it or clip the leaves. They also burrow into the ear and feed on kernels. (CIMMYT, 2016).

Already, the impact of the fall armyworm is being felt across Africa. Estimates from 12 African countries indicated that the pest is causing annual maize losses of between 8 – 21 million tonnes, leading to monetary losses of up to US$ 6.1 billion, while affecting over 300 million people in Africa, who, directly or indirectly, depend on the crop for food and well-being. The pest's impact is likely to be even higher when its damage on other crops is quantified (ICIPE, 2017). FAW-induced economic impacts can include reduction of income due to reduced maize sales; reduced food consumption because of reduced food availability from both crops and livestock, as crop residues are a major livestock feed source in rural areas; higher medical treatment expenditure for people exposed to insecticides and environmental damage related to insecticides contamination (Denberg and Jiggins, 2007; Midingoyi et al., 2018).

However, the biology of the pest in Africa was poorly documented compared to several studies of its biology in its native America, where different containers like vials, cups and dishes (Bokonon-Ganta *et al*., 2003; Sharanaba *et al*., 2018; Prasanna *et al*, 2018) were used for such studies. Therefore, this study is to determine which of the containers is the most suitable for rearing FAW individually.

3.3.3 Rearing on artificial diet

The rearing was carried out using the ingredients adopted from International Centre for Maize and Wheat Improvement (CIMMYT) which was adopted from Teferal*et al*. (2011).

Table 3.3: Composition of the diet

S/N	Ingredients	Quantity(g/ml)/1L diet
	Fraction A	
1	Maize powder	25.2 g
2	Cowpea powder	88.4 g
3	Brewers' yeast	27.7 g
4	Ascorbic acid	2.5 g
5	Sorbic acid	1.3 g
6	Methyl-p-hydroxybenzoite	2.0 g
7	Multivitamins	2.1 g
8	Sugar	35.1 g
9	Distilled water	403.1 ml
	Fraction B	
10	Agar (Tech no 3)	25.3 g
11	Distilled water	403.1 ml
	Fraction C	
12	Formaldehyde 40 %	2 ml

3.3.3.1 Preparation of artificial diet

The powdered ingredients in fraction A except methyl-p-hydroxybenzoite were mixed using a clean plastic spoon in a clean plastic container. Distilled water was added and thoroughly mixed with blender for 2 minutes. Methl-p-hadroxylbenzoite was then added to the mixture and blended for another 2 minutes. In a separate container agar powder was mixed with distilled water and blended for 2 minutes. The mixture was then boiled for 3 minute and thoroughly stirred using blender, after which it was allowed to cool and then added to the mixture of fraction A and both were thoroughly blended. Finally formaldehyde was added to the mixture of A and B and then blended for 2 minutes. The diet was later dispensed into the plastic containers

3.3.4 Protocol for rearing on artificial diet

3.3.4.1 Bulk rearing of *Spodopterafrugiperda* larvae

Forty gram of the diet was poured into a plastic jar (height; 16 cm; bottom dia, 11 cm; top dia,(10cm) with holes were punched on the solidified diet. One hundred neonate larvae were transferred into the jar using camel hair brush, and covered with tissue paper and tied with a cover lid. The jar was inspected daily for changes in the larval instars.

Rearing *Spodopterafrugiperda* individually in different containers

When most of the larvae (> 50%) had attained 2nd instar larval stage, they were selected and transferred individually into different containers to be reared

3.3.5 Research Design, Treatments and Replications Three different containers (cups, vials and dishes) were used for rearing the larvae individually to avoid cannibalism and each of the containers was regarded as a treatment and replicated ten times to give a total of thirty containers. All treatments were laid in a Completely Randomized Design (CRD).

The containers were

(I) C1-Plastic cup(9 cm, height;4 cm, bottom dia;7 cm top dia)

(ii) C2- Plastic vial (10 cm, height; 2.5 cm bottom dia; 2.5 cm top dia)

(iii) C3-Plastic dish (5 cm, height;6.5, bottom dia; 8 cm top dia)

Plate II : Rearing larvae individually in plastic cups

Plate III: Rearing larvae individually in plastic vials

Plate IV: Rearing FAW larvae individually in plastic dishes

3.3.5.1 Management of larvae

The 2rd instar larvae were placed individually in each container with 10 g of the diet inside and place in the laboratory benches under ambient temperature (max 30 ºC± 5 ºC; min 20°C ±5°) and relative humidity (80 % ±5%), with day light ratio of 13L: 11D.The larvae were observed daily and viewed under hand lens (Ø 90mm) for developmental changes. Sixth instar larvae were weighed using Citizon C4 204.

Management of pupae

When the larvae developed into pupae, they were removed from the container and placed into three separate small plastic containers (6 cm x 6.5 cm x 9 cm),which were lined with tissue paper at the bottom. The containers were placed in the oviposition room, and observed daily for the presence or absence offungal contamination till adult emergence

Management of the Adults

Newly emerged adults were paired (male and female) and carefully transferred into three different plastic buckets representing a treatment. Eachbuckets were lined with A4 white paper for the female as oviposition sites. Two petridishes were kept inside each bucket; one was half filled with water containing cotton wool inside to provide adequate humidity and the other was half filled with 5 % sugar solution as diet for the moths. The buckets were placed

inside the ovipostion room and monitored. Both the water and the sugar solution were changed after every two days to maintain their freshness and to avoid contamination.

Management of eggs

When the eggs were laid on the white paper, they were carefully counted and cut using scissors and placed inside three different plastic containers (9 cm x 8.5 cm x 7 cm). The containers were covered with tissue paper and tied with cover lid. The three containers were transferred into the oviposition room and monitored till the eggs were hatched.

Data Collection

The following parameters were recorded during the rearing process; Duration of larval stages,Length of larvae, Weight of larvae,Weight of pupae,Duration of pupation,Larval survival, Pupal survival, fecundity and Adult life span

Data Analysis

The data of the two experiments were combined, transformed and evaluated by Analysis of Variance (ANOVA). The means were separated using Least Significant Difference (LSD) at 5 % level of significance and the Statistical Analysis System (SAS version 9) packagewas used for the analysis.

RESULTS

The larval development of FAW on artificial diet using different rearing containers was reported in Table 4.16. The duration of first larval instar was the highest in plastic vials (4.0±0.27 days) and differed significantly (P<0.05) with what was obtained from the plastic cup (3.3±0.00 days) and plastic dish (3.0±0.27 days). A Similar trend was observed in the second, third, fourth and fifth instar larval instars. The duration of the final stage (6th instar) was the highest in plastic vials, plastic vials (5.0±0.00 days), followed by plastic dish (4.3±0.27 days) and plastic cup (4.0±0.27 day).which were themselves at par

The results of larval length and larval weight are shown on Table 4.17, length of larvae reared in plastic vial was the highest (41.1±0.27 mm) and was significantly different from those reared in plastic cup (39.0±0.27 mm) and plastic dish (38.5±0.19 mm). Similar trend was observed in larval weight, where it was the highest for the larvae reared in plastic vial had the highest weight (0.43±0.01 g).followed by those in plastic cup (0.39±0.01 g) and plastic dish (0.39± g). Pupal length and weight were also the highest for the pupae reared in plastic vials (17.5±0.27 mm and 0.33±0.01 g respectively) and are significantly different (P<0.05 %) from those reared in plastic cup (17.0±0.01 mm and 0.2±0.01 g respectively) and plastic dish (16.3±0.19 mm and 0.20±0.01 g respectively).

The percentages of larval and pupal survival were the highest in plastic vial (83.3±0.27 % and 86.7±0.27 % respectively) (Table 4.18), followed by those in plastic cup (76.7±0.27 % and 76.7±0.27 % respectively), while the lowest percentages of larval and pupal survival were recorded in plastic dish (73.3±0.27 % and 73.3±0.27 %).There was no significant difference (P<0.05) between plastic cup and plastic cup. Male and Female moths reared in plastic vials lived longer (15 7±0.27 days and 18.7±0.14 days respectively).While Males and females reared in plastic Cup lived for 14.3±0.27 days and 17.7±0.27 days respectively, which was not significantly different with those reared in plastic dish (14.7±±0.27 days and 17.7±0.27 days respectively). Females reared in the plastic vials had the highest fecundity (1210.3±0.16), followed by those reared in plastic dish (1133.0±33.77) and the least fecundity was recorded on those reared in plastic cup

(1109±18.32), and was significantly lower than plastic vial (P<0.05).

The duration of the life cycle of FAW is shown on Table 4.19 and fig 4.15. The incubation period obtained from plastic cup (4.3±0.27 days), plastic vials (4.0±0.47 days) and plastic dish (4.4±0.00 days) and there was no significant difference (P<0.05) among them. Duration of larval development in plastic vials was the highest (23.0±0.27 days), followed by those in plastic cup (20.5±0.19 days) and plastic dishes (20.3±0.19 days). Pupation was the highest in plastic cup (14.3±0.14 days), while it took those in Plastic vials and plastic dishes 11.3±0.14 days and 11.3±0.47 days respectively. The result, therefore showed that the moth spent the highest number of days when reared in plastic vials (38.0±0.00 days), followed by plastic cup (36.7±0.27 days) and plastic dish (36.3±0.27 days). There were significant differences (P<0.05) among the three rearing containers.

Table 4.16: Effect of rearing containers on larval development of fall armyworm reared on artificial diet

Treatments	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar
Plastic cup 4.0±0.27b	3.0±0.27b	3.3±0.27b	3.0±0.00ab	3.0±0.27b	3.6±0.27ab	
Plastic vial 5.0±0.00a	4.0±0.27a	3.7±0.00a	3.7±0.00a	4.3±0.00a	4.3±0.27a	
Plastic dish	3.3±0.00b	3.3±0.00b	3.3±0.27b	3.3±0.27ab	3.3±0.00b	4.3±0.27b
CV	15.12	14.02	13.46	13.76	12.70	12.30
SE±	0.50	0.47	0.40	0.40	0.39	0.38

Means in the column accompanied by the same letter(s) are not significantly different (P< 0.05), using Least Significance difference (LSD).

Table 4.17: Effect of rearing containers on length/weight of Larvae and Pupae of fall armyworm reared on artificial diet

Treatments	Larval length (mm)	Larval weight (g)	Pupal length (mm)	Pupal weight(g)
Plastic cup	39.0±0.27b	0.39±0.01b	17.0±0.27b	0.20±0.01b
Plastic vial	41.17±0.27a	0.43±0.01a	17.5±0.27a	0.33 ±0.01a
Plastic dish	38.50±0.19b	0.39±0.01b	16.33±0.19b	0.20±0.01b
CV	4.44	5.69	3.98	6.95
SE±	1.76	0.02	0.68	0.02

Means in the column accompanied by the same letter(s) are not significantly different (P< 0.05), using Least Significance Difference (LSD).

Table 4.18: Effect of rearing containers on larval and pupal survival, adult lifespan and fecundity of fall armyworm reared on artificial diet

	Larval survival (%)	Pupal survival (%)	Adult lifespan (days)		Fecundity
			Male	Female	
Platic cup 1109.7±18.32b	76.7±0.27b	76.7±0.27b	14.3±0.14b	17.7±0.27b	
Plastic viall 1210.3±1.63a	83.3±0.27a	86.7±0.27a	15.7±0.27a	18.7±0.14a	
Plastic dish 1133.0±33.77b	73.3±0.27b	73.3±0.27b	14.7±0.27b	17.7±0.27b	
CV	7.42	7.32	3.88	5.56	15.07
SE±	5.75	5.80	0.58	1.0	35.9

Means in the column accompanied by the same letter(s) are not significantly different (P< 0.05), using Least Significance Difference (LSD).

Table 4.19: Effect of rearing containers on the life-cycle of fall armyworm reared on artificial diet

Treatments	Egg incubation Period (days)	Duration of larval development (days)	Duration of pupal development (days)	Life- cycle (Egg-Adult(days)
Plastic cup 36.7±0.27b	4.3±0.27a	20.5±0.27a	12.3±0.41a	
Plastic vial 38.0±0.00a	4.0±0.47a	23.0±0.27b	11.3±0.14b	
Plastic dish 36.3±0.27c	4.4±0.0a	20.3±0.19b	11.3±0.47b	
CV	16.22	4.67	5.42	1.29
SE±	0.67	0.63	0.63	0.47

Means in the column accompanied by the same letter(s) are not significantly different (P< 0.05), using Least Significance Difference (LSD).

DISCUSSION

The larvae of FAW had shown cannibalistic tendencies the field (Samento, 2002; Farias, 2001) and when reared in the laboratory (Zenner-de-Polania et al., 2009; Gousan et al., 2001). To avoid this habit exhibited by the pest especially by the 3rd – 4th instar larvae, it was reared individually in different containers in different countries other than Nigeria by different researchers, such as glass tube (Modolon *et al*., 2017), petridishes (Pinto *et al*., 2019), plastic cups (Bokonon-Ganta *et al*.,

2003; Dasilva and Prasanna, 2013).breeding dish (Sharanaba *et al*., 2018), and vials (Prasanna *et al*, 2018).

This study revealed the influence of containers to the development of larvae during mass rearing of FAW, where six larval instars were recorded. The result was in conformity to what had been discovered by Pinto *et al*. (2018) and Leckah *et al*. (2018). The first instar larvae in plastic vial spent the highest number of days, which were 4 days. While it spent 3 days in the plastic cup.of all the larval instars, the sixth instar spent more days in the different containers. Five days was the highest number of days by the 6th instar larva, which was in the plastic vials, which was higher than what it has spent in plastic cup and plastic dish. Prasanna *et al* (2018) recorded 3.7 for the sixth instar larvae, which was lower than what had been recorded in this study. This could be attributed to the fluctuation of Temperature during the study. Lower temperatures tend to slow down the development of the larvae as reported by Leckha *et al* , (2020). The slower development in the plastic vial may not be unconnected with the condition of the diet inside, which remained moist and softer than the diet in the plastic cups and plastic dish. According to Pannizzi and Parra (2009), food quality is influence by physical attributes (hardness, surface pilosity and shape) that interfere with insect capacity to consume and digest the food, and consequently have a good quality larvae and pupa..

Larval length and larval weight were the highest for the larvae reared in the plastic vials and could be attributed to the water content of the diet, that remained higher throughout the rearing period when compared to the other two containers. Plastic vials are narrower in shape and the diet remains moist for longer peiod. Similarly pupal length and pupal weight were higher for the pupae obtained from larvae reared in plastic vials, compared to those in plastic cups and plastic dish. This quality was needed as Bernardi *et al*. (2014) reported that the heavier the pupa is, the higher the fecundity. Similarly, according to Wang et al (2020), there is positive relationship between weight of female pupae and fecundity of *Spodoptera frugiperda*.

Larval and pupal survival were also the highest from those reared in the plastic vials, and was within the range of 80 -90 % reported by different researchers (Mentezano et al, 2016; 2014b, 2015b; Specht and Roque-Specht, 2016), who showed that a survival rate above 75 % should be provided by an artificial diet.

Generally female life spans were higher than the male life spans and were higher than higher than those adults obtained from the larvae reared in plastic cup and plastic dish. Therefore, the survival rate recorded in this study was higher than what was reported by several scientists (Mentezano *et al*., 2019, Lekha *et al*., 2020, and Pinto *et al*., 2019). However it was at par with what was reported by Prasanna *et al*. (2018) where a range of 7-21 days was reported as ideal.

Highest fecundity was obtained from the female, which has their larvae reared in the plastic vials, though lower than what Pinto *et al*. (2018) reported ranging from 1600- 1800 eggs / female. Females that had their larvae reared in the plastic cup had the lowest fecundity. The high fecundity of female FAW, which was recorded in the plastic vials was as a result of the quality of the pupae obtained initially. This was because the quality of the food remained high for longer period, and this, according to Brawne (1995) affects the fecundity and dispersion of the adult.

Finally, the result of the total period of the life cycle had shown it was affected by the different containers used in rearing FAW in an artificial diet. Although the same type of the artificial diet was used, different values for the stages of the insect's life cycle were recorded. In all the three containers incubation period was a little bit higher than what was reported by Mentezero *et al*. (2019) that was within the rage of 2-3 days. The differences could be as a result of Temperature fluctuation during the rearing period. Because the lower the Temperature the higher the incubation period (Prasanna *et al*. (20180

Similarly the number of days that took the larvae to develop varies in the different containers. The longest was obtained in the plastic vial and the shortest period was recorded in the plastic dish.. However, the development period recorded in this study was longer than what was reported by Boregas *et al*.(2013) and Mentozero *et al* (2019). But Prasanna *et al* (2018) reported that larval development period may reach up to 30 days in cooler periods and this might have played well in this study, as a result of cooler night experienced during the rearing period of this study.

Consequently at the end of the rearing process, the number of days for FAW to complete it life cycle was the highest in which the larvae were reared in the plastic vials, and the shortest was recorded in which the larvae were reared in plastic dish. The result of this study had revealed that heavier larvae and pupae, and consequently female of higher fecundity were obtained when the larvae were reared in plastic vials. While shorter duration of the life cycle was recorded from the larvae reared on plastic dish.

In conclusion, the study had shown that plastic vial was the most suitable for rearing FAW individually, because it has resulted in obtaining heavier larvae and pupae, which gave females of highest fecundity. High fecundity is a trait needed for mass production of FAW larvae needed some other experiments on the pest.

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