RESPONSE OF COWPEA POD BORER, *Maruca vitrata* FABRICIUS (LEPIDOPTERA: CRAMBIDAE*)* LARVAE TO SPROUTED COWPEA SEEDS

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SUMMARY

The effect of two sprouted cowpea seeds varieties (SAMPEA 7 and 'Dan Shika') and artificial diet (control) on growth and development of *Maruca vitrata* was determined for two generations (1st and 8th) in the laboratory. The aim was to identify a cheap source of diet for laboratory rearing of *M. vitrata* for scientific research. Fifty grams each of the sprouted cowpea seed varieties and artificial diet were infested with twenty-five 1st instars larvae of *M. vitrata* which were allowed to develop to adult stage. Data was collected on the growth and developmental parameters of the insect. The result indicated that the mean percentage larval survival in artificial diet was significantly higher ($p<0.05$) than the larval survival on SAMPEA7 and 'Dan Shika' sprouted seeds. The mean weight of larva on SAMPEA 7 sprouted seeds and artificial diet were at par ($p>0.05$) but significantly higher ($p<0.05$) than 'Dan Shika' sprouted seeds. The mean percentage pupa survival in artificial diet and SAMPEA 7 sprouted seeds were at par but were significantly higher ($p>0.05$) than 'Dan Shika'sprouted seeds. The mean percentage pupal survival, larval survival and weight gain of the 8th generation was significantly higher ($p<0.05$) than the 1st generation. There was no significant difference ($p>0.05$) in mean percentage egg hatchability among the different *Maruca* diets and SAMPEA 7 sprouted seeds had the highest benefit-cost ratio (BCR) than other *Maruca* diets. The result indicates that SAMPEA7 sprouted seeds was as good as

artificial diet in rearing *M. vitrata*, thus could be used as an alternative to artificial diet.

Keywords: Cowpea, Sprouted, Maruca, Larvae, Podborer

COWPEA [*Vigna unguiculata* L (Walp)] is an important leguminous crop, grown and utilized in the semi-arid region of the world. In Nigeria the crop is mainly grown for its seeds, which are high in proteins (25%), several vitamins and minerals (1). The leaves and immature pods of the crop are consumed as a good source of nutrients and food security (2). One unique advantage the crop has over other crops is its ability to fix atmospheric nitrogen (3) thus requires a few mineral inputs for its cultivation. It grows and covers the top soil which in turn prevents soil erosion (2). These characteristics make cowpea a valuable crop for resource poor farmers.

In spite the nutritional and economic importance of cowpea, pests and diseases are generally major constraints to its production, causing yield losses of over 70% especially in untreated fields (4). *Maruca vitrata* (Lepidoptera: Crambidae) is one of the major field insect pests of cowpea and can cause between 40 to 90% yield losses (5); (6) especially in untreated fields. Various control options such as insecticides, cultural practices, biological and host plant resistance methods are used in managing *M. vitrata* infestation (7), although some of these methods are either not providing adequate control of the pests or are posing a threat to human and environmental health (8). Recently an environmentally friendly control option for *M. vitrata* based on a host plant resistance cowpea embedding a *Bacillus thuringiensis* (*Bt*) gene expressing Cry1Ab endotoxin has been developed (9). The essence was to provide a new control option for the pest. However, a successful development of such crop variety entails screening of the crop under high insect pressure with the aim to ascertain its resistance potential. This largely relies on consistent mass supply of healthy insect larvae which in turn depends on insect rearing diets (10). *Maruca vitrata* has been mass reared using several diets. The first rearing of *M. vitrata was* on fresh cowpea flowers and pods (11). Although this was the natural diet of the pest, the method was found not to be cost and labour effective for mass rearing of *M. vitrata* as it demands a larger land area for continuous cultivation of large fresh flowers and pods. Artificial diet containing different vital ingredients good for development of *M. vitrata* was developed by (12); (13); (14) to ease mass rearing. Since then, mass rearing of *M. vitrata* in the laboratory has largely relied on use of artificial diet with great success (15, 16). However, one of the major challenges with artificial diet is that, it is very expensive as ingredients for preparing a liter of diet can cost as high as $120/L which translates to ₦43,200/L. Moreover, some of these ingredients are not locally available which adds to the cost. This suggests the need for alternative cost-effective diets or ingredients for effective mass rearing of *M. vitrata*. A preliminary test and personal communication have shown that larvae of *M. vitrata* could be fed on sprouted cowpea seeds, thus providing a basis for a simple and cheap rearing diet. The objective of this study was to evaluate the effect of sprouted cowpea varieties on growth and development of *M. vitrata* as a cost-effective diet suitable for laboratory rearing of uniform *M. vitrata* of good vigor and reproductive capability.

MATERIALS AND METHODS

Establishment of *Maruca vitrata* colony

Maruca vitrata colony was established for the experiment at the insect rearing laboratory of the Crop Protection Department (CPD), Ahmadu Bello University, Zaria under a controlled laboratory condition of 25ºC ± 2ºC and 50 - 60% Relative humidity. The starter *Maruca* colony was obtained from a stock colony maintained at the CPD insect rearing laboratory. Mass rearing commenced with twenty pupae each placed in four opened petri dishes and later transferred into a wired mesh cage (30 x 30 x 30 cm). The cages were placed on top of a laboratory bench for adult emergence. The adults that emerged inside the cage were fed using filter paper moistened with 5% sugar solution. Four days after emergence, female moths were identified using their swollen abdomen containing eggs and were picked in groups of four into a transparent plastic cup (3cm diameter × 35cm depth) and kept for 24hrs to allow for oviposition (egg laying). Twenty-four hours later, the cups carrying eggs were kept under the same experimental conditions until the eggs hatched to larvae. A 24-hour old hatched neonate larva was used for the bioassay experiment.

Preparation of sprouted cowpea seeds

Two cowpea varieties seeds were used for the experiment; SAMPEA 7 and 'Dan Shika'. SAMPEA 7 was obtained from cowpea breeding unit of the Department of Plant Science, Institute for Agricultural Research ABU, Zaria and 'Dan Shika' was purchased from Samaru market, Zaria.

300g each of the cowpea varieties were weighed, damaged seeds were sorted out and removed, then the remaining seeds of each was soaked in 4 different 1liter arenas using 1000ml of clean water and then 1% (10 ml) sodium hypochlorite was added to prevent growth of mold before, during and after germination. The soaked seeds were left on laboratory bench for 4 hours, after which the water was drained using a sieve and rinsed with clean water. The drained seeds were sterilized in a micro flow for 30 minutes and excess moisture was also removed. Each cowpea variety seeds were used as treatment and were further divided into three different arenas containing 50g each to serve as repetitions of the treatments. Tissue paper was placed under each arena to absorb excess moisture. The arenas were taken to infestation room where seeds germinated (sprouted) within 24hrs for germination and used for larval infestation. Artificial *Maruca* diet prepared according to method of (14) and was used as control. All the ingredients used for preparing the artificial diet were purchased from IITA Ibadan, Nigeria in strict adherence to their protocol.

Infestation of *Maruca* Larva into the treatments

A total of three treatments were used comprising of two different sprouted cowpea varieties and artificial diet (control). Fifty grams each of the *Maruca* artificial diet and the sprouted cowpea treatments were arranged on laboratory bench in a completely randomized design (CRD) with three repetitions in the infestation room. Twenty-five 1st instars larvae of *M. vitrata* were infested into each of the treatment and larval development, pupal and adult emergence were observed and recorded.

Analysis of benefit cost ration of *Maruca* diet

The economics of using different diets was worked out using cost and mean larval output for each diet following a model used by (17). The larval output was determined by infesting 1000 1st instar larvae each into either 1L of artificial diet or 1kg of each sprouted cowpea seeds variety and then the number that enters pupal stage was counted and recorded at 12 days of infestation. The analysis was done based on prevailing market price of cowpea (N600/kg), cost of artificial diet (N45, 000/L) and larvae (N200/larva). Total Cost of larvae was obtained by multiply cost per larvae by total live larval output for each diet. Net benefit for each diet was obtained by subtracting cost of diet from total cost of larvae. Benefit of each sprouted cowpea variety over artificial diet was obtained by subtracting total cost of artificial diet from net benefit for each sprouted diet. The BCR was obtained by dividing the benefit of each sprouted cowpea by cost of preparation of each diet.

Data Collection

Data were collected on larval survival, larval weight, pupal survival, pupal weight and developmental period from larva to adult for up to 8^{th} generations. Number of surviving larvae were observed and recorded 10 days (5^{th} instars) after infestation and dead ones were discarded immediately. Weight of 10 randomly selected surviving larvae in each treatment was taken and recorded across the three repetitions. The same procedure was followed for pupal weight and survival, where 10 pupae were considered and the number that survived to adult were recorded. Data were also taken on effect of the treatments on percentage eggs hatchability in which fifty eggs laid by each of three adult females that emerged from each of the treatment were observed for hatching. The total number of eggs hatched within 7days were observed and recorded. To record the larval, pupal and adult developmental period, the number of days taken by 10 larvae to pupal stage was recorded. Similarly, the number of days taken by 10 pupae to emerge as adult was also recorded and then average of each was taken to get the mean developmental period. To know the longevity of the adult life, 10 unsexed newly emerged adults were observed on daily bases and the dead ones were counted and recorded until they all died. Data obtained was subjected to analysis of variance (ANOVA) using SAS software to determine significant difference and mean were separated using Duncan multiple range test at 5% level of probability.

RESULTS

The result in Table 1 indicates that there was a significant difference in percentage mean larval survival and weight among the different diets. Artificial diet had the highest mean percentage larval survival which was significantly ($p<0.05$) higher than percentage mean larval survival of SAMPEA7 and 'Dan Shika'sprouted seeds which were at par. In terms of larval weight, the artificial diet and SAMPEA 7 had the highest mean larval weights (85.0mg and 83.8mg respectively) and both were significantly ($p<0.05$) higher than the mean larval weight of 'Dan Shika' (72.3mg). The mean percentage larval survival of 8^{th} generation was significantly higher ($p<0.05$) than the 1^{st} generation. There was no significant ($p>0.05$) difference in weight between larva of 1^{st} and 8 generation.

Table 1. Mean larval survival and weight of *Maruca vitrata* reared on sprouted cowpea seed varieties over two generations

Treatments	Mean larval survival (%)	Mean larval weight (mg)
Maruca diet (MD)		
SAMPEA 7	71.2b	83.8a
'Dan Shika'	71.0b	72.3b
Artificial diet	88.2a	85.0a
SE $\pm$	8.1	5.2
Inset generation (IG)		
1^{st}	64.0b	78.8^{NS}
8^{th}	89.5a	81.8^{NS}
SE $\pm$	8.1	5.2
Interaction MD X IG	NS	NS

Means within the same column followed by same superscript are not statistically different at 5% level of probability ($p \leq 0.05$) using Duncan Multiple Range Test (DMRT).

Table 2 shows that the mean percentage pupal survival in artificial diet and SAMPEA 7 sprouted were at par but were significantly higher ($p>0.05$) than the mean percentage pupal survival of 'Dan Shika'sprouted seeds. The mean percentage generational assessment shows that the mean percentage survival of pupa in 8^{th} generation was significantly higher ($p<0.05$) than the mean percentage pupal survival in 1^{st} generation. There was no significant difference of mean pupal weight among the different treatments. The mean weight of pupa in the 8^{th} generation was significantly higher ($p<0.05$) than the mean pupal weight of 1^{st} generation. There was no significant difference ($p>0.05$) in mean percentage egg hatchability among the different treatments and in mean percentage egg hatchability between adults of 1^{st} generation and 8^{th} generation.

Table 2. Mean pupal survival, pupal weight and eggs hatchability of *Maruca vitrata* reared on sprouted cowpea seeds varieties over two generations

Treatments	Mean pupal survival (%)	Mean pupal weight (mg)	Mean egg hatchability (%)
Maruca Diet (MD)			
SAMPEA 7	88.4a	88.5^{NS}	78.7
'Dan Shika'	64.9b	84.2^{NS}	73.0
Artificial diet	84.7a	91.3^{NS}	86.3
SE $\pm$	7.5	6.9	6.11
Insect generation (IG)			
1^{st}	62.1b	68.2b	79.5
8^{th}	76.6a	93.7a	79.11
SE $\pm$	5.4	5.9	5.11
Interaction MD X IG	NS	NS	NS

Means within the same column followed by same letters are not statistically different at 5% level of probability ($p \leq 0.05$), Duncan Multiple Range Test (DMRT).

Table 3 shows range and percentage mean of larva, pupa and adult developmental periods. There was no significant difference in the developmental stages of *M. vitrata* between sprouted cowpea seeds and artificial diet.

Table 3. Developmental periods of *Maruca vitrata* reared on sprouted cowpea varieties

	Laval period (days)			Pupal period (days)			Adult longevity (days)		
Maruca Diet	Range	Mean	SD (±)	Range	Mean	SD (±)	Range	Mean	SD (±)
SAMPEA 7	10-13	10.5	0.8	7-9	8.3	0.6	8-10	8.9	1.6
'Dan Shika'	10-13	10.3	0.7	7-9	8.1	0.7	4-13	8.3	2.5
Artificial diet	9-11	10.1	0.3	7-9	7.6	0.7	7-13	9.7	0.4

Table 4 shows economic analysis of different *M. vitrata* diet tested. It indicated that SAMPEA 7 sprouted seeds had the highest Net benefit (₦107, 000kg^{-1}) and BCR (1:178.3) than 'Dan Shika' and Artificial diet. Artificial diet had the least Net benefit of ₦99, 000L^{-1} and BCR (1:2.2).

Table 4. Cost and economic benefit of *Maruca* diet

Maruca Diet	Cost of diet (₦)[1]	Mean Larval output (Number)	Cost/larva (₦)	Total cost of larvae (₦)[2]	Net Benefit (₦)[3]	Benefit over Artificial diet (₦/kg)[4]	BCR[5]
SAMPEA 7 (Kg)	600	540	200	108,000	107,000	62,000	1:178.3
'Dan Shika' (Kg)	600	460	200	92,000	91,400	46,400	1:152.3
Artificial diet (L)	45,000	720	200	144,000	99,000		1:2.2

[1]The cost was obtained based on prevailing market price of cowpea (N600/kg), cost of artificial diet (N45, 000/L) and larvae (N200/larva). [2]Total Cost of larvae was obtained by multiply cost per larvae by total of live larval output for each diet. [3]Net benefit for each diet was obtained by subtracting cost of diet from total cost of larvae. [4]Benefit of each sprouted cowpea variety over artificial diet was obtained by subtracting total cost of artificial diet from net benefit for each sprouted diet. [5]The BCR was obtained by dividing the benefit of each sprouted cowpea by cost of preparation of each diet.

DISCUSSION

A successful laboratory rearing of healthy insects for scientific research solely relies on insect rearing diets (10) and this depends on the use of artificial diet (13;14). However, one of the major challenges of artificial diet is that most ingredients used for formulation are not locally available which makes them very expensive. This study demonstrated the potential of sprouted cowpea seeds as a cheap diet for rearing *M. vitrata* in the laboratory. Although, larval survival was higher (88%) in Artificial diet than in sprouted SAMPEA 7 (71%) and 'Dan Shika' (71%), however the mean larval weight (85mg) and pupal survival (84%) and weight (91mg) of artificial diet was not significantly different with that of SAMPEA7 sprouted seeds which were 84mg, 88% and 89mg for larval weight, pupal survival and weight respectively. Thus, SAMPEA7 sprouted seeds is comparable with artificial diet for ensuring *M. vitrata* larval and pupal growth. This may be because sprouted seeds are high in important nutrients (protein, fiber, vitamins and minerals) required for good nourishment of larva for their survival (19).

Although there was a decline in *M. vitrata* larval performance after a number of successive generations (12), however, this study has found that the more *M. vitrata* was exposed to the diet the higher was their survival rate and live weight. This was indicated in the result of larval and pupal survival and weight where the larval and pupal performance of 8th generation was higher than that of 1st generation. This finding was supported by Unmole (18) who reported that larval survival and pupa rate increase as insects moved from one generation to another on sprouted Mung beans. Although no supporting literature was found to explain the reason for this phenomenon, however it could be that longer exposure of the larvae to the diet makes them more use to it and consume more of the diet. Cohen (19) reported continuous insect feeding on a nutritious diet supports robust feeding, development, growth, reproduction and sustain good populations.

Surprisingly no previous work was found comparing egg hatchability of artificial diet with sprouted cowpea, however, our finding agreed with (18) that the more insects were exposed to the sprouted Mung bean the better their egg hatchability. There was no significant difference found in percentage eggs hatchability between artificial diet and sprouted cowpea. This suggests that the sprouted cowpeas are as good as the artificial diet in *M. vitrata* egg hatchability.
The developmental periods of all the different life stages of *M. vitrata* in artificial diet were similar for all the sprouted cowpea (SAMPEA 7 and 'Dan Shika'**)**. The larval, pupal and adult longevity periods found in this study agrees with finding of (11); (20); (21) on artificial diet. Sprouted SAMPEA 7 had the highest benefit (₦107, 000kg^{-1}) and BCR (1:178.3) than other diets.

CONCLUSION

This study found that SAMPEA 7 sprouted cowpea was as good as artificial diet in terms of larval weight, pupal survival and weight, egg hatchability, developmental period and had the highest Benefit cost ration than all other diets tested. Thus, indicates the potential of sprouted cowpea seeds as a cheap alternative diet for rearing *M. vitrata* in the laboratory. SAMPEA 7 cowpea is locally available and cheaper. The result of this study has demonstrated a variation in response of *M. vitrata* larvae between sprouted cowpea varieties, thus more variety of cowpea should be tested. The effect of the sprouted cowpea on other biological parameters of the insect should be tested.

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