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Cocoon formation, pupation and adult emergence of African Palm Weevil (*Rhynchophorus phoenicis*) Reared on six different substrates

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Summary

The need for alternative substrate/culture media in captive management of the African Palm weevil (APW) *Rhyncophorus phoenicis* (Coleoptera Rhyncophoridae) necessitates assessment of the usefulness of the substrate in supporting complete life cycle of the insect. Following our work on "Preliminary studies on the performance of African Palm weevil (APW) (*Rhyncophorus phoenicis*) on eight different substrates and that of survivability, growth and nutrient composition of APW reared on four different substrates in which we discovered the usefulness of sugar cane and three fruits (watermelon, pineapple and pawpaw) in enhancing growth of the APW, in this study cocoon formation, pupation and emergence of adults were monitored in six substrates (Raffia palm log (RPL), Sugar cane tops (SCT), Palm Frond Petiole (PFP), Spoilt watermelon (SWM) and Ripe Paw paw (RPP) and Spoilt Pine apple (SPA)) for 50 days. The experimental design used was Completely Randomized Designed (6 treatments and 3 replicates per treatment) whereby each treatment had 30 APW larvae in three replicates of 10 larvae per replicate. For the culture media with the three fruits, the fruits were cut into sizeable chunks and placed in plastic sieves of 30-40 cm to facilitate draining out of moisture and the top was covered with mosquito net to avoid flies. The substrates in each housing unit were removed and replaced with fresh ones on every other day to minimize microbial attack. Two of the substrates, Palm frond Petiole (PFP) and Raffia Palm Log (RPL), were thoroughly watered every two days. The three substrates, PFP, RPL and Sugar cane tops (SCT), were carefully split open and loosely tied back using half inch binding wire for the raffia log and palm petiole and masking tape for the sugar cane. Ten APW larvae were introduced into the enclosure for each replicate of the experimental unit with RPL, PFP and SCT covered with a wire net box to prevent parasites and allow the larvae to burrow their way into the substrate. The experimental units were monitored every two days for signs of cocoon formation, pupation or adult emergence. The result showed that cocoon formation and subsequent pupation occurred in three substrates, RPL, SCT and PFP, and was absent in all the ripe fruits: SWM, RPP and SPA. Statistical analysis of the data on weight and linear body measurement of newly emerged adult from the three substrates showed that the records for the RPL and PFP were significantly better than those from SCT. Cocoons from PFP and RPL were light brownish in colour, similar and difficult to differentiate while those from SCT were cream coloured. The texture of the cocoon from PFP and RPL appeared tough and more articulately woven while that of SCT were rough and less firmly woven. It is therefore recommended that APW could be reared in alternative substrates such as off cut of sugar cane and palm fronds.

Key Words

Rhyncophorus phoenicis, Substrates, Cocoon formation, pupation, adult emergence

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Introduction

The widening gap between animal protein supply and burgeoning human population in developing countries has become worrisome to the global community. FAO/WHO/UNU (1991) recommended 34 g of animal protein consumption per person per day for normal growth and development, but in Nigeria animal protein consumption level is 7-10g/person/day (Ebenebe, 2005) corroborating the claims of Mmadubuike (2000) about failure of conventional meat proteins to meet the animal protein needs of the teeming population. In Nigeria, animal production is faced with numerous challenges: though there is large population of all livestock species, the meat supply is still generally low (Bamaiyi, 2013). Apparently, efforts to increase animal protein supply through various meat proteins have failed to meet the animal protein requirement of the populace (Ebenebe, 2005). A search for cheaper, environmentally friendly animal has directed research interest to insect protein. Insect protein could be a possible solution to animal protein deficiency in the country, due to their ability to provide cheap protein of high amino acid profile with little or no harm to the environment.

Recently, FAO/WUR Experts consultation (2012) recognized the nutritional potential of insects which they posited to be rich in protein and fat, providing ample quantities of minerals and vitamins, as the essential amino acids are often present. Other health benefits of insect consumption have also been outlined in FAO 2013 repository document titled “Edible insects: future prospect for food and feed security”. Nutritional and other health benefits of edible insect has been documented by many authors (Ekpo & Onigbinde, 2004; Banjo et al., 2006; Ebenebe et al., 2007; Edijala et al., 2009). The larva of the APW (*Rhynchophorus phoenicis*) is cherished as food among the many communities, especially in those places where palms (oil, raffia and coconut) are cultivated on commercial basis (Allote & Mpuchane, 2003). In Nigeria, communities in the Niger Delta area, western and southern states of Nigeria cherish the larva as a delicacy (Okaraonye & Ikewuchi, 2009). In fact, it can be seen hawked along major roads and markets in Edo and Delta states of Nigeria (Ekrakene & Igeleke, 2007) and in River states while in Bayelsa states it is tagged “Balyesa suya” as a symbol of how the community cherish the larva. The roasted larva of APW shares the same level of likeness with chicken and are both displayed for sale at Oba junction, along the Owerri to Onitsha express way in Anambra state, Nigeria (Fig. 1, authors observation). Thus, the wild collection of edible insect can no longer support the demand, especially as the need to use it to augment the animal protein needs is being promoted. There is need for captive rearing of edible insects like APW in Nigeria.

Several authors have reported successful rearing of *Rhynchophorus* spp. in laboratory conditions. Giblin-Davis et al. (1989) and Shashina et al. (2009) all reported that sugarcane stem is a good alternative for the rearing of *Rhynchophorus cruentatus*. Giblin-Davis et al. (1989) reported successful rearing of *Rhynchophorus cruentatus* with the syncarpium of pineapple though complete development of *Rhynchophorus cruentatus* did not occur in pineapple. These informed our earlier preliminary research work on rearing and multiplication of African Palm Weevil (APW) in captivity using eight different substrates, especially agricultural wastes. In the course of this previous experiment it was discovered that some of the substrates that supported larval growth and performance could not support pupation and subsequent metamorphosis to adult stage. Hence, the current research was conducted to test new substrates/culture media and to verify the result of the previous research work.

Material and Methods

The study was carried out at the Biological Conservation Unit of the Department of Zoology, Nnamdi Azikiwe University Awka (Anambra State lies between the latitudes 7°E and 7°9’E and longitudes 6°6’N and 6°17’N while its geographical coordinates are 6°10’0” North, 7°4’0” East), The experiment was conducted using one hundred and twenty African Palm Weevil (APW) *Rhyncophorus phoenicis* of similar weight hand - picked from rotting raffia palms at “Mgbo area” of Ebenebe town in Awka North Local Government of Anambra State. Six substrates used as alternative culture media in this study were collected within the state (Figs. 2–10):

- Sugar Cane Tops (*Saccharum officinarum*) (SCT) (Cut off tip of sugar cane stem usually thrown out as waste at Ose market, Onitsha)
- Raphia Palm Log (*Raphia africana*) (RPL) (Collected from the Mgbo swamps)
- Palm Frond Petiole (*Elaeis guineensis*) (PFP) (Collected from the University premises)
- Spoilt Watermelon (*Citrullus lanatus*) (SWM) (Collected from the heap of spoilt and discarded watermelon at the fruit and vegetable market, Onitsha)
- Ripe Pawpaw (*Carica papaya*) (RPP) (Collected from fruits market, Onitsha).

Figure 1. Fried APW larvae on display for sale on road sides.

• Spoilt Pineapple (*Ananas comosus*) (SPA) (Also collected from fruits markets, Onitsha.

The experiment was designed on 6 x 3 Completely Randomized Design whereby each experimental unit housed 30 larva and ten larva per replicated. For the culture media with the three fruits, the fruits were cut into sizeable chunks and placed into plastic sieves of 30 cm diameter by 40 cm high to facilitate draining out of moisture and the top was covered with mosquito net to avoid flies. The substrates in each housing unit were removed and replaced with fresh ones every other day to minimize microbial attack. Three of the substrates: Palm frond Petiole (PFP) (well watered PFP), Raffia Palm Log RPL (well watered RPL) and Sugar cane top (SCT) were carefully split open and loosely tied back using half inch binding wire for the raffia log and palm petiole and masking tape for the sugar cane. Ten APW larvae were introduced into the enclosure for each replicate of the experimental units with RPL, PFP and SCT covered with a wire net box to prevent parasites and allow the larvae to burrow their way into the substrate. The experimental units were monitored every two days for signs of cocoon formation, pupation or adult emergence. Dates of observation of cocoon, pupa and adult emergence were also recorded for each unit. Snap shots of cocoon, observation on their texture, colour were noted for each substrate. Emerged adults were weighed at each instance and the body length measured, while denoting the sex. The data on weight and linear body measurement of newly emerged adults from the three substrates were statistically analyzed with ANOVA using SPSS package version 20.

Results

Cocoon formation and pupation

The result of the records on the observation on cocoon formation and pupation are summarized in Table 1. Cocoon formation and pupation occurred in three substrates: PFP, RPL, and SCT. However, they did not take place in any of the fruits: SWM, RPP and SPA.

Body weight and linear body measurement of emerged adult APWs

The result of the statistical analysis of the data on weight and linear body measurement of newly emerged adults from the three out of the six substrates are presented in Table 2. There was no significant difference ($P > 0.05$) in the body weight of emergent adults raised in the three substrates (RPL, PFP and SCT). Numerical values of the mean body weight however showed the highest mean body weight record for the emergent adults raised on RPL (2.12 + 0.28 g) followed by those on PFP (1.81 + 0.21 g), with the least record for those raised on SCT (1.58 + 0.31 g). A similar trend was obtained for thorax width with RPL emergent adult attaining 1.23 + 0.09 cm, PFP emergent adult (1.18 + 0. 05 cm) and SCT emergent adult (1.17 + 0.00). For the body length, the highest mean body length was recorded for the emergent adult on RPL (4.32 + 0.14 cm), followed by those on SCT (4.12 + 0.11 cm) and the least mean body for the ones on PFP (4.06 + 0.25 cm).

Comparison of weight and linear body measurement of the emergent Adult

The result of the statistical comparison of the body weight and linear body measurement of the emergent adult are presented in Table 3. The result showed no significant difference ($P > 0.05$) between the mean body weights, mean body length, mean thorax width of the male and female emergent *Rhyncophorus phoenicis*, respectively. Numerically, the records appear higher in female with the mean body weight of the newly emerged APW as 2.03 + 0.27 g,

Culture Medium	Presence/ Absence of Cocoon	Pupation	Duration of Pupation (Days)
PFP	Present	√	22-28
RPL	Present	√	24-29
SCT	Present	√	21-27
SWM	Absent	-	X
RPP	Absent	-	X
SPA	Absent	-	X

Table 1. Observation on Cocoon Formation and Duration of Pupation.

Descriptives			
		Mean	**Std. Error**
Weight (g)	SCT	1.5800±0.31113	0.22000
	PFP	1.8100±0.21213	0.15000
	RPL	2.1200±0.28284	0.20000
Thorax (cm)	SCT	1.1700±0.00000	0.00000
	PFP	1.1850±0.04950	0.03500
	RPL	1.2350±0.09192	0.065000
BL (cm)	SCT	4.1200±0.11314	0.80000
	PFP	4.0600±0.25456	1.80000
	RPL	4.3200±0.14142	0.10000

Table 2. Weight and linear body measurements of larvae reared on six different substrates.

Descriptives			
		Mean	**Std. Error**
Weight (g)	Male	1.6467±0.28024	0.16180
	Female	2.0267±0.26633	0.15377
Thorax (cm)	Male	1.1633±0.01155	0.00667
	Female	1.2300±0.06557	0.03786
BL (cm)	Male	4.0467±0.17010	0.09821
	Female	4.2867±0.11719	0.06766

Table 3. Comparison of Weight and Linear body Measurement of the Male and Female Emergent Adult.

a torax of 1.23 + 0.07 cm, and BL of 4.27 + 0.12 cm, while the male records were 1.65 + 0.28 g, 1.16 + 0.11 cm, and 4.05 + 0.17 cm for mean body weight, mean thorax width and mean body length respectively.

Discussion

Cocoon formation, pupation and duration of pupation

Cocoon formation and pupation which failed in the ripe fruits confirms our earlier finding on the failure of cocoon formation and subsequent pupation in watermelon due to lack of fibrous strands in the melon (Ebenebe & Okpoko, 2015). This result is also in line with Giblin-Davis et al. (1989) who reported successful rearing of *Rhynchophorus cruentatus* with the syncarpium of pineapple though complete development to adult stage did not occur in pineapple. Cocoon formation in Sugarcane and Raffia palm log and Palm frond petiole was observed rather in the three substrates with seemingly fibrous materials. While RPL is a natural substrate for *Rhynchophorus* spp., cocoon formation and pupation which occurred in PFP and SCT can be attributed to the nature of the substrates, as each of them has fibrous material with which the weevil weaves its cocoon and so simulated the natural habitat. Giblin-Davis et al. (2013) reported pupation of the weevil in oil Palm (*E. guineensis*) and two raffia palms (*Raphia hookeri* and *Raphia monbuttorum*); while several authors Giblin-Davis et al. (1989), Weissling & Giblin-Davis (1994), and Shashina et al. (2009) reported successful rearing of *Rhynchophorus cruentatus* on sugarcane stem.

Body weight and linear body measurement of emerged adult APWs

The result of linear body measurements and body weight of the emergent adult obtained in this study appear to be a pioneer study because literature report is scarce in this area of study, as most literature reports are on the adult larva.

Comparison of weight and linear body measurement of the male and female emergent adult

The mean values for parameters measured showed higher values for newly emerged female APW, however, statistical analysis of these values showed no significant difference in body weight, body length and thorax width. Tambe et al. (2013) also reported

Figure 2. APW larva with the Raffia palm Log (*Raffia Africana*) and Net Box used as Cover. Figure 3. APW larvae boring into sugar cane Substrate (*Saccharum officinarium*). Figure 4. Pupa in sugar cane substrate. Figures 5, 6. APW larvae boring into the Palm Frond Petiole (*Eleais guineenesis*). Figure 7. APW Larvae boring into the Spoilt watermelon (*Citrullus lanatus*). Figures 8, 9. APW larvae boring into the papaya fruit (*Carica papaya*). Figure 10. APW Larvae ravaging Spoilt Watermelon (*Ananas cosmosus*).

that morphometric parameters like abdomen length, abdomen width, head size and length from tip of rostrum to antennal insertion were significantly greater in females than males. Wattanapongsiri (1966) has earlier reported that the APW male and female are very uniform in body size, except for the rostrum length. Sex-related differences in growth patterns according to Shine (1990) form one of the major proximate determinants of sexual size dimorphism. It is therefore imperative to assess such sex related differences in the morphometric parameters of the emergent adult and that of full grown adult to establish the level of such dimorphism in these species of insects.

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