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The Edibility, Methods of Preparation Of the Raphia Palm Beetle, Rhyncophorus Phoenicis [Coleoptera: Curculionidae] In the Niger Delta and Associated Microorganisms

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Abstract: *The microbiological quality of adult Rhychophorus phoeniciswas assessed revealing the presence of some species of bacteria and fungi. A comparative study was also done to determine the presence of bacteria in the larva, pupa, pith, adult Beetle (1)and adult Beetle(2). The pith had the highest bacterial count followed by the larva ,pupa ,adult B1 and adult B2.This same order was observed in the fungal count. Total bacterial and fungal counts of the chitin of the adult beetle(1) were* 1.24×10^{6}*cfu/ml and* 2.0×10^{4}*cfu/ml respectively. Six genera of bacteria, four genera of moulds and a species of yeast were observed. Bacterial isolates identified included Bacillus, Staphylococcus, Acinetobacter, Pseudomonas,Micrococcus and Proteus species while the fungal species included Penicillium, andAspergillus species. Saccharomyces cerevisae was the only species of yeast present. The implication of Staphylococcus aureus to public health is of great significance.The larva of Rhychophorus phoenicisis a rich source of protein.*

Keywords*: Edibility, :Microbiological quality, Larva, Pupa, adult Rhynchophorus phoenicis, palm pith,*

I. Introduction

Many edible insects are available in the Niger Delta of Nigeria and every ecological zone of the country .has edible insects of diverse types. Some edible insects are not documented because they are known. Some of the insects are eaten raw, cooked, roasted or fried. Some are boiled as porridges along with yams, coco yams or in stews where they serve as good sources of protein. Most of the insects are hunted by children both in the day and at nights during dry and rainy seasons. People hunt for edible insects in fallen palms and harvest up to 10 – 20kg of the grubs from fallen palms. Some of the edible insects of the Niger Delta include Rhynchophorus phoenicis, Oryctesmonoceros,O. boas, Gryllotalpa africana, Brachypterusspp, Macrotermes species, Bunaea alcinoe[**1**], Pachymeruscardo [**2**], Zonocerus variegatus,Pseudocreobotra spp and its eggs, Lixuscameranus, to mention a few. Depending on the tribes, different methods of preparation are employed in cooking the beetle. The domestication of some of the edible insects had been done [**3**, 4]. The nutrient composition of most of the edible insects had been determined [**5**, 6].

The objective of this study was to assess the edibility of the beetle within the Niger Delta ecological zone and also to determine the microbiological quality of the palm piths where the edible larvae live, the larvae, pupae and the adult beetle.

II. Materials And Methods

Adult beetles, larva, pupa and pith from Raphia palm trees were collected from Taabaa, Ogoniland, Rivers State ,Nigeria. The samples were used immediately after collection.The agar used were nutrient agar (Laboratory M. Bury,Lancashire,U.K.),MacConkey agar and Potato dextrose agar(**7**).

Bacterial and Fungal Viable Counts

The method used was the 10-fold dilution method of **{7**).Ten grams(10g) each of fresh adult beetle, ,larva, pupa and pit were aseptically transferred into 90ml of sterile saline in 150ml conical flasks .The flasks were shaken vigorously to dislodge the microbial flora. Further 10-fold dilutions were carried out by adding 1.0ml of the penultimate dilution to 9ml of fresh diluents. Finally,0.1ml of an appropriate dilution was placed on dried nutrient agar, evenly spread with a sterile glass spreader and incubated at 30◦c for 24hrs.At the end of the incubation period, counts were performed for the dilutions with counts between 30 to 300 colonies (**8**). All counts were performed in duplicates and the average taken.

Mould and yeast counts were enumerated by aliquots of appropriate diluted samples on acidified potato dextrose agar containing streptomycin (1mg / 100 ml) .The plates were incubated at 30◦c and counted after 48 hours for yeasts and 96 hours for moulds.

Similarly, 0.1ml of 10^{4} dilutions were inoculated on MacConkey agar.

Mean colony counts were calculated and expressed as colony forming units per gram(cfu/g) of the sample analyzed (**7**).Representative colonies of the ten-fold dilution of the skin were picked and sub cultured on nutrient agar until pure cultures were obtained.The pure cultures were stored on agar slants.

Identification and Characterization of Isolates

The methods described in **{9}**were adopted in characterization of isolates. Isolates were identified by standard methods (**10**). The identification of *Staphyloccus, Acinetobacter, Bacillus, Escherichia, Proteus and Pseudomonas* was done with reference to {**7, 9}.**.

Natives were interviewed on methods of preparation of the insects before consumption and other information collected from them are included in Table 1 according to tribes.

Statistical Analysis

Results were subjected to statistical analysis employing the student t-test at 95% probability levels.

III. Results And Discussion

Most of the edible insects of Nigeria are high in protein. The edible Lepidopterous moth larva of the Niger Delta, *Bunaea alcinoe* has a protein content of 55.4% and is harvested during the rainy and dry seasons of the year by children and youths [11]. Table 1 showed that *R. phoenicis* are consumed raw, boiled, fried or roasted by different tribes of the Niger Delta and eastern states . Some of the edible larvae have been domesticated [**3**] and *R. phoenicis* has been domesticated and the rearing procedures had also been demonstrated in the laboratory [12]. Uptake of crude oil had been reported in *Oryctesmonoceros [***13**]. Some of the metals have been found to be carcinogenic [**14**] and apart from the bacterial implications, higher health risk is involved in the consumption of *R. phoenicis* and other edible insects in their raw state or preparing them at temperatures which cannot kill the bacteria in them that are harmful to human health,

Table 1.Edibility Of The Raphia Palm Beetle, *Rhynchophorus phoenicis L*. In The Rural Tribes Of The Niger Delta/ Eastern States And Methods Of Preparation.

TRIBES	Insect stage eaten	Method of Preparation	Consumed as	Source of Insect	Enjoyed by
Ikwerre	Larva, Pupa & Adult	Fried, raw, boiled or roasted	Snacks& in food	Raphia palms	Children and adults
Ogoni	Larva & Adults	Fried, raw, roasted or boiled	Snacks and in food	Raphia Palms	Children and adults
Efiks	Larva, pupa &Adults	Fried, raw, roasted, boiled	Snacks and in food	Raphia Palms	Children and adults
Ibo	Larva, pupa, adults	Raw, fried, roasted & boiled			Children and adults
Efiks	Larva, pupa & Adults	Fried, raw, roasted, boiled	Snacks and in food	Raphia Palms	Children and adults
Urhobo	Larva, pupa, adults	Raw, fried, roasted & boiled	Snacks		Children and adults
Etche	Larva, pupa, adults	Raw, fried, roasted & boiled	Snacks & in Food	Raphia palms	Children and adults
Ekpeye	Larva, pupa, adults	Raw, fried, roasted & boiled	Snacks & in Food	Raphia Palms	Children and adults
Kalabari	Larva	Raw, fried, roasted & boiled	Snacks & in Food	Raphia Palms	Children and adults

The total heterotrophic bacteria [colonial characteristics] isolated from the adult beetle is shown on table 1.The total bacterial population of 1.24×10^6 and 1.21×10^6 cfu/ml were obtained on nutrient and Mac Conkey agar respectively., {**15, 16]**)and they also observed a total bacterial count of 1.68×10^5 cfu/ml from processed edible weevil caterpillar (*Rhynchophorus phoenicis*) and 4.49×10^7 cfu/g from an edible caterpillar of Emperor moth *(Bunaea alcinoe*)**{15,16}.**while[**5]** had a bacterial count of 1.86×10^6 cfu/g on the chitin of edible larva (*Bunaea alcinoe*). Recently, microbial populations for the chitin in *R. phoenicis* were obtained [**17]** as follows: $9.2x\ 10^5$ cfu/ml (total heterotrophic bacteria) The observation in this work is quite similar to their findings.

The total fungal population of 2.0×10^4 cfu/g was obtained on SDA medium.This is higher than the findings of [**15]** whichwas 1.92×10^2 .A higher fungal count of 9.5×10^6 was observed**[16]**while a fungal count of $7.30\ x10^5$ cfu/ml was obtained [**17].**Two genera of moulds namely *Penicillium* and *Aspergillus* and one species of yeast,*Saccharomyces cerevisae* were isolated from the adult beetle based on their colonial morphology and microscopic characteristics (Table 3). The identification of the fungal isolates were cross-matched with those described in **[7]**. .The microbial species isolated recently were *Acinetobacter, Bacillus, Klebsiella, Pseudomonas, Saccharomyces, Serratia* and *Staphylococcus* spp [**17].**

The Microbial flora observed in this study were similar to those in the works of **[5, 15, 16, 17]** but some species that were not found in this study were found [17]. .*Penicillium* and *Aspergillus* were not found in the study [17\] but were observed in our study.

Table 1: Colonial characteristics of bacteria isolated from adult Beetle

Cultural Morphology	Microscopy
Golden yellow colonies on NA , pink on MCA	Gram +ve cocci, predominantly in clusters
Cream moist colonies on MCA,discrete	Gram –ve short rods
Colonies Cream moist colonies on MCA,	
growth spread over surface on NA	
Rose pink ,point colonies on MCA	Gram –ve rods, predominantly singles
Cream moist pin-point colonies on NA	Gram positive cocci in chains
Green blue colonies on Na,crem irregular	Gram –ve rods in singles ,few in short chains
Surface on MCA	
Cream, dull and dry wavy colonies on NA	Gram positive beads like rod in short chains
NA-Nutrient agar; MCA-Mac Conkey agar	

Table 2 : Cultural morphology and characteristics of fungi isolated from adult Beetle.

Cultural Morphology	Microscopy	Probable identity
White filamentous mycelia	Conidial head are radical,conidiophores	Aspergillusniger
growth with visible dark	are unbranched,norhizoid;hyphae is	
brown spores above septate.		
mycelia		
Simple upright unbranched conidiophores		Aspergillussp.
With tappers developing into an enlarge		
Globase swelling at the apex. Conidiophore		
was hyaline and conidia were seen borne on		
Short chain of sterigma.		
Green pigmentation with white	Hyphae is septate;conidiophores branched	Penicillium
background, powdery surface	to form brushlike conidial head with whorl caseicolum	
	Of phialides	
Dull,cream mucoid and butyrous	Gram positive large spherical .oval	Saccharomyces
Large colonies on NA and pink	oval budding cells	cerevisae
on MCA		
NA-Nutrient agar; MCA-MacConkey agar		

The nutritional composition revealed a high moisture content(56.82%) followed by crude protein (32.71%),total carbohydrate,fat, fibre and ash were 0.88%,4.17%,8.59% 0.89% respectively[17].`
Some strains of *S.aureus* and a species of *Bacillus,B.cereus,* are known enterotoxin producers [5]..Their presence in this insect used as food is of public health concern.The cooking process applied to these beetles before consumption employs temperatures that can eliminate *S.aureus* but not *B.cereus* which associated with high protein content foods though they rarely give rise to food borne infections but generally lower the nutritional quality of contaminated food [**18].** *Pseudomonas aeruginosa* produces protease and lipase that catalyze reactions that results in the degradation of proteins and fats,hence ,producing an undesirable flavor of the food products**[15]**.

Fungi are widely distributed in soil and air .*Aspergillus* spp.are frequently isolated from food.[**15]**). Aflatoxins produced by *Aspergillus* may be implicated in hepato-cellular carcinoma. *Penicillium* spp produce toxins (ochratoxin-A) which is a potent nephrotoxin and causes damage in pigs and experimental animals[**19]**.
The microbiology of edible insects and their products have been discussed **[20].** .In Africa, Asia and other parts of the world ,insects and their products are used as food and feeds **[21]** so efforts in improving the finished products should be intensified as some natives in Africa eat them raw and also because of the presence of the aforementioned microorganisms discovered in this study.

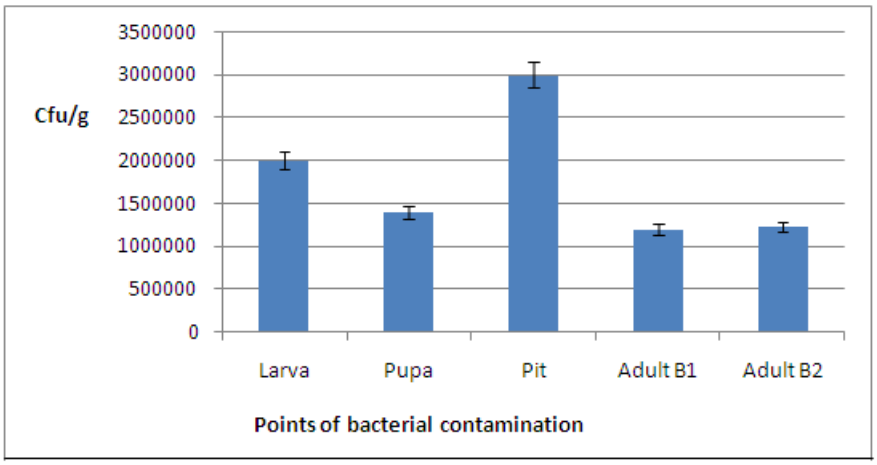

Fig 1: Bacterial counts in different points of contamination.

A comparative study was done to observe the bacterial load present in the larva ,pupa, pit ,adult B1 and B2.From figure 1,it is shown that the pit had the highest bacterial load. In the descending order, it is as follows: Pit>larva>pupa>adultB1>adult B2. The fungal load showed the same pattern like that of the bacteria (fig. 2).

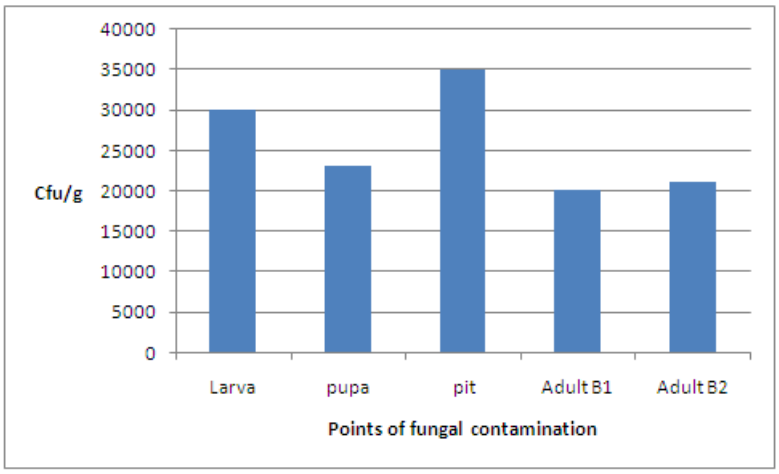

Fig. 2: Fungal counts in different points of contamination.

From the students' T-test table , there was no significant difference at 0.05 probability levels between the bacterial and fungal loads found in the larva ,pupa, pith, adult B1 and B2.

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