Dual Purpose Edible Insect Larva (Rhynchophorus Phoenicis) In South South Nigeria – Microbiological Assessment Of Body Parts

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Abstract: This study assessed the microbial load (Bacteria and Fungi) of the various parts of the body of the dual purpose edible insects (Rhynchophorus phoenicis) from South South Nigeria. Live samples of the insects (Maggot) were collected from the three project locations namely: Asarama in Andoni Local Government Area of Rivers State, Kalioko (Ogoni community) in Khana Local Government Area of Rivers State and Ikot Akpanudo community in Oruk Anam Local Government Area of Akwa Ibom State where the insects are abundantly available. Methodology employed; Samples of decaying palm grubs from Raphia palm trees containing maggots were surveyed and the developed maggots collected with sterile forceps and kept in a deep freezer separately for 30 minutes to be immobilized to ease further dissection. Media used were nutrient agar, MacConkey agar and Sabouraud dextrose agar. Ogoni sample had the highest bacteria load while the highest fungal load was observed in Akwa Ibom sample. Result revealed that Ogoni sample recorded the highest bacterial load (2.89 x 10^6 cfu/g) on the head while the lowest was observed in the intestine (4.0x 10^4 cfu/g). Akwa lbom sample recorded the highest fungal load $(1.11 \times 10^6 \text{cfu/g})$ in the body fluid while the lowest $(3.0 \times 10^4 \text{ cfu/g})$ was in the external body. Bacteria such as Bacillus species, Staphylococcus species, Serratia marcasans, Enterobacter species and fungi such as Aspergillus, nidulans, Aspergillus niger, Aspergillus vesicolor, Aspergillus fumigatus Aspergillus tarreus, Penicilium funiculosum, Penicilliuin camemberti, Penicilium merneffei, Mucor racemosus, Candida species, Alternria spp, Fusarium spp, Absidia corymbifera, Pulularia pullans, Phialophora vertucosum, Histoplasma capsulatun, Aureobasidiuin pullulans, Scopulariopsis spp, Epidemophyton spp, Trichophyton spp Physarum cinereum, Fusarium spp, were isolated from this insect (four (4) species of bacteria and 21 species of fungi). The fungal species, Mucor racemosus had the highest frequency with the count of 5.0x 10^{7} cfu/g in the body fluid while Abasidia corymbifera had the least count of $1.0x10^{5}$ cfu/g from Akwa Ibom Maggot. Also, Aspergilius (niger, fumigatus and vesicolor,) had the highest frequency with $5.0x10^7$ cfu/g count on the head. In Conclusion, this insect contains huge microbial (Bacteria and fungi) load, hence adequate hygienic practices and proper processing are needed before they can be consumed. Nevertheless, the vast microbial loads, in these species of insect could serve as a ready source of microbes in some processing and pharmaceutical industries. It is worthy to note that the insect larva serves a dual purpose; as a high source of high protein-delicious food in South South, Eastern and Western Nigeria and usually harbours important pharmaceutical antibiotic producing fungi – Penicillium sp

Keywords: Edible insect larva, Rhynchophorus phoenicis, insect body parts, Penicillium sp.

Date of Submission: 04-10-2018

Date of acceptance: 16-10-2018

I. Introduction

Consumption of insects as food (entomorphagy) in Nigeria is not new (DeFoliart, 2002). The larva and adult of the Raphia palm wine weevil (Rhynchophorus phoenicis) (F.) are cherished as food among the many communities in Nigeria and around the world, especially in those places where palms (oil, raphia and coconut) are cultivated on commercial basis (De.Foliart, 1992; Allotey and Mpuchane, 2003; Choon-Fah et al., 2008). In the South South and Eastern States of Nigeria, this larva is often a cherished delicacy. In fact, it can be seen hawked along major roads and markets in Edo and Delta States of Nigeria (Ekrakene and Igeleke, 2007). From Sapele where it is called edible worm or maggot to Warri where they call it diet, down to Bayelsa, Rivers, Cross Rivers, Akwa Ibom and all the Eastern states of Nigeria, it is widely consumed either raw, boiled, fried, smoked and sometimes used in the preparation of stews and soups, as part of a meal or as a complete meal. In a research, Abia state recorded the highest percentage for Termite and Grasshopper consumption with percentages 17.46% and 36.57% respectively (Oghale, 2014). It was only from Abia that the raphia palm weevils (maggots) were listed as consumed unintentionally. Nine species were listed. The highest percentage for consumption of larvae

of Lepidopteran species (33.33%) was from Imo state. The consumption of praying mantids was solely in this state and it was also in this state that the unintentional consumption of the rice weevil was recorded.

The high protein content observed in the insect larva, Rhynchophorus phoenicis implies that larva meal can contribute significantly to the daily human protein requirements, usually about 23-56 g (FAO/WHO/UNU, 1991; Chaney, 2006a). According to Ene (1963) many educated and urbanized West Africans were either ignorant or reluctant to admit the existence of certain indigenous customs such as the consumption of insects. By means of a questionnaire survey, Ene (1963) reported that a high percentage of West Africans had some knowledge of entomophagy among the population. The bulks of respondents to the questionnaire were from Nigeria, Ghana and Cameroun undergraduate students of Agriculture, Medicine and Zoology and included a few Nigerian lecturers and their wives. In another report, Ivbijaro (1990), however, expressed delight that information was being gathered on food insects, which, in Nigeria, were important sources of high food protein among rural dwellers and a growing delicacy to many city dwellers (Ogbalu and Nrior, 2014). The results of a study showed that Rhynchophorus phoenicis larva is a rich source of good quality protein, iron, manganese, copper and relatively rich and safe oil-since insect oils have low cholesterol contents (De Foliart, 1992). Fasoranti and Ajiboye (1993) and Banjo et al., (2006) studied insect consumption by humans in western parts of Nigeria. Banjo et al., (2006) reported that fourteen insect species were consumed as food in south western Nigeria and they include, Macrotermes bellicosus, (termites: Isoptera); Brachytrypes spp., Zonocerus variegates, Cytacanthacris naeruginosus unicolor (Uvarow), (Orthoptera); Analeptes triafasciata, Oryctes boas (Fabr.), Rhynchophorus phoenicis (Fabr.) (Coleoptera); Anaphes infracta (Walsinghan), A. Recticulata (Walker), A. Venata (Butler) and Cirina forda (Westwood) (Lepidoptera). These insects are consumed either in one or more of the stages of development in the life history of an insect (egg, larva, pupa and adult). Grubs of the Raphia palm weevil, Rhyncophorus phoenicis (Fabr.) are fried and eaten in several parts of western Nigeria especially in Edo, Delta and Anambra states. Active marketing of the fried grubs takes place in these states.

The moisture content of food is an index of water activity (Olutiola et al., 1991) and is used as a measure of stability and susceptibility to microbial contamination (Uraih and Izuagbe, 1990). Therefore, the shelf-life of Rhynchophorus phoenicis larval meal can be improved by processes such as sun-drying, frying or roasting, which brings about dehydration and have earlier been reported to extend the keeping quality of pork meals by reducing microbial activities.

Microbial growth encourages spoilage and for peasants in particular economic loss during storage. Besides, it is important that peasants who consume these insects are enlightened that consumption of poorly processed and cooked insects such as maggot could be predisposed to health hazards such as typhoid, urinary tract infection, cholera and related infection amongst others. The presence of enterobacteria in these edible insects is indicative of possible sewage pollution, the common contaminant in polluted littoral zones, a report which has been highlighted by Akamatsu (1983); Nrior et al., (2017a,b).

A number of microbiological tests of foods (plants and animal origin) and their products are used by authorities to check that the microbiological status is satisfactory. The purpose of these tests is to detect pathogenic bacteria (Salmonella, Staphylococcus aureus, E. coli) or indicator organisms of fecal pollution (fecal coliforms, fecal streptococci) or other types of general contamination or poor handling practices (coliform bacteria, faecal streptococci, total viable count) (Nrior et al., 2017b). Microbiological testing can be costly and time-consuming. Estimation of bacterial numbers in plants and animal products are frequently used to retrospectively assess microbiological quality or to assess the presumptive safety of the product. The number, size and nature of the samples greatly influence the results and even the most elaborate sampling cannot guarantee the safety of the product. However, it is still worthwhile; if substandard consignments are found, the psychological effect on the seller is high, especially if the consignment is deemed for export to countries that have established microbiological criteria (Nrior and Ogbalu, 2014).

Consumption of wild animals including insects such as Raphia palm weevils (Rhynchophorus phoenicis) for food has been of immense importance in maintaining the health status of rural inhabitants over the years through their nutrient contents. However, the microbiological characteristics of the various species of the insects with respect to body parts and regions were not put into consideration hence the need for this research. More so information on the type and nature of microbes precisely bacteria and fungi species are yet to be documented. Reports on the microbial evaluation of commonly consumed insects are however scanty. There is need to study these groups of insects to undertake a comparative microbial evaluation of these edible insects in order to ascertain their safety for consumption and their possible value in food processing industries.

The major objectives of this study are therefore to evaluate the species of microbes (bacteria and fungi) present in these edible insects (maggots), determine the microbial population in the edible insects from the various areas of sampling and to assess the population and species of microbes in the various parts of the body of the insects.

II. Materials and Methods

Sample Collection and Preparation

Some samples of decaying palm grubs from Raphia palm trees containing maggots were surveyed and the maggots were collected and brought to the laboratory for further analyses. The developed maggots were collected with sterile forceps. Aseptic procedures were carried out so as to minimize contamination from bacteria not associated with the collected maggots.

Live samples of the insects (Maggot) were collected from the three project locations namely: Asarama in Andoni Local Government Area of Rivers State, Kalioko (Ogoni community) in Khana Local Government Area of Rivers State and Ikot Akpanudo community in Oruk Anam Local Government Area of Akwa Ibom State where the insects are abundantly available. The insects were gotten from the marketers in the respective communities who specialized in the harvest of the particular species of insects and the larvae.

The samples after collection were brought to the laboratory for storage in a sterile plastic container. The samples were stored in the refrigerator at 4^{0} C for 24 hours.

Media Used.

The used media include Nutrient agar (Biomark Laboratory India), MacConkey agar (Micro Master India) and Sabouraud dextrose agar (Biomark Laboratory India). The various media were prepared according to the instructions of the manufacturers. Diluents used were physiological saline prepared by adding 0.85g of sodium chloride (Nacl) to 100ml of sterile distilled water for use as diluents.

Enumeration of microbial population

Internal samples of the maggot (intestine and body fluid) and the external samples (head) from the various project sites were prepared according to the methods described by Adedire (2002) and Nrior et al., (2017a,b). The maggots from the various sites were kept in a deep freezer separately for 30 minutes to be immobilized before clipping the wings to ease further dissection. Half gram of the internal and the external contents were aseptically transferred into 4.5ml of diluents (physiological saline) in a 150ml conical flask which were vigorously shaken to dislodge the microbes (bacteria and fungi) present in each of the parts (head, intestine and body fluid) seperately. Ten-fold serial dilutions were carried out by adding 1.0ml of the initial dilution to 9ml of fresh diluents according to Harrigan and MaCance (1990); Nrior et al., (2017b) method.

After which, 0.1ml of an appropriate dilution were inoculated on dry nutrient agar, MacConkey agar and Sabouraud dextrose agar plates in duplicates and evenly spread with a sterile spreader and incubated at 37^oC for 24 hours.

At the end of incubation, counts were performed on the dilution which will show counts between 30 and 300 colonies (Anon, 1994).

According to Ogbalu and Nrior (2015), Nrior et al (2017); Colony forming Unit per milliliter or gram (Cfu/ml or Cfu/g) is equal to number of colonies on media plate after incubation divided by sample dilution used multiplied by volume plated (aliquot) as shown in the formula below:

For liquid sample

Cfu/ml = <u>Number of colonies (Colonies counted on plate)</u> Dilution x Volume plated

OR

Cfu/ml = <u>Number of colonies x Dilution factor</u> Volume plated

Note: Dilution factor is the reciprocal of sample dilution used for plating.

That is, Dilution factor = $\underline{1}$

Dilution

For solid sample Cfu/g = <u>Number of colonies(Colonies counted on plate)</u> Dilution x Volume plated

OR

Cfu/g =<u>Number of colonies x Dilution factor</u> Volume plated N/B: From 10-fold serial dilution; **Dilution** used for Total Heterotrophic Bacteria on Nutrient Agar = 10^{-6} , Total Heterotrophic Fungi on Sabouraud Dextrose Agar = 10^{-4} , Enteric Bacteria on MacConkey Agar = 10^{-3} **Isolation and Identification of Microbes**

Bacteria: Representative colonies were picked and inoculated onto nutrient agar to obtain pure cultures. The pure cultures were stored as frozen 10% (v/v) glycerol suspensions at -35° C in a refrigerator (Wellington and Williams, 1978). This glycerol serves as a means for fresh working cultures.

Identification of the isolates were carried out according to the schemes of Cowan and Steel (1966) and Buchanam and Gibbon (1974). The tests employed include morphological tests, catalase, coagulase, indole and methyl red, growth on MacConkey agar, fermentation/oxidation of glucose, sucrose and mannitol.

Fungi: Isolation and identification of fungi was based on their macroscopic morphology - best growth temperature, growth rate, colour on SDA, colour on reverse side, texture and special feature while the microscopic morphologies and identities of the different species of the fungal isolates based on characteristic features of conidiopore, phialides, vesicle, sclerotia, hulle cells, sporangiophore, apophysis, columella, sporangium and rhizoids.

III. Result and Discusion

MICROBIOLOGY OF MAGGOT: The total heterotrophic bacteria count of Raphia palm weevil

(maggot) in Akwa Ibon is as shown in fig. 1. Heterotrophic count varies with region of the body such that the content were 2.97×10^6 cfu/g for the head, 1.89×10^6 cfu/ml for the body fluid, 3.02×10^6 cfu/g for the intestine and 2.4×10^5 for the external body. The intestine had the highest bacteria load while the least load was observed in the external body. The total bacteria load observed in the maggot was 8.12×10^6 cfu/g.



Fig. 1: Total Heterotrophic Bacterial count (cfu/g/ml) in Akwa Ibom isolate

[NB: HD=Head, BF=Body Fluid, IT= Intestine, EB=External Body]





[NB: HD=Head, BF=Body Fluid, IT= Intestine, EB=External Body]

Fig. 2 shows the heterotrophic bacteria count of Raphia palm maggot in Asarama in Rivers State. A total of 1.96×10^6 cfu/g, 1.42×10^6 cfu/ml and 4.0×10^4 cfu/g were observed from the head, body fluid and intestine respectively. The highest count of bacteria was observed on the head. In all a total of 3.42×10^6 cfu/g bacteria were observed in this area which was the lowest bacteria site. Intestine had the lowest bacteria count.

Figure 3 shows the heterotrophic bacteria count in Ogoni area of Rivers State. About 2.89 x 10^6 cfu/g, 1.10 x 10^6 cfu/g, 2.88 x 10^6 and 2.70 x 10^6 heterotrophic bacteria were observed on the head, intestine, body fluid and external body respectively. The highest count (2.89 x 10^6 cfu/g) was observed in the head. Maggot in Ogoni area had the highest bacteria load (9.57x 10^6 cfu/g) among all the areas studied.



Fig. 3: Total Heterotrophic Bacterial count (cfu/g/ml) in Ogoni isolate [NB: HD=Head, BF=Body Fluid, IT= Intestine, EB=External Body]

A total of four (4) bacterial isolate were isolated from the samples consisting of four (4) from Akwa Ibom (Bacillus spp, Serratia marcesans, Staphylococcus spp, and Enterobacter spp), three (3) from Asarama Isolates (Staphylococcus sp, Serriata spp and Bacillus spp) and four (4) from Ogoni Isolates (Bacillus spp, Serratia marcesans, Bacillus spp and Staphylococcus spp). Total Fungi Count

Fig. 4 - 6 showed the fungal count of Akwa Ibom, Asarama and Ogoni areas respectively. A total of 4.5 x 10^6 cfu/g, 3.0 x 10^4 cfu/g, 7.1 x 10^5 and 1.11 x 10^6 fungi were observed on the head, external body, intestine and body fluid of Akwa Ibom maggot respectively (Fig. 4).



Fig. 4: Total Heterotrophic Fungi (THF cfu/g/ml) in different body parts of the maggot from Akwa Ibom [NB: HD=Head, BF=Body Fluid, IT= Intestine, EB=External Body]



Fig. 5: Total Heterotrophic Fungi (THF cfu/g/ml) in different body parts of the maggot from Asarama [NB: HD=Head, BF=Body Fluid, IT= Intestine]



Fig. 6: Total Heterotrophic Fungi (THF cfu/g/ml) in different body parts of the maggot from Ogoni [NB: HD=Head, BF=Body Fluid, IT= Intestine, EB=External Body]

In all, Akwa Ibom had a total of 6.35×10^{6} cfu/g The highest fungal load $(1.11 \times 10^{6}$ cfu/g) was observed in the body fluid. Fungal load was also highest $(1.11 \times 10^{6}$ cfu/g) in the body fluid of Asarama maggot just like that of Ogoni maggot $(1.08 \times 10^{6}$ cfu/g)(Fig 5). In all, Ogoni maggot had the highest fungal load of $(2.0 \times 10^{4}$ cfu/g) on their body fluid. The lowest fungal count was observed in the external body of Ogoni maggot (Fig. 4-6). Asarama maggot had a total of 3.42×10^{6} cfu/g while Ogoni had the least value of fungal count of 2.2×10^{6} cfu/g. Asarama maggot had the highest fungal count $(1.96 \times 10^{6}$ cfu/g) on the head while the lowest count (4.0 x 10^{4} cfu/g) was observed in the intestine (Fig. 6).

The bacterial load or count in this study varies with regions/parts of the body of the maggot and the area sampled. This is in line with the findings of Bassey et al (2014) who disclosed that microbial load or count differs with respect to the part of the body of the organism (molluscs and arthropods) and also with environmental difference or geographical location and the state of the organism. The bacterial count observed in this study differs from that reported by Bassey et al (2014) but in agreement with Ogbalu and Williams (2015) who reported total count of 1.68×10^5 cfu/g from processed edible weevil caterpillar (Rhynchophorus phoenicia) and 4.49×10^6 cfu/g from an edible caterpillar of emperor moth (Bunae alcinoe).



Fig. 7: Comparative Total Heterotrophic Bacterial (THB cfu/g) Load between the two states of Nigeria.



Fig. 8: Comparative Total Heterotrophic Fungi (THF cfu/g) Load between the two states of Nigeria.

Comparative evaluation revealed Total Heterotrophic bacteria (THB - cfu/g) to be highest from samples from Rivers State (9.57 x 10⁶ cfu/g) and least with samples from Akwa Ibom state (8.12 x 10⁶ cfu/g) while Total Heterotrophic fungal count took the reverse trend with Akwa Ibom State having the highest fungal load (6.06 x10⁶ cfu/g) and least with Rivers State sample (1.9 x 10⁵ cfu/g) (Fig. 7-8).

According to Omotoso and Adedire (2007), high bacterial load and protein content of a particular food indicates high nutritional quality and susceptibility to spoilage micro organisms. This statement is in agreement with this finding since the maggot has high microbial load and therefore susceptible to quick microbial deterioration.

The fungal load/count observed in this study is in agreement with the 3.6 x 10^6 cfu/g reported by Robinson (2014) and 2.2 x 10^6 cfu/g reported by Bassey et al (2014). The result of this finding is also in conformity with the earlier statement that microbial load/count varies with part of the body of organisms, the geographical location and the feeding habit or the life style of the organism.

	0	
	Fungal isolates in	lentified
1	Physarum cinered	ım
2	Penicillium	funiculosum
3	Penicillium	cememberti
4	Aspergillus	nidulans
5	Fusarium spp	
6	Aspengillus	versicolor
7	Aspengillus	niger
8	Alternaria spp	
9	Mucor racemosus	3
10	Aspergillus fumi	gatus
11	Absidia corymbit	era
12	Pulularia pullans	
13	Phialophora verru	ıcosum
14	Penicillium mern	ettei
15	Aspergillus tarren	15
16	Histoplasma caps	sulatum
17	Aureobasidium p	ullulans

	18	Scopulariopsis spp
	19	Epidemophyton spp
	20	Trichophyton spp
	21	Candida spp
Table 2	Bact	eria isolated from Insect larva (maggot)
Bact	erial Is	solates Identified
1.]	Bacillus spp.

3

4

The Isolates observed/identified in this study (Table 1-2) are in conformity with the reported count of
Wachukwu et al (2002) who worked on the weevil larvae.

Staphylococcus spp.

Serratia spp. Enterobacter spp

The observed microbial or bacteria load in the gastrointestinal tract (gut) of the Rhynchophorus phoenicis (maggot) especially Staphylococcus species in this study is similar to that reported by (Wachukwu et al 2020) which was attributed to the feeding habit or life style of the organism in the area. According to Wachukwu et al (2002) the species such as Bacillus and Staphylococcus are known enterotoxin producers which therefore portray danger especially for people who may want to eat the maggot in raw form. Although this insect maggot is a rich source of protein but the presence of Staphylococcus aureus is of public health significance.

Rhynchophorus phoenicis from Asarama had just 4 fungal species distributed as follows - Penicillium funciculosum (head), penicillium funiculosum (body fluid), Aspergillus nidulans and Scopulariopsis spp (intestine). Sample from Ogoni had a total of 8 fungal species consisting physarum cinereum and mucor racemosus (head), Physarium cinereum, Penicillium merneffei and Phialophora verrucosum (body fluid) and Aspergillus tarreus (intestine) (Fig.7).





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According to Omotoso and Adedire (2007), high bacterial load and protein content of a particular food indicates high nutritional quality and susceptibility to spoilage micro organisms. This statement is in agreement with this finding since the maggot has high microbial load and therefore susceptible to quick microbial deterioration.

Aspergillosis species observed in this study has been reported by Ogbalu and Williams (2014); Nrior et al (2017b) in their study and was said to be responsible for aflatoxins which may be implicated in hepato-cellular carcinoma. Ogbalu and Williams (2014) also reported the Penicillium species present in this study to be attributed to ochratoxin-A production which is a potent nephroxin and causes damage in the body system. However, some isolated fungi in this study are of high pharmaceutical importance.

IV. Conclusion and Recommendation

There has been increase in the rate of consumption of this insect maggot among people in Niger delta and other parts of this country due to its high nutritional quality and medicinal value especially among those inhabiting the rural areas owing to their cultural background.

It is of importance to note that this highly desired and consumed insect has high microbial load or population in different parts of the bodies that are consumable. The microbial flora such as Bacillus species, Staphylococcus species, Enterobacter species and host of other fungi such as Mucor racemosus, Candida specie fusarium species, Aspergillus nidulans etc are present in this insect. The head and the body fluid were observed to have higher load of the microbes. Therefore, it is also of interest to note that microbial load is highest in the maggot obtained from Ogoni which could be attributed to their unhygienic practices and some environmental factors which must have made the environment suitable or favourable for microbial growth. The bacterial load of the insect in the areas studied is higher than the fungal load except the Akwa Ibom Isolate where the fungal load is higher than that of Asarama area of Rivers state. This is to say that the insects in these areas were more contaminated with bacteria than fungi except that of Asarama community in Rivers state.

Based on the result of this finding, the following recommendations were made:

- (i) This insect contains huge microbial (Bacteria and fungi) load, hence adequate hygienic practices and proper processing are needed before they can be consumed.
- (ii) The inhabitants of these areas should avoid eating the insects (maggots) raw to avoid pathogenic invasion especially those in Ogoni axis.
- (iii) The maggot should be properly cleaned and subjected to intense heat before consumption.
- (iv) A credible microbiological test should be carried out (conducted) on these insects (maggot) so as to ascertain the health status before consumption.

Nevertheless, the vast microbial loads in these species of insect could serve as a ready source of microbes in some processing and pharmaceutical industries.

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DOI: 10.9790/2402-1209035968
