Research Journal of Food Science and Quality Control E-ISSN 2504-6145 P-ISSN 2695-2459
Vol 7. No. 1 2021 www.iiardjournals.org

Microflora and Health Implications of African palm weevil (*Rhynchophorus phoenicis*) in Idemili Local Government Area of Anambra State, Nigeria

[1]Thomas, Commander N., [2]Disegha, G. C.; [3]Green, M.E.
[1]Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.[2,3]Department of Microbiology, Rivers State University, Port Harcourt, Nigeria.

Abstract

Microflora of the larvae of African palm weevil (Rhynchophorus phoenicis) and its habitat was investigated. Live R. phoenicis larvae and tissue of decaying palm used in the study were collected from a local market at Oba in Idemili Local Government Area of Anambra State, Nigeria. The samples were subjected to microbiological and biochemical analyses. The results showed that the bacterial load of R. phoenicis larvae was the highest at 5.05 x 10^8 CFU/g, while the highest fungal count of 3.5 x 10^8CFU/g was observed in the internal gut content. The internal gut of R. phoenicis contained more bacteria than fungi. Bacterial species isolated include: Bacillus spp., Escherichia coli, Serratia spp., and Pseudomonas spp., while fungal species isolated were Aspergillus fumigatus, Cladosporium sp., Penicillium spp. and Microsporum canis. Four genera of bacteria and four species of fungi were isolated in all from the larvae. It was observed that R. phoenicis larvae are highly contaminated with bacteria and fungi, hence adequate hygienic practices and proper processing are required before consumption, as the presence of E.coli of greater than 3 per gram is undesirable in foods. However, no health issues have been reported among the local people for consuming the raw larvae.

Key Words: Rhynchophorus phoenicis, microbial load, and health implications.

INTRODUCTION

Rhynchophorus phoenicis commonly known as African Palm Weevil, belonging to the family Curculionidae and order Coleoptera, is a pest of palms (oil, raphia and coconut). The female lay eggs in wounds which hatch to small larvae that feeds on the sap and develops to be big grubs by several molts within the trunk of the palms. The final larvae construct cocoons with fibres where they pupate and then new adults emerge from it (Thomas, 2003). *R. phoenicis* causes harm in palm trees which is characterized by yellowish to brownish discharges from tunnels created by the activities of the weevils. There are also decaying tissues which produce odour of fermentation (Hill, 1983). The weevil is considered as a devastating pest in palm plantations (Muafor*et al*., 2014). The larvae of *R. phoenicis* is a delicacy and well-known for its high nutritional value, which makes it generally known as edible Suya in Bayelsa State in the Niger Delta region of Nigeria and other countries in Africa (Amadi and Kiin-Kabari, 2016). They are mostly processed, sold, and consumed in Akwa Ibom, Bayelsa, Delta, Edo, Rivers, Imos and Anambra states of Southern Nigeria (Thomas, 2003, 2019). The method of preparation for consumption differs in different localities and tribes. In some places, it is boiled while others fry, smoke and eaten raw

(Thomas and Briyai, 2019). It is sometimes consumed as part of a meal or as a complete meal along with bread or cooked plantain. Some tribes suggest it to their pregnant women, possibly as a source of essential nutrients for healing purposes (Ekpo, 2003). The consumption of different species of the edible larvae of palm weevils (*Rhynchophorus* spp.) has been confirmed as highly nutritious globally. According to the World Health Organization (WHO), every 100 g of palm weevil larvae contains 182 kcal of energy, 6.1% of protein, 3.1% of fat, 9.0% of carbohydrates, 4.3 mg of iron, 461 mg of calcium, and other important vitamins and minerals" (Bernad*et al.*,2018).

The production and harvesting of *R. phoenicis* has greater difficulties in the rainy season due to the annual floods that cover the swamps in the palm belt of Niger Delta. The larvae develop in a moist environment with temperature of about 25°C to 30°C. The method employed in harvesting the *R. phoenicis* larvae is cumbersome due to the way it burrows into the tissues of the palm tree. Methods employed in harvesting larvae include cutting down palm tree, digging of the inner sap of the dead palm tree and detecting of feeding sounds of the larvae. Sometimes, harvesters cut the stump into pieces and extract the larvae from tunnels in the trunk (Muafor*et al.*, 2014). The edible larvae harvested from the field are routinely preserved using different methods, such as salting, drying, freezing, smoking, and boiling with water. Drying and salting are used to preserve freshly harvested palm weevil in areas around Itokin and Epe in Nigeria (Adeoye*et al.,* 2014). A natural way of preserving live *R. phoenicis* larvae for extended period of time is to keep the larvae with the decaying tissues of the raphia in dark aerated containers by controlling the humidity by sprinkling water occasionally (Séré*et al.*, 2018).

Palm weevils being secondary pests of palms can pose health risks in humans and animals when consumed in raw form, as they are potential carriers of pathogenic bacteria and fungi which have negative impact when consumed (Ebenebe and Okpoko, 2015). The aim of the study was to carry out microbiological investigation on the larvae of African Palm weevil (*R. phoenicis*), to cultivate, isolate and identify the bacteria and fungi associated with the larvae and decayed tissues of the host plant.

MATERIALS AND METHODS

Samples of live *R. phoenicis* larvae were bought from a local market at Oba in Idemili Local Government Area of Anambra State, Nigeria. The larvae were collected with the decaying tissues of palm in an aerated plastic bucket and transported to the microbiology laboratory of Rivers State University, Port Harcourt, Nigeria.

Microbiological examination was carried out using Nutrient and Sabouraud dextrose agars, which were prepared according to their respective manufacturer's instructions. Physiological saline was prepared by adding 0.85g of sodium chloride (NaCl) to 1000ml of Sterile distilled water for use as diluents. Internal parts of the live *R. phoenicis* larvae were dissected using a sterile dissecting blade and the decaying tissues of palm were homogenized separately using sterile mortar and pestle. Serial dilution was made with one gram (lg) of each sample by adding into 9ml of sterile saline contained in a test tube. This was vortexed to form a stock solution of 10^{-1} concentration. Then a series of ten-fold dilutions were prepared from the stock. An aliquot of 0.1ml each from 10^{-2}, 10^{-4} and 10^{-6} was pipetted and inoculated on the surfaces of dried Nutrient agar and Sabouraud dextrose agar plates using sterile 1ml pipettes. The inoculum was spread evenly using a sterile glass rod spreader which was further sterilized by dipping into 98% ethanol and flamed with a Bunsen burner. All plates were inverted and incubated at 37°C for 24 hours. The number of total heterotrophic bacteria and fungi colonies were counted and recorded. Morphological identification was carried out by studying the colonial morphology with consideration of size, shape, margin and elevation of

the colonies. To obtain pure cultures, different colonial morphology observed on each plate was sub-cultured in dried freshly prepared Nutrient and SDA agar plates using a sterile wire loop and inoculating needle to obtain pure cultures. The stock cultures obtained from the pure isolates of bacterial and fungal colonies were stored in the refrigerator for future laboratory use. 2.52g of nutrient agar was dissolved in 100ml of distilled water and 3.25g of Sabouraud Dextrose agar was dissolved in 100ml of distilled water and heated for homogeneity. Then 10ml of the media was dispensed into McCartney bottles and sterilized in the autoclave for 15 minutes at 121 °C, after which the media contained in the McCartney bottles were slanted and allowed to solidify. Each pure culture isolate was streaked on the surface of an agar slant using a sterile wire loop and inoculating needle and incubated for 24 to 48hours at 37°C. The stock cultures were then preserved in the refrigerator (Disegha, G. C. and Onuegbu, 2018).

Biochemical tests were carried out using standard techniques (Cheesebrough, 2006). The bacterial isolates were identified using ABIS Online (Stoica and Sorescu, 2012), while the fungi isolates were identified based on their morphological and microscopic characteristics by examination of prepared glass slides on transparent sticky tape and methylene cotton blue reagent.

RESULTS

The results (Table 1 and Table 2) depicts the total heterotrophic bacterial and fungal counts of various samples of *R. phoenicis* larvae and its habitat, while Table 3 depicts the bacterial isolates identified through morphological identifications and biochemical reactions. Table 4 is showing the characterization and identification of fungal isolates associated with *R. phoenicis larvae* in its habitat.

Table 1: Total heterotrophic bacterial count associated with internal gut and decaying tissue of habitat of *R. phoenicis*.

Sample	Total Bacterial Count	Mean Bacterial Count	CFU/g
IGC_1	188	94	$9.4 x10^4$
IGC_2	137	68.5	$6.85 x 10^6$
IGC_3	115	55	$5.5 x 10^2$
DTH_1	356	178	$1.78 x 10^5$
DTH_2	182	91	$9.1 x 10^6$
DTH_3	101	50.5	$5.05 x 10^8$

Key: IGC - Internal Gut Content; DTH– Decaying Tissue of Habitat**.**

Table **2: Fungal count associated with *R. phoenicis* larvae in it's habitat.**

Sample)	Total Fungal Count	Mean Fungal Count	CFU/g
IGC_1	17	8.5	$8.5 x10^4$
IGC_2	8	4	$4.0 x 10^6$
IGC_3	7	3.5	$3.5 x 10^8$
DTH_1	151	75.5	$7.5 x 10^5$
DTH_2	66	33	$3.3 x 10^6$
DTH_3	43	21.5	$2.15 x 10^8$

Key: IGC - Internal Gut Content; DTH– Decaying Tissue of Habitat**.**

The species of bacteria and fungi identified are given on Table 3 and Table 4 respectively.

Table 3: Bacterial isolates identified through Morphology and Biochemical Reactions

	IGC_1	IGC_2	IGC_3	IGC_4	DTH_1	DTH_2	DTH_3	DTH_4	DTH_5
Test	*Bacillus sp.*	*Pseudomonas sp.*	*Bacillus sp*	*Bacillus sp*	*Pseudomonas sp.*	*Pseudomonas sp.*	*Escherichia coli*	*Bacillus sp.*	*Serratia sp.*
GR	+	+	+	+	+	+	+	+	+
Cat	+	+	+	+	+	+	+	+	+
St	+	+	+	+	+	+	+	+	+
Oxi	+	+	+	+	-	+	-	+	+
Ci	+	+	+	+	+	+	+	+	+
Mtl	+	-	+	+	+	+	+	+	+
Ur	-	-	-	-	-	-	-	-	-
SH	+	+	-	+	+	-	+	-	-
VP	+	+	-	+	+	-	-	-	+
MR	+	+	+	+	+	+	+	+	-
Ind	+	+	+	+	-	+	+	+	+
Glu	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Lac	+/+	-/-	+/+	+/+	+/+	+/+	+/+	+/-	+/-
Mal	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-
Man	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+

KEY: GR: Gram Reaction (all Rod); Gnr: Gram Negative Rod; Cat: Catalase; St: Salt Tolerance; Oxi: Oxidase; Ci: Citrate; Mt: Motility; Ur: Urease; SH: Starch Hydrolysis; VP: Voices Proskaeur; MR: Methyl Red; Ind: Indole; Glu: Glucose; Lac: Lactose; Mal: Maltose; Man: Manitol; +: (Positive reaction); - (Negative reaction); +/+:(positive for acid/gas); +/-:(Acid positive/gas negative)

Table: 4 Characterization and Identification of Fungal Isolates associated with *R. phoenicis larvae.*

Identification	*Penicillium sp.*	*Clasdosporium sp.*	*Microsporum canis*	*Aspergillus fumigatus*
Macroscopic	Radially spread furrowed blue-green, velvety colony, with white periphery with reverse side white colour.	Radially furrowed dark-brown velvety growth with white periphery; reverse side yellow in colour.	White, coarsely fluffy colony, with yellowish white centre and deep yellow reverse side.	Dark green colony, generally powdery or granular. The growing margin appears as white apron with dark reverse side.
Microscopic	Septate hyphae, with branched conidiophares bearing phialides, with streptate conidia on phialides.	Septate hyphae, branching conidiospore, spores	Septate aerial hyphae, with solitary conidia.	Conidial heads are columnar and smoth.

DISCUSSION

The results from this study revealed that the internal gut content of *R. phoenicis larvae* and its habitat contained four species of bacteria: *Bacillus spp., Escherichia coli, Pseudomonas* spp. and *Serratia* spp. The total bacterial count was 9.4 x 10^4 CFU/g while total fungal count was 7.5 x 10^5 CFU/g. The presence of *Escherichia coli* was an indication of faecal contamination of the *R. phoenicis* larvae and its habitat was undesirable because many rural dwellers use the larvae for medicinal purposes and consume it raw (Wachukwu *et al.*, 2002). CSMF (1986) reported that *E. coli* detected in foods is tolerated when it is less than 3 per gram, which was the case in this study. *E. coli* is a faecal coliform and it is a specific indicator of faecal pollution of higher probability than other faecal coliforms (Odonkor and Ampofo, 2013). According to the Microbiological Guidelines for food, the permissible level of raw ready-to-eat meat and fish of<10 is satisfactory, $10^6<10^7$ is borderline, and > 10^7 is unsatisfactory (Centre For Food Safety, 2014). *Escherichia coli* infection can cause disease such as urinary tract infection, bacteraemia and meningitis. *Bacillus spp.* observed was also associated with food borne disease. ICMFS (1986) showed that *Bacillus spp.* presenting cooked food is as a result of inadequate temperature controls.

The presence of *Pseudomonas* spp. in the larvae of *R. phoenicis* was also undesirable, though Kruick (2013) noted that *Pseudomonas* spp. was a common environmental organism that poses no health risk to healthy people, but is most severe in patients who are already hospitalized with other disease condition. Besides, the presence of *Pseudomonas* spp. can have some negative effect on the nutritional quality of the weevil, because it reduces protease and lipases that can catalyze reactions leading to degradation of proteins and thus produce an unacceptable flavor to the food when eaten(Nester *et al.*, 1998).

This study also revealed that the internal gut content and the tissues of its habitat associated with *R. phoenicis* also contains four species of fungi: *Aspergillus fumigatus, Cladosporium* spp., *Microsporum canis* and *Penicillium* pp.The observation of these of fungi in the internal gut content of the larvae of *R. phoenicis* and its habitat was not out of place because the weevils are sold uncovered in an open place and the unsold larvae were kept for sale in subsequent days. *Cladosporium* spp. is ubiquitous with its spores distributed in the air, water and soil which causes deterioration and spoilage of foods. Rafal *et al.* (2012) showed that *Cladosporium* spores are dominant in the in the air (80%) in various parts of Africa. *Cladosporium spp.* can cause allergic reactions in human which sometimes lead to asthma. *Aspergillus fumigatus* are acutely toxic to man and animals. Yu et *al.* (2005) reported that *Aspergillus fumigatus* is the cause of invasive and noninvasive aspergilloses in humans, animals and insects.

CONCLUSION

The rate of consumption of edible larvae *of R. phoenicis* among people in Niger Delta and other parts of the world has increased in recent times, due to its high nutritional and medicinal values, especially among those dwelling in the rural areas. It is important to note that larvae of *R. phoenicis* is a highly desired and consumed insect but has high microbial load in different parts of the body. The microbial flora which consisted of *Bacillus* spp.*, Staphylococcus* spp.*, Serratia* spp. and other fungi such as *Aspergillus fumigatus., Cladosporium* spp.*, Penicillium*spp.*,* and *Microsporum*_spp. were isolated from this insect. The habitat of the larvae had higher loads of the microbes which may be due to the excretion from the larvae and other environmental factors. Hence it requires adequate hygienic practices and proper processing before consumption. It is strongly advised that people should avoid eating these larvae in raw form to avoid contracting any infection, as the microbial load may be high.

REFERENCES

Adeoyeet *al.* (2014).Eco-Diversity of Edible Insects of Nigeria and Its Impact on Food Security.*Journal of Biology and Life Science*, 5(2):175. DOI: 10.5296/jbls.v5i2.6109.

Amadi, E.N. and Kiin-Kabari, D.B. (2016). Nutritional Composition and Microbiology of Some Edible Insects Commonly Eaten in Africa, Hurdles and Future Prospects: A Critical Review. *Journal of Food Microbiololgy: Safety and Hygiene*. 1: 107. doi:10.4172/2476-2059.1000107.

Bernad, Q. *et al.* (2018). Nutritional Potential and Microbial Status of African Palm Weevil (Rhynchophorus phoenicis) Larvae Raised on Alternative Feed Resources. *American Scientific Research Journal for Engineering, Technology, and Sciences* (ASRJETS). p. 45-52.

Centre For Food Safety (2014). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items). Risk Assessment Section Centre for Food Safety Food and Environmental Hygiene Department, Hong Kong.

Cheesebrough, M. (2006).District laboratory practice in tropical countries.(2nd Ed.). Cambridge: Cambridge University Press.

Disegha, G. C. & Onuegbu, C.M. (2018). Bacteriological Quality of Fresh Prawn (*Macrobracium rosenbergii*) Sold in Port Harcourt. Research Journal of Food Science and Quality Control, 4 (2): 18-26.

Ebenebe, C. I. and Okpoko, V.O.(2015). Microbiological quality of raw and roasted African palm weevil (*Rhynchophorus phoenicis*) consumed in the South Eastern Nigeria. *Animal Research International*,12(2): 2159 – 2165 2159 ISSN: 1597 – 3115 ARI 2015 12(2): 2159 – 2165.

Ekpo, K.E. &Onigbinde, A.O.(2005). Nutritional potentials of the larvae of *Rhynchophorusphoenicis.* Pakistan Journal of Nutrition, 4(5): 287 – 290.

Hill, D.S, (1983). Agricultural Insect Pests of the Tropics and their Control.2nd edition. Cambridge, UK: Cambridge University Press.

ICMSF (1986).Sampling for microbiological analysis; principles and specification. International Commission on Microbiological Specifications for Food (ICMSF). New York: Balckwell Publications.

Kruick, G. (2013). *Pseudomonas aeroginosa*.www.healthline.come/health/pseudomonas. Access 13th June, 2014.

Muaforet *al.* (2014). Exploitation, trade and farming of palm weevil grubs in Cameroon. Center for International Forestry Research. Cameroon.

Nester, E. W. Roberts, C. E., Pearsall, N.N., Anderson, D.G. and Nester, M.T. (1998). Microbiology: A human perspective, 2nd Edition. New York: WBC/McGraw Hill.

Odonkor, S.T. and Ampofo, J.K.(2013). Escherichia coli as an indicator of bacteriological quality of water: an overview. Microbiology Research, 4(1): 5-11.

Opara, M.N. *et al.*2012). Studies on the production trend and quality characteristics of palm grubs in the tropical rainforest zone of Nigeria. *Journal of Agricultural Technology*,

8(3): 851-860.

Rafal, O., Legman, A., Pusz, W., Miluch, A. and Miodynska, P. (2012).Characteristics and taxonomy of Cladosporrium fungi.*Medical Mycology/Mikologia,* 19(2): 80.

Séré*et al.* (2018) Traditional knowledge regarding edible insects in Burkina Faso. *Journal of Ethnobiology and Ethnomedicine*, 14:59https://doi.org/10.1186/s13002-018-0258-z

Stoica C. and Sorescu, I. (2012). ABIS online - Advanced Bacterial Identification Software, https://www.tgw1916.net, Accessed 2015.

Thomas, C.N. (2003).Biology, utilization and rearing of Rhychophorusphoenicis in palms of the Nigeria Delta of Nigeria.Ph.D. Thesis, Rivers State University, Port Harcourt.

Thomas, C.N. &Briyai, F. O.(2019). Traditional Consumption of *Rhynchophorus phoenicis* Larvae (F.) as Human Food in Niger Delta Area, Nigeria. *Journal of Biology and Genetic Research*, 5(1):38-44.

Wachukwu C.K.1, Thomas C.N., Kigigha L.T. (2002). Microorganisms associated with Palm weevil larvae (Rhynchophorus_phoenicis). *Asian Journal of Dairy and Food Research,* 21(1): 44-47.

Yu, J., Cleveland, Nierman, W.C. and Benneth, J.W. (2005).*Aspergilus flavus* genomics: gateway to human and animal health, food safety, and crop resistance to diseases. *RevistaIbero americana de Micologia*, 22(4): 195 – 202.