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Original article

Microbial Diversity of the Edible Dung Beetle (*Aphodius rufipes*) in Relation to Conventional Post-harvest Processing Practices in Minna, Nigeria

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ABSTRACT

Dung beetle larva is a traditional delicacy of the Gbagyi people of Niger State, Nigeria. The microbial load of dung beetle larva was assayed using the pour plate method while enumeration of organisms was done using the colony counter. The highest aerobic bacterial load was obtained from the external surface of fried dung beetle larvae obtained from the market, FDBM (6.53×10^6 cfu/mL) while the least was from fried dung beetle larva processed in the laboratory, FDBL (0.25×10^6 cfu/mL). Similar bacterial count was obtained from external surface of fresh dung beetle, FDB (3.0×10^6 cfu/mL) and sundried dung beetle, SDB (3.0×10^6 cfu/mL). Higher aerobic bacterial load was recorded on the whole body of FDBM (2.03×10^6 cfu/g) while FDB (4.9×10^6 cfu/g), SDB (5.1×10^6 cfu/g) and FDBL (5.6×10^6cfu/g) had similar bacterial load on their whole body. The highest anaerobic bacteria count was obtained from the external surface of FDB (9.3×10^6 cfu/mL) and SDB (8.7×10^6 cfu/mL) followed by FDBL (2.9×10^6 cfu/mL). The least anaerobic count was recorded on external surface of FDBM (2.5×10^6 cfu/mL). The highest anaerobic bacteria count was obtained from whole body of FDBM (10.9×10^7 cfu/g) while the least was from FDB (4.2×10^6 cfu/g) and FDBL (4.4×10^6 cfu/g). The highest fungal count was obtained from external surface (9.00×10^4 cfu/mL) and whole body (48.00×10^4 cfu/g) of FDB while the least was from FDBL external surface (5.67×10^4 cfu/mL) and whole body (5.00×10^4 cfu/g). The bacterial counts in dung beetle larva except for FDBM were within the acceptable limits when compared with the recommended limits (10^4 – 10^6 cfu/g) by International Commission on Microbiological Specification for Foods (ICMSF). The total

fungal count obtained from the present study was above the recommended limit (10^3cfu/g). Species of *Bacillus, Pseudomonas* and *Streptococcus* were isolated from external surface of FDB and SDB respectively while FDBL had species of *Bacillus* and *Proteus* sp. on its external surface. Only *Bacillus* sp. was isolated from the external surface of FDBM. From the whole body of FDB and SDB, *Bacillus* was isolated while species of *Bacillus* and *Proteus* were isolated from FDBM. Species of *Pseudomonas* and *Micrococcus* were isolated from whole body of FDBL. *Bacillus* sp. was the most frequently isolated bacterium while *Streptococcus* sp. was the least. *Aspergillus niger* was the most frequent fungus isolated while *Aspergillus fumigates* was the least. The results of the study revealed that fried dung beetle larva prepared in the laboratory had the least microbial count. Samples of dung beetle larva studied contained both pathogenic and non-pathogenic microorganisms. Thus adequate precaution should be taken during processing and handling.

Keywords: Aerobic, Anaerobic, Bacterial counts, Dung beetle larva, Frying, Sundrying.

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INTRODUCTION

Globally, there is an increase in the cases of malnutrition as a result of high costs and inadequate protein diets, specifically, in Africa and developed countries require the consumption of insects as new alternative food sources that enhance the basic diet of man [1]. Dung beetle larvae (*Aphodius rufipes)* have been reported as a traditional delicacy of the Gbagyi people in Niger State. It is an alternative protein source [1]. Dung beetles are commonly found in rice fields, dung of horses, sheep and cow, where their tunnel nests are built underneath. Dung beetle adults feed on fluid extracted from cattle dung, while the larvae live on undigested plant fibers in the dung. The females are characterized as the roller as they fashion a brood ball which both male and female roll away from the dung path. A single egg is deposited in the brood ball where the larva develops through its in star which burrows into the ground before entering the pupa stage. It is this larval stage that is often consumed as a delicacy. It is commonly consumed boiled, grilled, smoked or fried and served as snacks or taken with carbohydrate foods. Only some of the species of dung beetles are edible, especially, the larval stage [2].

Insects have microorganisms in their gut that are essential for their metabolism, behavior and survival but may also influence their safety as food. These microorganisms are a reflection of the mode of life of insects in the wild as well as under rearing conditions. Some of the microorganisms may become pathogenic to the insects under stress conditions. Also, like other animals, insects tend to have microorganisms on their surface and some of these are pathogenic to them [3]. The methods of harvesting, preparation, storage and marketing practiced by the locals are often unhygienic and susceptible to contamination. During processing, the edible insects may come in contact with soil and other rotting materials and may become re-contaminated by microorganisms that can cause spoilage during drying and storage.

It is, therefore, imperative to determine the sanitary quality of this edible insect.

[4] and [5] reported bacterial isolates belonging to the genera, *Staphylococcus*, *Bacillus, Proteus*, *Micrococcus,* and *Acinetobacter*on the cuticle and intestinal tract of edible insects. Some of these isolates are known to produce enterotoxins while others cause food borne diseases and lower nutritional quality of contaminated insects [6]. The fungal species, isolated from edible insects include species of *Cladosporum, Fusarium, Aspergillus* and *Penicilium*which are food contaminants and produce mycotoxins particularly aflatoxin and ochratoxin whose primary target is the liver and are potent carcinogens, mutagens and teratogens and are acutely toxic to animals and human [6]. This study was aimed at evaluating the microbiological quality of processed (sundried and fried) dung beetle larva with a view of revealing its potential for use as human and animal food supplement.

MATERIALS AND METHODS

Sample Collection and Identification

Fresh samples of dung beetle larvae (*Aphodius rufipes)* of average weight 450.9g were purchased from Kure market, Niger State, Nigeria. The larvae were handpicked and transferred to sterile perforated containers. The insects were identified and authenticated by an Entomologist in Department of the Biological Sciences, Federal University of Technology, Minna. Insects were either used immediately or within 24hours of storage in refrigerator.

Sample preparation

Two hundred gram (200 g) each of fresh sample, sundried, fresh sample fried at 60°C for 15 minutes in the laboratory and fried sample obtained from market were designated as FDB, SDB, FDBL and FDBM respectively.

Microbiological analysis of dung beetle larva

Total microbial load in whole insect and on the cuticle (surface) were determined using the pour plate method of [7]. A gram of each crushed insect sample was transferred into (10ml) of sterile distilled water to obtain a stock solution. A millilitre (1ml) of the stock solution was dispensed into 9ml of sterile distilled water to obtain 10^{-1} dilution. Further dilution was made to10^{-7}. A millilitre (1ml) of the 5^{th}, 6^{th} and 7^{th} diluents were aseptically inoculated in triplicates into freshly prepared molten nutrient agar and the 3^{rd} and 4^{th} diluents were aseptically inoculated into sabouraud dextrose agar. Cultured plates were incubated at 37°C aerobically in an incubator and anaerobically in an anaerobic candle jar for 24 hours for bacteria and at28°C for 5 days for fungi. The resulting colonies were counted using a coulter counting chamber and calculated using the formula;

Colony forming unit (cfu) = number of colonies × volume of diluent × reciprocal of dilution

Microbial load on external surface of the insects was similarly determined as described above, but in this case, the edible insect sample was soaked in normal saline for 5 minutes. One milliliter (1 ml) was dispensed into 9 ml of sterile distilled water to obtain 10^{-1} dilution. Further dilution was made to 10^{-6}. A millilitre (1ml) of the 5^{th} and 6^{th} diluents were aseptically inoculated into freshly

prepared molten nutrient agar in triplicates as mentioned above and the 3rd and 4th diluents were aseptically inoculated into sabouraud dextrose agar. Mannitol salt agar (MSA) and blood agar were further inoculated and incubated appropriately to selectively isolate microorganisms which were sub-cultured until pure isolates were obtained. The pure isolates were preserved in agar slant bottles for further identification using standard bacteriological and mycological methods [8].

Confirmation of Identity of microorganisms

Bacterial isolates were characterized and identified by staining technique (Gram staining), macroscopic examination, motility and biochemical tests (catalase, coagulase, citrate utilization, oxidase, indole, methyl red, Voges-Proskauer, starch hydrolysis, urease production and sugar fermentation (glucose, lactose, maltose and sucrose) [9]. Fungal isolates were identified by macroscopic and microscopic techniques as described by [10]. Growth pattern, pigmentation and presence of septa were used for the macroscopic identification of fungal species while mounting preparation technique was used to view the fungi microscopically.

Data Analysis

Results were expressed as mean values $\pm$ standard deviation (S.D). Within groups, comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by Duncan's Multiple Range Test [11].

RESULTS

Bacterial Load of dung beetle larva

The aerobic bacterial load from the external surface and whole body of the insect samples obtained ranged from 2.5×10^5 to 6.53×10^6 cfu/ml and 4.9×10^6 to 2.03×10^8 cfu/g respectively (Table 1). Fried dung beetle larva obtained from the market (FBDM) had the highest microbial load of 6.53×10^6 cfu/ml and 2.03×10^8 cfu/ml on the external surface and whole body, respectively. Fried dung beetle larva prepared in the laboratory (FDBL) had the least value of 2.5×10^5 cfu/ml on the external surface while fresh dung beetle larva (FDB) had the least value of 4.9×10^6 cfu/g from its whole body of the insects. The aerobic bacterial load on the whole body was higher than that on the external surface for all the edible insect samples.

The anaerobic bacteria load from the external surface and whole body of the insect samples ranged from 2.9×10^6 to 2.5×10^7 cfu/ml and 4.2×10^6 to 1.09×10^7 cfu/g respectively (Table 2). Fried dung beetle larva obtained from the market (FBDM) had the highest microbial load of 25×10^6 cfu/ml and 10.9×10^6 cfu/g on both the external surface and whole body. Fried dung beetle larva prepared in the laboratory (FDBL) had the least value of 2.9×10^6 cfu/ml on the external surface while fresh dung beetle larva had the least value of 4.2×10^6 cfu/g on the whole body of the insects. The anaerobic bacteria load on the external surface was higher than that on the whole body for all the insect samples.

Fungal load of dung beetle larva

The fungal load from both the external surface and whole body of the edible insect samples obtained ranged from 6.0×10^4 – 9.00×10^4 cfu/ml and 5.00×10^4

– 48.00×10^4 cfu/g respectively (Table 3). Fresh dung beetle larva (FDB) had the highest fungal load on both the external surface and whole body respectively while fried dung beetle larva prepared in the laboratory (FDBL) had the least values for both the external surface and whole body of the edible insects respectively. The fungal load from the whole insects was higher than that on the external surface for all the insect samples.

Table 1: Aerobic bacterial load of dung beetle larvae

Post-harvest treatment	Source of Bacteria	
	External surface ($\times 10^6$cfu/ml)	Whole body ($\times 10^6$cfu/g)
FDB	3.0 ± 0.5^{ab}	4.9 ± 1.0^{b}
SDB	3.0 ± 0.2^{ab}	5.1 ± 0.4^{b}
FDBM	6.53 ± 6.47^{a}	203 ± 32.6^{a}
FDBL	0.25 ± 0.05^{b}	5.6 ± 1.5^{b}

Values are means ± standard deviation of triplicates. Means with the same superscripts in a column are not significantly different from each other ($P > 0.05$) using DMRT.
FDB - Fresh dung beetle larva SDB - Sundried dung beetle larva FDBL - Fried dung beetle larva prepared in the laboratory FDBM - Fried dung beetle larva obtained from the market

Table 2: Anaerobic bacteria load of dung beetle larva

Post-harvest treatment	Source of Bacteria	
	External surface ($\times 10^6$cfu/ml)	Whole body ($\times 10^6$cfu/g)
FDB	9.3 ± 0.3^{b}	4.2 ± 0.7^{c}
SDB	8.7 ± 1.0^{b}	7.6 ± 1.0^{b}
FDBM	25 ± 3.0^{a}	10.9 ± 1.4^{a}
FDBL	2.9 ± 0.3^{c}	4.4 ± 1.4^{c}

Values are means ± standard deviation of triplicates. Means with the same superscripts in a column are not significantly different from each other ($P > 0.05$) using DMRT.
FDB - Fresh dung beetle larva SDB – Sundried dung beetle larva FDBL - Fried dung beetle larva prepared in the laboratory FDBM - Fried dung beetle larva obtained from the market

Table 3: Fungal load of dung beetle larva

Post-harvest treatment	Source of Fungi	
	External surface ($\times 10^6$cfu/ml)	Whole body ($\times 10^6$cfu/g)
FDB	9.00 ± 2.30^c	48.00 ± 22.74^c
SDB	6.66 ± 2.18^b	15.33 ± 1.20^b
FDBM	7.33 ± 0.8^b	14.3 ± 0.88^b
FDBL	5.67 ± 1.15^a	5.00 ± 1.15^a

Values are means $\pm$ standard deviation of triplicates. Means with the same superscripts in a column are not significantly different from each other ($P > 0.05$) using DMRT.
FDB - Fresh dung beetle larva SDB - Sundried dung beetle larva FDBL - Fried Dung beetle larva prepared in the laboratory FDBM - Fried Dung beetle larva obtained from the market

Table 4: Associated bacteria on the external surface and whole body of the dung beetle larva

Edible insects	Code	External surface	Whole body
Fresh Dung beetle larva	FDB	*Bacillus* sp, *Pseudomonas* sp *Streptococcus* sp	*Bacillus* sp
Sundried Dung beetle larva	SDB	*Bacillus* sp, *Pseudomonas* sp *Streptococcus* sp	*Bacillus* sp
Fried Dung beetle larva Obtained from the market	FDBM	*Bacillus* sp	*Bacillus* sp, *Proteus* sp
Fried Dung beetle larva Prepared in the laboratory	FDBL	*Bacillus* sp, *Proteus* sp	*Pseudomonas* sp, *Micrococcus* sp

Table 5: Associated fungi on both the external surface and whole body of the dung beetle larva

Edible insects	Code	External surface	Whole body
Fresh Dung beetle larva	FDB	*Aspergillus flavus*, *A.niger*, *A. fumigatus*	*A. flavus*, *A. niger*, *A. fumigatus*
Sundried Dung beetle larva	SDB	*A. flavus*	*A. flavus*, *A. niger*
Fried Dung beetle larva obtained from the market	FDBM	*A. niger*	*A. flavus*
Fried Dung beetle larva prepared in the laboratory	FDBL	*A. flavus*, *A. niger*	*A. flavus*

Frequency of occurrence of microorganisms in dung beetle larva

The frequency of occurrence of bacterial isolates in dung beetle larva is presented in Table 6. *Bacillus* sp was the most frequently isolated organisms from all the edible insects (59%) while *Streptococcus* sp was the least (4%). *Aspergillus niger*was the most frequently isolated fungal species on the dung beetle larva (56%), followed by *A. flavus*(33%) while *Aspergillus fumigatus*(11%) was the least isolated (Table 7).

Table 6: Frequency of occurrence of bacterial isolates in dung beetle larva

Bacterial isolates	Number	% Frequency of occurrence
Bacillus sp	16	59
Micrococcus sp	4	15
Pseudomonas sp	3	11
Proteus sp	3	11
Streptococcus sp	1	4

Table 7: Frequency of occurrence of fungal isolates in dung beetle larva

Fungal isolate	Number	% Frequency of occurrence
Aspergillus niger	5	56
Aspergillus flavus	3	33
Aspergillus fumigatus	1	11

Characteristics of Bacterial isolates

The morphological characteristics of all the bacterial isolates obtained on nutrient, MacConkey and blood agar are presented on Table 8. The organisms isolated include, species of *Bacillus*, *Micrococcus*, *Proteus, Streptococcus* and *Pseudomonas.* The microscopic and biochemical characteristics of the bacterial isolates are shown in Table 9. Five genera of bacteria which were: *Bacillus, Micrococcus, Proteus, Streptococcus* and *Pseudomonas* were isolated from the edible insect samples.

Morphological Characteristics of Fungal isolates from the dung beetle larva

Two moulds namely, *Aspergillus niger* and *A. fumigatus* were isolated from the edible insects. The identities of the fungal isolates were cross-matched with those of standard taxa. The morphological characteristics of fungal isolates from the edible insects are presented in Table 10.

Table 8: Morphological characteristics of bacterial isolates from the dung beetle larva

Nutrient Agar	MacConkey Agar	Blood Agar	O/FA	Suspected organisms
Large irregular dull, white (cream) spreading colony	ND	Beta-hemolysis	FA	*Bacillus* sp
Oval, mucuid, raised center, diffusible green colony with fruity odour	ND	ND	OA	*Pseudomonas* sp
Circular, entire convex, bright yellow non-diffusible pigment	ND	Beta-hemolysis	OA	*Micrococcus* sp
Large, irregular, wavy rounded, white/dull opaque dry colony	ND	Beta-hemolysis	OA	*Bacillus* sp
White to cream, rhizoid/ spreading colonies	ND	Beta-hemolysis	FA	*Bacillus* sp
Pinpoint colonies, circular	ND	Beta-hemolysis	FA	*Streptococcus* sp
Round & small, entire convex, yellowish brown colour (tan) mucoid colonies	small, regular, colourless colony	ND	FA	*Proteus* sp

ND - Not determine OA - Obligate aerobes FA - Facultative anaerobes

Table 9: Biochemical characteristics of bacteria isolated from the dung beetle larva

S/N	Grm	Sh	Cat	Coa	Cit	Oxi	Ure	Mot	Sth	Hem	MSA	Glu	Suc	Lac	Mal	H_2S	MR	VP	IndSus org
	+	Rod	+	-	+	-	+	+	+	*β*	ND	+	+	-	+	ND	-	+	- *Bacillus* sp
	+	Rod	+	-	+	-	-	+	+	*β*	ND	+	+	-	+	ND	-	+	*Bacillus* sp
	+	Cocci	-	-	+	-	-	-	ND	*β*	-	+	+	+	+	ND	+	-	- *Streptococcus* sp
	-	Rod	+	-	+	+	-	+	ND	ND	ND	-	-	-	-	ND	+	-	- *Pseudomonas* sp
	+	Cocci	+	-	+	+	ND	-	-	*β*	-	-	-	-	ND	ND	-	-	- *Micrococcus* sp
	+	Rod	+	-	+	-	-	-	+	*β*	ND	+	+	-	-	ND	-	+	- *Bacillus* sp
	-	Rod	+	-	-	-	+	+	-	*β*	-	+	+	-	ND	+	+	-	+ *Proteus* sp

Sh:-Shape, Grm:-Gram reation, Cat:-catalase, Coa:- coagulase, Cit:-citrate, Oxi:- oxidase, Ure:- urease, Suc:- sucrose, Mal:- maltose, Lac:-lactose, Mot:- motility, Sth:-starch hydrolysis, Hem:-hemolysis, MSA:- mannitol salt agar, Glu:- glucose, MR:- Methyl red, VP:- VogesProskauer, Ind:- indole, Sus org:- suspected organism, H_2S:- Hydrogen sulfide Production, ND:- Not determined.

Table 10: Morphological characteristics of fungal isolates from the dung beetle larva

Cultural characteristics	Microscopic characteristics	Suspected organisms
Black colony with white mat On SDA, splitting into several loose columns when aging.	Dark brown to black conidia heads Conidiosphores are smooth walled, turning dark towards the vesicle. Conidia brown & rough walled.	*Aspergillus niger*
Blue-green colony with white edge on SDA. vesicle is curved & roughly parallel to each other.	Columnar conidia heads and unbranched/uniseriate. The	*Aspergillus fumigatus*
Yellow-green colony with white edge on SDA	Yellow-green colonies with rough walled. Septate hyphae.	*Aspergillus flavus*

DISCUSSION

The microbial contaminants obtained from the study of dung beetle larva samples are heterotrophic bacteria and some fungi. Some of the heterotrophic bacteria in nature are pathogenic while some are non-pathogenic. The total aerobic and anaerobic bacteria counts obtained from the whole body of dung beetle larva ranged from 4.9×10^6 to 2.03×10^8 cfu/g and 4.2×10^6 to 10.9×10^6 cfu/g, respectively, while that obtained from the external surface of the dung beetle larva ranged from 2.5×10^5 to 6.53×10^6 cfu/ml and 2.9×10^5 to 25×10^6 cfu/ml, respectively. The acceptable limit of bacterial count is (10^4-10^6 cfu/g) recommended by [12]. The bacterial counts obtained in the present study were within the acceptable limits except for FDBM.

The total heterotrophic aerobic bacterial population count of 10^7cfu/g obtained from the insect *Gryllotalpa africana* (cricket) and 4.49×10^7cfu/g obtained from an edible caterpillar of Emperor moth *(Bunaea alcinoe*) by[13] and [14], respectively were above the recommended limits and higher than the aerobic count obtained in the study. [15] obtained total aerobic bacterial plate count of about 10^3cfu/g from dried larva of *Cirina forda* which was lower than the result obtained from this study. [11]obtained aerobic bacterial count of 1.68×10^5 cfu/g from processed edible weevil caterpillar (*Rhynchophoru sphoenicis*) which was similar to the aerobic bacterial count obtained from fried dung beetle larva prepared in the laboratory (FDBL) in this study.

[16]obtained anaerobic bacteria counts of $2.84\times10^3\pm0.76\times10^3$cfu/g and $7.16\times10^2\pm2.83\times10^2$ cfu/g from corned beef and canned sardine, respectively, values which were lower than the anaerobic counts obtained from this study. The high number of microorganisms from the external surface and whole body of some of the dung beetle larva samples indicated that they were contaminated. The external surface of edible insects harbours a variety of microorganisms which may be introduced from farming, substrates used, soil, wash water, manures, clothing of the workers (handlers), and physical facilities like containers, utensils, entomological nets for collection [17]; [18] while microorganisms from the whole body might have been introduced through the environment by exposure, cross contamination, processing and post handling practices and from human contacts which could potentially lead to a wide range of health problem and can cause food borne diseases [19].

The total fungal counts from the whole and external surface of the dung beetle samples analyzed ranged from 5.00×10^4 cfu/g to 48.00×10^4 cfu/g and 6.00×10^4 cfu/ml to 9.00×10^4 cfu/ml, respectively. The total fungal count of the edible insect was within the recommended limit. Similar fungal population of (10^4) was obtained from the insect; *Gryllotalpa africana* (cricket) by [13] while higher fungal count of (10^6) was obtained in smoked fish sold in Benin City, Nigeria reported by [20].

In general, edible insects are highly rich in protein sources [21] and therefore may support the growth and proliferation of a large population of microorganisms, especially proteolytic bacteria and fungi. In addition, according to [22], edible insects can be contaminated with both pathogenic and spoilage microorganisms during harvesting, processing, packaging, storage factors, distribution and marketing. Contamination can occur via

storage in the market in contaminated bins and other containers that possibly are in contact with decaying products. During storage of edible insects in the refrigerator, cross contamination can take place when it is not properly packaged. Contamination can also take place when the insects are not kept at the right temperature, thus promoting the temperature danger zone [22].

Furthermore, crushing of dung beetle larva during enumeration of the total microbial count resulted in the release of microbiota present in the gut of the insects which is essential for the metabolism, behaviour and survival of the insects [23].This microbiota is normally a reflection of the lifestyle of insects in the wild as well as under rearing conditions. The gut content passage time varies depending on the species of insects and their kinds of diet [14]. Insects are processed both as food and feed with their intestinal contents, and even if the intestine is emptied before harvesting, frass (waste) will remain in the substrate and can contaminate the insects. Some of the microbiota may become pathogenic to the insects under stress conditions. Also, like other animals, insects have microbiota on their surface and some of these are pathogenic in nature [18].

In the present study, different aerobic and anaerobic microbial contaminants were isolated in different percentage viz: *Bacillus* sp. (59%), *Micrococcus* sp. (15%), *Pseudomonas sp.* (11%), *Proteus* sp. (11%), *Streptococcus* sp. (4%), *Aspergillus niger* (56%), *Aspergillus flavus* (33%), *A. fumigatus* (11%). *Bacillus* species were isolated from fresh, sundried and processed dung beetle larvae. They were the most frequently isolated bacterial contaminants from the edible insect analyzed. They are associated with food borne disease [24].

Earlier, *Bacillus* spwas reportedly isolated from live and processed African palm weevil *Rhynchophorus phoenicis* ([14]; [25]). *Bacillus* sp main primary habitat is the soil, are abundant, aerobic, spore-formers and ubiquitous in the environment. They are mostly found in decaying organic matter, air, plant, water, dust and vegetables. Some of the species are normal flora. *Bacillus* sp produce spores that are resistant to cold, heat and common disinfectants, and survive on the surface of the environment for a long period of time [26]. They also produce enterotoxins that have the ability to withstand high temperatures ([27];[28]; [29]). They are known to cause diseases such as septicemia, infection of the wound, eyes, ears, urinary, diarrheal syndrome (watery diarrhea and abdominal cramps) and respiratory tracts when they are consumed. [30]reported the incidence of *Bacillus* sp. from both meat and fish products.

The study also revealed the presence of *Micrococcus* sp in the edible insect samples analyzed. They are common in places, which include human skin, which are the normal microbial flora, soil, water and dust [31]. They are strict aerobes that grow well in environments where there is little water or high abundance of salt. Generally, *Micrococcus* species is assumed as harmless bacterium, but there have been reports on rare cases of micrococcal infections in people with compromised immune systems, as with HIV patients. Recently, this organism was implicated as an opportunistic pathogen and has been related with recurrent bacteremia, septic arthritis, septic shock, meningitis, endocarditis, intracranial suppuration and cavitating pneumonia in immunosuppressed patients [6]. Presence

of *Micrococcus* sp was reported in the microbiological investigations of edible dried *Cirina forda* larvae [15]. Also, [32] isolated *Micrococcus* sp in smoked *Trachurus trachurus.* Their results are similar to those obtained in this investigation.

Pseudomonas sp was also isolated from dung beetle larval samples investigated in this study. They are common environmental bacterial and microbial contaminants of food with high moisture contents. They are associated with high level of food contamination and cause moist spoilage of dung beetle larva. The organism reduces the nutritive and eating quality of dung beetle larval samples ([28]; [6]). [33] also isolated *Pseudomonas* sp from imported frozen fish samples and [34] noted that the isolates were undesirable but their presence had no health risk to healthy individuals except people who were already hospitalized with another disease condition.

The results also revealed the presence of *Proteus* sp (11%) in the dung beetle larval samples analysed in this study. Food products contaminated with *Proteus* sp. is an indication of feacal contamination. *Proteus* sp degrades foods with high protein contents and reduces the nutritive quality of the foods [6]. [25] isolated *P. vulgaricus* from live African palm weevil *Rhynchophorus phoenicis* and [35] isolated *Proteus* sp in the microbial flora found in smoked fish sold at Tombia and Swali market in Yenagoa metropolis, Nigeria. [24] noted that *Proteus* sp. hardly cause food-borne infections but reduces the nutritive value of foods.

In this study, *Streptococcus* sp (6%) was isolated from the edible insect samples analysed. *Streptococcus* sp is less often involved in food spoilage but possess several factors such as exotoxins and streptokinase production that increase its virulence. These toxins are responsible for causing fever, scarlet fever and rashes [36]. [14] isolated *Streptococcus mitis* from processed caterpillar of a lepidopteran, *Bunaea alcinoe. Streptococcus* sp. is one of the leading pathogens of nosocomial infections, particularly associated with foreign body infections. Septicemia and endocarditis are also diseases associated with *Streptococcus* sp. Their symptoms are fever, headache, and fatigue to anorexia and dyspnea [37]. These findings are in agreement with those of [38] who isolated *Streptococcus* sp from raw meat samples sold in the Open Markets of city in Kolkata. Most of the pathogenic microorganisms, especially, bacteria that invade edible insect samples could be detrimental to human health when such insects are consumed.

During handling and processing, the insects may come in contact with soil, faecal matters, gut contents and consequently become re-contaminated with microorganisms that cause spoilage during drying and storage. Adequate precautions should be taken during processing and handling of the insect samples as this will go a long way in reducing the health risk associated with microbial contamination.

The fungi isolated from the dung beetle larva samples include *Aspergillus niger* (56%), *A. flavus* (33%) and *A. fumigatus* (11%). *A. flavus* and *A. niger* were isolated from sundried dung beetle larva (SDB), fried dung beetle larva obtained from the market (FDBM) and fried dung beetle larva prepared in the laboratory (FDBL) while from fresh dung beetle larva (FDB), *A. flavus, A. niger* and *A. fumigatus* were isolated. Fungi are commonly dispersed in soil and air and

frequently isolated from foods. These organisms might have contaminated the edible insects through the deposits of their spores during sun-drying or from soil during harvesting [39]. The majority of fungal isolates identified in this study could produce mycotoxins. The fungi isolated in the edible insect studied are of great health concern because they grow at low water activity; thus making them important in post-harvest/processed contamination and decay [40]. *Aspergillus flavus* are the cause of invasive and non-invasive aspergillosis in humans, animals and insects, and it also causes allergic reactions in humans reported by [41].

Aspegillus species produce mycotoxins such as aflatoxins, ochratoxins and sterigmato-cystines[42]. In human acute aflatoxicoses has been reported in different parts of the world and are associated with abdominal pain, vomiting, pulmonary oedema, convulsions, coma and death with cerebral oedema resulting in the damage of the liver, kidney, and heart [43]. The consumption of foods contaminated with mycotoxins could lead to liver disease [42], which is the primary target organ [43]. Generally, toxin-producing fungi cause diseases in immuno-compromised individuals, and as such, the diseases caused by these fungi are rare.

The presence of these fungi in the samples of dung beetle larva examined in this study could be as a result of handling processes and cross contamination during storage or during sales of the insects. The finding in this study is in agreement with the findings of [25], who isolated *Aspergillus flavus* from African palm weevil (*Rhynchophorusphoenicis*). [15]also isolated *Aspergillus niger*from edible dried *Cirinaforda*larvae in Makurdi metropolis of Nigeria. Storage and packaging are also important factors to be considered in ensuring the safety of insect foods. Fungal infestations of edible insects cause loss of dry matter through utilization of proteins and lipids, leading to loss of nutritional value [6],

CONCLUSION

Results obtained from this study suggest that edible dung beetle larva contained heterotrophic microorganisms which may be pathogenic or non-pathogenic in nature. Adequate precautions should therefore be taken during processing and handling of the insect.

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