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# Microbial analysis and quantitative assessment of aflatoxins from edible dried insects (Palm Weevil, Cricket and Shea Tree Caterpillar) consumed in southwestern and southeastern regions of Nigeria

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ABSTRACT

Currently in Nigeria, a large number of edible insects are known, and they are globally consumed. The aim of this research was to investigate the microbial load and aflatoxin levels in palm weevil, cricket, and shea butter caterpillars. Six different dried edible insect samples were obtained from different States in the country, (5 palm weevils from Ibadan and Owerri State, 5 shea tree caterpillars from Owerri, 10 palm weevils from Ogun, and 10 crickets from Ibadan and Ondo state). All the samples were packaged in sterile zip lock bags, microbial load analysis was carried out using Standard Microbial Technique, and aflatoxin quantification was done using High-Performance Liquid Chromatography (HPLC). Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version. Microbial analysis of cricket from Ibadan and Ondo showed high counts of total aerobic bacterial load ($104.0 \times 10^7$ cfu/g and $91.0 \times 10^7$ cfu/g respectively), the highest aflatoxin quantification of fungi present had values of (17.00 µg/kg and 12.00 µg/kg) in cricket from Ondo and Ibadan respectively. The aflatoxin level was above the permissible limits for ready-to-eat edible dried insects (AFB1; 2µg/kg, Total aflatoxins; 4 µg/kg). Microbial Identification of bacteria and fungi colonies isolated from the palm weevils, crickets, and shea tree caterpillar revealed 3 dominant species of bacteria (*Staphylococcus aureus, Escherichia coli, and Proteus mirabilis*), three mycotoxin-producing fungi were isolated which includes; *Aspergillus niger, Aspergillus terreus, and Aspergillus flavus*. The presence of *E. coli* signifies a potential risk to food safety. Also, the presence of *Aspergillus flavus* in most of the edible insect sample suggests a potential risk for aflatoxin production. The findings of this study indicate an urgent need for strict quality control measures to ensure the safety of edible insects consumed in Nigeria. Additionally, research into effective processing methods to reduce contamination is recommended.

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## 1. Introduction

The consumption of insects as food by any organism is referred to as entomophagy. In recent years, entomophagy has gained attention as a sustainable and protein-rich nutritional source with a lower environmental impact compared to traditional livestock (1). The most commonly eaten insect groups

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are caterpillars, termites, bees, flies, wasps, crickets, ants, grasshoppers, beetles, leaf hoppers, dragonflies, plant hoppers, scale and true beetles. These insects are prepared through roasting, drying, frying, or incorporation into other dishes.The caterpillar of the lepidopteran *Bunaea alcinoe* is both edible and a valuable source of amino acids and proteins (2).

Edible insects have been part of the human diet throughout history and their consumption has been traditionally recognized in some countries and is mostly collected from natural habitats (3). The recent high demand for insect-based foods in the Western part of the country has triggered the mass-rearing systems (4). Edible insects have played a significant role in providing nutritious diets across various African countries (5). In addition, edible insects serve as a crucial natural resource and are often utilized as a coping mechanism, particularly during periods of food scarcity and crisis (6). Adverse climatic conditions in Africa affect small-scale production of animal protein, so the diet is supplemented with edible insect protein. Edible insects have great socioeconomic and environmental benefits for developing countries (7). Insects are part of the human diet in many parts of the globe. It is often eaten whole, but it can also be made into granules or a paste. In traditional cultures, insects are treated in a variety of ways to improve their organoleptic and nutritional properties, as well as their keeping qualities. They include: Roasting, frying, steaming, smoking, blanching, curing, and stewing (8, 9). Global interest in utilizing edible insects as a food is on the rise, with a gradual increase in Western countries, raising serious concerns about food security and safety and the consumption of potentially harmful insect species (10).

Imathiu, (11) opined that; to establish food safety, the processing and storage of insects should adhere strictly to the same hygiene standards as those applied to conventional foods and feeds. Common insect hazards include pathogenic microorganisms and parasites (12), toxins, toxins, and other chemical contaminants. (13).

Mycotoxins are secondary metabolites produced by many phytopathogenic fungi. They are notable food contaminants with acute and chronic health effects on human. Mycotoxins can emerge from the contamination of the substrate used for rearing edible insects. Various mycotoxins have been discovered in edible insects which are of greatest health concern (14).

Despite the numerous benefits of entomophagy, consumer acceptance continues to be a major barrier to adopting insect-based diets in Western countries. Whether entomophagy can become a part of the Western diet is dependent on the availability of edible insects and overcoming peoples negative attitudes towards them. Specifically, ensuring the food safety of edible insects can help shift perceptions and encourage consumer acceptability of the insects. Ready-to-eat edible insects are generally consumed as snacks and food in the southern part of the country so it is imperative to determine the microbial and aflatoxin levels to ensure their safety to consumers.

## 2. Materials and Methods

### 2.1. Study area

Six different edible insect samples were collected from four distinct locations in Nigeria: Ibadan, Ondo, Ogun, and Owerri. In each location, the insect samples were sourced from three different vendors to ensure representative sampling across the regions.

2.2. Sample collection

All dried edible insect samples were collected and transported to the laboratory in sterile zip lock bags, sealed, labeled, and stored in a cool, dried environment at room temperature 27°C until use.

2.3. Preparation of media

The media used were Nutrient agar, Potato dextrose agar, and MacConkey agar which were prepared in line with the manufacturers' instruction, dispensed into conical flasks, and sterilized in an autoclave at 121°C for 15 min, and then poured aseptically into petri dishes.

2.4. Determination of microbial counts

One-tenth milliliters (0.1 mL) of the fifth (5th) and seventh (7th) dilutions gotten from all samples were added to a freshly prepared surface-dried potato dextrose agar (PDA), MacConkey agar (MAC), and nutrient agar (NA) were each aseptically inoculated to the agar plates. A sterile inoculation loop was used to spread the inoculum evenly over the surface of the plate. PDA culture plates were incubated at room temperature for 5 days, while NA and MAC culture plates were incubated at 37°C for 48 h. Once growth was visible, the number of bacterial colonies on each plate was counted. Fungal counts were recorded numerically. The average counts from all individual plates were calculated and multiplied by the corresponding dilution factor to determine the total viable cells per sample unit weight, expressed as colony-forming units per gram of sample (cfu/g) (15).

2.5. Bacteriological analysis of edible dried insects

Each insect samples were placed in a clean dry mortar and pestle, then crushed. One gram of each insect sample was aseptically introduced into 9 mL of sterile distilled water, mixed properly, and 10-fold serial dilutions were made. 1 mL of the $10^7$ and $10^5$ dilutions was pipetted into a sterile Petri dish and then 10 mL of freshly prepared chilled Nutrient Agar and MacConkey Agar was added to the sterile Petri dish. The Petri dish was mixed well and allowed to set. Bacterial plates were incubated at 37° C. for 24-48 h.

The colonies that emerged was enumerated to determine the total viable bacterial count. Each colony was subsequently sub-cultured onto fresh agar plates for accurate identification. The bacterial isolates were examined macroscopically by assessing their color, growth characteristics, surface morphology, and colony shape

2.6. Mycological analysis of edible dried insects

One gram of the crushed sample was aseptically suspended in 10 mL of sterile distilled water, thoroughly mixed, and serially diluted in 10-fold increments. Then, 1 mL of the $10^7$ and $10^5$ dilutions was transferred into a sterile Petri dish, followed by the addition of 10 mL of freshly prepared chilled potato dextrose agar supplemented with 1% streptomycin (16).

All Petri dishes were well mixed and allowed to be set. Fungal plates were kept at room temperature for 72 h. Fungal colonies were examined macroscopically and microscopically and maintained in pure culture. Fungal isolates were examined macroscopically by observing colony characteristics for texture, shape, and color according to the scheme of (16).

2.7. Isolation of fungal colonies on yeast extract slant

All colonies from potato dextrose agar plates were picked with a sterile loop, sub-cultured, washed and

finally maintained on yeast extract slant agar by refrigeration.

2.8. Aflatoxin quantification of edible dried insects

Aflatoxins analysis was carried out using high-performance liquid chromatography (HPLC), and was treated with chemicals and solvents in grade or equivalence determinations. All water used was distilled and passed through a Milli-Q purification system "Millipore, London, UK" for HPLC. Acetonitrile used as mobile phase was HPLC grade and supplied by Merck, Darmstadt, Germany.

Edible insect samples were prepared for analysis using a method adapted from the Official Methods of Analysis (2000), 17th Edition, with minor modifications. A 5 ± 0.05 g portion of each ground sample was weighed into a 250 mL Erlenmeyer flask, to which 30 mL of 80% methanol was added. The mixture was stirred for 30 min at 120 rpm using a magnetic stirrer, then filtered through Whatman paper (St. Louis, MO, USA). A 20 mL portion of the filtrate was diluted with a 2:1 ratio of Phosphate Buffer Solution (PBS), centrifuged at 3500 rpm for 20 min, and then filtered again using Whatman filter paper (pore size 0.45 µm). From the extract, 50 mL was injected into AflacleanTM immunoaffinity columns (LC Tech, Alpha, Germany) at a flow rate of 0.5 mL per minute. The aflatoxin-containing fraction was slowly eluted with methanol and evaporated at 45°C under nitrogen. The residue was dissolved in 2 mL of a mobile phase mixture of water, methanol, and acetonitrile (60:30:15, v/v/v), filtered, and injected into an HPLC system with fluorescent detection at 356 nm (17).

2.9. Statistical analysis

Data obtained were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 20.0 (18). Mean values were compared using a one-way analysis of variance (ANOVA). Results were presented as Mean±Standard deviation. Post hoc test was done using the Student-Newman-Keuls (SNK). A probability value (P – value) less than 0.05 was considered statistically significant.

**3. Results**

3.1. Total fungal count (tfc) of dried palm weevil, cricket and shea tree caterpillar

Table 1 below shows the total fungal count of the dried palm weevil, cricket, and shea tree caterpillar samples. The fungal colonies were counted using a hematocytometer.

The table shows total fungal counts (cfu/g) from dried insect samples across different locations. Palm weevils from Ibadan and Owerri have the same fungal count of $10.0 \times 10^7$, while the sample from Ogun is higher at $41.0 \times 10^7$. Cricket samples from Ondo and Ibadan have the highest fungal counts, with $91.0 \times 10^7$ and $104.0 \times 10^7$, respectively.

**Table 1.** Total number of fungal counts from all dried insect samples

| name of sample | total fungal count (cfu/g) |
|---|---|
| Palm Weevil Ibadan | $10.0 \times 10^7$ |
| Palm Weevil Ogun | $41.0 \times 10^7$ |
| Palm Weevil Owerri | $10.0 \times 10^7$ |
| Cricket Ondo | $91.0 \times 10^7$ |
| Cricket Ibadan | $104.0 \times 10^7$ |
| Shea tree caterpillar | Too numerous to count |

3.2. Total bacterial count (tbc) of dried palm weevil, cricket and shea tree caterpillar

Table 2 shows the total bacterial count of the dried palm weevil, cricket, and shea tree caterpillar samples.

The table presents total bacterial counts (cfu/g) from dried insect samples. Palm weevils from Ibadan and Shea tree caterpillar samples had bacterial counts that were too numerous to count, while the Palm Weevil from Owerri showed a count of 19.0 $\times 10^7$. No bacterial growth was observed in Palm Weevil (Ogun) or Cricket samples from Ondo and Ibadan.

**Table 2.** Total number of bacterial counts from all dried insect samples

| name of sample | total fungal count (cfu/g) |
|---|---|
| Palm Weevil Ibadan | Too numerous to count |
| Palm Weevil Ogun | No growth |
| Palm Weevil Owerri | 19.0 $\times 10^7$ |
| Cricket Ondo | No growth |
| Cricket Ibadan | No growth |
| Shea tree caterpillar | Too numerous to count |

3.3. Morphological characteristics of isolated bacteria from dried palm weevil, cricket, and shea tree caterpillar

Table 3 below shows the morphological characteristics of bacteria isolated from dried palm weevil, cricket, and shea tree caterpillar samples. Colony color, shape, boundaries, opacity, and surface area characterized each bacterium.

The table describes the morphological characteristics of bacteria isolated from dried Palm Weevil, Cricket, and Shea tree caterpillar samples. Bacteria such as *Streptococcus sp., Escherichia coli, Alcaligens sp., and Micrococcus sp.* were identified, showing variations in colony color, shape, optical density, surface texture, and elevation. *Escherichia coli* colonies consistently appeared as pink and circular on MAC agar, while *Streptococcus sp.* colonies were white and circular on NA agar.

3.4. Biochemical characteristics of the bacteria isolates from the edible insect samples

Table 4 shows variations in citrate utilization and fermentation patterns. Most isolates demonstrated complete sugar fermentation with negative results for sulfur production, indole formation, and motility, except for isolate PWI, which exhibited incomplete fermentation and positive citrate utilization.

3.5. Morphological properties of fungi isolated from dried palm weevil, cricket and shea tree caterpillar

Table 5 and 6 below shows the morphological characteristics of fungi isolated from dried palm weevil, cricket, and shea tree caterpillar samples. Each fungus was characterized based on hyphae, underside color, spore type, and colony morphology (color, shape, height, surface area, margins, and opacity).

The table outlines the colony morphological characteristics of fungi isolated from dried Palm Weevil, Cricket, and Shea tree caterpillar samples. Fungi colonies displayed diverse colors, including white, bluish-green, black, and green, with forms ranging from circular to filamentous. Elevation varied from flat to raised or umbilicate, and surfaces were either smooth, glistening, or wrinkled. Colony opacity was either translucent or opaque, and margins were mostly entire or filiform.

**Table 3.** Morphological and cultural characteristics of bacteria isolated from dried palm weevil, cricket and shea tree caterpillar

| agar plate | isolate code | color | shape | opacity | surface | elevation |
|---|---|---|---|---|---|---|
| NA | PWI | White | Circular | Opaque | Smooth | Flat |
| MAC | | Yellow | Circular | Opaque | Flat | Smooth, Glistening |
| NA | PWOG | White | Circular | Transparent | Smooth | Flat |
| MAC | | Pink | Circular | Opaque | Flat | Smooth, Glistening |
| NA | PWOW | White | Irregular | Opaque | Smooth | Raised |
| MAC | | Pink | Circular | Opaque | Flat | Smooth, Glistening |
| NA | CO | White | Circular | Opaque | Smooth | Flat |
| MAC | | Pink | Circular | Opaque | Flat | Smooth, Glistening |
| NA | CI | White | Circular | Opaque | Smooth | Flat |
| MAC | | Pink | Circular | Opaque | Flat | Smooth, Glistening |
| NA | STC | White | Irregular | Opaque | Smooth | Raised |
| MAC | | Yellowish-pink | Circular | Opaque | Flat | Smooth, Glistening |

**KEY: PWI** - Palm Weevil Ibadan, **PWOG** - Palm Weevil Ogun, **PWOW**- Palm Weevil Owerri, **CO** - Cricket Ondo, **CI** - Cricket Ibadan, **STC** - Shea tree caterpillar, **NA** – Nutrient agar, **MAC** – MacConkey agar

**Table 4.** biochemical characteristics of the bacteria isolates from the edible insect samples

| isolate code | citrate test | Tripple Iron test | | Sugar | | | SIM test | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $H_2S$ | $CO_2$ | slant | butt | sugar | sulfur | indole | motility |
| PWI | +VE | -VE | -VE | Yellow | Red | Incomplete fermentation | -VE | -VE | -VE |
| PWOG | +VE | -VE | -VE | Yellow | Yellow | Complete fermentation | -VE | -VE | -VE |
| PWOW | -VE | -VE | -VE | Yellow | Yellow | Complete fermentation | -VE | -VE | -VE |
| CO | -VE | -VE | -VE | Yellow | Yellow | Complete fermentation | -VE | -VE | -VE |
| CI | -VE | -VE | -VE | Yellow | Yellow | Complete fermentation | -VE | -VE | -VE |
| STW | +VE | -VE | -VE | Yellow | Yellow | Complete fermentation | -VE | -VE | -VE |

**Table 5.** Colony morphological characteristics of fungi isolated from dried palm weevil, cricket and shea tree caterpillar

| isolate code | colour of colony | form | elevation | surface | margin | opacity |
|---|---|---|---|---|---|---|
| PWI | White | Circular | Flat | Glistening | Entire | Translucent |
| PWOG | Bluish Green and Black | Filamentous | Flat | Rough | Filiform | Opaque |
| PWOW | Green | Filamentous | Raised | Wrinkled | Filiform | Opaque |
| CO | Whitish-yellow | Irregular | Convex | Smooth | Entire | Opaque |
| CI | White | Circular | Flat | Smooth | Entire | Translucent |
| STC | Black and Whitish-yellow | Filamentous | Umbilicate | Wrinkled | Filiform | Opaque |

**KEY: PWI** - Palm Weevil Ibadan, **PWOG** - Palm Weevil Ogun, **PWOW**- Palm Weevil Owerri, **CO** - Cricket Ondo, **CI** - Cricket Ibadan, **STC** - Shea tree caterpillar

**Table 6.** Morphological characteristics of fungi isolated from dried palm weevil, cricket and shea tree caterpillar

| isolate code | hyphae | type of spore | color of reverse side | organism |
|---|---|---|---|---|
| PWI | Aseptate | Conidia | White | *Saccharomyces cerevisiae* |
| PWOG | Septate | Conidia | Sulfur-yellow | *Penicillium chrysogenum, and Aspergillus niger* |
| PWOW | Septate | Conidia | Sulfur-yellow | *Aspergillus niger* |
| CO | Septate | Ascospore | White and golden Yellow | *Aspergillus terreus, and Aspergillus fumigatus* |
| CI | Aseptate | Conidia | Pale yellow | *Saccharomyces cerevisiae* |
| STC | Septate | Conidia | Brownish yellow | *Aspergillus niger and Aspergillus flavus* |

**KEY: PWI** - Palm Weevil Ibadan, **PWOG** - Palm Weevil Ogun, **PWOW**- Palm Weevil Owerri, **CO** - Cricket Ondo, **CI** - Cricket Ibadan, **STC** - Shea tree caterpillar

Table 6 details the morphological characteristics of fungi isolated from dried Palm Weevil, Cricket, and Shea tree caterpillar samples. Fungi exhibited both septate and aseptate hyphae, with conidia being the predominant spore type. Aspergillus species, including *Aspergillus niger, A. flavus*, and *A. terreus*, were commonly isolated, with sulfur-yellow or brownish-yellow reverse colony colors. *Saccharomyces cerevisiae* was also identified in some samples with white or pale-yellow reverse side coloration.

Figure 1 Frequency occurrence of fungi count isolated from dried palm weevil, cricket and shea tree caterpillar Figure 1 presents the frequency of occurrence of fungal counts isolated from common edible insects, including Palm Weevil, Cricket, and Shea Tree Caterpillar, in various Nigerian markets. The frequency was determined by calculating the percentage of insect samples that showed fungal contamination out of the total number of samples collected for each species. The chart highlights the distribution and prevalence of fungi across these insect samples, indicating which species were most commonly affected by fungal contamination.

3.6. Aflatoxin quantification of dried palm weevil, cricket and shea tree caterpillar

Table 7 shows the quantification of aflatoxin in dried palm weevil, cricket, and shea tree caterpillar samples. The table shows aflatoxin concentrations of the different types of aflatoxins (AFB1, AFB2, AFG1, AFG2) using the high performance liquid chromatography in dried Palm Weevil, Cricket, and Shea tree caterpillar samples from Nigerian markets. Cricket samples had the highest total aflatoxin levels, with 28.50 µg/kg from Ondo and 22.00 µg/kg from Ibadan. Palm Weevil samples showed lower levels, with 7.00 µg/kg in Ogun and 1.00-3.00 µg/kg in Ibadan and Owerri. Shea tree caterpillar had a total aflatoxin concentration of 10.00 µg/kg.

Figures 2, 3, 4, 5, 6, and 7 show the frequency of occurrence of aflatoxin quantification of common edible insects in some markets in Nigeria.

Figures 2 to 7 display the frequency of aflatoxin quantification and fungal counts in common edible insects from Nigerian markets. Figures 2, 3, and 4 illustrate the occurrence of Aflatoxins B1, B2, and G1, respectively, across insect samples, highlighting variations in toxin levels. Figure 5 presents fungal counts of these insects, showing statistical comparisons between samples. Figure 6 summarizes the total aflatoxin levels, while error bars across these figures represent the standard deviation, with significant differences marked by varying alphabet letters ($p > 0.05$).

Figure 7 illustrates the frequency of occurrence of total aflatoxins in common edible insects from various markets in Nigeria. It highlights how often aflatoxins were detected across different insect samples, indicating their prevalence and potential risk of contamination in these edible insects. The chart provides insight into the distribution of total aflatoxins across the studied markets.

**Table 7.** Concentrations of aflatoxin b1, b2, g1 & g2 (µg/kg) of dried palm weevil, cricket and shea tree caterpillar in some markets in Nigeria

| sample code | AFB1 | AFB2 | AFG1 | AFG2 | total aflatoxin |
|---|---|---|---|---|---|
| Palm Weevil Ibadan | 2.00±0.42$^{d}$ | 0.00±0.00$^{b}$ | 1.00±0.14$^{c}$ | 0.00±0.00$^{a}$ | 3.00±0.56$^{d}$ |
| Palm Weevil Ogun | 7.00±0.71$^{c}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{a}$ | 7.00±0.71$^{c}$ |
| Palm Weevil Owerri | 1.00±0.14$^{d}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{a}$ | 1.00±0.14$^{d}$ |
| Cricket Ondo | 17.00±2.83$^{a}$ | 2.00±1.13$^{a}$ | 9.50±0.71$^{a}$ | 0.00±0.00$^{a}$ | 28.50±4.67$^{a}$ |
| Cricket Ibadan | 12.00±1.41$^{b}$ | 0.00±0.00$^{b}$ | 10.00±0.99$^{a}$ | 0.00±0.00$^{a}$ | 22.00±2.41$^{b}$ |
| Shea Tree Caterpillar | 3.00±0.71$^{d}$ | 0.00±0.00$^{b}$ | 7.00±0.57$^{b}$ | 0.00±0.00$^{a}$ | 10.00±1.28$^{d}$ |

$^{abcd}$Means (±Standard deviation) in the same column having similar superscripts are not significantly different ($p > 0.05$)

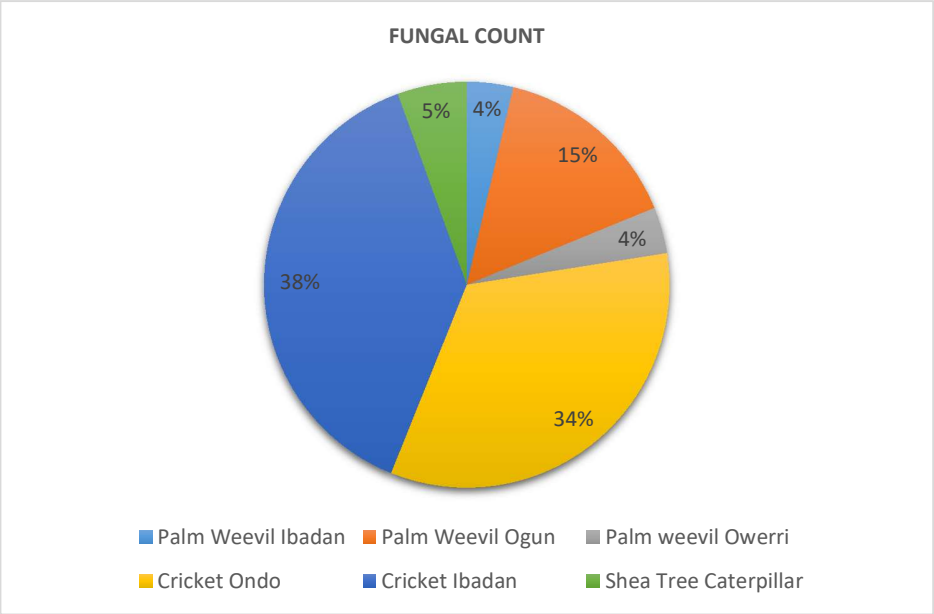


**Figure 1.** Frequency of occurrence of fungal count isolated from common edible insects in some markets in Nigeria

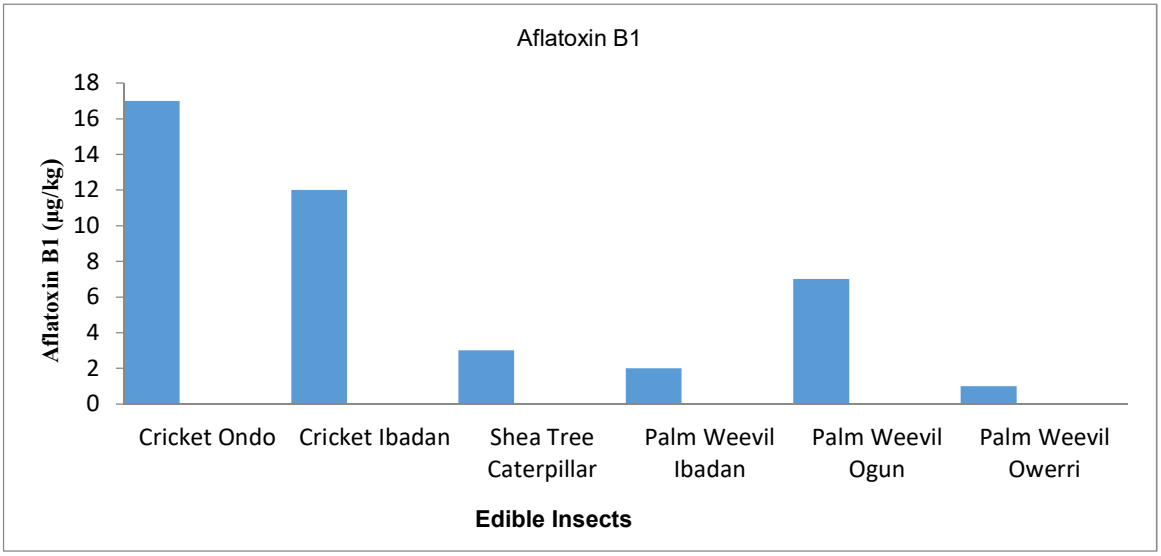


**Figure 2.** Aflatoxin B1 in common edible insects in some markets in Nigeria


**Figure 3.** Aflatoxin B2 in common edible insects in some markets in Nigeria

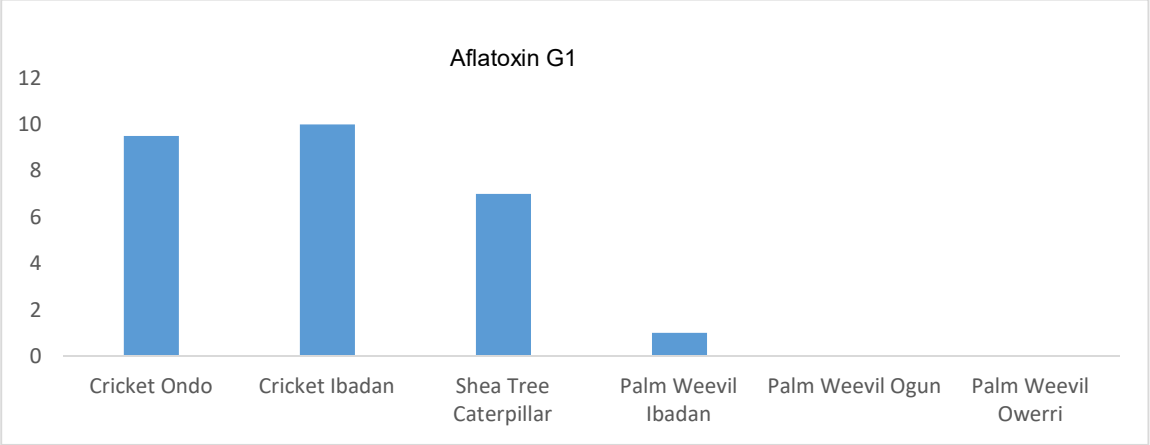


**Figure 4.** Aflatoxin G1 in common edible insects in some markets in Nigeria

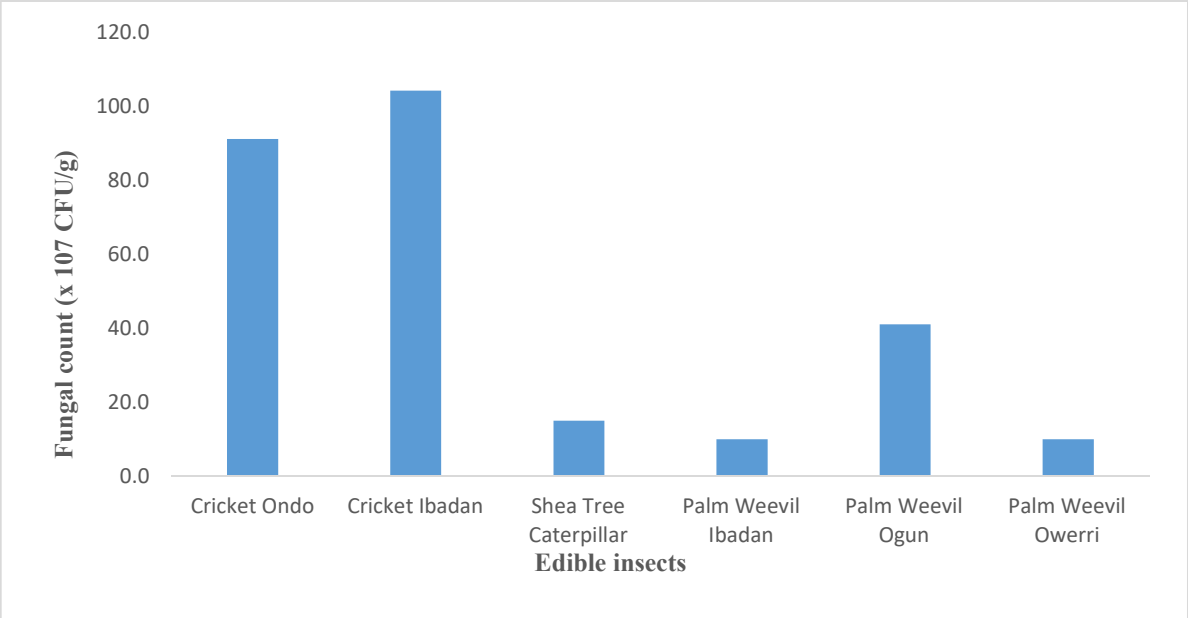


**Figure 5.** Fungal count of common edible insects in some markets in Nigeria; Mean (bars) having similar alphabets are not significantly different ($p > 0.05$); Error bars represent standard deviation.

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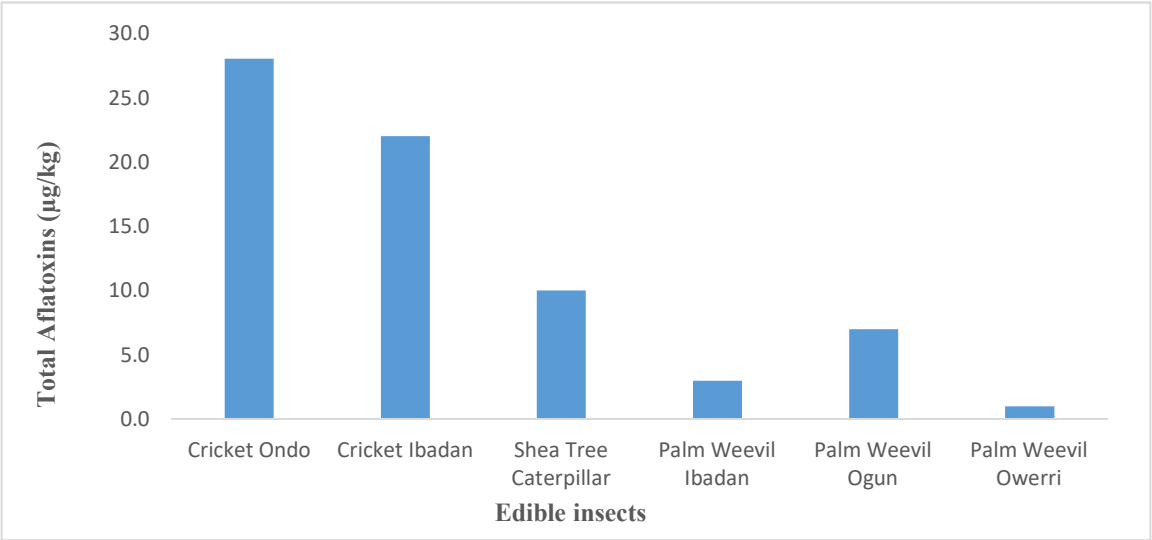



**Figure 6.** Total aflatoxins of common edible insects in some markets in Nigeria; Mean (bars) having similar alphabets are not significantly different ($p > 0.05$); Error bars represent standard deviation.

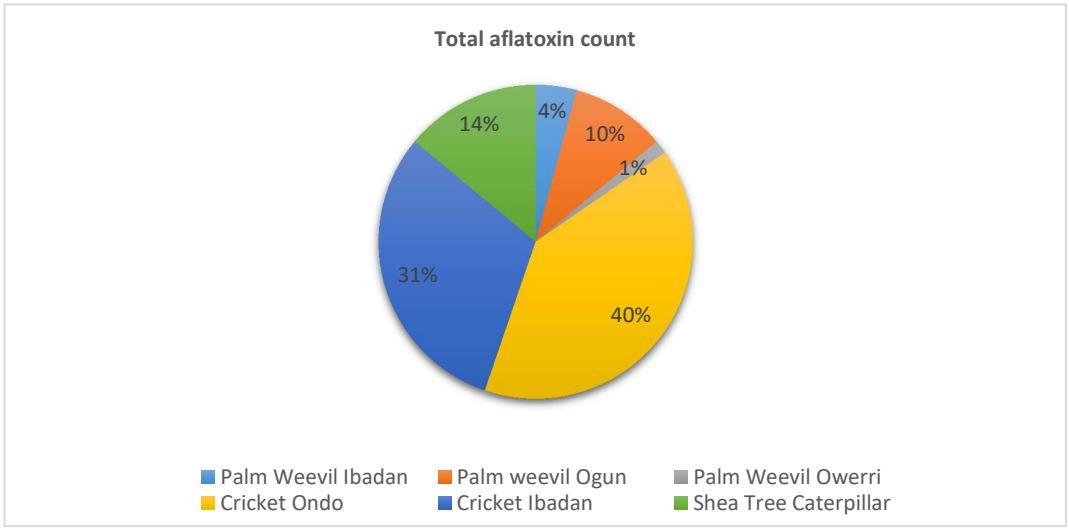
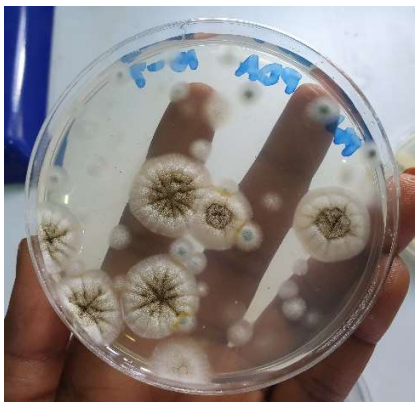



**Figure 7**. Frequency of occurrence of total aflatoxins isolated from common edible insects in some markets in Nigeria

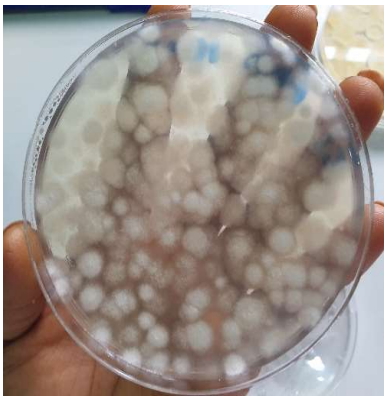
**Plate 1.** Cultural Characteristics of Fungi on PDA Plate

Shea Tree Caterpillar (STC)

Cricket Ondo (CO)



Palm Weevil Ogun (PWOG)



Cricket Ibadan (CI)

Palm Weevil Ibadan (PWI)

Palm Weevil Owerri (PWOW)

**4.Discussion**

The high bacterial count recorded for Cricket Ibadan (CI) at 104.0 ×$10^7$ cfu/g suggests a significant microbial load, potentially linked to environmental or handling conditions.

This is followed by Cricket Ondo (CO), with a viable bacterial count of 91.0 ×$10^7$ cfu/g, indicating similar contamination levels. In contrast, Palm Weevil Ibadan (PWI) and Palm Weevil Owerri (PWOW) exhibited the lowest bacterial counts, both at 10.0 ×$10^7$ cfu/g, which may reflect better preservation or less exposure to contamination. These findings align with Fong et al. (19) who also reported lower bacterial loads in palm weevils, suggesting that certain insects may naturally harbor fewer microorganisms under similar conditions. The viable bacterial counts of palm weevil Ibadan and shea tree caterpillar were too numerous to count (having the highest bacterial counts). The least viable bacterial count occurred in palm weevil Owerri which had a count of 19.0 ×$10^7$ cfu/g, while Palm weevil Ogun, Cricket Ondo, and Cricket Ibadan had no growths. The dominant bacteria isolated from Palm weevil, Cricket and Shea tree caterpillar are *Staphylococcus aureus*, *Escherichia coli*, and *Proteus mirabilis*, which agrees with the findings of Fong et al. (19).

Fungi isolated from Palm weevil, Cricket, and Shea tree caterpillar were; *Penicillium chrysogenum, Aspergillus niger, Aspergillus terreus and Aspergillus flavus.*

The levels of microbial contaminants were significantly higher than the acceptable limits for ready-to-eat foods, implying the likely presence of food pathogens, confirmed by molecular biology techniques Ferone et al. (20).

The highest level of aflatoxin (AFB1) was detected in Cricket Ondo (CO) containing 17.00 µg/kg and Cricket Ibadan (CI) containing 12.00 µg/kg which is above the European Union's Aflatoxin Acceptable Limit (AFB1; 2µg/kg, total aflatoxins not beyond; 4 µg/kg) Abdul-Ra'oof et al. (21) and which is below the United States Aflatoxin Maximum Acceptable Limit (AFB1; 20 µg/kg). While other insect samples had minimal aflatoxin levels. The presence of microbial contamination and aflatoxins in palm weevils, crickets and shea tree caterpillars appears to be a significant health problem in Nigeria and this is in agreement with the work of Zhou et al. (22) who opined on the safety level of aflatoxins in edible insects.

**5. Conclusion**

The study underscores significant concerns regarding the safety of edible insects in Nigerian markets, highlighting notable microbial contamination and varying aflatoxin levels. The presence of hazardous microorganisms and aflatoxins poses potential threats to consumers, particularly with higher levels detected in cricket samples. To ensure food safety, it is crucial to implement rigorous quality control measures for edible insects. Regular monitoring of microbial contamination and aflatoxin levels should be conducted in markets. Additionally, creating awareness amongst producers and consumers about proper handling and storage practices can help mitigate contamination risks. Research into effective processing methods to reduce fungal and aflatoxin levels in edible insects is also recommended to enhance food safety and consumer confidence.

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**Authorship contribution**

**EOO** - Lead researcher, Conception, Design, Drafting of the manuscript, **JB**- Supervisor, Design, Data analysis and Data interpretation, **CD** – Sample collection and Literature review, **BO** - Design, Literature review, **AOB** - Literature review and Revision of manuscript, **AA** - Design, and Revision of manuscript, **OO** - Literature review, Data analysis analysis, **AA** – Lead Professor, Approval of the final version of the manuscript

**Declaration of competing interest**

The authors affirm they have no conflicts of interest to disclose.

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