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MICROBIAL ASSESSMENT OF CONTAMINANTS OF AFRICAN LOCUST BEAN (*PARKIA BIGLOBOSA* (JACQ.) G.DON) SOLD IN SELECTED MARKETS IN NORTH CENTRAL NIGERIA

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ABSTRACT

The African Locust bean (Parkia biglobosa), popularly known as 'Iru' in Lokoja and 'Nune' in Makurdi, is prone to contamination during possessing and sale. The study evaluated the microbial contaminants of the locust bean condiment sold in ten locations in Lokoja and Makurdi, North Central Nigeria. The locust bean condiment samples from the two locations were randomly collected using the purposive sampling technique and assessed for possible microbial contaminants. The laboratory screening was laid out in a completely randomised design and replicated three times. Bacterial and fungal contaminants were isolated following standard procedures. The result showed that the locust bean condiment samples were contaminated with five bacteria and fungi genera, namely: Klebsiella, Proteus, Salmonella, Shigella and Staphylococcus*;* Aspergillus, Fusarium, Mucor, Penicillium and Rhizopus. *In Lokoja, the total viable count(TVC) ranged from 80.00 × 103 cfu/ g to 226.33 x 103 cfu / g, with the highest total coliform count recorded in samples from the Federal University Lokoja Campus (110.00 × 103 cfu/g) and the lowest in Barracks (30.00 × 103 cfu/g). In Makurdi, condiments from the North bank market recorded the least TVC of 48.33 × 103 cfu/g, while Akpehe market samples had the* *highest TVC of 65.33 × 103 cfu/g. The total fungal count ranged from 15.50 × 103 cfu/ g in samples from the international market to 46.00 × 103 cfu/g in samples from the Adankolo market. The locust bean samples in the study area were contaminated above the acceptable limit, and processors and traders need to ensure hygienic handling along the value chain to prevent future contamination of the condiment.*

Keywords: Locust bean, ‘Iru’, Microbes, contaminants, condiment, markets.

INTRODUCTION

The African locust bean (*Parkia biglobosa* (Jacq.) G. Don) belongs to the family Fabaceae, subfamily Mimosoideae and genus *Parkia* with about seventy species distributed all over the world (Heyman *et al*., 2012; Nayak *et al*., 2022). The locust bean tree grows in the wild in Nigeria's rainforest and savannah regions. The pod contains a sweet pulp that can be eaten as a snack and crushed and fermented seeds to produce a condiment. This condiment from the fermented locust bean seeds is popularly known as 'Iru' among the Yoruba of South West Nigeria and 'Nune' among the Tiv people of North Central Nigeria. *Parkia biglobosa* bean is an important economic forest tree for fodder, timber, and food. The seed provides a livelihood for women who ferment and process it into a condiment sold in open containers in rural and urban markets (Koura *et al*., 2011; Aderounmu *et al*., 2019). The leaves of *P. biglobosa* are fed to animals as fodder while the wood is used as timber (Aderounmu *et al*., 2019). The fermented and processed locust bean condiment contains protein, minerals, vitamins and other nutrients. It is popularly used to season sauces and soups in Nigeria (Makanjuola and Ajayi, 2012). *Parkia biglobosa* juice, produced from the powdery fruit pulp, is added to orange juice to make a drink (Adeloye and Agboola, 2020). It is reported to exhibit antidiabetic, antihypertensive and antimicrobial properties linked to the presence of terpenoids, phenolic acids and flavonoids (Heyman *et al*., 2012).

The marketing of the locust bean is a profitable business in various parts of Nigeria (Joseph *et al*., 2021). The stem bark and leaf of *Parkia biglobosa* are medicinal and are used in treating hypertension and Malaria (Karou *et al*., 2011; Builders *et al*., 2012). The methanol extract of the stem bark of the locust bean tree is reported to possess anti-malarial activity by inhibiting the growth of the plasmodium (Builders *et al*., 2012). The locust bean plant also treats skin infections and incision wounds (Shao, 2002). The decoction of the bark and root is used to treat toothache, mouth wash, diarrhoea, eye infections and dysentery (Shao, 2002). The locust bean condiment 'Nune' or 'Iru' is common in the South West and North Central parts of Nigeria, where it is used as a substitute for the chemically formulated seasoning cubes. The locust bean condiment is of plant origin and has been reported to help lower blood sugar in diabetic patients (Airaodion *et al*., 2019). It is nutritionally richer than the synthetic condiments. One hundred grams of the locust bean seed contains 37.34% protein, 17.0% carbohydrate, 24.21% crude fibre, 5.30% fat and 3.55% ash. It is also rich in mineral elements: calcium 9.01mg/100g, potassium 20.5mg/100g, sodium 35.00 mg/100g, magnesium 35.00mg/100g, iron 3.31mg/ 100g, phosphorus 73.00mg/100g and moisture content of 42.80% (Olalude *et al*., 2021). The condiment is a good source of dietary fibre, which helps in reducing obesity and stroke (Olalude *et al*., 2021). The high magnesium content is advantageous in the reduction of high blood pressure.

The processing of the African locust bean condiment involves boiling the seeds for 12 -24 hours, dehulling manually in a mortar or through the application of abrasives while rubbing the boiled seeds in between the palms and fermentation of the cotyledons packed in jute bags and placed in earthen pots for 18 to 72 hours depending on the locality. The characteristic smell of the fermented bean results from the mucilage and ammonia produced from the protein and amino acids (Okpara and Ugwuanyi, 2017; Samyal, 2022). The fermented seed of *P. biglobosa* has a good safety index of 10.76 in laboratory trials and is considered safe for consumption (Ouedraogo *et al*., 2012; Okoye *et al.,* 2014). Although locust bean condiments are a good alternative to synthetic condiments and seasonings, there is speculation about their possibility of contamination, which scares potential consumers from using them in their meal preparations.

This study assessed the microbiological contaminants of *Parkia biglobosa* condiment sold in Lokoja and Makurdi in Kogi State and Benue State, North Central Nigeria.

MATERIALS AND METHODS

Study location

The study was conducted with locust bean samples from Makurdi and Lokoja Metropolis Nigeria. Makurdi is located between latitude 6° 22'N and 7° 56' N and longitude 7° 37' E and 9° 5'E 98 m above sea level while Lokoja is located between latitude 7°45'N and 7°51'N and longitude 6°41'E and 6°45'E, 125 m above sea level.

Sample collection

Sixty samples of fermented *Parkia biglobosa* seeds displayed for sale were randomly purchased in June 2021 from retailers in twenty locations in Makurdi and Lokoja metropolises. These Markets were: Wadata Market, High Level Market, Modern Market, Wurukum Market, Lafia Junction, Northbank Market, Ichwa, Federal University of Agriculture, Makurdi (FUAM) Junction, Akpehe and International Market in Makurdi. Fermented locust bean samples from Lokoja were purchased from New Market, Felele, Lokongoma Market, Kpata Market, Adankolo area, Fehintolu Street, Adankolo Market, Barracks Market, Federal University Lokoja Campus and Old Market. All the samples were labelled and transported immediately to the Microbiology Laboratory of the Federal University of Agriculture, Makurdi. They were stored in the refrigerator at 4°C and analysed for microbial contaminants within 48 hours.

Microbial Analysis of Locust Bean Samples

The microbial analysis of samples was done following the method of Egberongbe *et al*. (2021). One gram of fermented locust bean condiment sample from each market was serially diluted. For the serial dilution, 1 mL of the homogenous locust bean sample was serially diluted into 9 mL of sterile distilled water in a test tube to obtain an eightfold dilution. One millimetre each from 103-105 strengths was pipetted onto the appropriate media, and the media plates were incubated for 24 hours at 37ºC. Discrete colonies were sub-cultured on appropriate medium, which included Nutrient agar, Mannitol Salt Agar, Salmonella-Shigella Agar, MacConkey, which were obtained in dehydrated forms and prepared according to the manufacturer's instructions. One millimetre each from 103 was pipette into the nutrient medium and uniformly spread using the bent sterile glass rod. The spread plate media were incubated for 24 hours at 37º ± 2 °c. Fungi were isolated using Potato Dextrose Agar and incubated for 7 days. Colonies of microbes were counted and recorded for each sample that was incubated. Bacteria were identified based on their cultural, morphological and biochemical characteristics on the different agars and confirmed using the reference manual (Bergey, 1984).

Enumeration and Detection of Coliform Bacteria Contaminating Locust Bean Condiment

The enumeration and detection of coliforms on Nune samples were done following the method of Onyeze *et al.* (2013). One millilitre of diluted sample from the 1:10 dilution of samples was added into test tubes containing 9 ml of lactose broth to make a 1:100 and serially diluted subsequently to a 1:1000 strength. The test tubes were then incubated at 37±2ºC for 48 h without gas (presumptive test) to confirm faecal coliform (Onyeze *et al*., 2013).

Gram Staining and Microscopy

Gram staining was conducted on bacterial isolates to determine their reaction using the method of Onyeze *et al.* (2013). Biochemical identification was done using catalase test, motility test, indole test, citrate utilisation test, and urease test, following the method described by Cheesbrough (2005).

Determination of Microbial Load

The fungal load was determined by counting the colonies on the medium with a colony counter. The colony-forming unit per gram (cfu/g) was calculated as a proportion of the number of colonies counted in a plate relative to the volume of the sample plated multiplied by the dilution factor.

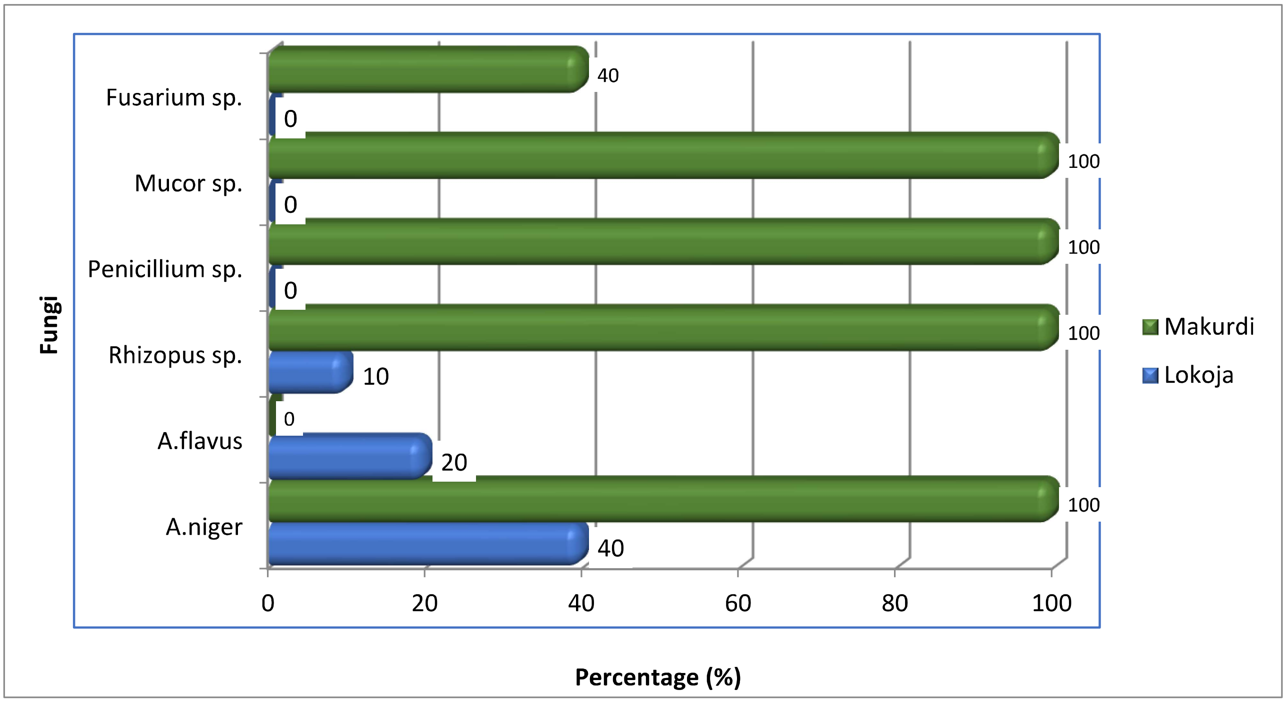
Data Analysis

The data on microbial load were subjected to Analysis of Variance (ANOVA) using SPSS version 16 at a 5% significance level and presented as bar charts. The data on the incidences of fungi and bacteria were presented as percentages of occurrences.

RESULTS

Five fungal genera, *Penicillium, Rhizopus, Mucor*, *Aspergillus*, *Fusarium* and eight bacterial genera, *Bacillus*, *Micrococcus*, *Sacharomyces*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Shigella*, and

Salmonella, were isolated from the fermented locust bean samples across the two locations. *Aspergillus niger*, *Rhizopus* sp, *Mucor* sp and *Penicillium* sp recorded percentage occurrence of 100% in Makurdi and were absent in Lokoja. *Aspergillus flavus* had a percentage occurrence of



20 % in Lokoja (Figure 1). *Parkia biglobosa* condiments from Lokoja were contaminated with *Bacillus* sp with 100% occurrence, *Sacharomyces* sp. 60% occurrence, *and Klebsiella* sp. 20% occurrence. *Staphylococcus* sp*., Shigella* sp*., Micrococcus* sp. *Proteus sp. and Salmonella* sp. had the least percentage occurrence of 10% in Lokoja (Figure 2). *Parkia biglobosa* condiments from Makurdi had a higher percentage occurrence of *Proteus* sp. (100 %), *Klebsiella sp.* (90 %), *Salmonella sp* (80 %) and *Shigella sp.* (60 %). In contrast, *Bacillus* sp., *Saccharomyces* sp., *Staphylococcus sp.*, *and Micrococcus* sp. recorded the lowest occurrence of 10 % each.

Figure1: Percentage occurrence of fungi isolated from African locust bean samples

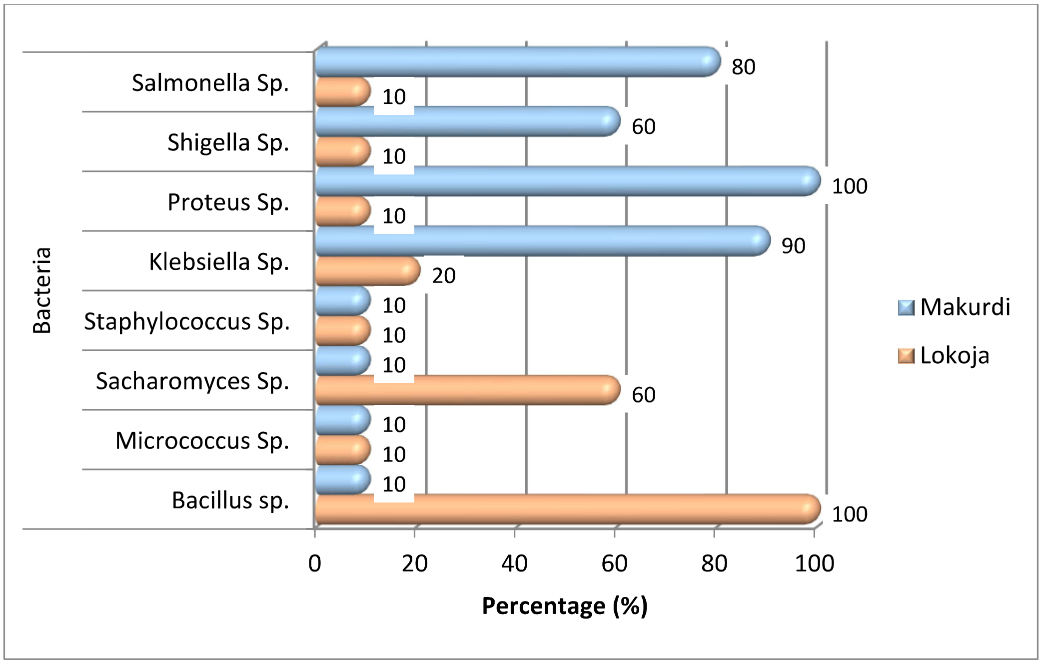


Figure 2: Percentage occurrence of bacteria isolated from African locust bean samples

The microbial load of locust bean condiment sampled from locations in Lokoja is presented in Figure 3. The data showed that locust bean samples from Adankolo market had the highest heterotrophic count of 226 × 10^5cfu/g, followed by samples from Felele with 212 × 10^5 cfu/g, FUL campus with 182 × 105cfu/g and International Market with 175 × 10^5cfu/g cfu/g while samples from Barracks had the least 80 × 10^5 cfu/g. The total fungi count was highest in samples from the Adankolo market, 46 × 10^5 cfu/g, the FUL campus, and the Kpata market, 34 × 10^5 cfu/g. The highest Total Coliform Count of 110 × 10^5 cfu/g was recorded by samples sold at the FUL campus, followed by samples sold at Felele 96×10^5 cfu/g, Lokongoma 87.5 × 10^5 cfu/g and the old market 72 × 10^5 cfu/g.

Figure 4 shows the microbial load of locust bean samples sold in locations in Makurdi. Locust bean condiments sold in the Akpehe market recorded the highest THC of 65.33 × 10^5 cfu/g, followed by the Wurukum market at 58.67 × 10^5 cfu/g, Ichwa at 57.67 ×10^5 cfu/g, and the international market at 53.33 × 105 cfu/g. The locust bean samples from the Federal University of Agriculture Makurdi (FUAM) campus had a microbial load of 51.67 × 10^5 cfu/g. In comparison, locust bean condiments from North Bank Market and Modern Market recorded lower microbial loads of 48.33 × 10^5 cfu/g and 42.33 × 10^5 cfu/g, respectively. The total coliform count of locust bean condiments was highest in samples collected from the Modern market (60.33 ×10^5 cfu/g), Wurukum (52.00 × 10^5 cfu/g), high-level market (51.00 × 10^5 cfu/g), and Akpehe market (4.00 × 10^5 cfu/g). Locust bean condiments from Wadata had microbial load of 35.00 ×10^5 cfu/g, International Market (2833 × 10^5 cfu/g), Ichwa 26.00 × 10^5 cfu/g, FUAM Junction (22.00 × 10^5 cfu/g), Lafia Junction 14.33 × 105 cfu/g, while North bank recorded the least TCC of 8.67 × 10^5 cfu/g. The Total fungi count was low in condiments from Akpehe Market (4.00 × 105 cfu/g) and the weakest in condiments sold at North bank (2.67 × 10^5 cfu/g), high level (2.67 × 10^5 cfu/g), Lafia junction (2.67 × 10^5 cfu/g) and Modern market (2.33 × 10^5 cfu/g).

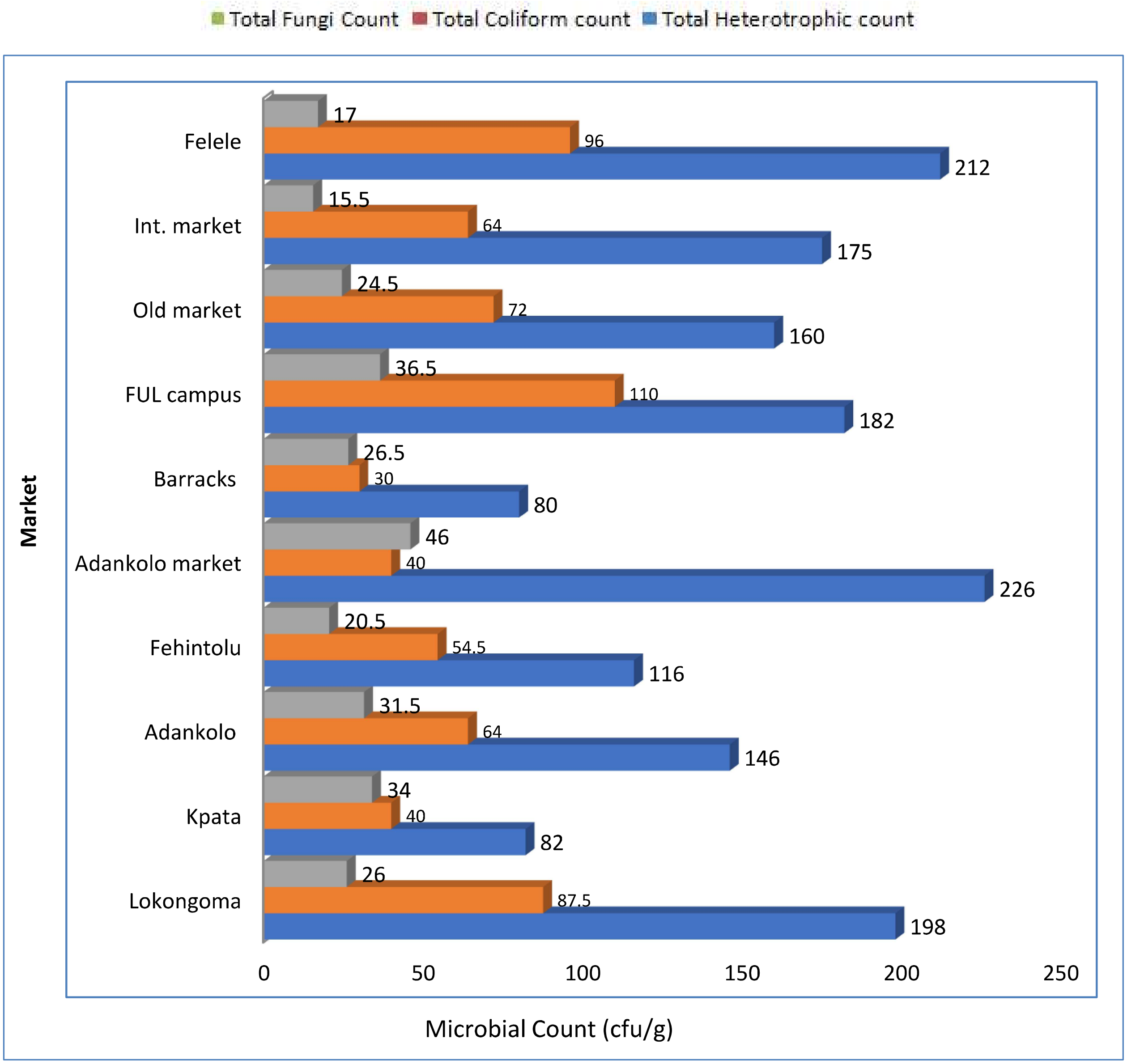


Figure 3: Microbial load of locust bean samples from markets/ locations in Lokoja

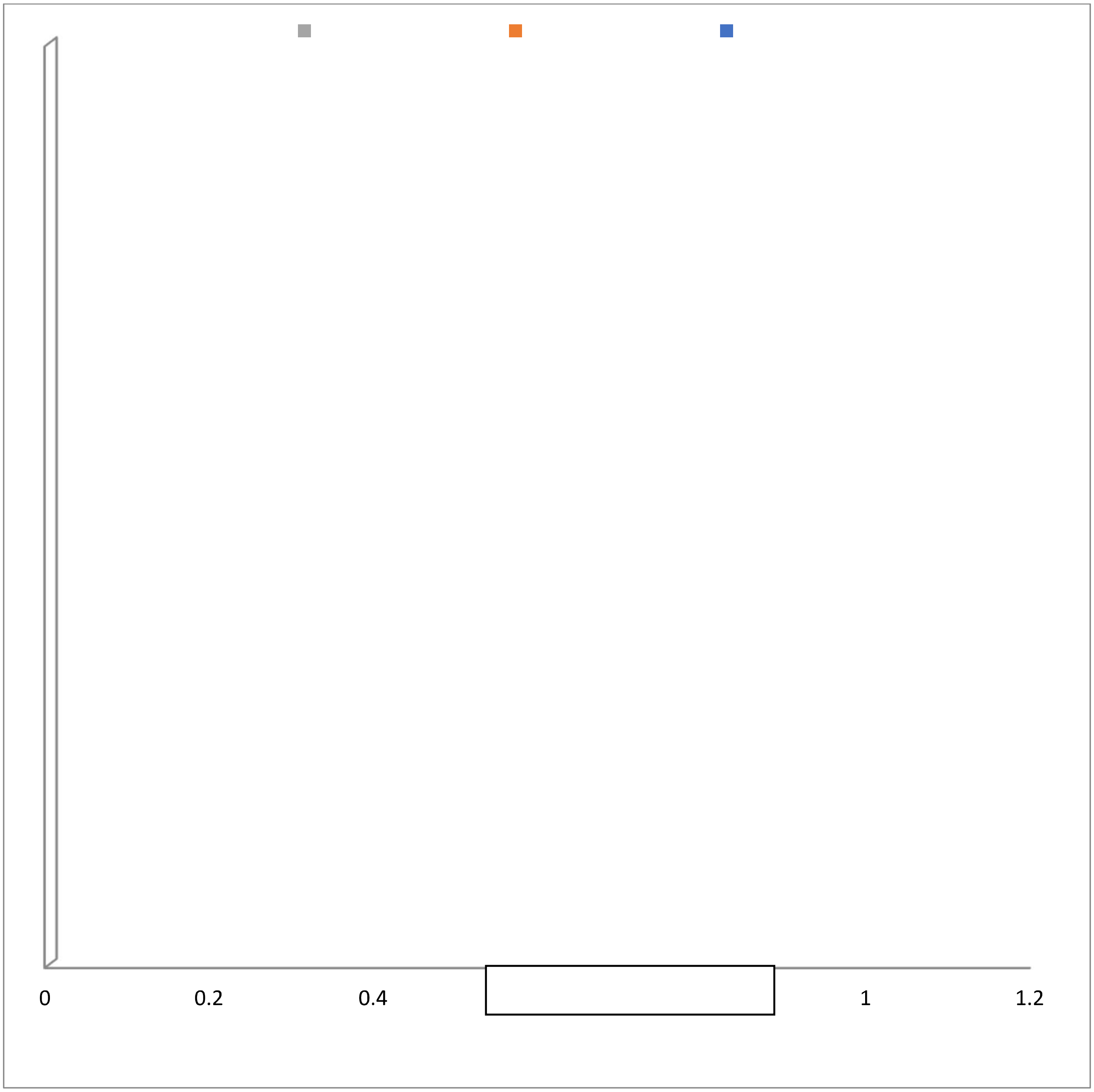


Figure 4: Microbial load of locust bean samples from markets/ locations in Makurdi

DISCUSSION

The present study assessed the microbial contaminants of the African locust bean condiment, which is popularly used as an alternative to the seasoning cubes. The range of microbes isolated from *Iru* condiments shows that the quality of the condiments sold on the market is compromised.

This suggests the possibility of poor hygiene during the condiment's handling, processing and sale in the two locations. Aderounmu *et al*. (2019) report noted that the traditional method of processing the locust bean into condiment exposes it to impurity contamination and constitutes food-borne hazards to consumers. The result of the present study indicates a lower microbial load in locust bean condiments from Makurdi compared to the microbial load of condiments from Lokoja. The differences in the microbial load in the two locations could be attributed to the marketers' handling and storage processes. Contamination of the condiments could be caused by the processing and handling of the condiments and by variations in environmental conditions, such as temperature and moisture, which support the growth of different pathogens (Nevalainen and Seuri, 2005). Okpara and Ugwuanyi (2017) observed that the differences in the drying period of the locust bean condiment could be responsible for the variation of microbes contaminating 'Iru' in different locations where it is consumed.

Similarly, Nayak *et al*. (2022) noted that the fermentation process of the locust bean condiment usually enhances the multiplication of microorganisms and usually results in deterioration of the fresh and un - dried condiment Onyeze *et al.* (2013) further observed that microorganisms that are involved in the fermentation process of fermented products could contribute to spoilage due to improper storage. The isolation of *Salmonella* spp from the condiment in this study suggests poor hygiene of the processors and traders. Percival and William (2014) reported that the dissemination of *Salmonella* spp is enhanced through using contaminated water, handling food items with unwashed hands or subjecting food to inadequate cooking time and improper storage. Kumar *et al.* (2021) listed *Aspergillus* spp, *Penicillium* spp, *Staphylococcus aureus* and *Bacillus* spp as possible microorganisms usually spread by human activities.

Although Kumar *et al.* (2021) opined that the air in clean shops and places where items are sold could contain as much as 25 spores/m3 of pathogens, some human activities such as coughing and sneezing by traders of food items contribute to the spread of pathogens leading to contamination of the items displayed for sale. This is likely to be the case in this study because most condiments were sold along dusty roads and shops, which could have easily exposed the condiments to contamination by microbes. This observation is in line with the report of Zekeri and Usman (2017), which noted that 27.1 % of locust bean consumers in Kano State, Nigeria, reported that poor hygiene was evident and recommended an improvement in the hygiene habits of the locust bean processors and traders.

Earlier investigations by Antai and Ibrahim (1986) on microbes contaminating *Parkia filicoidea* (Welw), a close relative of *P. biglobosa*, indicated the presence of *Bacillus subtilis, Staphylococcus* sp, *Micrococcus* sp, *Proteus* sp, *Leuconostoc mesenteroides* and *L. dextranicus* and the absence of fungi. Furthermore, the report showed that after 48 hours of fermentation of *P. filicoidea* condiment, the total viable count of *B. subtilis* was 3.6 × 10-10 per gram, while that of *Staphylococcus* spp was 4.4 × 104 per gram. The presence of some soil bacteria, such as *Bacillus subtillis,* could be because the soil contains a wide range of seed-transmitted microorganisms (Johnston-Monje *et al*., 2021).

This assertion is supported by the report of Kwee *et al*. (1986), which opined that microbiological quality of processed food was mainly based on the presence or absence of bacteria, coliforms and moulds, hence the reference to acceptable limits. Coliform counts of 102 cfu/mL is considered safe for food (Kucukoner and Tarakci, 2003). The presence of coliforms and mould is used to determine the quality of a food product. The high coliform count indicates high food contamination, especially during processing and packaging, which may result in food deterioration and spoilage (Eromo *et al*., 2016).

The *P. biglobosa* condiment samples evaluated in this study were contaminated with bacteria and fungi, although the samples were not visibly infected. This is corroborated by the report of Adetunji *et al*. (2018), who observed that mould contamination could result in biodeterioration of nuts without showing any sign of mouldiness. Similarly, Musa *et al*. (2011) observed that isolates of *A. niger* from 'Okpehe' condiment from Anyigba Kogi State Nigeria elaborated mycotoxins. However, these were not lethal to rats fed with the crude extracts of the secondary metabolites produced. Ruqayyah and Latifat (2020) also isolated *Staphylococcus, Salmonella*, *Enterobacter, Klebsiella, Fusarium, Rhizopus and Aspergillus* species from yaji spice mixed with *Parkia biglobosa* (locust bean), garlic, ginger, black pepper, red pepper, cloves and seasoning cubes sold in Kano, Nigeria. Moh *et al*. (2017) also found *Shigella* sp, *Klebsiella pneumonia* and *Proteus penneri* in fermented yoghurt in Cameroon.

Dietary intake of food contaminated with these bacteria and fungi poses serious health problems such as diarrhoea and Aspergillosis. Paudyal *et al*. (2017) reported Salmonella in raw and ready-to-eat foods. Curutiu *et al*. (2019) noted that packaging, handling and transport contribute to increased microbial contamination of food and drinks. In his study, Kumar *et al.* (2021) linked the presence of *Penicillium* spp to Penicilliosis in persons with weakened immune systems.

Shigella sonnei and *Salmonella enteric* are the main causal agents for gastrointestinal illness and bloody diarrhoea, while *Klebsiella pneumonia* incites bronchopneumonia in humans (Curutiu *et al*., 2019). Similarly, Moh *et al*. (2017) linked *Shigella* sp to food contamination that can result in stomach cramps, vomiting, diarrhoea, dysentery, fever and flatulence.

Tominaga *et al*. (2020) observed that the presence of coliform bacteria in food items could be due to insufficient heat application during processing or to some secondary contaminants after processing. According to Balali *et al.*(2020), the consumption of food contaminated with bacteria and fungi elicits typhoid fever, dysentery, diarrhoea, and cholera.

CONCLUSION

The study has shown that *P. biglobosa* condiment sold in selected markets in the study area was contaminated with five bacteria and three fungal genera, namely: *Penicillium, Rhizopus, Mucor*, *Aspergillus, Fusarium* and eight bacterial genera viz: *Bacillus* spp., *Micrococcus* spp., *Sacharomyces* spp., *Staphylococcus* spp., *Klebsiella* spp., *Proteus* spp., *Shigella* spp. and *Salmonella* spp. Although there are no Nigerian Standards available to the public for fermented condiments, the range of microbial contaminants recorded in this study is above the recommended World Health Organisation (WHO) standard of 20 cfu ml^{-1}.

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