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MYCOFLORA OF FERMENTED LOCUST BEANS (*Parkia biglobosa*) SEED, "IRU" SOLD IN LOKOJA MARKETS, KOGI STATE, NIGERIA

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ABSTRACT

Fermented locust beans are a popular nutritious food seasoning that are prone to microbial contamination due to unhygienic handling and improper storage. This research aimed to evaluate the mycological quality of locust beans sold in Lokoja markets, Kogi State, Nigeria. Thirty (30) samples of locust beans were collected from five different markets in Lokoja, analyzed for inherent fungi which were identified using standard mycological techniques. The results indicated variations in the mean fungal loads across the sampled markets. Old market had the highest mean fungal load (4.33×10^5 CFU/g), followed by Adankolo market (3.67×10^5 CFU/g) and Kpata market (3.0×10^5 CFU/g) while Lokongoma market had the lowest mean fungal load (2.0×10^5 CFU/g). The mean fungal load of the fermented locust beans from Old market was significantly different ($p>0.05$). A total of 55 fungal isolates that include: *Aspergillus niger* (29.1 %), *Rhizopus arrhizus* (25.5 %), *Fusarium cladosporium* (18.2 %), *Fusarium cummunis* (12.7 %), *Aspergillus nudilans* (9.1%), *Aspergillus tamari* (7.3 %) and *Aspergillus terreus* (1.8%) were obtained from the samples. *Aspergillus niger*, *F. communis*, *F. clamadosporium* and *R. arrhizus* were present in all the sampled markets, suggesting they are the predominant fermenter of locust beans. The presence of mycotoxigenic fungi in the locust beans seeds poses a significant public health risk. Therefore, it is important to ensure that locust beans are processed and stored under hygienic conditions at all times to safeguard consumer health.

Keywords: Locust beans, mycological quality, fungal contamination, public health risk

INTRODUCTION

Parkia biglobosa, commonly known as the locust beans plant, is a perennial tree species that falls under the family Fabaceae (Saleh *et al.,* 2021). It is commonly found in the tropical regions of Africa, particularly in countries such as Nigeria, Ghana, Senegal, and Cameroon. Locust beans, also known as African locust bean in Nigeria is highly valued for its culinary and medicinal uses in various African culture. The locust beans plant is a medium-sized tree that can reach heights of up to 20 meters and produce elongated pods that are woody and dark brown in color. The pods can grow to be about 30 centimeters in length and contain several seeds embedded in a sweet pulp (Lompo *et al*., 2018; Saleh *et al.*, 2021). These pods are a significant component of the plant's economic value, as they are harvested for their edible seeds, which are commonly referred to as locust beans. The seeds of the locust beans plant are flat, oval-shaped, and dark brown in color. They are surrounded by a fibrous pulp which can be extracted by boiling the pods and fermenting them for several days (Saleh *et al.,* 2021). The resultant product is a popular condiment used in various traditional African dishes. Locust beans are known for their aroma and distinctive flavor, which is often described as nutty and savory.

Fermented locust bean seed also known locally as "dawadawa" or "iru" in Nigeria play a significant role in culinary tradition and offer various health benefits. Fermented locust beans are commonly used as a flavor enhancer and seasoning in traditional African cuisines. The fermentation process develops a rich, savory, and umami taste, which adds depth and complexity to dishes. Fermented locust beans contribute to the unique taste and aroma of African soups, stews, sauces, and condiments (Akubor *et al*., 2017). Their incorporation in cooking brings diversity to flavor and contribute to the overall sensory appeal of cuisine (Akubor *et al*., 2017; Ezugwu *et al*., 2018). Fermentation further enhances their nutritional profiles by increasing the availability and digestibility of nutrients (Esenwah and Ikenebomeh, 2008). Fermented locust beans contain significant amounts of protein, dietary fiber, vitamins (such as thiamine and riboflavin), and minerals (including calcium, iron, and magnesium) (Iheke *et al*., 2017; Olasupo and Okorie, 2018; Abdurraman and Haliru, 2019; Termote *et al.*, 2022). Fermented locust beans also possess bioactive compounds that exhibit antioxidant and antimicrobial properties (Eboma *et al*., 2020). According to Daramola, (2014) and Saleh *et al.*, (2021), fermented locust beans have strong antioxidant activity, which help combat oxidative stress and reduce the risk of chronic diseases. Furthermore, studies suggest that the fermentation of locust beans promotes the growth of lactic acid bacteria, such as *Lactobacillus* species (Muhammad and Araoye, 2016). These bacteria can confer health benefits by improving gut health, enhancing nutrient absorption and boosting the immune system. Moreover, locust beans have demonstrated various health benefits, including blood sugar regulation (Nasrallah *et al.*, 2023), wound healing by influencing the proliferation of dermal fibroblasts (Kuma *et al.*, 2022), antimalarial activity (Builders *et al*., 2012), and antimicrobial properties for managing bacterial infections (Olukunle *et al.*, 2019; Akpor *et al.*, 2021) and treating coccidiosis in poultry (Heuze *et al.*, 2019).

In Kogi State, locust beans serve as a staple ingredient in the local cuisines. Currently, the demands remain consistently high with consumers showing a strong preference for this culinary ingredient (Ijewere, 2019). However, little attention is given to the potential dangers posed by microbial contaminants. Despite the nutritional benefits, poor handling practices during processing, exposure to dust and flies and inappropriate storage conditions contribute to the accumulation of fungi in locust beans, further compromising their quality and safety (Ojiego *et al*., 2022). Fungi, such as *Aspergillus* spp. and *Fusarium* spp. may proliferate in the fermented locust beans, producing mycotoxins, which are toxic compounds harmful to human health. Mycotoxins, such as aflatoxins, ochratoxins, and fumonisins which are known to have detrimental effects on human and animal health, including carcinogenic, hepatotoxic, nephrotoxic, and immunosuppressive properties (Zain, 2011; Awuchi *et al.*, 2021). This raises concerns regarding the potential ingestion of fungi and possibly their mycotoxins in improperly processed food which is seasoned with contaminated fermented locust bean seeds. It is, therefore, pertinent to investigate the mycoflora of fermented locust bean seeds so as to provide valuable insights into the extent of fungal contamination and associated risk. This will inform the establishment of appropriated processing and storage methods to minimized fungal growth and mycotoxin production, thereby, ensuring public health safety.

MATERIALS AND METHODS

Study Area

The study was conducted in Lokoja, the capital city of Kogi state, Nigeria; situated at coordinates 7.80230°N latitude and 6.73330°E longitude (Okolo *et al.,* 2022). Lokoja is a significant confluence city in the north-central region of Nigeria, where the Niger and Benue rivers meet. It falls within the tropical savanna climate zone, experiencing distinct wet and dry seasons. The wet season extends from April to October; characterized by increased rainfall and higher humidity, while the dry season lasts from November to March, with less rainfall and lower humidity. The average annual temperature ranges from 25 to 35 ºC (Alab, 2009; Adetunji, 2018). Lokoja has an estimated population of around 500,000 residents and is a bustling hub attracting people from neighboring areas for economic and social activities (Audu, 2012). The city has a diverse population, with various ethnic groups residing there. It is known for its thriving agricultural sector, with crops such as yam, cassava, maize, and vegetables being cultivated. Lokoja also serves as a trading center with vibrant markets contributing to the local economy (Okolo *et al.,* 2022).

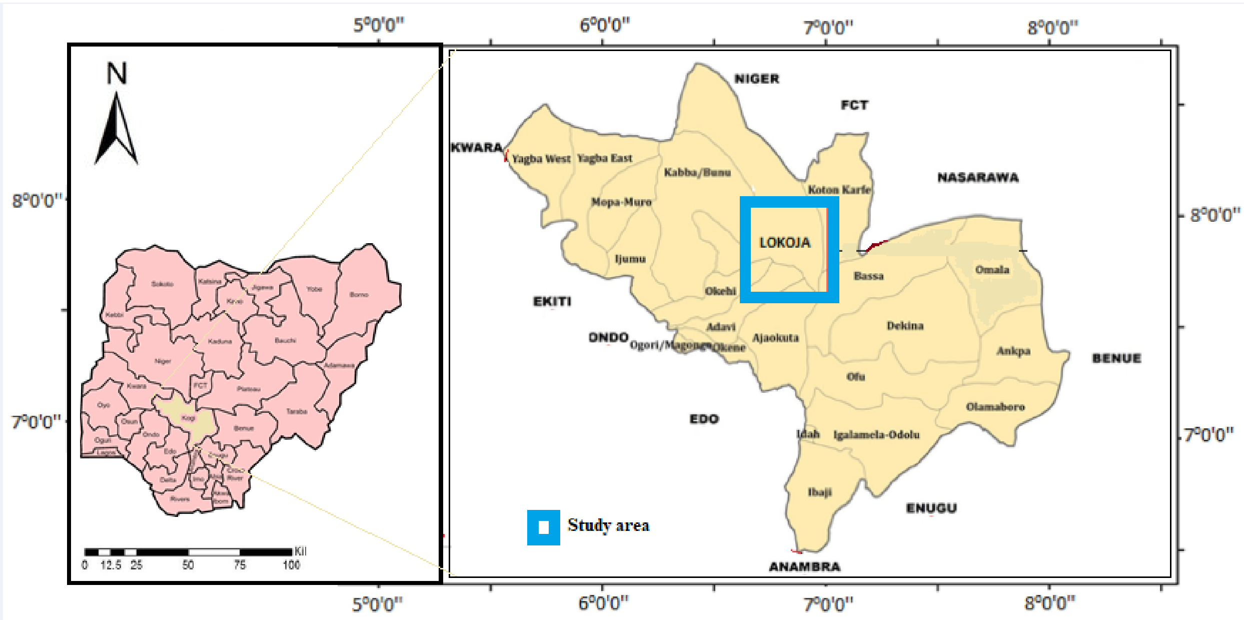

Figure 1. Map of Nigeria showing Kogi State and Lokoja the study area (Okolo *et al.,* 2022)

Collection of samples

In this study, a total of 30 plastic-polythene-wrapped locust bean (iru) samples were randomly purchased from five major markets in Lokoja: New market, Old market, Adankolo market, Lokogoma market and Kpata market between August and September, 2022. The samples were carefully placed in ice-packed polythene bags and transported to the Biological Sciences Laboratory of Federal University Lokoja for laboratory analysis within one hour of sample collection.

Isolation, Enumeration and Identification of Fungi from the Samples

The procedure described by Mailafia *et al.* (2017) was utilized with minor adjustments for the isolation, enumeration and identification of fungi. A gram (1 g) of locust bean seeds was weighed and transferred into a test tube containing 9 mL of sterile distilled water. The tube was thoroughly shaken, and a 5-fold serial dilution was performed. To isolate fungi from the samples, pour plate method was used. This involved adding potatoes dextrose agar prepared according to the manufacturer's instructions to 1 mL of the serially diluted sample (10^{-2} and 10^{-4} dilutions). The medium and inoculum were swirled and left to solidify before incubation at 28 °C for 7 days in an incubator. To determine the fungal load of the locust bean seed samples, the colonies on the medium were counted using a colony counter. The colony forming units per gram (CFU/g) were calculated. The microscopic morphology of the fungi isolates were observed by placing a drop of lactophenol cotton blue onto a grease free slide and then placing a small fragment of fungal mycelium in the drop of lactophenol cotton blue stain. The slide was covered with a cover slip and viewed under a microscope at ×10 and ×40 objectives for the identification of the fungal isolates obtained from the locust bean samples. The colonial morphology and microscopic characteristics of the fungal isolates were used to identification the fungi according to these fungi identification guides (Klich, 2002; Samson and Varga, 2007; Watanabe, 2010).

Date analysis

The data were analyzed using descriptive statistics. Additionally, the mean fungal load obtained from various markets was examined using Analysis of Variance (ANOVA) in SPSS 20, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Fermented African locust beans, also known as "dawadawa" or "iru," hold significant cultural and culinary importance in West Africa. However, fungal contamination can occur during the fermentation, packaging, retailing and/or storage processes, which affects both the quality and safety of the product. Understanding the significance of fermented locust beans and addressing fungal contaminations is crucial for ensuring their beneficial utilization. This study investigated the mycological quality of "iru" sold in the five major markets within Lokoja metropolis. The results of the fungal load analysis in the major markets of Lokoja, Kogi State are presented in Table 1. Among the markets, Old market had the highest fungal count, with a maximum count of 7.01×10^5 CFU/g. On the other hand, Kpata market displayed the lowest fungal count (1.0 × 105 CFU/g). When the mean fungal loads were compared, Adankolo market and Old market had the highest means, with values of 3.67×10^5 CFU/g and 4.33×10^5 CFU/g, respectively (Table 1) These results suggest that these markets may have higher levels of fungal contamination compared to others. In contrast, Lokongoma market and New market had relatively lower mean fungal loads of 2.01×10^5 CFU/g and 2.33×10^5 CFU/g, respectively. The standard deviation values reflected variation in fungal load values within each market. Old market samples had the highest standard deviation, indicating greater variation in the fungal load. This suggests that fungal contamination in Old market may vary significantly across different samples. Lokongoma market had the lowest standard deviation, indicating relatively less variation in fungal load among the samples collected from that market. Statistical analysis revealed that the mean fungal load of fermented locust bean seeds from Old market was significantly different ($p>0.05$). The results of the fungal load analysis in the major markets of Lokoja, Kogi State are consistent with findings from previous studies conducted in similar backgrounds. For instance, a study by Bello (2010) on fungal contamination in fermented food products reported varying fungal counts across different markets, with some markets exhibiting higher levels of contamination compared to others. This agrees with our findings where the Old Market showed the highest fungal counts. Moreover, the higher mean fungal load observed in Adankolo market and Old market is in line with studies by Adekoya *et al.* (2018) and Bello (2010) that reported elevated fungal levels in markets where hygiene practices and storage conditions were suboptimal.

Table 1: Fungal load of fermented locust beans from major markets in Lokoja, Kogi State, Nigeria

Sampling sites	Fungal counts (CFU/g)			
	Minimum	**Maximum**	**Mean***	**SD**
Adankolo Market	2.01×10^5	5.11×10^5	3.67×10^{5ab}	1.04×10^5
Kpata Market	1.00×10^5	5.01×10^5	3.02×10^{5ab}	1.63×10^5
Lokongoma Market	1.01×10^5	3.02×10^5	2.01×10^{5bc}	0.82×10^5
New Market	2.03×10^5	3.00×10^5	2.33×10^{5bc}	0.47×10^5
Old Market	a. $\times 10^5$	7.01×10^5	4.33×10^{5a}	2.49×10^5

* Values with similar superscript letters of the alphabets are not significantly differently ($p<0.05$), SD: Standard deviation.

The findings of this study on the mycofloral of fermented Locust beans provide insight into the fungal species present in the product. A total of 55 fungal isolates that belong to three genera: *Aspergillus*, *Fusarium* and *Rhizopus* were isolated. The predominant species identified included *Aspergillus niger* (29.1 %), *Rhizopus arrhizus* (25.5 %), *Fusarium cladosporium* (18.2 %), *Fusarium cummunis* (12.7 %), *Aspergillus nudilans* (9.1%), *Aspergillus tamari* (7.3 %), and *Aspergillus terreus* (1.8%) (Figure 2). The results indicated that the genus, *Aspergillus* was the most prevalent among the fungal isolates. *Aspergillus* is a diverse genus of filamentous fungi known for its ability to colonize various substrates, including food products (Perrone *et al*., 2007). In this study, the most predominant species within the *Aspergillus* genus was *Aspergillus niger*, accounting for 29.1 % of the total isolates. *Aspergillus niger* is a commonly occurring black mold species known for its wide range of metabolic capabilities and its involvement in various industrial applications, including food fermentation and enzyme production (Yu *et al*., 2021). *Rhizopus arrhizus* was the next most abundant species identified, comprising 25.5% of the isolates. *Rhizopus arrhizus* is a filamentous fungus that is often associated with the fermentation of foods. It has the ability to break down carbohydrates and contribute to the production of organic acids, flavors, and textures in related fermented products (Cara *et al*., 2018). Two species from the genus, *Fusarium* were also prevalent in the fermented Locust bean seeds. *Fusarium cladosporium* and *Fusarium cummunis* accounted for 18.2% and 12.7% of the isolates respectively. *Fusarium* species are known for their ability to produce mycotoxins, secondary metabolites that can be detrimental to human consumers of the products in significant amounts (Munkvold, 2017; Piacentini *et al.,* 2019). These mycotoxins can contaminate food products, posing potential risk to consumer health. Therefore, the presence of *Fusarium* species in fermented Locust beans seeds highlights the importance of monitoring and controlling their growth during fermentation processes. In addition to the predominant species mentioned above, other species of *Aspergillus* were also identified, albeit in lower proportions. These included *Aspergillus nidulans* (9.1 %), *Aspergillus tamarii (*7.3 %), and *Aspergillus terreus* (1.8 %). Each of these species have unique enzymatic characteristics (de Vries and Visser, 2001) and may contribute to the sensory attributes and biochemical changes occurring during fermentation. The fungi genera reported in this study are in concordance with the previous studies on African locust beans and other related products (Adelekan, 2012; Nwadiaro *et al*., 2015; Osuntokun *et al.,* 2020).

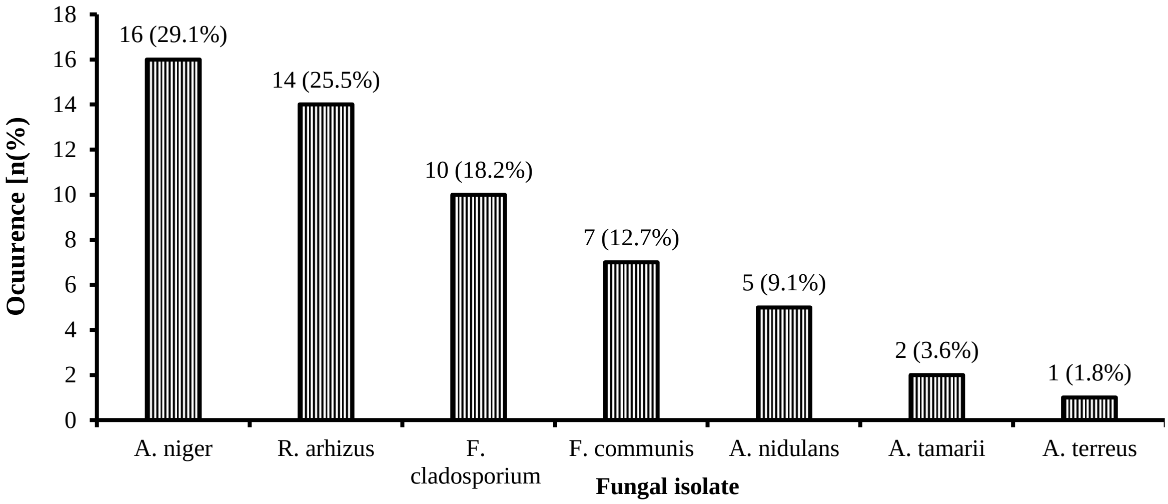

Figure 2: Occurrence of fungal isolates from fermented locust beans from Lokoja markets

The findings from Table 2 reveal the distribution of fungal isolates in fermented locust beans from various markets in Lokoja. *Aspergillus tamarii* was detected in the Adankolo and Lokongoma markets but absent in Kpata, New, and Old markets. *Aspergillus terreus* was not found except in the Old market. *Aspergillus nidulans* was present in Adankolo and Lokongoma but absent in Kpata, New, and Old markets. *Aspergillus niger*, *Fusarium communis*, *F. clamadosporium*, and *Rhizopus arrhizus* were all present in all sampled markets, suggesting they are the predominant fermenter of locust beans. These fungi are commonly found in the environment and have the capacity to grow on various organic substrates. Previous studies made similar submissions and thus in line with our study (Adelekan, 2012; Nwadiaro *et al*., 2015; Osuntokun *et al.,* 2020). While some species, such as *Aspergillus niger* and *Rhizopus arrhizus*, play a positive role in food fermentation, contributing to the development of desirable flavors and textures, others species of *Aspergillus* and *Fusarium* species, can potentially produce harmful mycotoxins (Munkvold, 2017; Gil-Serna *et al*., 2019; Kagot *et al.,* 2019; Piacentini *et al.,* 2019). Under favourable conditions, mycotoxins are produced by some species of *Aspergillus* and *Fusarium* species, which can contaminate food and pose health risks to humans and livestock. Exposure to mycotoxins can have varying effects, including immediate illness, long-term health consequences like cancer, and damage to organs such as the liver and kidneys (Awuchi *et al.,* 2021). The identification and characterization of these fungal species in fermented locust beans have significant implications for food safety and quality.

Table 2: Distribution of fungal isolates from fermented locust beans from Lokoja markets

Fungal isolate	Market (Sampling site)				
	Adankolo	**Kpata**	**Lokongoma**	**New**	**Old**
A. niger	(+)	(+)	(+)	(+)	(+)
A. tamarii	(+)	(-)	(+)	(-)	(-)
A. terrus	(-)	(-)	(-)	(-)	(+)
A. nidulans	(+)	(-)	(+)	(-)	(-)
F. communis	(+)	(+)	(+)	(+)	(+)
F. clamadosporium	(+)	(+)	(+)	(+)	(+)
R. arrhizus	(+)	(+)	(+)	(+)	(+)

Key: (+) = present, (-) = absent

CONCLUSION

In conclusion, this study revealed significant variation in fungal load across different markets retailing fermented locust beans (*Parkia biglobosa*) seed "iru" in Lokoja, Kogi State, Nigeria. The predominant fungal genera identified were *Aspergillus*, *Fusarium* and *Rhizopus*, with *Aspergillus niger* being the most prevalent species. These findings emphasize the importance of implementing hygienic processing and storage practices to minimize fungal growth and mycotoxin production in locust beans. Further research is needed to understand the factors contributing to variations in fungal load and to develop standardized protocols for ensuring the safety and quality of fermented locust beans seeds.

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