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Fungal biodiversity associated with groundnuts stored in Nasarawa State

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Abstract

Various food commodities such as groundnuts are prone to fungal contamination in favourable environmental conditions. The purpose of this study was to isolate and identify fungi associated with stored groundnuts. A purposeful and random sampling was employed to collect three hundred (300) samples of groundnuts in storage for more than six (6) months from local storage facilities known as ‘rumbun’ from the three agricultural zones (Nasarawa South, Nasarawa North and Nasarawa West) in Nasarawa State. The samples were grounded and cultured in potato dextrose agar (PDA) under sterile conditions, with the aid of a microscope and the fungal flora were determined using taxonomical schemes relying on their morphological and cultural characteristics. The total heterotrophic fungal ranged from 1.4×10^2 to 2.9×10^5 with stored groundnut from Nasarawa South being the most contaminated (4.8×10^4 CFU/g) followed by Nasarawa West (1.6×10^4) and Nasarawa North was the least contaminated (3.3×10^3 CFU/g). Fungal diversity from this study included *Rhizopus stolonifer* (65.3%), with the highest prevalence followed by *Mucor* spp (48.5%), *Aspergillus niger* (43%) and *Aspergillus flavus* (39 %) while *Neosartorya fisheri* (0.6 %). The findings of this study suggests that the groundnuts in storage from the three agricultural zones are heavily contaminated by fungi capable of producing mycotoxins which could present a public health challenge to the consumers. It is therefore recommended that groundnuts for long term storage should be properly dried to reduce the attack of fungi with reduced moisture.

Keywords: Agricultural zone; Contamination; Fungi; Groundnuts; Storage facility

1. Introduction

Groundnuts (*Arachis hypogea*) belong to the family of Fabaceae and are grown in all parts of the world and various names, such as African nuts, monkey nuts and peanuts [1,2]. Many tribes in Nigeria have different local names for groundnuts such as “isagwe” (Edo), “epa” (Yoruba), ‘ayayaa” (Hausa), “okpa or opapa” (Ibo), and “omizaguo” (Owan) [3]. It is a good source of protein, carbohydrates, minerals, vitamins, and fat for humans and animal [4]. Mubiru (2016) stated that groundnuts are rich in folate, tryptophan, niacin and beta-sitosterol. They are widely consumed in different ways, as they can be consumed as boiled nuts, roasted and salted nuts, as well as groundnut cake (kuilikuili) as butter, cheese and oil and other bakery products. In the early to mid-1990s Nigeria was the largest producer of groundnuts in Africa and among the largest producers in the world, it was major source of foreign exchange for the country [6]. However, groundnut production has suffered a major setback due to its vulnerability to fungal attack especially stored groundnuts. This has been a major threat to the income of these farmers in Nigeria as groundnut farmers do not have good storage facilities, improved varieties and drying systems to reduce the moisture content of the commodity [6]. This issue is well dealt with in developed countries as there are good storage facilities, drying systems, improved varieties and the commodities are properly screened [7]. Fungi cause many groundnut diseases such as necrosis, seed

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rot, black mould, wilt, leading to poor yield [8]. Fungi also produces toxins as secondary metabolites that are poisonous and carcinogenic to humans and animals [9]. There are several classes of these mycotoxins which include; Aflatoxins, ergot, alkaloids, amanitins, ochratoxins, cyclopiiazonic acid, tricothecenes, citrinin and slaframine etc., and these toxins are primarily produced by *Fusarium* spp., *Aspergillus* spp., *Alternaria* spp, *Penicillium* spp and *Emericella* spp. [9]. The hazardous health in humans and declining productivity in livestock consuming groundnuts contaminated with mycotoxins have pushed many nations and regional blocks to set allowable limits for mycotoxin; however, most poor farmers in Nigeria cannot meet these set limits resulting in their inability to export their produce [4]. Food safety is a major international issue as stated by the World Health Organisation [10], who reported a devastating economic burden resulting from an annual destruction of 25 % or more food crops from Africa due to high mycotoxin incidence in such food. The mycological quality of groundnuts and its by-products has been documented in several parts of Nigeria such as in Benin [11]. Tobin-West et al. [12] also reported the high incidence of moulds from groundnut seeds from four major markets in Port-harcourt south-south Nigeria. The mycological quality of groundnuts was reported in Lagos state [13], another study was also carried out in Bauchi State (roasted groundnut sold in Wunti, Yelwa and Railway) [14]. In Nasarawa State North Central Nigeria data on the mycological diversity of stored groundnuts are scarce. Therefore, this study aimed to determine the fungal diversity of groundnuts in storage in Nasarawa state.

2. Methodology

2.1. Study Area and Design

Nasarawa state has thirteen (13) Local Government Area's (LGA's) which are zoned into three Agricultural clusters, consisting of Nasarawa South zone (Obi, Awe, Keana, Lafia and Doma), Nasarawa West zone (Kokona, Karu, Keffi, Toto and Nasarawa) and Nasarawa North zone (Akwanga, Nasarawa-Eggon, and Wamba). According to the Nigerian Investment Promotion Commission [15], all agricultural zones are known to produce groundnut in commercial quantity. A combination of simple random technique and purposeful sampling was employed to determine the population size of farmers that own local mud barns called 'rumbun' in Hausa with groundnuts of up to six months in storage and to collect the samples from the zones, with aid of the local government agriculture department and traditional authorities.

2.2. Sample Size

A total of 300 stored groundnut samples of 4-5 kg of each sample were obtained from the different storage mud barns in the three agricultural zones of Nasarawa state. The sample size was determined using the Yamane [16] sample size calculation method:

$$n = \frac{N}{(1 + N(e)^2)}$$

Where:

n= signifies the sample size;

N= signifies the population under study (1,170);

e= Signifies the Margin of error (which was taken to be 0.05).

2.3. Sampling

A total of three hundred (300) stored groundnut seeds samples (about 4-5 kg each) were obtained from the storage facilities (rumbun) in the three Agricultural zones. Purposefully one hundred and sixteen (116) were collected from Nasarawa south zone, one hundred and fifteen samples were collected from Nasarawa west zone while sixty-nine samples were collected from Nasarawa north zone. The sample were collected, packaged, labelled and sealed using a sterile plastic bag and place in a cooler containing ice packs and were transported to the microbiology laboratory Federal University of Lafia for further analysis.

2.4. Isolation and identification of fungal isolates

Total heterotrophic fungal count was performed as described by Dachoupakan *et al.* [17]. A stock suspension of the sample was prepared by homogenising 10 grams of stored groundnuts in 90 mL of peptone water, and ten-fold serial dilutions of the homogenate were prepared. One tenth milliliter (0.1 mL) of each dilution was pipetted onto a potato dextrose agar plate and uniformly distributed on the plate using a sterilized glass spreader. The entire procedure was repeated for duplication. The plates were incubated at 28°C for three to five days. Colonies on the surface of the agar

plate ranging between 30-300 were counted and recorded, the heterotrophic plate count was determined using the following formula:

$$CFU/g = \frac{\text{number of colonies counted}}{\text{volume of inoculum used}} \times \text{recipocal of the dilution factor.}$$

The fungal isolates were identified as described by Chuku *et al.* [18] using macroscopic features such as shape, appearance, colour, conidia arrangement, and other vegetative structures. The fungi were also identified microscopically using lactophenol in cotton blue and a light compound microscope aided with some identification keys [19].

2.5. Statistical Analysis

The data obtained were analyzed using the R Console software (Version 3.2.2). The Shapiro-Wilk normality test was performed on the means of the total heterotrophic fungi counts (CFU/g), which showed that the data were not normally distributed. Hence, the Kruskal-Wallis Chi-square test was employed to compare the mean counts of the fungi in relation to the locations. Kruskal-Wallis Chi-square test was followed by a post-hoc Wilcoxon rank sum test with Bonferroni correction, which was used for multiple pairwise comparisons of means where there were significant differences between the locations. Pearson's Chi-square test was used to compare the proportion of fungal species in relation to the agricultural zones and LGAs. The level of significance was set at $P < 0.05$.

3. Results

The total heterotrophic fungi mean counts in stored groundnuts from Nasarawa South (47888.000 ± 4807.9871 CFU/g) were the highest compared to other agricultural zones as shown in Figure 1, with Nasarawa North having the lowest mean count (3334.694 ± 203.7812 CFU/g). The mean count in relation to locations showed a very significant difference (Kruskal-Wallis $\chi^2 = 84.817$, $df = 2$, $P < 2.2 \times 10^{-16}$ or $P < 0.001$).

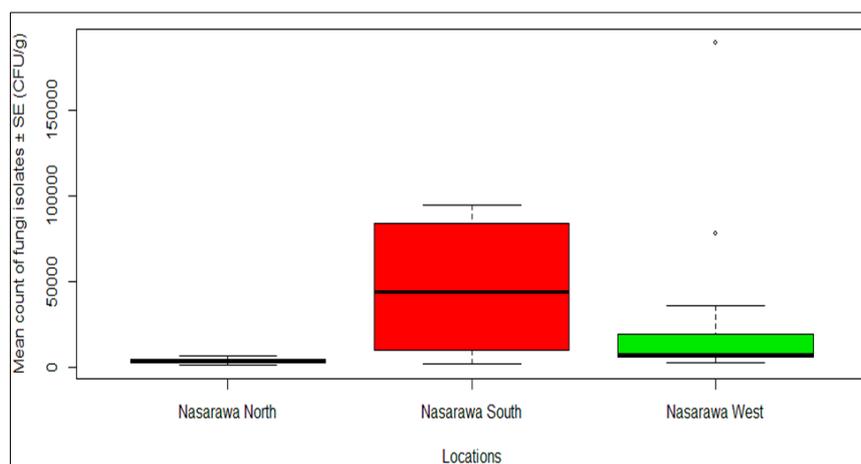


Figure 1 Mean count of fungi isolates from stored groundnuts in relation to the agricultural zones

The pairwise multiple comparison of the averages showed a highly significant difference ($P < 0.001$) for all pairings, as shown in Table 1.

Table 1 Pairwise comparison of heterotrophic mean counts of fungi isolates from stored groundnuts in relation to locations using Wilcoxon Rank Sum Test

Locations	Nasarawa North	Nasarawa South
Nasarawa South	1.1×10^{-13} *	-
Nasarawa West	1.1×10^{-12} *	5.3×10^{-7} *

Means with * are statistically significant, $P > 0.05$. No comparison as the location cannot be paired with itself

Table 2 Frequency and distribution of fungal isolates from stored groundnut in relation to the various Agricultural zones

Fungal isolates	Agricultural Zones			Total N=300 (%)	χ^2	df	P-value
	N. South N=116 (%)	N. West N=115 (%)	N. North N=69 (%)				
<i>Aspergillus arachidicola</i>	2 (1.7)	18(15.7)	0(0)	20 (6.6)	25.597	2	2.766 x 10 ⁻⁰⁶
<i>Aspergillus candidus</i>	0(0)	8(6.9)	0 (0)	8 (2.6)	5.2	2	0.07427
<i>Aspergillus carbonarius</i>	7 (6.0)	9 (7.8)	2 (2.8)	18 (6.0)	2.3181	2	0.3138
<i>Aspergillus clavatus</i>	10 (8.6)	25 (21.7)	6 (8.7)	41 (13.6)	8.7338	2	0.01269
<i>Aspergillus flavus</i>	49(42.2)	41 (35.7)	27(39.1)	117 (39.0)	0.54205	2	0.7626
<i>Aspergillus minisclerotigenes</i>	2 (1.7)	8 (34.8)	1(1.4)	11 (3.6)	50.341	2	1.171 x10 ¹¹
<i>Aspergillus niger</i>	53 (45.7)	49(42.6)	27 (39.1)	129 (43.0)	0.49882	2	0.7793
<i>Aspergillus parasiticus</i>	16 (13.8)	8 (34.8)	11(15.9)	35 (11.6)	12.444	2	0.001986*
<i>Aspergillus tamarii</i>	5 (4.3)	9 (7.8)	6(8.7)	20 (6.6)	1.5587	2	0.4587
<i>Aspergillus terreus</i>	1 (0.9)	13 (56.5)	0 (0)	14 (4.6)	110.28	2	2.2 x 10 ⁻¹⁶
<i>Aspergillus wentii</i>	2 (1.7)	0(0)	4 (5.8)	6 (2.0)	1.7543	2	0.416
<i>Cladosporium spp.</i>	0(0)	11(9.6)	6 (8.7)	15 (5.0)	9.2164	2	0.00997
<i>Emericella spp.</i>	23 (18.6)	8(34.8)	1 (1.4)	32 (10.6)	30.295	2	2.64 x10 ⁻⁷
<i>Fusarium spp.</i>	4 (3.4)	21(18.3)	5 (7.2)	30 (10)	12.445	2	0.001984*
<i>Monascus ruber</i>	2 (1.7)	2 (1.7)	8(34.8)	12 (4.0)	57.362	2	3.5 x 10 ⁻¹³
<i>Mucor micheli</i>	2 (25)	30 (26.1)	4 (5.8)	36 (12.0)	13.742	2	0.001037*
<i>Mucor racemosus</i>	13 (7.8)	30 (26.1)	7(10.1)	40 (13.3)	13.55	2	0.001142*
<i>Mucor spp.</i>	51 (43.9)	62 (53.9)	32 (46.4)	145(48.3)	1.1269	2	0.5692
<i>Neosartorya fisheri</i>	1 (0.9)	1 (0.9)	0(0)	2 (0.6)	0.075	2	0.9632
<i>Penicillium spp.</i>	13 (50)	15(13.0)	5(7.2)	33 (11.0)	31.556	2	1.405 x 10 ⁻⁰⁷
<i>Rhizopus stolonifera</i>	68 (58.2)	95(82.6)	33(47.8)	196(65.3)	10.151	2	0.006247*
<i>Trichoderma spp.</i>	1 (0.9)	0(0)	5 (7.2)	6(2)	11.4	2	0.003346*
Yeast	28(24.1)	22 (19.1)	20 (28.9)	70(23.3)	1.9983	2	0.3682

Rows with * superscripts are significantly different at $P < 0.05$

Table 2 showed the fungi distribution of from stored groundnuts in relation to the three agricultural zones of Nasarawa state, *Rhizopus stolonifera* (65.3%), *Mucor* spp (48.5%) and *Aspergillus niger* (43%) showed the highest frequency of occurrence, *Neosartorya fisheri* (0.6 %) the least frequency of occurrence.

4. Discussion

Groundnut is widely consumed in Nigeria especially Nasarawa state because of its nutritional value and health benefits, however, it is also well reported that they are likely vehicles for the transmission of pathogenic foodborne microbes [20]. The fungal organisms isolated in this study included ; *Aspergillus arachidicola*, *Aspergillus candidus*, *Aspergillus carbonarius*, *Aspergillus flavus*, *Aspergillus minisclerotigenes*, *Aspergillus clavatus*, *Aspergillus niger*, *Aspergillus tamarii*, *Aspergillus terreus*, *Aspergillus wentii*, *Aspergillus parasiticus*, *Cladosporium* spp., *Emericella* spp., *Fusarium* spp., *Monascus ruber*, *Mucor micheli*, *Mucor racemosus*, *Mucor* spp. *Neosartorya fisheri*, *Penicillium* spp. *Rhizopus stolonifer*, *Trichoderma* spp and yeast and most the organisms isolated have also been isolated in previous studies both in Nigeria and elsewhere. Abuga [21] reported *Aspergillus* spp. *Penicillium* spp, *Mucor* spp *Rhizopus* spp, *Fusarium* spp; were present in the Aliero capital market, Kebbi State. Another study conducted in south western Nigeria by Ezekiel *et al.* [22] reported the incidence of *Aspergillus flavus*, *Aspergillus tamarii*, and *Aspergillus parasiticus* in roasted groundnuts. Aliyu and Kutama [23] isolated 25 different species of fungi in Kano State including *Aspergillus parasiticus*, *Cladosporium* spp., and *Emericella*. *Aspergillus niger*, *Aspergillus tamarii*, *Fusarium* spp., had the highest prevalence. The presence could be a result of poor storage conditions and high moisture content.

This study revealed the prevalence of fungi in stored groundnuts in Nasarawa state. *Rhizopus stolonifer* (65.3%), *Mucor* spp. (48.5%) and *Aspergillus niger* (43%) showed the highest frequency of occurrence whereas *Neosartorya fisheri* showed the least occurrence (0.6 %). This finding is consistent with that of Xing *et al.* [20], who reported a high frequency of *Rhizopus* spp. (30 %); and a similar study conducted in Benin, by Akinnibosun and Osawaru [11] which reported high a prevalence of *Rhizopus stolonifer*, *Mucor* spp, and *Aspergillus tamarii*. The high incidence of fungal abundance and species richness can be attributed to high humidity levels and other abiotic factors such as weather, elevation, geographic location, and soil characteristics, which is similar to the findings of Talley *et al.* [24] who reported a consistent trend for fungi to be less abundant and diverse as temperatures increase and moisture decreases. Deterioration of groundnuts in storage by fungi is determined by many factors which can range from intrinsic nutritional factors, microclimatic factors and implicit factors [25]. Any of these factors or in combination can influence the proliferation of the fungal diversity in groundnuts during storage. The species richness and fungal abundance found in all the groundnut samples in storage goes to show that groundnuts are very susceptible to fungal attack especially to *Rhizopus stolonifer* and *Aspergillus* species, which agrees with findings of Salau *et al.* [25] who reported that groundnuts are highly susceptible to *Aspergillus* species contamination. The presence of *Rhizopus stolonifera* is capable of causing zygomycosis in humans and produces a secondary metabolite known as rhizonin A, which is dangerous and fatal [26]. *Penicillium* spp and *Aspergillus clavatus* are fungi that were recovered from the samples from this study. These fungi produce secondary metabolites known as Patulin and frequent exposure to this toxin can lead to neurological, immunological and gastrointestinal disorders such as ulceration, distension and malformation of the embryo [27]. Most *Aspergillus* species are known to produce mycotoxin, most notably aflatoxins and ochratoxins which causes negative health effects to man and animal [28].

A highly significant difference ($P < 0.001$) in the total heterotrophic fungi mean counts of stored groundnuts was observed in this study with the highest mean count observed in Nasarawa South agricultural zones (4.8×10^4 CFU/g), followed by Nasarawa West (1.6×10^4) and Nasarawa North had the least mean count (3.3×10^3 CFU/g). The possible reason for such a high statistically significant differences in the means could be the microclimatic conditions of the Nasarawa south agricultural zone compared to other zones and the poor agricultural practices carried out by the farmers. This study does not disagree with a similar study conducted in Sokoto State by Salau *et al* [29] that analyzed groundnut kernels from the three agricultural zones of which Tambuwal zone had the highest total fungal count of 2.8×10^4 CFU/g while samples from Isa zone had the lowest fungal load (1.7×10^3 CFU/g). However, the researcher attributed his results to post harvest practices.

5. Conclusion

This study revealed the fungal abundance in stored groundnuts in the three Agricultural zones of Nasarawa state. Among the various fungi, *Rhizopus stolonifer* had the highest of 65.3 % highest and *Neosartorya fisheri* (0.6 %). Due to high levels of fungal contamination found across the three agro-zones including contamination with *Rhizopus stolonifer*, *A. flavus* and *A. niger*, which are notable mycotoxin producers. Stored groundnuts in Nasarawa state may not be safe for human and animal consumption. This is not unrelated to the poor agricultural practices of these farmers. Based on these

findings, it is recommended that government should engage more agricultural extension workers to enhance awareness among these farmers and develop a set of rules and guidelines for proper handling from planting to harvesting down to storage to ensure food safety.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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