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Isolation and Identification of Fungi Associated with Raw Groundnut Seeds Sold at Four major markets in Port Harcourt Metropolis, Rivers State

Tobin-West, M. D.[1] Dimkpa, S. O. N.[1] Osakwe, J. A.[1]
1.Department of Crop/Soil, Rivers State University, Port Harcourt, P.M.B 5080, Rivers State, Nigeria
2.Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, P.M.B 5080, Rivers State, Nigeria

Abstract
The isolation and identification of fungi associated with raw groundnut seeds obtained from four major markets in Port Harcourt Metropolis was carried out in the Department of Plant Science and Biotechnology Laboratory (Pathology unit) in a Completely Randomized Design (CRD) using standard procedures. The identified fungal isolates include *Aspergillus*, *Penicilium*, *Mucor*, *Rhizopus* and *Fusarium* species. *Aspergillus spp.* with a mean value of 2.0 was the predominant fungi ($P<0.05$) identified followed by *Fusarium spp.* (1.1) and the least was *Penicillium spp.* (0.5). Mile three market recorded the highest ($P<0.05$) fungal incidence with a mean value of 1.44, followed by Mile one market (1.40) and the lowest was Rumuokoro market (0.70). Large seeds were more prone to fungal contamination with a mean of 1.2 than small seeds (1.0). Similarly, unwholesome seeds recorded the highest ($P<0.05$) amount of fungal incidence with a mean of 1.7 than wholesome seeds (0.5). Public awareness should be carried out on personal hygiene of food vendors, market sanitation, storage conditions and management practices for improving the quality of raw groundnut seeds sold in Port Harcourt markets to reduce contamination level.
Keywords: Raw groundnut, seed size, healthiness, market and Fungi.

INTRODUCTION

Groundnut *(Arachis hypogaea* L.*)* a member of the family *Fabaceae*; is also known and called with many names such as peanut, African nut, monkey nut, Chinese nut, manila nut, goobers pea, earthnut and ground bean (Subrahmanyam *et al.,* 1992). In Nigeria, it has many local names such as "epa" (Yoruba), 'ayayaa" (Hausa), "okpa or opapa or asibpko" (Ibo), "omizaguo" (Owan), "isagwe" (Benin) (Olayinka *et al.,* 2013). Groundnut originated in South America and was introduced to Africa by the Portuguese (Prasad *et al.,* 2009).

Groundnut is a tropical plant and requires a long warm growing season. The favourable climate for groundnut is a well distributed rainfall of at least 500mm during the crop-growing season, with abundance of sunshine and relative temperature within the range of 25 to 30°C for optimum plant development (Weiss 2000). Temperature lower than 25^{o}C during the sowing period increases disease/pest infestations and also delays germination (Ajeigbe *et al.,* 2014). Groundnut grows best in a well-drained sandy loam soil with a pH of 6.5-7.0. Sandy loam soil is preferable for groundnut production than the clay soil as it result to pod loss and difficult harvest (Ajeigbe *et al.,* 2014). Groundnut seed contains reasonable amounts of nutritionally important minerals including about 12% carbohydrate, 35% protein and most especially 50% non-drying edible oil (Onwueme and Sinha, 1991).This crop is important not only in the seeds food value for man but also supplies proteinous fodder for livestock and replenishes soil nitrogen due to the presence of nitrogen fixing bacteria in its root nodules (Jambunathan, 1991). A large number of food products can be prepared from groundnut namely boiled nuts, roasted nuts, salted nuts, groundnut milk, groundnut bars, groundnut butter, groundnut cheese, bakery products etc. (Opeke, 2006).

Sullivan (1984) stated that groundnut is highly susceptible to mycoflora diseases as they are rich in nutrients, such fungi includes *Rhizopus spp*., *Penicillium spp.*, *Aspergilllus niger* and *Aspergillus flavus*. These fungi cause several diseases such as seed rot, necrosis, leaf spot, rust, crown rot, damping off, wilt, grey mold, black mold etc., and the end result of these infections is a severe qualitative and quantitative yield loss (Hubert and David, 1983). The susceptibility of groundnut seeds to fungal pathogens is usually enhanced by poor methods of processing and storage of the seeds, which often lead to the release of toxins that are carcinogenic and when consumed by humans and animals cause lungs/liver cancer as well as other cardiovascular diseases (American Encyclopaedia, 1998). Groundnut pods are continuously dried till moisture level is reduced below 10% to avoid aflatoxin contamination caused by *Aspergillus flavus* and also preserves seed viability. Aflatoxin contamination is a major hazard/constraint to animal and human health and also to groundnut trade. Risks related to human health, death and declining productivity in livestock after consuming aflatoxin-contaminated feeds led to groundnut importing countries setting standards that allow only extremely low level of contamination and this low level of contamination is not achievable by most poor groundnut farmers (Ajeigbe *et al.,* 2014). It has also been reported by Olayinka *et al.,* (2016) and Atasie *et al.,* (2009) that larger groundnut seed size is prone to

deterioration by microbial agent due to their high moisture content. Elegbede (1998) reported that the activities of microorganisms often lead to the release of toxins that are harmful when consumed by human and animals. Food-borne illness of microbial origin is a major international health problem associated to food safety in developing countries (WHO, 2002).

Mycological assessment of groundnut seeds consumed in several states of Nigeria has been reported. Some of these states include Benin City, Edo state (Akinnibosun and Osawaru, 2015), unpeeled groundnut sold in Yenagoa metropolis (Kigigha *et al.,* 2016), Lagos state (Kayode *et al.,* 2014); roasted groundnut sold in three major areas (Wunti, Yelwa and Railway) in Bauchi State (Adebesin *et al.,* 2001), groundnut vendors on the highways of Onitsha-Owerri, Southeast Nigeria (Oranusi and Braide, 2012), groundnut seeds sold in Aliero Central market, Ekiti state (Ibrahim, 2014). Thus, the aim of this research is to isolate, evaluate and identify the fungal species associated with the raw groundnut seeds sold in four local markets in Port Harcourt.

2 MATERIALS AND METHODS

2.1 Experimental site

The experiment was carried out at the Department of Plant Science and Biotechnology (Pathology unit), Faculty of Science, Rivers State University, Port Harcourt.

2.2 Collection of Experimental materials and seed sorting

Groundnut seeds were purchased from the four local markets all within Port Harcourt Metropolis. The markets are Mile one, Mile three, Rumuokoro and Town market. Seed sorting was done manually by hand to separate wholesome from unwholesome seeds following the procedure of Kawube *et al.,* 2005.

2.3 Preparation of culture media and normal saline

Sabouraud Dextrose Agar was used in this research to isolate and identify the fungi; and the media was prepared according to manufacturer's instruction 11g of Sabouraud Dextrose Agar and 2g of Antibiotics was weighed and dissolved into 160ml of distilled water in a conical flask. Cotton wool was then placed to cover it and wrapped with aluminium foil. After which the solution was then autoclaved at 121^{o}c for 15 minutes, it was then allowed to cool and dispensed into Petri-dishes.8.5g of Sodium chloride (NaCl) was weighed and tipped into 100ml of distilled water after which it was sterilized in the autoclave. 1ml of the sample was dropped into 9ml of normal saline and shake vigorously to form a uniform solution of 10^{-1} concentration. The stock was subjected to a decimal dilution using sterile pipette to form 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} concentration (Harrigan, 1998).

2.4 Inoculation and Incubation

1ml pipette was employed to drop 0.01ml of the inoculums into the Petri-dishes and evenly spread all over the surface of the agar plate using stirring rod. All plates were incubated immediately after inoculation and placed upside down to prevent drops of condensations from collecting on the inoculated surface. Sabouraud Dextrose Agar plates were incubated for 28^{o}C for 72hrs, after which pure culture was prepared from the distinct fungal isolate observed (Harrigan, 1998).

2.5 Characterization and Identification of Isolates

The fungal growth observed in the pure culture was identified as described by CMI (2010).

2.6 Experimental Design and Statistical techniques

The experimental design used in this research is 4×2×2 Factorial arranged in a Complete Randomization Design. The treatments were divided into three categories which are markets, size and healthiness.

The factors and levels that were involved in this research were:

- Market: Mile one, Mile three, Town and Rumuokoro
- Size: Large and Small seeds
- Healthiness: Wholesome and Unwholesome

Analysis of variance (ANOVA) was used to determine the treatment effects and means were tested using Tukey Pair wise comparison of grouping at 5% level of probability (Minitab, 2010).

3 RESULTS

Table 1 revealed five genera of Fungi isolated from the groundnut samples purchased from four different markets which are *Aspergillus spp. Rhizopus spp., Penicillium spp., Mucor spp.* and *Fusarium spp*. Unwholesome mile-one-market large seeds (M_1+L+U) recorded a mean of 2.7 representing the highest incidence ($P<0.05$) of fungi presence on the sampled groundnut seeds across the various markets, size and healthiness followed by unwholesome mile-three-market small seeds (M_3+S+U) with a mean of 2.5 and the least was 0.3 for wholesome town-market large seeds (TO+L+W). *Aspergillus spp.* was highest ($P<0.05$) with a mean value of 5.5

in unwholesome mile-three-market large seeds, followed by unwholesome mile-three-market small seeds (4.9) and the lowest was wholesome mile one-market small seeds (0.8) but was completely absent in wholesome rumuokoro-market large and small seeds (Ru+L+W and Ru+S+W), wholesome town-market small and large seeds (TO+L+W and TO+S+W) and unwholesome town-market large seeds (TO+L+U). Unwholesome mile-one-market large seeds (M_1+L+U) with a mean value of 5.7 had higher ($P<0.05$) occurrence of *Rhizopus spp.* compared to the other groundnut samples. *Fusarium spp.* was high in unwholesome rumuokoro-market large seeds (Ru+S+U) and unwholesome mile-three-market small seeds (M_3+S+U) with a mean count of 3.1 followed by wholesome rumuokoro-market large seed (RU+L+W). *Mucor spp.* was high in unwholesome mile-one-market small seeds (M_1+S+U) with a mean value of 4.0 followed by unwholesome rumuokoro-market small seeds (Ru+S+U) with a mean of 3.1. Unwholesome town-market large seeds (TO+L+U) were significantly high in *Penicillium spp.* incidence but recorded no presence of *Aspergillus spp., Rhizopus spp.* and *Mucor spp.* Cumulatively, *Aspergillus spp.* with a mean microbial incidence of 2.0 was the predominant fungi ($P<0.05$) affecting the raw groundnut seeds sold in Port Harcourt metropolis followed by *Fusarium spp.* (1.1) and the least was *Penicillium spp.* with a cumulative mean value of 0.5 (Table 2).

However, the experimental result on healthiness (Figure 1) revealed significant higher fungi incidence of 1.7 on unwholesome seeds than wholesome seeds (0.5) irrespective of the sizes. The result further showed that fungi loads were significantly higher in larger seeds with mean value of 1.2 than smaller seeds (1.0). Similarly, fungi incidence on unwholesome seeds sourced from mile-three-market were significantly higher followed by unwholesome mile-one-market seeds and the lowest was wholesome seeds sourced from rumuokoro-market and town-market (Table 3). Generally, the fungi loads on seeds sourced from mile-three-market were significantly higher compared to town and rumuokoro markets, although not different from mile-one-market (Figure 2). The result from the pure culture (Table 4) showed six distinct growths with two belonging to the same genus *Aspergillus.* Also on Table 4 are the physical/morphological characteristics and Microscopic appearance of the isolated mycoflora.

Table 1: Occurrence of the fungal isolates in the raw groundnut seed sizes collected from different markets and sorted in various healthiness

Treatment	*Aspergillus spp.*	*Rhizopus spp.*	*Fusarium spp.*	*Mucor spp.*	*Penicillium spp.*	Mean
M_1+L+U	4.7	5.7	0	1.2	1. 9	2.7^{a}
M_1+L+W	2.2	1.97	0	1.1	0	1.1^{e}
M_1+S+U	2.3	0	1.1	4.0	0	1.5^{c}
M_1+S+W	0.8	0	1.0	0	0	0.3^{fg}
M_3+L+U	5.5	2.6	1.7	0	0	2.0^{b}
M_3+L+W	2.1	0	0.9	0	0	0.6^{fg}
M_3+S+U	4.9	2.0	3.1	2.2	0	2.5^{a}
M_3+S+W	2.1	0	0	1	0	0.6^{f}
RU+L+U	3	0	3.1	0	0	1.0^{e}
RU+L+W	0	0	2.0	0	0	0.4^{fg}
RU+S+U	2.7	0	0	3.1	0	1.2^{de}
RU+S+W	0	0	0	1.8	0	0.4^{fg}
TO+L+U	0	0	3	0	2.8	1.2^{de}
TO+L+W	0	0	0	0	1.7	0.3^{g}
TO+S+U	2	3.1	1	0	0.8	1.4^{cd}
TO+S+W	0	0.8	0. 9	0	0.7	0.5^{fg}

Means with same letters are not significantly different $P<0.05$ by Tukey's Pair wise comparisons at 95% confidence intervals.

KEY

M_1 – Mile one market, M_3 - Mile three market, RU - Rumuokoro market, TO - Town market, L - large seeds, S – Small seeds, W – Wholesome, U – Unwholesome.

Table 2: Mean occurrence of the isolated microorganism

Microbial Isolate	**Mean values**
Aspergillus spp.	2.0^{a}
Fusarium spp.	1.1^{b}
Rhizopus spp.	1.0^{bc}
Mucor spp.	0.9^{c}
Penicillium spp.	0.5^{d}

Frequency with same letters are not significantly different ($P<0.05$) using Tukey's test at 95% confidence interval

Table 3: Combined effect of market and healthiness of the raw groundnut seeds sourced from different markets

Market	Healthiness	Total Mean
Mile one	Unwholesome	2.1[a]
Mile one	Wholesome	0.7[d]
Mile three	Unwholesome	2.2[a]
Mile three	Wholesome	0.6[d]
Rumuokoro	Unwholesome	1.1[c]
Rumuokoro	Wholesome	0.4[e]
Town	Unwholesome	1.3[b]
Town	Wholesome	0.4[e]

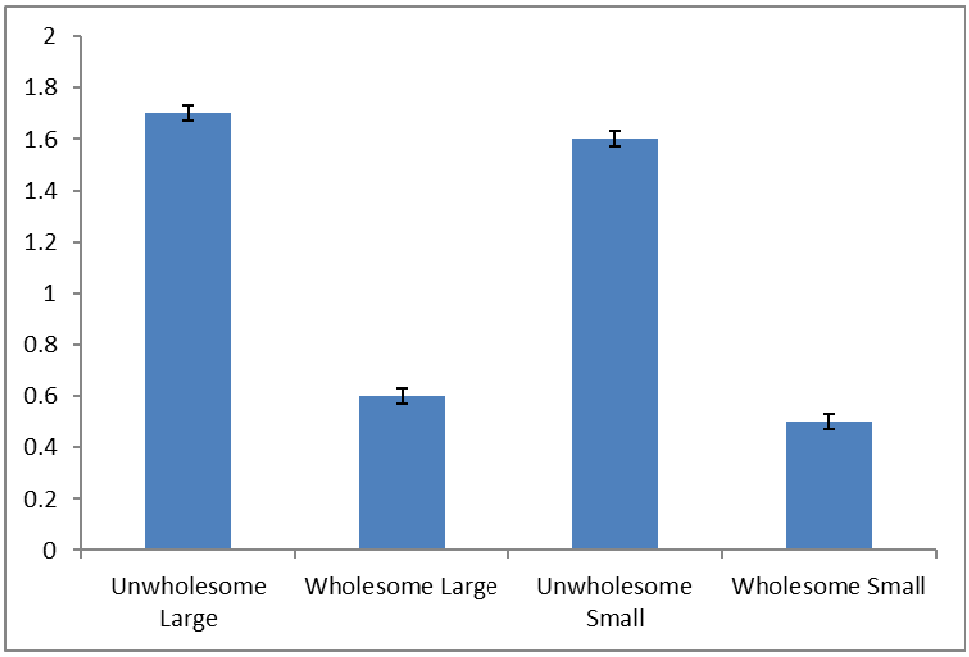

Figure 1: The combined effect between Seed size and Healthiness of the groundnut samples

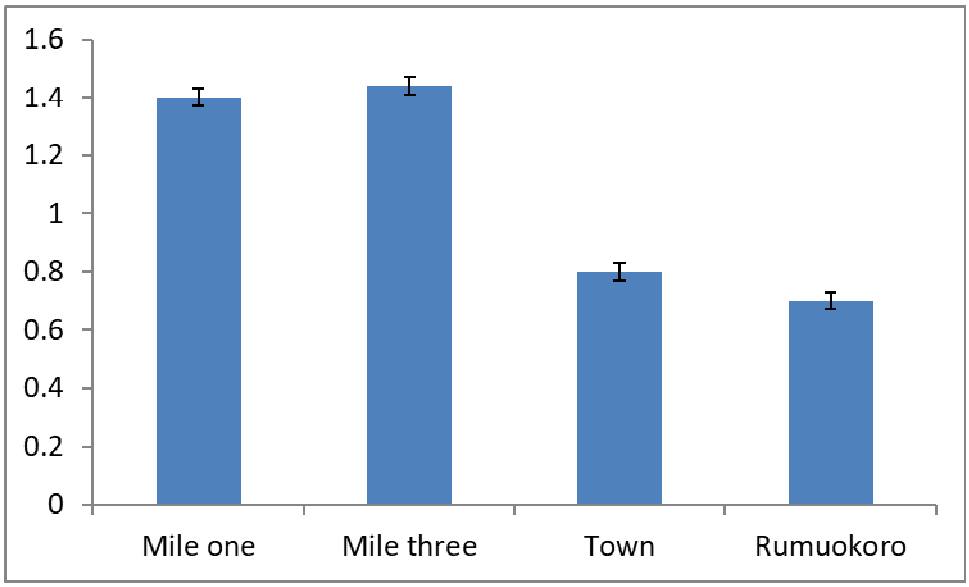

Figure 2: The difference in the fungal loads between four local markets in Port Harcourt Metropolis

Table 4: Identified Fungi with their Morphological Characteristics

Isolates	Morphological Characteristics	Microscopic Appearance	Probable Organisms
1	Initial growth is white, later becoming black with pale yellow on the reverse side	Erect conidiophores and hyphae is septate	*Aspergillus spp.*
2	White edge/margin, velvety furrowed centre with bluish green color	Brush-like conidiophores with septate hyphae	*Penicillium spp.*
3	White margin with cream yellow color in the centre	Non-septate mycelia that bears branched sporangiosphore	*Mucor spp.*
4	Yellow-green powdery center with white margin and pale yellowish on reverse	Long erect conidiophores with septate hyphae	*Aspergillus spp.*
5	White cottony colony with dense growth	Short crescent shaped conidiophores, septate hyphae with abundant micro-conidia	*Fusarium spp.*
6	Brownish grey color colony fast growing with whitish margin	Short rhizoids, sporangiophore unbranched and hyphae is coenocytic	*Rhizopus spp.*

4 DISCUSSIONS

Fungi had been known to affect groundnut production for a long time. Five fungal species were identified in this research: *Aspergillus spp.*, *Mucor spp.*, *Rhizopus spp.*, *Penicillium spp.* and *Fusarium spp.* These fungi were among the microorganisms isolated from previous findings; Fagbohun and Faleye (2012) isolated *Aspergillus niger, Aspergillus flavus, Rhizopus spp. Mucor spp.* and *Aspergillus fumigatus* in sundried groundnut, Adebesin *et al.,* (2001) isolated *Aspergillus flavus, Aspergillus niger, Aspergillus tamarii*, *Penicillium Citrinum, Rhizopus stolonifer* and *Macrophomina phaseolina* in roasted groundnut sold in Bauchi State, Ibrahim (2014) observed *Mucor spp., Aspergillus spp., Penicillium spp., Curvularia spp., Fusarium spp.* and *Rhizopus spp.* in groundnut seeds sold in Aliero central market, Ekiti state. Also, *Aspergillus spp., Penicillium spp., Fusarium spp*., *Mucor spp*. and *Rhizopus spp*. were isolated from unpeeled groundnut sold in Yenagoa metropolis (Kigigha *et al.,* 2016). Akinnibosun and Osawaru (2015) isolated *Neurospora spp., Aspergillus niger, Aspergillus flavus, Mucor spp. Rhizopus spp., Penicillium spp., Trichoderma spp*. and *Fusarium spp.* in peeled and unpeeled groundnut seeds sold in Benin City.

From this study, *Aspergillus spp.* was more prevalent in the samples collected from four markets in Port Harcourt Metropolis while *Penicillium spp.* was less prevalent. This finding is in line with Akinnibosun and Osawaru (2015) and Kigigha *et al.,* (2016) who identified *Aspergillus spp.* as the predominant fungi that affect the quality of groundnut seeds sold in Benin City and Yenagoa Metropolis respectively. *Aspergillus spp.* was prominent due to the fact that it contaminates groundnut at various stages right from harvest to production (Dange and Patel, 1984). The effect of seed size on fungi incidence revealed that large seeds were significantly higher in fungi incidence than small seeds and this agrees with the report of Olayinka *et al.,* (2016) and Atasie *et al.,* (2009) who reported that larger groundnut seeds are more prone to contamination and have low shelf-life because of their high moisture content. Higher loads of fungi were identified in unwholesome seeds than wholesome seeds and this is probably caused by physical bruising during harvesting, packaging, poor storage facility and physiological factors.

It is noteworthy to mention that this study revealed variation in the fungi occurrences on groundnut seeds sold at different markets in Port Harcourt metropolis. The present experimental result recorded higher fungi load on groundnut seeds sourced from mile three market followed by mile one market and the lowest was found in rumuokoro market. The variation in the fungi occurrence across the various markets could be attributed to exposure to environmental pollution, poor market sanitation, handling and poor personal hygiene of the food vendors (Mensah *et al.,* 2002). The fungi isolated in this research are toxin producing organisms and they cause series of health challenges ranging from vomiting to cancer of major organs and sometimes death (Akinyemi *et al.,* 2011).

5 CONCLUSIONS

This study, isolated and identified various toxin producing fungi such as *Aspergillus spp.*, *Fusarium spp.*, *Mucor spp.*, *Penicillium spp.* and *Rhizopus spp*. associated with raw groundnut seeds sold in some major markets in Port

Harcourt metropolis, Rivers State, Nigeria. The isolated mycoflora poses health hazard to indigenous consumers through the toxins they produce. Large and unwholesome seeds had more fungal contamination than the small and wholesome seeds respectively. Public awareness should be carried out on personal hygiene of food vendors, market sanitation, storage conditions and management practices for improving the quality of raw groundnut seeds sold in Port Harcourt markets to reduce contamination level.

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