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Mycoflora associated with Groundnut Seeds Collected from the three Senatorial Districts of Kwara State, Nigeria

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Abstract
Groundnut seeds are of numerous benefits to mankind which can be reduced through contamination by fungal species, depending on the fungal species and their degree of contamination of the seeds. Occurrence of fungal species on groundnut seeds is of great public health concern. Therefore, it is essential to examine the different fungal species present. This study employed both the phenotypic and molecular methods to isolate and characterize the fungal species attributed to groundnut seeds. The Zymo Research Group's recommended protocols were followed in order to extract the genomic DNA of each of the fungal species recovered. The PCR amplification and sequencing of the ITS region from the total genomic DNA of fungal isolates were conducted to molecularly identify them, using the NCBI database for comparison. The commonest mycoflora isolated from the stored groundnut seeds were *Aspergillus flavus*, *A. niger*, *Alternaria alternate*, *Fusarium graminearum*, *Macrophomina phaseolina*, *Penicillium digitatum* and *Rhizopus stolonifer*. The total incidence of each of them ranged from 8 - 32%. Among the three surveyed districts, Kwara Central exhibited the highest incidence of major mycoflora at 40%, while Kwara North had the lowest at 27%. *Aspergillus niger* demonstrated the highest incidence among the fungal species found in groundnut seeds collected from all districts. Failure to adequately dry these seeds before storage could elevate the risk of mycotoxin contamination. Implementing management approaches targeting different fungi is essential to preserve nutritional value of groundnut seeds during storage.

Keywords: Contamination, Fungal species, Genomic DNA, Molecular tools, Mycotoxins.

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Introduction

Farmers cultivate groundnut (*Arachis hypogaea* L.), an annual leguminous crop with geocarpic characteristics, in different regions worldwide, especially in West Africa. Its significance lies in serving as a crucial source of both food and fodder within farming systems (Sinare *et al*., 2021). The global ranking places it as the fourth most crucial oilseed crop and the thirteenth most vital food crop (Asare Bediako *et al*., 2019). In Nigeria, it goes by various names such as "epa" (Yoruba), "jeda" (Hausa), "Ahụekere" (Igbo), "omizaguo" (Edo), and "isagwe" (Benin) (Tobin-West *et al*., 2018). Groundnut seeds hold significant value as a cash crop, contributing to both domestic and global commerce in numerous industrialized as well as developing countries (Mothiba *et al*., 2023).

Groundnut seeds are a nutritional powerhouse, offering a wealth of minerals, including phosphorus, copper, magnesium, calcium, iron, manganese, potassium and zinc, as well as niacin, riboflavin, tocopherol and phylloquinone (Arya *et al*., 2016).

Groundnut seeds are eaten raw, boiled or roasted; oil extracted from the seeds is used for cooking, and as industrial raw material (for making fertilizer and oil cakes). After removing the seeds, the plants are also used as straw, animal feed and green manure. A significant hindrance to the worldwide production and availability of high-quality groundnut yields is the damage and degradation inflicted by bacteria, fungi, viruses, insects and parasitic weeds (Isalar *et al*., 2021). Any of the above listed plant pathogens associated with the groundnut seeds as contaminants may result in seed dormancy, rot, diminishment of seed viability, impairment of seedlings coupled with the secretion of chemical substances that can lead to disease incidence in humans and animals (Goko *et al*., 2021).

Groundnut seeds, susceptible to invasion by fungi, are frequently eaten by many individuals unaware of the harmful species associated, which can pose health risks. Although, most of these fungal species grow on the surface of the groundnut seeds, exhibiting various macro-morphological characteristics, their identification is challenging and may lead to error in diagnosis (Fang *et al*., 2023). Moreover, many fungal species exhibit similar macro-morphological and micro-morphological characteristics. Relying solely on morphological methods in fungal systematics might prove insufficient for classifying at the species level, presenting a challenge unless complemented by molecular tools. This study sought to isolate fungal species present in groundnut seeds and then identify these pure fungal isolates morphologically and molecularly.

Materials and Methods

Collection of Groundnut seeds

Two major Markets with high patronage for agricultural produce were selected from each of the three senatorial districts in Kwara State for this study, i.e. Kaima (Latitude: 9° 36' 19.08" N, Longitude: 3° 56' 27.64" E) and Tsaragi (Latitude: 8° 48' 42.56° N, Longitude: 4° 53' 68. 28 E) in Kwara North, Alapa (Latitude: 8° 29' 52" N, Longitude: 4° 32' 49" E) and Ipata (Latitude: 8° 28' 42" N, Longitude: 4° 30' 10" E) in Kwara Central, as well as Ganmo (Latitude: 8° 9' 35" N, Longitude: 4° 43' 28" E) and Share (Latitude: 8° 49’ 0” N, Longitude: 4° 59’ 0” E) in Kwara South. In each of the markets, four warehouses of groundnut seeds were randomly selected, and from each warehouse, 250 g of stored groundnut seeds were weighed and put in a sterile polythene bag. The four bags of 250 g of stored groundnut seeds were then bulked to make 1000 g of stored groundnut seeds in another sterile polythene bag labeled according to name of the market. These gave six samples of 1000 g of stored groundnut seeds representing the six markets (Esan *et al*., 2020). The samples were taken to the Biology laboratory at the University of Ilorin, Ilorin, for further examination.

Isolation and Morphological Identification of Fungi from Groundnut Seeds

The procedures of Adetunji *et al*. (2018) were followed. Seven whole groundnut seeds were picked randomly from each of the samples, with each of them split into 2 equal parts by hand. After a 30-second surface sterilization with 2.5% sodium hypochlorite solution and rinsing in three cycles of sterile distilled water, four of the seeds, having undergone this process, were evenly placed on Potato Dextrose Agar (PDA) plates. These plates were amended with 1 ml of streptomycin BP and were arranged in triplicates. Subsequently, the plates were put in an incubator at a temperature of 25°C for a duration of 7 days, with daily observations made to track the development of fungal growth. In order to have pure isolates, the isolates recovered were further purified on fresh plates. These isolates were then kept at room temperature for additional investigation while being maintained on PDA slant in McCartney bottles. The isolates were identified through an examination of colony and cell morphology using wet mount preparation with reference to Fawole and Oso (2007) and Kidd *et al*. (2023).

Molecular Identification of the Fungal Isolates

Zymo Research DNA kit was used to extract all of the fungal isolates' genomic DNA according to step-wise protocol available online at www.zymoresearch.com. The amplification of the ITS region of the extracted DNA of all the fungal isolates was performed at the Unilorin Central Research Laboratory employing primer pairs ITS1: 5' (TCCGTAGGTGAACCTGCGG) 3' and ITS4: 5' (TCCTCCGCTTATTGATATGC) 3'. The PCR procedure involved an initial denaturation at 95°C for 5 minutes, followed by denaturation at 94°C over 30 cycles for 30 seconds, annealing at 50°C for 1 minute, extension at 68°C for 1 minute, and a final extension at 68°C for 10 minutes. To identify the products obtained from amplification, 1% agarose gel electrophoresis was utilized. Sequencing services were provided by Inqaba Biotec-africa's Genomics Company, West Africa LTD, located in Ibadan, Oyo State. The obtained sequencing results were utilized for determining the percent identity of the sequences using the on-line tool, Basic Local Alignment Search Tool (BLAST-n) available on https://www.ncbi.nlm.nih.gov/.

Results

Morphological Identification of the Fungal Isolates

Seven fungal species were isolated from the groundnut seeds. They were initially labeled as Isolates A to G, which were morphologically identified tentatively as *Aspergillus flavus*, *A. niger*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum*, *Alternaria alternate* and *Fusarium graminearum* and based on their observed macroscopic and microscopic features (Table 1). Their pictures are presented in Plate 1.

Table 1: Morphological Identification of the Fungal Isolates

Fungal Isolates	A	B	C	D	E	G	F
Colony Colour	yellowish-green	dense layer of black colour	White to grey	White to brownish-grey fluffy growth	greenish or olive-colored	greenish-black surface	cotton-like
Hyphal Type	septate	septate	Septate	Aseptate	septate	Septate	Septate
Spore Type	Conidia	conidia	Conidia	Sporangiospores	conidia	Conidia	Conidia
Tentative Identity	*Aspergillus flavus*	*A. niger*	*Macrophomina phaseolina*	*Rhizopus stolonifera*	*Penicillium digitatum*	*Alternaria alternate*	*Fusarium graminearum*

Percentages of Occurrence of the Fungal Isolates

The percentage of occurrence of *Aspergillus niger* was the highest in this study (32%), followed by that of *Macrophomina phaseolina* (22%), with *Alternaria alternate*, *Fusarium graminearum* and *Penicillium digitatum* sharing the lowest value (8%) (Table 2). The Total Fungal Count (TFC) for the groundnut seeds collected from Kwara Central was the highest (40%), while the lowest value of the same parameter (27%) was observed in the groundnut seeds collected from Kwara North (Table 2).

Molecular Identification of the Isolated Fungi

Table 3 displays the outcomes of the BLAST analysis, showing the percent identity and the matched accession number of the DNA sequences obtained from each of the fungal isolates. The consensus DNA sequences for the seven fungal isolates are presented in the Appendices A to C.

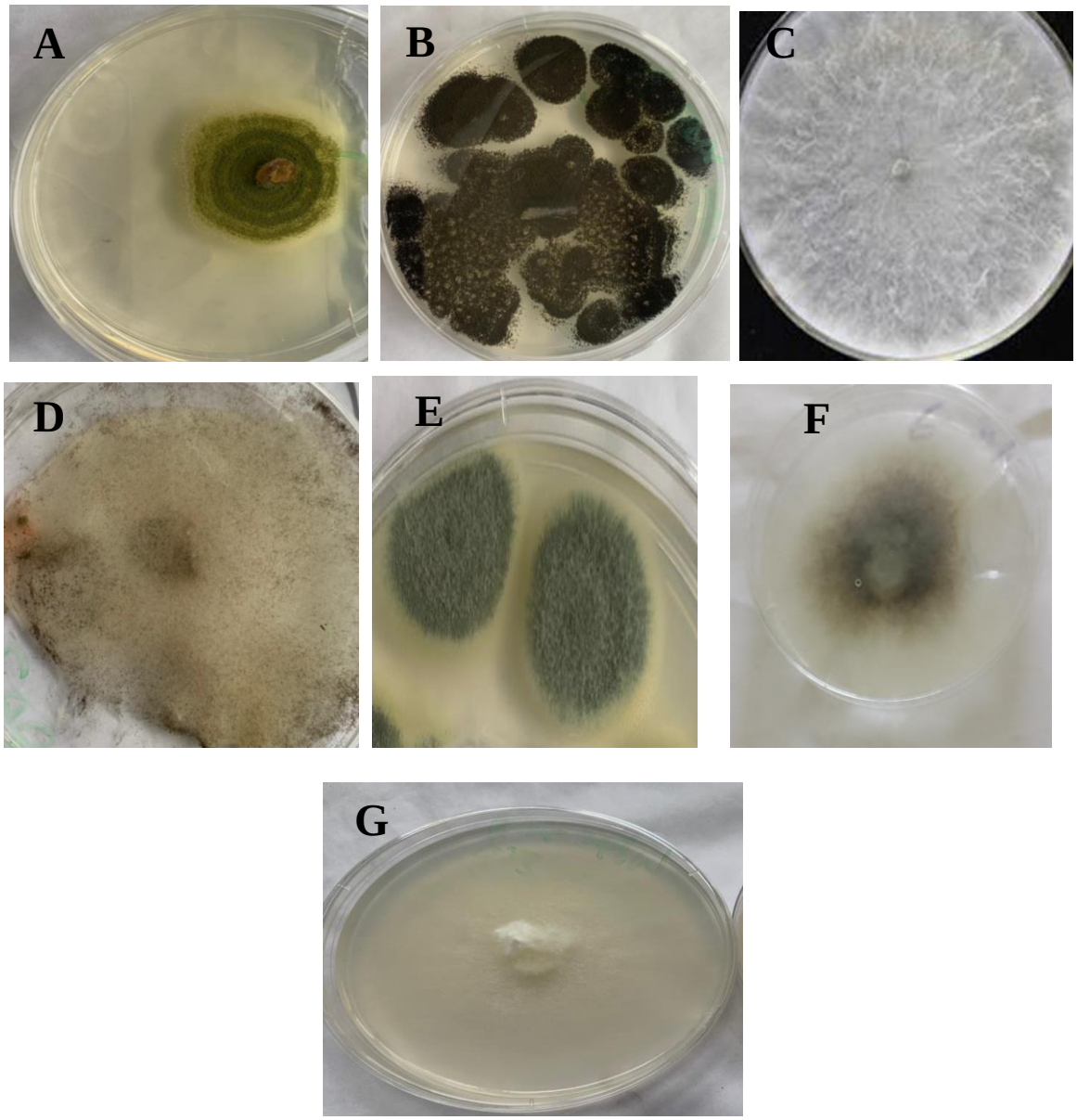

Plate 1: Pure fungal cultures isolated from groundnut seeds on PDA plates. Letters A-G correspond to the isolates, with isolate A being identified as *Aspergillus flavus*; B- *Aspergillus niger*; C- *Macrophomina phaseolina;* D- *Rhizopus stolonifer*; E- *Penicillium digitatum*; F- *Alternaria alternate*; G- *Fusarium graminearum.*

Table 2: Occurrence Percentage of Fungal Species (%) and Total Fungal Count (%) in Groundnut Seeds collected from the Three Senatorial Districts

Senatorial – Districts	*Aspergillus flavus*	*A. niger*	*Macrophomina phaseolina*	*Rhizopus stolonifer*	*Penicillium digitatum*	*Alternaria alternate*	*Fusarium graminearum*	TFC
Kwara Central	4	12	8	4	4	4	4	40
Kwara North	2	8	6	4	0	4	3	27
Kwara South	4	12	8	4	4	0	1	33
Total	10	32	22	12	8	8	8	100

Table 3: Species identified through BLAST searches of the DNA sequences.

Isolates	Organisms (Accession numbers)	Percent Identity (%)
1	*Aspergillus flavus* (KX067890.1)	100
2	*Aspergillus niger* (ON208695.1)	99
3	*Macrophomina phaseolina* (MN067769.1)	100
4	*Rhizopus stolonifer* (MK990551.1)	99
5	*Penicillium digitatum* (MK450692.1)	100
6	*Alternaria alternate* (MW723773.1)	100
7	*Fusarium graminearum* (MT742829.1)	100

Discussion

In this study, *Aspergillus flavus, A. niger*, *Alternaria alternate*, *Fusarium graminearum*, *Macrophomina phaseolina*, *Penicillium digitatum* and *Rhizopus stolonifer* were recovered from groundnuts collected from 2 markets each from the 3 senatorial districts of Kwara State, North Central Nigeria. All the above listed fungi have also been reported in earlier studies conducted in Nigeria and other places. Akharenegbe *et al*. (2022) isolated *Aspergillus flavus*, *A. niger*, *Mucor* sp. and *Rhizopus stolonifer* from stored groundnut seeds obtained from Nasarawa State. Tobin-West *et al*. (2018) similarly identified *Aspergillus*, *Fusarium*, *Mucor*, *Penicilium* as well as *Rhizopus* from groundnut seeds collected from four different local marketplaces in Port Harcourt, River State. Ezekiel *et al.* (2018), in their study on roasted groundnuts in southwestern Nigeria, observed the occurrence of *A. flavus*, *A. tamarii*, and *A. parasiticus*. Similarly, Abuga (2014) isolated various fungal genera including *Aspergillus*, *Fusarium, Mucor*, *Penicillium* and *Rhizopus* from seeds of groundnuts obtained from capital market in Aliero, Kebbi State. In a study conducted by Aliyu and Kutama (2010) in Kano State, 25 distinct fungal species were identified in groundnut seeds, among which were *A. niger*, *A. parasiticus*, *A. tamari*, *Cladosporium* spp. and *Emericella* spp. These fungi are probably the vehicles responsible for the transmission of pathogenic foodborne diseases (Aladhadh, 2023).

The percentage of occurrence of the isolated fungal species varied among the three districts surveyed in this study, with *A. niger* being predominant (32%) and *Penicillium digitatum*, *Alternaria alternate* and *Fusarium graminearum* being the least (8%). The dominance of *A. niger* in our study corresponds with the observations made by Akinnibosun and Osawaru (2015) as well as Kigigha *et al*. (2016). Both studies identified *Aspergillus* spp. as the primary fungal genus influencing the nutritional value of groundnut in the city of Benin, and Yenagoa city in Bayelsa State respectively.

Omugo *et al*. (2021) also reported the dominance of *A. niger* among isolates recovered from groundnut seeds collected from Owerri metropolis, Nigeria. Anjorin *et al*. (2021) also reported *A. niger* as the predominant species obtained from improved groundnut varieties grown in Ahmadu Bello University, Samaru Zaria, Kaduna State. Similarly, Adithya *et al*. (2017) also reported *A. niger* as the dominant fungal species on groundnut seeds collected from specific regions in Telangana, a state in India that grows groundnuts. *A. niger* is known to be a very notorious contaminant of agricultural produce at various stages of their production, especially storage, groundnut seeds inclusive (Madilo *et al*., 2023). Out of the three districts surveyed, the total fungal count was highest in Kwara Central (40%) and lowest in Kwara North (27%). This could be as a result of unhygienic conditions of the storage facilities in Kwara Central.

Conclusion and Recommendation

The isolation of *Aspergillus flavus*, *A. niger*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum*, *Alternaria alternate* and *Fusarium graminearum* from stored groundnut seeds obtained from the 3 senatorial districts of Kwara State, Nigeria is an eye-opener to fungal contamination of stored agricultural produce. The predominant fungal species is *A. niger*, a toxigenic fungus that secretes deadly mycotoxins, notably ochratoxin A. There is therefore, the need for proper storage of groundnut seeds to prevent fungal contamination.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Contributions

This manuscript originates from the M.Sc. dissertation of the first author, under the supervision of the second author. Both contributors extensively reviewed, edited, and sanctioned the ultimate version of the manuscript.

References

Abuga, I. (2014). Isolation and identification of fungi associated with groundnut seeds sold at Aliero Central Market. *International Journal of Biological Sciences*, 1: 56-62

Akinnibosun, F. I. and Osawuru, E. E. (2015). Quality Assessment of Peeled and Unpeeled Roasted Groundnut (*Arachis hypogaea* L.) sold in Benin City, Nigeria. *International Research Journal of Natural and Applied Sciences.* 2(3): 18-32

Adetunji, M. C., Alika, O. P., Awa, N. P., Atanda, O. O. and Mwanza, M. (2018). Microbiological Quality and Risk Assessment for Aflatoxins in Groundnuts and Roasted Cashew Nuts Meant for Human Consumption. *Journal of Toxicology*, 2018: 1–11. https://doi.org/10.1155/2018/1308748

Adithya, G., Rajeshwari, B., Keshavulu, K., Sudini, H. and Swathi, Y. (2017). Mycoflora Associated with Groundnut Seeds Collected from Selected Groundnut Growing Districts of Telangana State, India. *International Journal of Current Microbiology and Applied Sciences*, 6(7): 4335–4342. https://doi.org/10.20546/ijcmas.2017.607.451

Akharenegbe, P., Chuku, A., Mawak, J., Sani, B., Nsemoh, H.E. and Fadayomi, F.K. (2022). Fungal biodiversity associated with groundnuts stored in Nasarawa State. *GSC Biological and Pharmaceutical Sciences*, 18(3): 023–029. https://doi.org/10.30574/gscbps.2022.18.3.0087

Aladhadh, M. (2023). A Review of Modern Methods for the Detection of Foodborne Pathogens. *Microorganisms*, 11(5): 1111. https://doi.org/10.3390/microorganisms11051111

Aliyu, B. S. and Kutama, A. S. (2010). Isolation and Identification of fungal flora associated with groundnut in different storage facilities. *Science*

World Journal, 2(2). https://doi.org/1004314/swj.v2i2.51738

Anjorin, S.T., Usman, A., Fapohunda, S. O., Kwanashie, A., Sulyok, M. and Krska, R. (2021). Mycobiota and fungal metabolites in improved groundnut varieties in Nigeria. *Jordan Journal of Biological Sciences*, 14(4): 719-726. https://doi.org/10.54319/jjbs/140412

Arya, S. S., Salve, A. R. and Chauhan, S. (2016). Peanuts as functional food: A review. *Journal of Food Science and Technology*, 53(1): 31-41. https://10.1007/s13197-015-2007-9

Asare Bediako, K., Dzidzienyo, D., Ofori, K. Offei, S. K., Asibuo, J. Y., Adu Amoah, R. and Obeng, J. (2019). Prevalence of fungi and aflatoxin contamination in stored groundnut in Ghana. *Food Control*, 104: 152-156. https://doi.org/10.1016/j.foodcont.2019.04.034

Esan, A. O., Fapohunda, S. O., Ezekiel, C. N., Sulyok, M. and Krska, R. (2020). Distribution of fungi and their toxic metabolites in melon and sesame seeds marketed in two major producing states in Nigeria. *Mycotoxin Research*, 36(4): 361-369.

Ezekiel, C.N., Oyeyemi, O.T., Oyedele, O.A., Ayeni, K.I., Oyeyemi, I.T., Nabofa, W., Nwozichi, C.U. and Dada, A. (2018). Urinary aflatoxin exposure monitoring in rural and semi-urban populations in Ogun state, Nigeria. *Food Additives & Contaminants*, 35: 1565–72

Fang, W., Wu, J., Cheng, M, Zhu, X., Du, M., Chen, C., Liao, W., Zhi, K. and Pan, W. (2023). Diagnosis of invasive fungal infections: Challenges and recent developments. *Journal of Biomedical Science*, 30(1): 42. https://doi.org/10.1186/s12929-023-00926-2

Fawole, M. O. and Oso, B. A. (2007). *Laboratory Manual of Microbiology*. Spectrum Books Limited, Ibadan, Nigeria. pp. 127.

Goko, M. L., Murimwa, J. C., Gasura, E., Rugare, J. T. and Ngadze, E. (2021). Identification and Characterisation of Seed-Borne Fungal Pathogens associated with Maize (*Zea mays* L.). *International Journal of Microbiology*. 2021: 1-11. https://doi.org/10.1155/2021/6702856

Isalar, O.F., Ogbuji, N.G., Okungbowa, F.I. and Ataga, A.E. (2021). Fungal contaminants associated with groundnut (*Arachis hypogaea*) seeds. *Journal of Bioinformatics and Systems Biology*, 4: 182-193

Kidd, S. E., Abdolrasouli, A. and Hagen, F. (2023). Fungi Nomenclature: Managing Change is the Name of the Game. *Open Forum Infectious Diseases*, 10(1): 0fac559. https://doi.org/10.1093/ofac559

Kigigha, L.T., Igoya, U.O. and Izah, S.C. (2016). Microbiological quality assessment of unpeeled groundnut sold in Yenogoa metropolis, Nigeria. *International Journal of Innovative Biochemistry and Microbiology Research*, 4(4): 11-22

Madilo, F.K., Glover, R.L.K., Nazrul Islam, M.D., Roy, N. and Letsyo, E. (2023). Microbiological assessment of groundnut (*Arachis hypogaea* L.) sold for consumption in Ghana. *Journal of Food Quality*, 1-10. https://doi.org/10.1155/2023/7836774

Mothiba, M., Mthombeni, D. and Antwi, M. (2023). Determinants of commercialization and choice of market channels among smallholder groundnut farmers in the Capricorn District, Limpopo Province, South Africa. *African Journal of Food, Agriculture, Nutrition and Development*, 23(8): 24443–24458. https://doi.org/10.18697/ajfand.123.23380

Omugo, J.E., Amadi, J.E., Okigbo, R.N., Ukpai. K.U., Evans-Kemka, C.I. and Nguma, M.O. (2021). Fungal isolates of Groundnut (*Arachis Hypogaea* L.) seeds in Owerri metropolis, Nigeria. *NJB*, 34(1): 69-82.

Sinare, B., Miningou, A., Nebié, B., Eleblu, J., Kwadwo, O., Traoré, A., Zagre, B., & Desmae, H. (2021). Participatory analysis of groundnut (*Arachis hypogaea* L.) cropping system and production constraints in Burkina Faso. *Journal of Ethnobiology and Ethnomedicine*, 17(1): 2. https://doi.org/10.1186/s13002-020-00429-6

Tobin-West, M. D., Dimkpa, S. O. N. and Osakwe, J. A. (2018). Isolation and Identification of Fungi Associated with Raw Groundnut Seeds Sold at Four major markets in Port Harcourt Metropolis, Rivers State. *Journal of Biology, Agriculture and Healthcare*, 8(6): 29-35