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Original Article

Isolation and Identification of Fungal Contaminants Associated with the Spoilage of Groundnut (*Arachis Hypogaea*) Seeds from Local Markets in Tubah Subdivision

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Abstract - *Groundnuts are consumed worldwide for their nutritional, health, and economic benefits. Fungi remain major contaminants on postharvest groundnuts, resulting in significant economic losses and health risks to humans. The study aimed at isolating and identifying the different fungi associated with contamination and spoilage of groundnuts sold in markets in Tubah Subdivision. Groundnuts were purchased, surface-sterilized in 70% alcohol, rinsed with distilled water, and cultured on Potato Dextrose Agar. Gentamicin was added to suppress bacterial growth. Seeds were inoculated in three replicates for 5 days at a temperature of 25 °C. Isolated fungi were again sub-cultured to obtain pure cultures. Fungal identification was done based on morphological and microscopic features. Results revealed five fungal genera, including Aspergillus, Rhizopus, Mucor, Penicillium, and Fusarium, as prominent in the spoilage of groundnuts. Aspergillus niger had the highest frequency of occurrence (42.1%), followed by Rhizopus stolonifer (23.6%), Mucor sp (15.3%), and Penicillium sp (13.8%). And Fusarium oxysporum (4.9%). The pathogenicity test revealed that the isolated fungus caused spoilage on groundnuts. Poor storage practices by vendors were the main reason for spoilage. Public awareness on storage methods should be encouraged as these contaminants are primary sources of mycotoxins, which are detrimental to human health.*

Keywords - *Fungi, Groundnuts, Pathogenicity, Spoilage, Tubah.*

1. Introduction

Groundnut (*Arachis Hypogaea*) is a leguminous annual crop grown mainly for its edible seeds. It is the 13th most important food crop and the 4th most important oilseed crop in the world (Isalah et al. 2021; Tseday et al. 2020). Also known as peanuts, groundnut seeds are a popular source of food worldwide. It is cultivated in more than 100 countries on all six continents and consumed either as fried or boiled grains (Tseday et al. 2020). A greater majority is crushed and processed into oil, which is commonly used as a form of cooking oil in many parts of the world, especially in China, India, and Nigeria (Murali et al. 2017).

A majority of groundnut cultivation is confined to the developing countries of Asia and Africa, which together account for 95% of the global groundnut area and 91% of global groundnut production (Murali et al., 2017). Due to this restriction, coupled with high local consumption, groundnuts are no longer exported, making them the main crop for farmers in these regions (Tseday et al., 2020). For this reason, groundnut production is receiving more and more attention because it has become an excellent cash crop for domestic markets as well as for foreign trade in several developing countries (ICRISAT, 2016).

Postharvest losses in groundnuts are increasingly becoming an urgent problem, particularly acute in developing countries that tend to lose about 30% of their annual harvest (Sarwar, 2020). In Cameroon, the production of groundnuts is estimated at 250,000 tonnes per year, with Northern Cameroon accounting for more than 56% of the national production (Hamasselbé et al. 2008). Other regions include the Northwest, West, and Centre. According to Hamasselbé et al. (2008), Cameroon lost about 50,000 tonnes out of the 250,000 tonnes of groundnut produced in 2006. This loss makes the cultivation and supply of groundnut seeds fall below local and even international demands. The consequence is a price hike.

The spoilage of groundnut seeds is predominantly caused by fungi (Tseday et al. 2020). *Mucor* and Rhizopus have been reported by Aliyu and Kutama (2007) as prevalent

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fungal contaminants of groundnuts. These groundnut seeds are primarily consumed without knowledge about the existence of these fungal contaminants, and the consequence is exposure to mycotoxins secreted by these fungi. Mycotoxins, especially Aflatoxins, are generally life-threatening with acute and chronic effects on the health of humans and livestock (Danicke, 2002). According to the 2019 statistics of the Global Cancer Observatory Committee, Aflatoxin B1 alone accounts for 25,200-155,000 cases of liver cancer each year, 40% of which occur in sub-Saharan Africa. These disturbing figures represent 1/3 of annual liver cancer cases (Benkerroum, 2020).

Despite the devastating effects of these fungi on human health and the economy at large, Ngoko et al. (2008) admitted that documented data on fungal contamination of cereals in Cameroon remains largely limited and inadequate. This inadequacy further compounds the weakened system of postharvest management and preservation that prevails. As it holds for the fight against every infection, the first and most important step is a correct diagnosis. Until we effectively isolate and identify the various fungal species responsible for the spoilage of groundnut seeds, we cannot determine what measures to put in place as preventive and control strategies.

Vendors of groundnuts in Tubah subdivision constantly experience huge economic losses in groundnuts due to a lack of modern storage facilities. Poor storage conditions favour fungal growth, often resulting in the release of mycotoxins that are highly detrimental to health. This study, therefore, aimed to deepen existing knowledge of the possible fungal species associated with the spoilage of groundnut seeds, to create awareness and pave the way for the effective development and implementation of prevention and control strategies.

2. Materials and Methods

The research was conducted in the Tubah Subdivision, Mezam Division, of the Northwest Region of Cameroon (Figure 1). Tubah is located approximately 15km from Bamenda, the regional capital of the Northwest Region. It is located between latitude 4°50'- 5°20'N and longitude 10°35'-11°59'E with a total population of about 52,635 inhabitants (Melle et al. 2016). The altitude ranges between 950 and 1500m above sea level, with a flat woody lowland. The zone has 2 seasons, the dry and the rainy seasons, ranging between November - April, and May - October, respectively. During this period, places are damp and activities are cautious. The mean annual rainfall is about 2200mm, with July and August registering the highest rainfall (Melle et al. 2016). The mean annual temperature is about 20.67 °C, with January and February registering the highest. July, August, and September are by far lower. In January and February, the ground temperatures fall and the ground becomes very cold from nightfall till dawn. This relapse is usually overcome shortly by the harsh sunshine that follows during the day. The soils in this area are very fertile for agricultural activities. This fertility is a result of dark humus volcanic soils coming from upland areas.

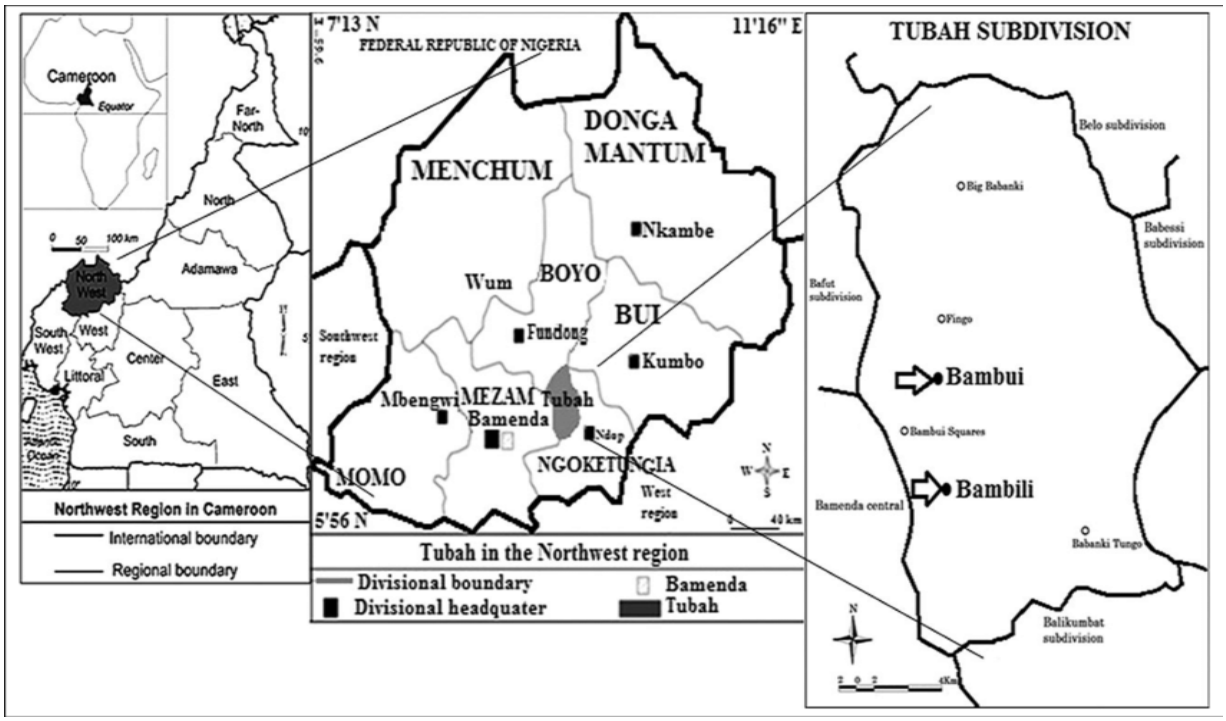

Fig. 1 Map of Tubah Subdivision Showing the Different Villages (Source: Ngwa and Fonjong, 2002a)

2.1. Collection and Selection of Groundnut Samples

Groundnut seeds were purchased from 10 vendors in two open-air markets in the Tubah subdivision, namely, Bambili and Bambui. From each market, five vendors were selected at random, and two cups of groundnut seeds were purchased from each. The groundnuts were taken to the Biological Science laboratory of the Faculty of Science at the University of Bamenda for selection, culture, isolation, and identification.

In the lab, infected seeds were sorted from each of the 10 samples and grouped and labelled on A4 papers (Figure 2).

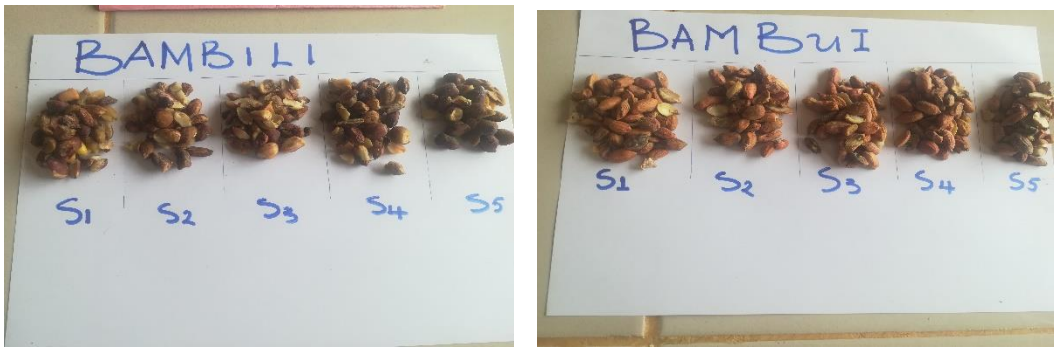

Fig. 2 Selection of Infected Groundnut Seeds from the Different Samples Collected from the Bambili and Bambui Markets in the Tubah Subdivision

2.2. Preparation of the Culture Media and Inoculation

The culture medium used throughout this study was Potato Dextrose Agar (PDA) prepared according to the manufacturer's instructions. It was prepared by adding 39 g of commercial PDA powder to 1 liter (1000 ml) of distilled water in a conical flask and boiling under a water bath in a pressure pot for about 15 minutes for the PDA to dissolve well. After that, the solution was allowed to cool to body temperature. 100 g of gentamicin antibiotics were then added to inhibit bacterial growth, and about 20 ml of the medium was dispensed to each petri-dish. Pouring was done close to the heat source in order to prevent contamination.

Among the selected groundnut seeds, infected portions of attacked grains were cut using surgical blades. The cut pieces were placed on a gauze and disinfected with 70% alcohol for 2 minutes and then washed in sterilized distilled water. Finally, they were inoculated on freshly prepared potato dextrose agar in labelled petri-dishes (five seeds per dish) in three replicates (Figure 3).

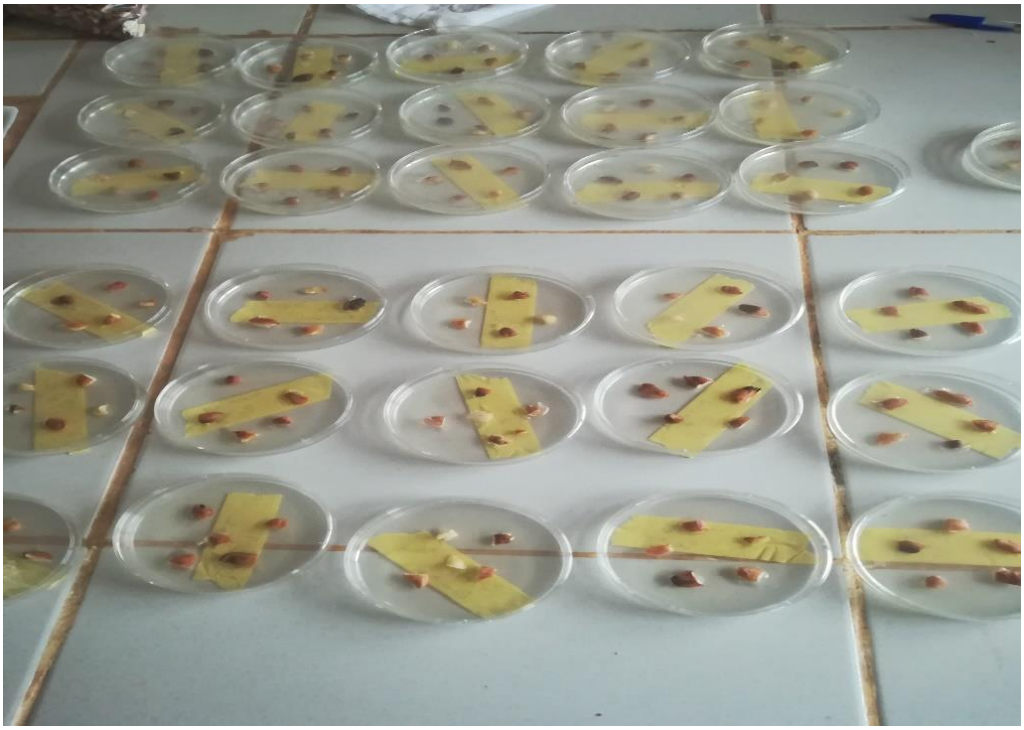

Fig. 3 Inoculated Petri Dishes Ready for Incubation

The petri dishes were sealed using paper seal tape and incubated in an incubator for five days at 25 °C. After 5 days, the plates were observed for the different colours of fungal colonies, and their frequencies were recorded. These colonies were again sub-cultured to obtain pure isolates.

To the sub-culture, another Potato Dextrose Agar (PDA) was prepared and allowed to solidify. A small portion of the different colonies was teased out and placed in the centre of PDA plates, and the petri dishes were sealed using a paper seal tape and incubated in an incubator for five days at 25 °C.

2.3. Morphological and Microscopic Analysis of Pure Cultures

After five days of pure culture incubation, the cultures were observed and identified to a genus level based on Morphological (Phenotypic) and Microscopic features as inferred from the mycological atlas described by Sarah et al. (2016). For microscopic analysis, clean, labelled, grease-free glass slides were used for identification. A drop of water was placed at the centre of each slide, and a small portion of the fungi culture was cut out with the aid of a sterilized inoculating needle. The cut piece was put directly in the water droplet, and a cover slip was then used to cover the teased portion. It was mounted on the microscopic stage and viewed. The viewing was first done with lower magnification (x4) and later with higher magnification (x40). The nature of hyphae (septate or not), the types of the fruiting bodies, and the spore structure served as criteria for the identification of the isolates.

2.4. Pathogenicity Test

A pathogenicity test was conducted to assess the potency of the fungal isolates. Healthy groundnut seeds were washed with distilled water and sterilized with 70% ethanol. The seeds were air-dried and inoculated with three 5 mm mycelial plugs from actively growing 7-day-old pure cultures of each of the five fungal isolates in three replicates, including a control. They were grown on PDA and incubated at 25ºC for 7 days and observed for spoilage. The results were recorded accordingly.

3. Results

Primary cultures were observed after 5 days of incubation and found to have different colours, textures, and structures of fungal colonies. Accordingly, the number of groundnut seeds inoculated and the number that showed positive fungal growth were recorded (Tables 1 and 2) as shown below. It was observed that all the seeds in all the triplicates made from the 10 samples of groundnut collected from the Bambui and Bambili markets showed fungal growth. The growth was mixed, with most plates showing more than one fungus colony.

Five groundnut seeds were inoculated per replicate, giving a total of 15 groundnut seeds per sample. All the seeds inoculated showed fungal growth.

Table 1. The Number of Seeds Plated and the Proportion that Showed Fungal Growth in Samples Collected from Bambui Market in Tubah Subdivision

Sample number	No of grains plated	No of grains positive for fungi	% of grains positive for fungi
1	5 + 5 + 5 =15	15	100
2	5 + 5 + 5 =15	15	100
3	5 + 5 + 5 =15	15	100
4	5 + 5 + 5 =15	15	100
5	5 + 5 + 5 =15	15	100

No= number, %= percentage

Table 2. The Number of Seeds Planted and the Proportion that Showed Fungal Growth in Samples Collected from Bambili Market in Tubah Subdivision

Sample number	No of grains plated	No of grains positive for fungi	% of grains positive for fungi
1	5 + 5 + 5 =15	15	100
2	5 + 5 + 5 =15	15	100
3	5 + 5 + 5 =15	15	100
4	5 + 5 + 5 =15	15	100
5	5 + 5 + 5 =15	15	100

No= number, %= percentage

3.1. Morphological and Microscopic Identification of Pure Cultures

After sub-culturing using freshly prepared potato dextrose agar, the pure cultures were analysed by Macroscopic (Morphological) and Microscopic techniques. The morphological analysis revealed five fungal genera: *Aspergillus, Rhizopus*, *Mucor, Penicillium, and Fusarium*. The morphological identification was based on the colony characteristics such as appearance, texture, and nature of mycelia.

The Microscopic observation revealed the following fungi organisms: *Aspergillus niger, Rhizopus stolonifer*, *Mucor sp, Penicillium sp, and Fusarium oxysporum*.

Table 3. Morphological and Microscopic Features of the Different Fungal Colonies and the Species Observed from Groundnut Samples Collected from Bambui and Bambili Markets in Tubah Subdivision

Morphological features	Microscopic features	Species
Initial growth is white, later becoming black. Seen as pale yellow on the reverse side of the dish. Colonies are compact and dense.	Hyphae are septate, and conidiophores are erect with swollen tips	*Aspergillus niger*
Whitish or grey in colour, colonies are fast-growing with hyphae well ramified. Hyphae turn blackish at maturity, producing black spores.	Short rhizoids and unbranched hyphae. Sporangiophores arise singly from nodes and bear sporangia on the tips.	*Rhizopus stolonifer*
White becomes brownish green at maturity with a cream-yellow center. The white hyphae are more pigmented with little ramification.	Non-septate mycelia that bear branched sporangiophores	*Mucor spp*
Colonies are white, fast-growing, and concentrated around the seeds. Turns bluish-green colour at maturity.	Brush-like conidiophores with septate hyphae	*Penicillium spp*
Colonies are pink to reddish with dense growth.	Short, crescent-shaped conidiophores, septate hyphae with abundant microconidia	*Fusarium oxysporum*

3.2. Frequency of Occurrence of Fungal Genera

The total number of colonies of each genus per sample was counted from the different samples, and a proportion of each genus was calculated (Tables 4 and 5). *Aspergillus* was the most abundant genus, followed by *Rhizopus, Mucor,* and *Penicillium,* while *Fusarium* was the least.

Table 4. The Proportion of Fungal Genera Identified from Groundnut Samples Collected from the Bambui Market.

Sample number	*Aspergillus*		*Rhizopus*		*Mucor*		*Penicillium*		*Fusarium*	
	No	%	No	%	No	%	No	%	No	%
1	12	48.0	06	24.0	04	16.0	02	08.0	01	04.0
2	09	50.0	04	22.2	02	11.1	03	16.7	00	00.0
3	08	40.0	06	30.0	03	15.0	03	15.0	00	00.0
4	10	55.6	04	22.2	03	16.7	01	05.6	00	00.0
5	09	47.4	02	10.5	02	10.5	05	26.3	01	05.3

Aspergillus was the most abundant genus, followed by *Rhizopus, Mucor,* and *Penicillium,* while *Fusarium* was the least.

Table 5. Proportion of Fungal Genera Identified from Groundnut Samples Collected from the Bambili Market.

Sample number	*Aspergillus*		*Rhizopus*		*Mucor*		*Penicillium*		*Fusarium*	
	No	%	No	%	No	%	No	%	No	%
1	07	38.0	05	27.0	04	20.0	01	05.0	01	05.0
2	07	30.0	06	28.5	03	14.3	03	14.5	02	09.5
3	09	40.9	05	22.7	04	18.0	04	18.0	00	00.0
4	08	34.8	06	26.1	02	08.7	03	13.0	04	17.4
5	07	36.8	04	21.1	04	21.1	03	15.8	01	05.3

The relative frequencies of occurrence of the different fungal genera identified from groundnut collected in the Bambili and Bambui markets were calculated and represented in Table 8.

Comparative results further showed that, apart from *Penicillium,* which showed equal abundance in both markets, all the other fungal genera were more abundant in Bambili than in the Bambui market.

A variation was seen between the relative frequencies of different fungal genera in seeds obtained from Bambili and Bambui. Apart from *Penicilium,* all the other genera were more abundant in Bambili than in Bambui.

Table 6. Fungal Genera Present in Groundnut from each of the two Markets (Bambui and Bambili) in Tubah Subdivision

Market	*Aspergillus*	*Rhizopus*	*Mucor*	*Penicillium*	*Fusarium*	Total
Bambui	48	22	14	14	02	100
Bambili	38	26	17	14	08	103
Total	86	48	31	28	10	203

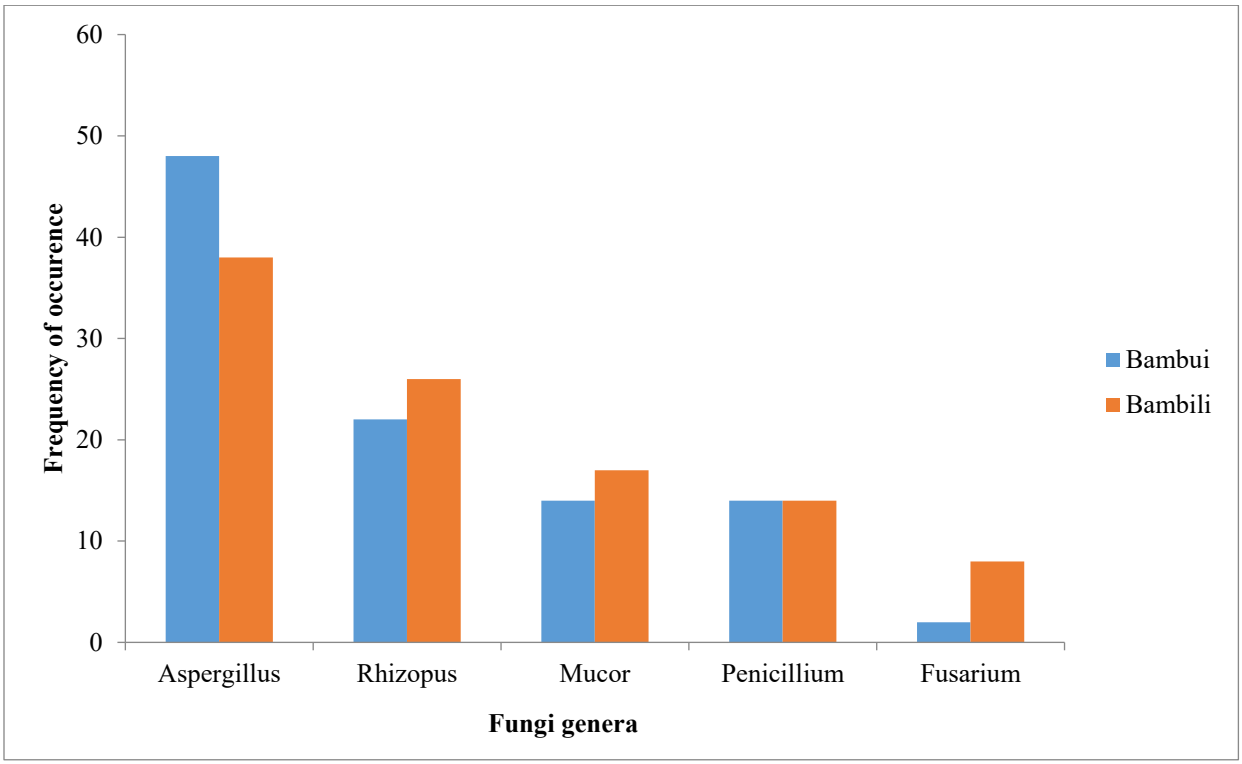

Fig. 4 The Frequency of Occurrence of the Five Fungal Genera Identified from Groundnut Collected in Bambili and Bambui Markets in the Tubah Sub-division.

Figure 4 summarises the variations in the relative frequencies of occurrence of the different fungal genera identified from groundnut collected in the Bambili and Bambui markets.

The frequency of occurrence of the different genera reveals that *Aspergillus* was the most prevalent, while *Fusarium* was the least.

Table 7. Identified Fungi Genera with their Frequency of Occurrence and Percentages.

S/N	Fungi genera	Frequency of occurrence	Percentage
1	*Aspergillus*	86	42.1
2	*Rhizopus*	48	23.6
3	*Mucor*	31	15.3
4	*Penicillium*	28	13.8
5	*Fusarium*	10	04.9
Total		203	≈100

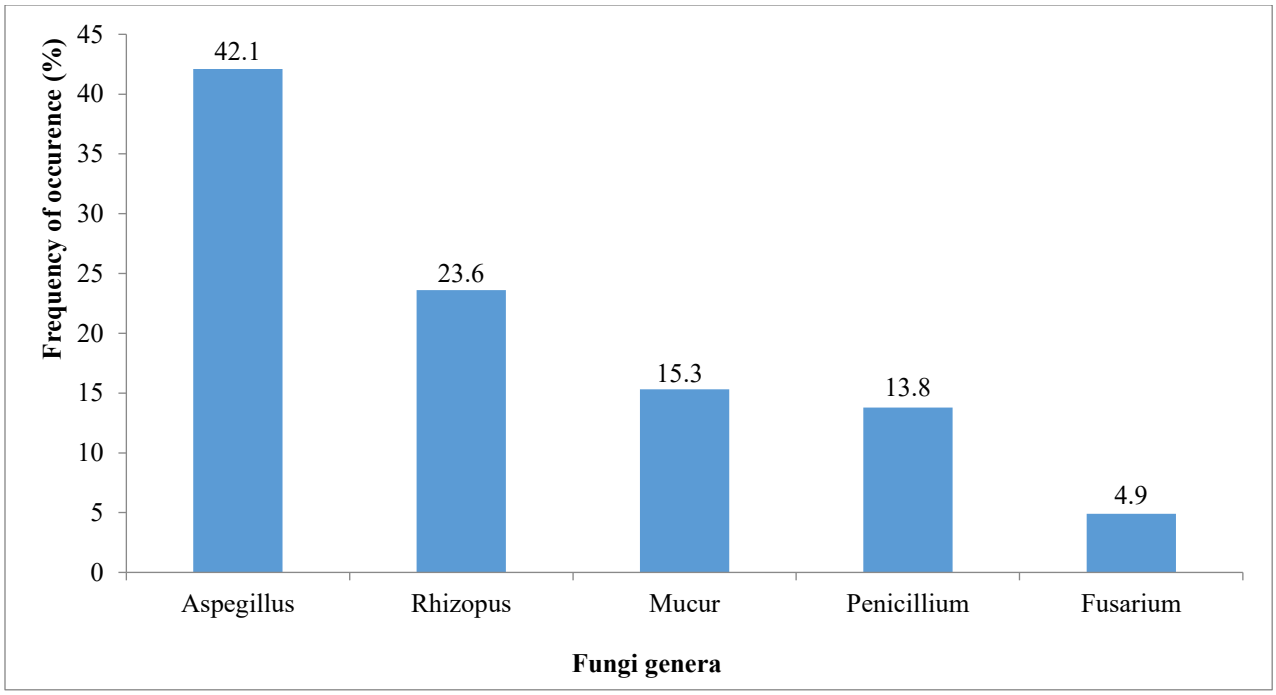

Fig. 5 The Overall Frequency of Occurrence of the Five Fungal Genera Identified from Groundnut Collected in Bambili and Bambui Markets in the Tubah Sub-division.

Figure 5 summarises the overall frequency of occurrence of the five fungi genera (*Aspergillus, Rhizopus, Mucor, Penicillium,* and *Fusarium*) that have been analysed from the 10 groundnut samples collected from Bambui and Bambili markets in Tubah subdivision.

Results from the pathogenicity test showed that all petri dishes containing seeds inoculated with the identified fungal isolates were pathogenic to the groundnut seeds, causing spoilage and inhibiting growth. The fungal isolates obtained from the pure cultures were able to induce the same symptoms when inoculated on healthy groundnut seeds. However, growth was observed in the petri dishes incubated with healthy seeds.

4. Discussion

Food contamination by fungi continues to pose long-term health complications to the public (Abdulrazzaq et al. 2004). Fungi have been known to affect groundnut production for a long time. This study revealed five fungal genera responsible for postharvest contamination in groundnut seeds. These include: *Aspergillus, Rhizopus, Mucor, Penicillium,* and *Fusarium*. These findings were similar to those of Tobin-West et al. (2018), who identified these same five fungi genera from groundnut seeds sold at four major markets in Port Harcourt Metropolis, Rivers State, Nigeria, though with different frequencies of occurrence. Ngoko et al. (2008) and Isalar et al. (2021), in their findings, identified *Fusarium* sp, *Penicillium* sp, and *Aspergillus* sp as fungal contaminants associated with groundnuts in Cameroon and Nigeria, respectively. These findings confirm that these fungal genera are major threats both to economic losses and to hazards to humans through mycotoxin production.

In addition to the five genera, Ibrahim (2014) in his study identified an additional genus (*Curvularia*) from groundnut seeds sold at Aliero central market in Nigeria. In another related study conducted by Tseday et al. (2020), *Rhizopus* was not found. During the collection of samples from the Bambili and Bambui markets, it was observed that vendors store their stocks in small rooms where there is congestion, poor ventilation, and a lack of hygienic measures. We suggest that these factors might have created favourable environmental conditions for the fungal contamination and growth.

From this study, the genus *Aspergillus* was the most prevalent, followed by *Rhizopus* in the samples collected from the Bambili and Bambui markets in Tubah Subdivision, while *Fusarium* was the least prevalent. This finding is in line with that of Tobin-West et al. (2018) and Ibrahim (2014), who identified *Aspergillus spp*. as the predominant fungi that affect the quality of groundnut seeds sold in four major markets in Port Harcourt Metropolis, Rivers State, and at Aliero central market in Nigeria, respectively. *Aspergillus spp*. was prominent due to the fact that it contaminates groundnut at various stages, right from harvest to storage. On the other hand, Tseday et al. (2020) reported that *Aspergillus* species are well-adapted to a broad range of environmental conditions, thus making them a prominent fungal contaminant.

This study also revealed variations in the frequency of occurrences of the different fungal genera on groundnut seeds sold at the Bambui and Bambili markets in the Tubah Subdivision. Groundnut seeds obtained from the Bambili market showed a higher frequency of fungal contaminants than those from the Bambui market. This might have been because of the fact that the Bambili market is located at a higher altitude with high humidity that provides favourable conditions for growth and development of fungal pathogens (Mirza et al. 2020). We equally suggest that since the Bambili market is a daily market, unlike Bambui, traders tend to stock larger quantities of groundnut in order to meet the daily demands of the large population. These larger stocks might have orchestrated the growth of fungi.

5. Conclusion

This study revealed that almost all groundnut seeds collected from both the Bambui and Bambili markets were highly infested with a number of fungal seed-borne pathogens. Poor storage and handling practices employed by vendors appeared to be the main reason for the pronounced growth of fungi on the groundnuts. Isolation, culture, and identification of contaminants using morphological and microscopic procedures revealed that five fungal genera (*Aspergillus, Rhizopus, Mucor, Penicillium, and Fusarium*) were distributed in all collected seed samples.

Aspergillus niger was the most dominant fungi with 86(42.1%), while *Fusarium oxysporum* exhibited the lowest 10(4.9%). The prevalence of *Aspergillus* on groundnut from the present should create awareness on proper handling and storage in order to safeguard the health of local consumers. Public sensitization should be made on the dangers of fungal contamination on food, as these continue to cause health risk through the production of deadly mycotoxins.

Acknowledgements

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