Original Research Article

Contamination of Locally Compounded Poultry Feeds and Ingredients by Fungi of the Genus Penicillium and Aspergillus Within Three Agro-Ecological Zones of Nigeria

ABSTRACT

Introduction: Fungi generally are field and storage contaminants of poultry feeds and feed ingredients, the presence of these fungi in poultry feeds affects the quality negatively by causing caking, reducing nutrients, and eventually producing secondary metabolites known as mycotoxins in these products when the environment is conducive. This study was carried out to isolate Aspergillus and Penicillium species from locally compounded poultry feed and feed ingredients that arrived at poultry farms within 30 days.

Method: A total of 52 samples of feed (n=13) and feed ingredients (n=39) were collected across 3 agroecological zones (involving 6 states) of Nigeria. Different mycological media was used for isolation, by deploying the pour plate method. The identity of Aspergilli and Penicilli isolates was characterized using macroscopic and microscopic observed features.

Results: Nine species of Aspergillus and five species of *Penicillium were* detected in the feed and feed ingredients. The highest occurring fungal genus was *Aspergillus* with *Aspergillus nige*r and *Aspergillus flavus* species appearing as the highest occurring species. *Penicillium glabrum* was the most predominant *Penicillium* species isolated from the compounded poultry feed and feed ingredient samples*.*

Conclusion: All the samples of feed and feed ingredients are contaminated with several species of Aspergillus and Penicillium.

Keywords: Fungal contaminants, feed ingredients, poultry feeds, feed quality

Comment [Ma1]: It is better to replace with "The fungal contamination of"

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1. INTRODUCTION

The nutritional quality of poultry feeds, their appearance and organoleptic properties are affected by the presence of microfungi. The nutrients in the feeds and their raw materials are utilized by these organisms in the process of metabolism which leads to the loss of nutrients in the feeds this will in turn have negative effects on animal health and productivity [1]. The presence of fungi in feed sometimes leads to low consumption or total rejection of feeds by poultry birds.

The genera Aspergillus, *and Penicillium* are ubiquitous and many of them have a strong ecological link with human food supplies as they cause spoilage during storage They are natural mycoflora usually found in the food production process [2]. Some species of *Aspergillus* and *Penicillium* are also plant pathogens or commensals, but these genera are more commonly associated with commodities and food during drying and storage [1]. A wide variety of fungal species, produce mycotoxins as secondary metabolites while *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. are said to be the primary producers of these toxins. *Stagonospora nodorum*, *Pyrenophora tritiirepentis* and *Alternaria alternata,* are also capable of synthesizing an array of mycotoxic compounds during disease development in crops [3].Though micro fungi exist in temperate zones, they are more often found in tropical climates such as those existing in Nigeria which is hot, humid and favourable for the growth of moulds. The abundance of mycotoxigenic moulds in these warm climates makes them major food spoilage agents. These fungi are most abundant in the tropics and as such, are major food spoilage agents in these warmer climates [2]. Many toxigenic fungi achieve the best growth on seeds between $24^{o}C$ and $28^{o}C$, with a seed moisture content of at least 17.5%which is also the optimum condition for toxin production even though these temperature ranges may vary among different fungal species. Crops used in feed production mature in seasons characterized by these conditions therefore the chances of contamination by fungi are high [4]. The high prevalence of toxins reported on the African continent is a result of ambient climatic conditions in most parts of the continent which encourages the growth of fungi and subsequently toxin production. Higher fungal contamination of farm produce in Nigeria has been reported during the rainy season as compared to the dry harmattan season [5]. This study aimed to determine the levels of contamination by various species of Aspergillus and Penicillum contaminating poultry feeds and feed ingredients in three agro-ecological zones of Nigeria.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

A total of 52 samples of locally compounded poultry feed (n=13) and the raw materials (corn, groundnut cake, fish meal, bone meal, palm kernel, soya beans, and wheat) (n=39) used for their production were collected for analysis. The samples were collected based on availability using the convenience sampling method from the Southern Guinea Savannah (22 samples), Northern Guinea Savannah, (10 samples) and Arid/Semi-Arid Zone (20 samples). Samples of the feeds and the same quantity of the raw materials were collected from selected farms across the three agro-ecological zones. One kilogram (1 Kg) of each sample was collected from three points (top, middle and bottom) of the bulk feed/ingredient bag using a probing pointer. The three 1 Kg samples collected from each bulk feed/ingredient bag were mixed thoroughly and a subsample measuring 1 Kg was collected in zip lock bags to form each sample lot. Samples were transported to the laboratory at the Department of Microbiology University of Maiduguri and stored at $4^{o}C$ for further analysis.

2.2 Isolation and morphological identification of fungi

The feed samples were aseptically plated on Potato Dextrose Agar and Malt Extract Agar (Oxoid, Hampshire, United Kingdom) supplemented with chloramphenicol, using the pour plate technique were used for the isolation of fungi from the samples. One gram of each sample was transferred into 9 ml sterile distilled water and thoroughly

mixed using a stomacher [6]. A ten-fold serial dilution was carried out, and 1 ml aliquots of selected dilutions were transferred into empty sterile Petri dishes and the molten malt extract agar was added, swirled carefully, and allowed to solidify. This was done in triplicates. Subsequently, the plates were incubated at 28^0C for 3-5 days and the isolates were subculture on PDA to obtain pure cultures. The pure cultures were maintained on PDA slants and kept in the refrigerator at 4^0 C [7].

Identification was carried out using by observing the macroscopic and microscopic

characteristics of the isolates. Colonial characteristics such as mycelia color and reverse color of the pure cultures were examined. Small portions of the culture were picked using inoculating needles, placed on a glass slide, teased out and stained with lactophenol in cotton-blue. The conidial heads**,** stipe, color, length, vesicles shape, conidia shape and other features were observed with the aid of a light microscope using the x10 and x40 objective lens and published guidelines [8].

3.0 RESULTS

3.1 Profile of Feed Samples and Fungi Identified from three agro-ecological zones of Nigeria

A total of 52 samples made up of 13 feeds and 39 ingredients were collected in this study. Analyses of these samples for *Aspergillu*s and *Penicillium* species revealed the presence of nine species of *Aspergillus* and five species of *Penicillium*. At least one species of fungi was isolated from all the samples with the exception of 2 bone meal samples from which no fungus was recovered. *Aspergillus* species were isolated from all the locally compounded poultry feed samples and ingredients while *Penicillium* species were isolated from 63% of them.

In the northern guinea savannah, 4 feed samples were collected and both Aspergillus and *Penicillum* were isolated in the feed samples from this agro-ecological zone. In the southern guinea savannah, *Aspergillus* species and *Penicillium* species were isolated from all the 5 feed samples collected from this agro-ecological. In the arid savannah, *Aspergillus* species was isolated from all (100%) the 5 feed samples collected from this agro-ecological zone while *Penicillium* species was isolated from only one sample (20%). In the derived savannah, Aspergillus species was isolated from all (100%) the 8 feed samples while *Penicillium* species was isolated from 7 (87.5%) of them.

Aspergillus and *Penicillum* species were common isolates in the feeds from all the agro-ecological zones.

The percentage of feed and feed ingredients from which fungi were isolated in the three different agro-ecological zones of Nigeria reveals that at least one fungus species was isolated from all the samples of feeds and feed ingredients collected in this study. This results in fungal isolation from 100% of the samples with the exception of the bone meal samples where fungi were isolated from 78% of it (Figure 1).

The percentage of 13 feed samples from which Aspergillus and Penicillium were recovered is presented in Figure 4.2. At least one *Aspergillus* species was isolated from 100% of the feed samples, followed by *Penicillium* species from 63%. *Aspergillus flavus* and *Aspergillus niger* species were isolated from 100% of the feed samples making the two species the most predominant species isolated from the feed samples. This is followed by *Aspergillus tamarii* isolated from 53% of the feed samples, while *Aspergillus fumigatus* and *Aspergillus nidulans* were each isolated from 50% of the feed samples. *Aspergillus terrus* and *Aspergillus ochraceus* each were isolated from 40% of the feed samples, while *Aspergillus parasiticus* and *Aspergillus oryzae* were isolated from 30% and 27% of the feed samples respectively making these two the least isolated species from the feed samples (Figure 3).

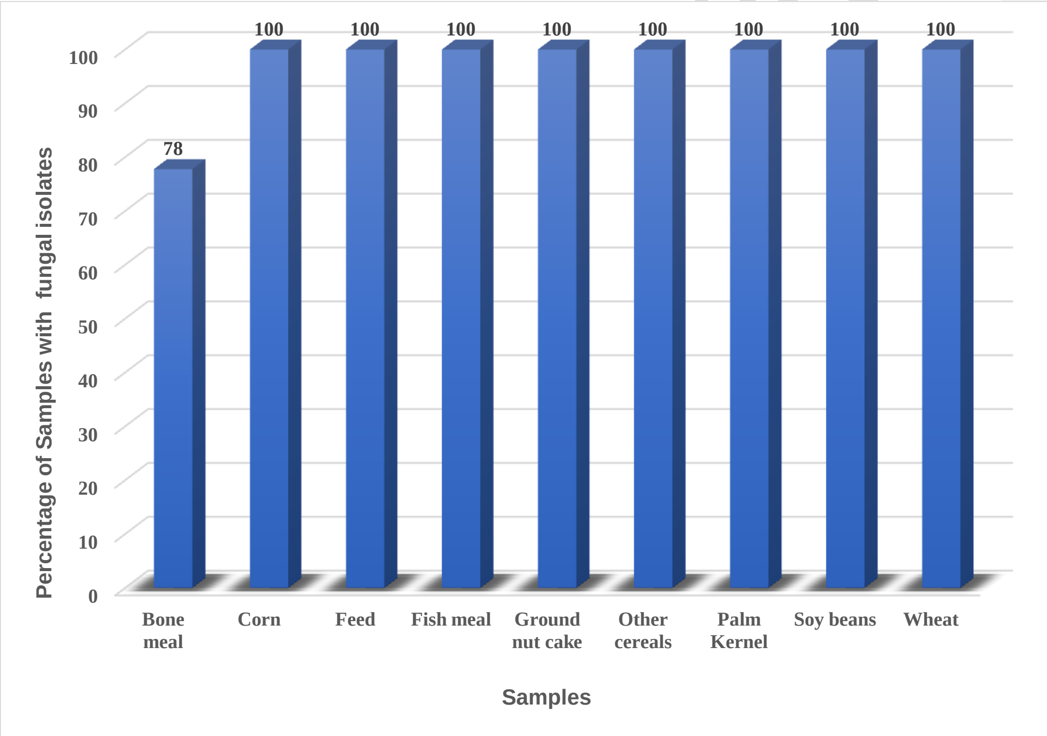

Figure 1: Percentage of poultry feed and feed ingredient samples from which fungi were isolated

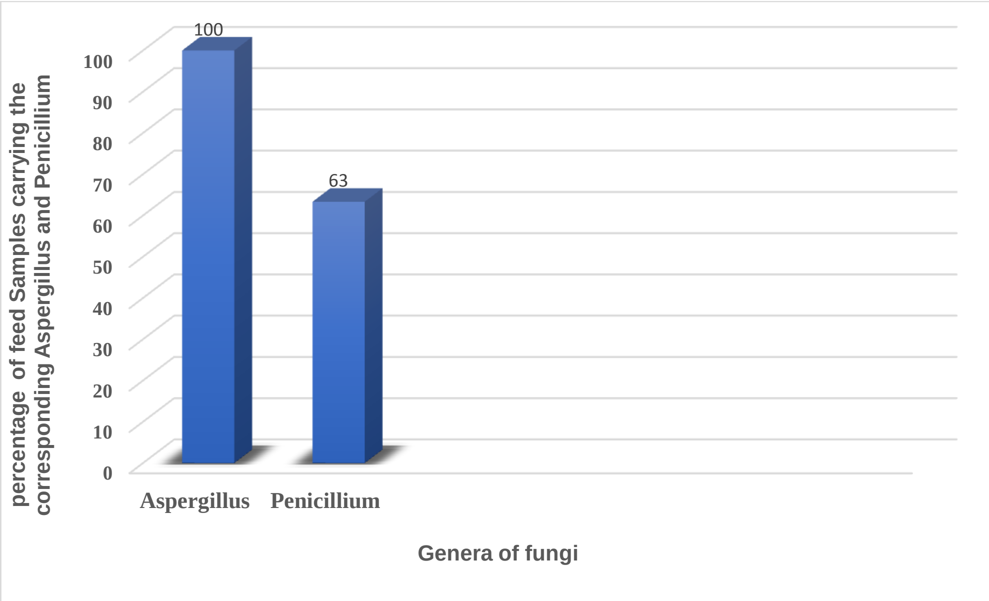

Figure 2: Percentage of 13 feed samples from which Aspergillus and Penicillium were isolated

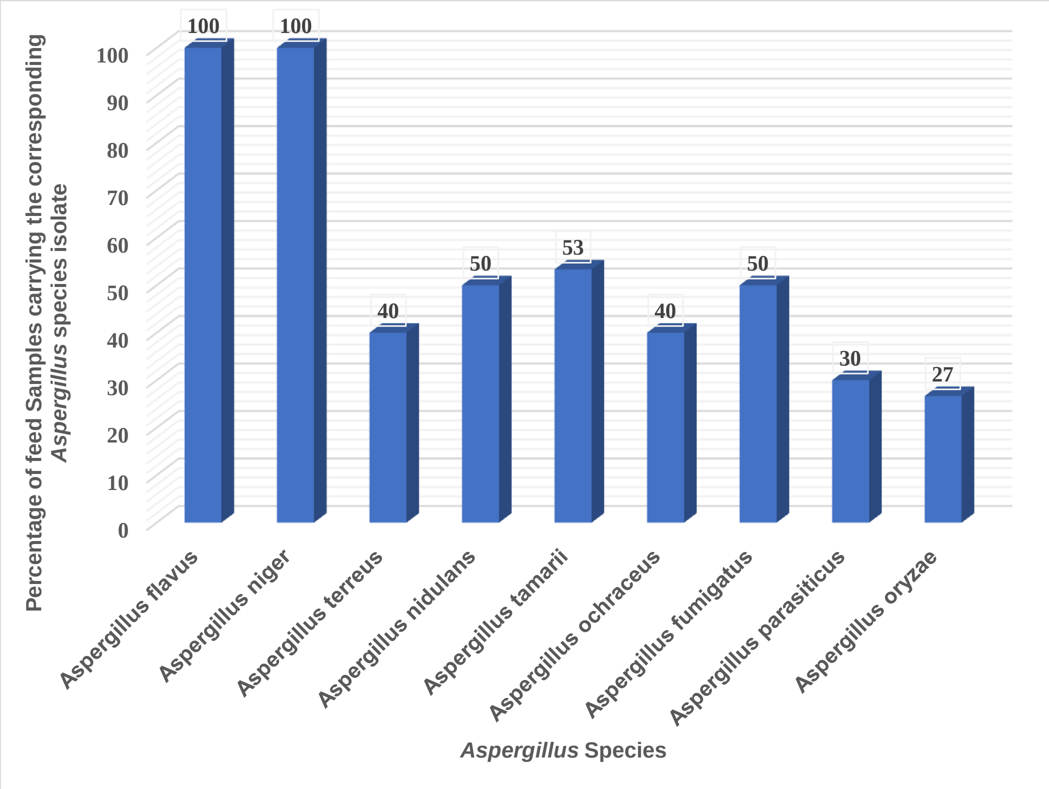

Figure 3: Percentage of feed samples from which different *Aspergillus* species were isolated.

The percentage of some of the feed ingredients from which different *Aspergillus* species were isolated is presented in Figure 4 and Figure 5. *Aspergillus niger* was isolated from 100% of corn, fish meal, groundnut cake and soya beans samples. It was also isolated from 90% of the wheat samples, 83% of others cereals, 67% of palm kernel and 33% of the bone meal samples. *Aspergillus flavus* was isolated from 100% of the corn, groundnut cake, other cereals, wheat and palm kernel. It was also isolated from 82% of the soya beans, 60% of the fish meal and 33% of the bone meal samples. *Aspergillus terreus was* isolated from 50% of other cereals, 47% of corn samples, 36% of soya beans samples, 33% of palm kernel samples, 27 % of groundnut samples, 20% of fish meal and wheat samples and 11% of bone meal samples. *Aspergillus tamarii* was isolated from 53% of corn samples, from 50% of other cereals samples, 45% of groundnut and soya beans samples, 40% of wheat samples, 33% of palm kernel samples and 11% from bone meal samples. *Aspergillus ochraceus* was isolated from 53% of the corn samples, 40% of the fish meal and wheat samples, 36% of soya beans, 33% of other cereals and palm kernel 18% of groundnut cake and from11% of the bone meal samples. *Aspergillus nidulans* was isolated from 50% of the wheat samples, from 45% of the groundnut cake samples, 35% of the corn samples, 33% of the palm kernel samples, 27% of the soya beans samples and 11% of the bone meal samples. *Aspergillus nidulans* was not isolated from fish meal samples and other cereals samples. *Aspergillus oryzae* was isolated from 70% of the wheat samples, 67% of the palm kernel samples, 55% of the groundnut samples, 50 % of other cereals sample, 45% of soya beans samples, 40% of fish samples, 11% of bone meal samples and from 6% of the corn samples. *Aspergillus parasiticus* was isolated from 50% of the wheat samples, 40% of the fish meal, 27% of the groundnut cake and soya beans samples and 12% from the corn samples. *Aspergillus parasiticus* was not isolated from palm kernel, other cereals and bone meal samples.

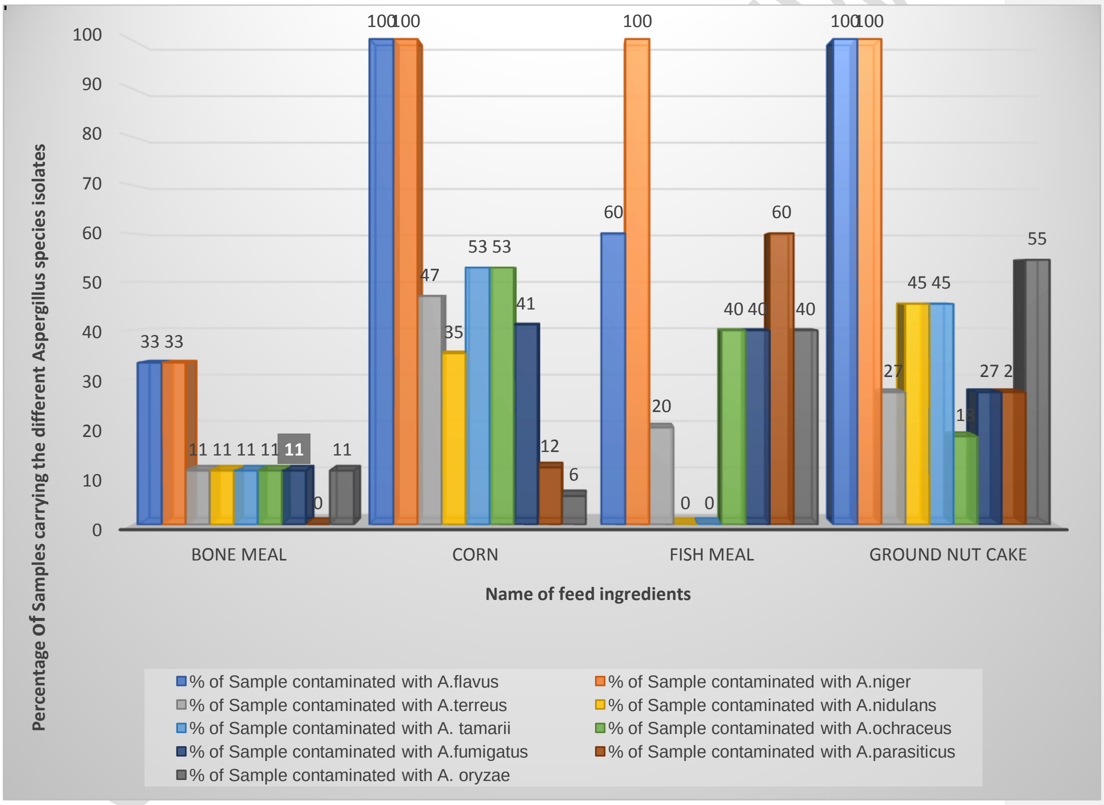

Figure 4: Percentage of feed ingredients from which different *Aspergillus* species were isolated

Figure 5: Percentage of feed ingredients from which different *Aspergillus* species were isolated

Penicillium glabrum was the most predominant *penicillium* species isolated from the compounded poultry feed samples. *Penicillium glabrum* was isolated from 27% of the feed samples followed by *Penicillium digitatum* which was isolated from 23% of the feed samples and *Penicillium italicum* was isolated from 20%. *Penicillium brasillanum* and *Talaromyces funiculosus* were each isolated from 17% of the feed samples while *Penicillium verruosum* was isolated from 13% of the feed samples making it the least isolated *Penicillium* species (Figure 6)

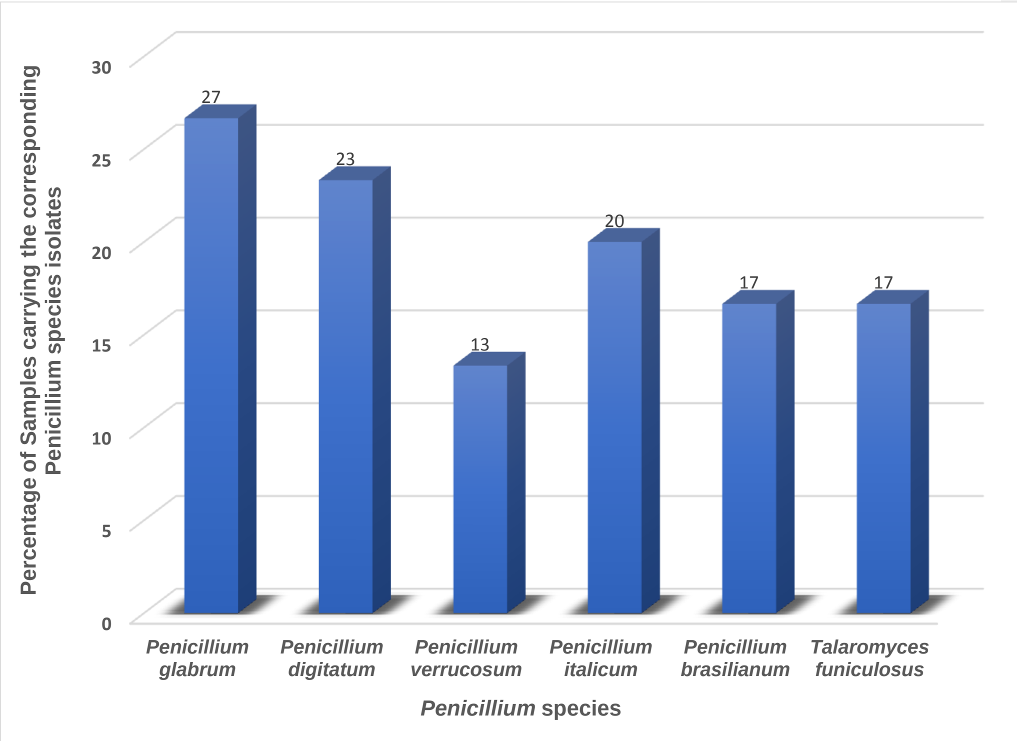

Figure 6: Percentage of feed samples from which different *Penicillium* species were isolated.

The percentage of feed ingredients from which different *Penicillium* species were isolated are presented in Figures 7 and 8. *Penicillium digitatum was isolated from* 40% of the fish meal samples making it the most frequently isolated *Penicillium* species from the fish meal samples. It was also isolated from 24% of the corn samples, 17% of other cereals samples, and 11% of bone samples. *Penicillium digitatum* was not isolated from the groundnut cake, palm kernel soya beans, and wheat samples. *Penicillium glabrum* was isolated from 24% of the corn samples, 20% of the fish meal and wheat samples, and 17% of the other cereals samples, and none from bone meal, groundnut cake, palm kernel, and soya beans samples. *Penicillium verrucosum* was isolated from 35% of the corn samples, 33% of other cereals samples, 30% of the wheat samples, 27% of the soya beans samples, 11% of the bone meal samples, and 9% of the groundnut cake samples. It was not isolated from the palm kernel samples. *Penicillium italicum* was isolated from 33% of other cereals samples, from 27% of groundnut cake samples, 22% of the bone meal samples, 20% of the fish meal samples, and 12% of the corn samples. *Penicillium italicum* was also isolated from 10% of the wheat samples and 9% of the soya beans samples but it was not isolated from the palm kernel samples. *Penicillium brasilianum* was isolated from 67% of the palm kernel samples, this was the only *Penicillium* species that was isolated from the palm kernel samples. It was also isolated from 24% of the corn samples, 20% of the fish meal samples, 17% of the other cereals samples, 10% of the wheat samples, and 9% of the groundnut cake samples. *Penicillium brasilianum* was not isolated from soya beans and bone meal samples. *Talaromyces funiculosus* was isolated from 20% of the fish meal and wheat samples, 18% of the soya beans samples, 17% of the other cereals sample 11% of the bone meal samples, 9% of the groundnut cake samples, and 6% of the corn samples. *Talaromyces funiculosus* was not isolated from the palm kernel samples.

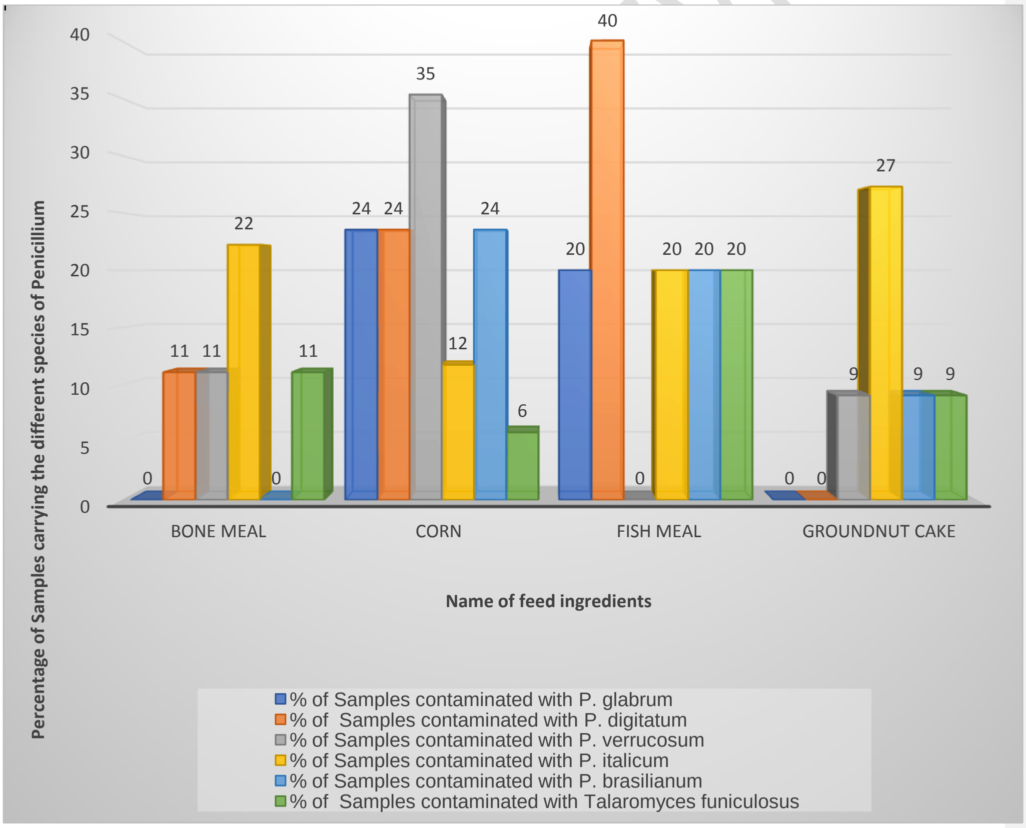

Figure 7: Percentage of feed ingredient samples from which different *Penicillium* species were isolated

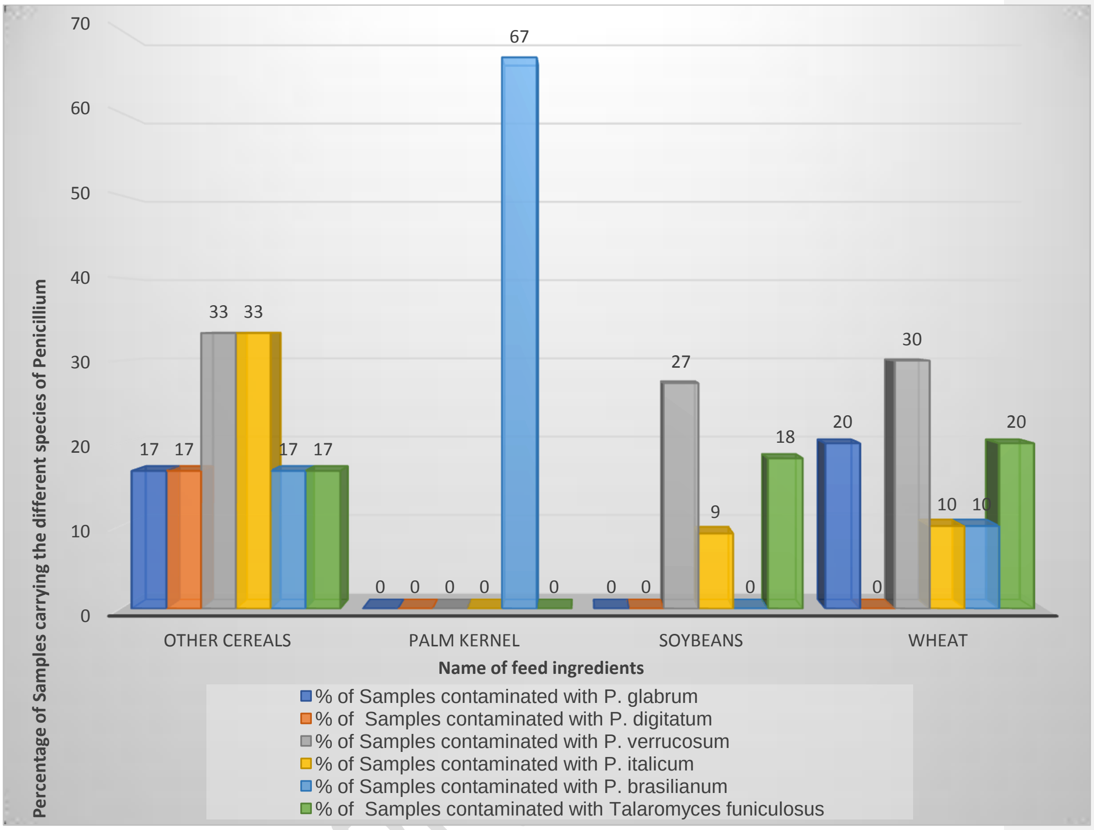

Figure 8: Percentage of feed ingredient samples from which different *Penicillium* species were isolated.

DISCUSSION

In this study, fungi belonging to the genus *Aspergillus, Penicillium,* were isolated from the samples of compounded poultry feeds and feed ingredients from three different agro-ecological zones of Nigeria. All the samples had a 100% isolation rate except the bone meal sample which had 78 % isolation. The genus *Aspergillus* was isolated from 100 % of the feed samples. This agrees with the works of [1] Nigeria [7] Serbia and [9] Yemen. They all reported that the genus *Aspergillus* was the most predominant genus isolated from poultry feeds and feed ingredients. [10] Pakistan also reported (83.33%) fungal isolation of farm mixed poultry feeds with *Aspergillus* being the most frequently isolated genus (54%) this is however contrary to the findings of [11] who reported the recovery of *Penicillium* as the most dominant species in poultry feeds.

Aspergillus flavus and *Aspergillus niger* were isolated from 100% of the compounded feeds in this study, making them the most predominant species in the samples. This is in line with the findings of [12] and [13] who reported *A. flavus* and *A. niger* as the most predominant species isolated from poultry feeds southwest Nigeria and Iran respectively. Similarly, the results of this study are also in consonance with the work of [14]; [15] Pakistan and [16] Brazil who reported *A. flavus* and *A. niger* as predominant species contaminating poultry feeds whereas the study by [10] describe only *Aspergillus niger* as the most frequently isolated species in feed samples and ingredients. Other fungal species isolated from feeds in this present study include *A. tamarii* (53%), *A. nidulans* and *A. fumigatus* (50%), A*. terreus* and *A. ochraceous* (40%), A*. parasiticus* (30%) and the least isolated *Aspergillus* species was *A. oryzae* (27%). These species were also isolated from poultry feeds by [17] in Argentina and [18] from poultry feed ingredients and finished feeds in Iran.

Penicillium species were isolated from 63% of the feed samples. This is in line with the results obtained by [19] Algeria, [20] Pakistan, who reported *Penicillium* species as having a high percentage of isolation from poultry feed and ingredients after *Aspergillus* species. This present finding is contrary to the reports of [21] Nigeria and [22] Bangladesh where *Penicillium* isolation was not reported. The most predominant *Penicillium* species contaminating the compounded poultry feeds in this study was *Penicillium glabrum*. This does not tally with the report of [23] who reported *Penicillium brevicompactum, Penicillium purpurogenum* and *Penicillium oxalicum* as the most predominant *Penicillium* species isolated from poultry feeds in Argentina, while *Penicillium chrysogenum* and *Penicillium novlgiovense* were the most predominant in feeds from Brazil. Additionally, the isolation of *Penicillium verrucosum* in this study supports the report by [10] who observed *Penicillium verrucosum* as the most frequently isolated *Penicillium* species in feeds. Several *Penicillium* species were isolated from all the feed ingredients at different rates. *Penicillium digitatum, Penicillium italicum,* and *penicillium brasilianum* are other species of *Penicillium* that were isolated from feed and feed ingredients in this study. Many of these fungi are toxigenic in nature producing various toxins in feeds and stored grains under conducive conditions. Some of the toxins produced by *Penicillium* species include Ochratoxins which are nephrotoxic and immunosuppressive [19]. A very serious problem with the contamination of agricultural produce by toxigenic fungi is the accumulation of mycotoxin to injurious concentrations in foods and feeds [24].

Apart from the effects of mycotoxins, mold-infested feeds have poor nutritional value and organoleptic properties, which affect the intake of feed by the animals [2].

Moulds have the ability to lower the value of feed ingredients through biochemical changes and physical damage all of which are deleterious to poultry health. [25]. They are responsible for a high rate of morbidity and mortality of birds, they cause diarrhea, fetal encephalitis, and stunted growth in young birds resulting in serious economic loss in the industry. In the older layers, contamination of feeds with fungi results in a significant drop in egg production [1]

CONCLUSION

This research has revealed the diversity of Aspergillus and Penicillium species that contaminate locally compounded poultry feeds and the individual ingredients used in the production of these feeds in Nigeria. It has also exposed the level to which each ingredient is contaminated bringing to limelight the quality of feeds and the danger to which the birds that consume these feeds are exposed. In the light of this, concerned authorities and stakeholders need to put quality control measures in place that must be adhered to during the process of feed formulation, transportation, storage and use.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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