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Full Length Research Article

FUNGAL CONTAMINATION OF AIR AND WATER SAMPLES FROM POULTRY FARMS THAT PRACTICE FLOOR AND CAGE HOUSING SYSTEMS IN ANAMBRA STATE, NIGERIA.

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Abstract

The industrialization of the poultry sector poses negative impact on air and water. Investigation on fungal contamination of 100 samples each of air and water were carried out in each of the ten poultry farms that practice floor and cage housing systems in Anambra State. The air mycoflora by plate exposure method and water samples (100ml) collected from tap water and drinkers in sterile sample bottles, were spread inoculated on Sabouraud Dextrose Agar plates supplemented with 0.05mg/ml Chloramphenicol, incubated at 25ºC for 3 – 7 days. The isolates were identified based on their macroscopic, microscopic and genetic features. Fungi isolated include the following genera, *Aspergillus*, *Penicillium*, *Fusarium*, *Curvularia*, *Trichoderma*, *Scopulariopsis*, *Acremonium*, *Syncephalis*, *Lichtheima*, *Paecilomyces*, *Cunninghamella*, *Starchybotrys*, *Chrysonila* and Yeast. The fungal loads of air and water samples during dry and rainy seasons were higher in poultries that practice floor system than cage system. The poultry with floor system had the highest mean fungal loads of 1.29×10^2 cfu/ml for air and 1.22×10^5 cfu/ml for water during dry season which is statistically significant (p=0.024). Most of the identified fungal species are characterized as allergens and an exposure of their spores may provoke adverse health effects.

Keywords: Poultry farms, fungi, floor system, cage system, fungal load.

Introduction

The rearing of domesticated birds (poultry) for meat, eggs and feather have been an important part of food production since the emergence of agriculture (Grzinic *et al*., 2023). It is a source of stable protein supply as well as food and nutritional security for many populations especially in rural areas in developing countries (Vaarst, *et al*., 2015).

Global poultry meat output has been roughly calculated to 137.7mil. t in 2021 (FAO, 2022), with the US (22.705 mil.t), China (19.500 ml.t), Brazil (14.076mil.t) and the EU (13.769 mil.t) being the biggest poultry meat producers as of 2020 (AVEC, 2021).

Poultry farming is developed according to the conditions laid out by each country (Saenz, 2021). The two major housing systems practiced in poultry farming are cage system and the floor system. The caged system involves intensive farming, where birds are kept in cages in several thousand in one or several buildings and close proximity. This system requires manpower in large number to carryout different operations and very high infrastructure and is associated with high production rates of up to 300 eggs/bird/year. In floor system, birds have an outdoor and some covered areas. It has moderate infrastructure and reduced labour demand (Sun *et al*., 2023).

The composition of the air of livestock buildings differs remarkably from that of atmospheric air (Chmieloweic-Korzeniowska *et al*., 2021). Some studies have shown that the number of microorganisms in air from animal husbandries can be as high as 3,000m^3 (de Rooij *et al*., 2019a), with animal excreta and secretions as main sources of contamination (Chmielowiec-Korzeniowska *et al*., 2018). Inside the poultry houses, the number of fungi is generally lower than bacteria (Grzinic *et al*., 2023).

Among microbiological threats for poultry sector workers are moulds growing in high humidity conditions on various organic materials or in the breeding rooms themselves. Fungi often implicated in air contamination of poultry house include, *Aspergillus*, *Penicillium*, *Mucor*, *Cladosporium*, *Fusarium*, *Alternaria*, *Scopulariopsis*, and *Acremonium*. Yeasts such as *Geotrichum candidum*, *Cryptococcus neoformans*, *Rhodotorula* and *Candida* species may be present in humid breeding premises, causing a lot of adverse health effects (Popescu *et al*., 2014; Grzinic *et al*., 2023).

Drinking water is an important nutrient for poultry and has a significant impact on poultry health, bird weight, feed conservation ratios and overall performance of the birds (AVEC, 2021; Chmielowiec-Korzeniowska, 2021; Mustedanagic *et al*., 2023). Poultry health and water intake are directly influenced by microbial water quality (Chmielowiec-Korzeniowska, 2021). Fungal genera associated with water pollution in poultry farms include *Aspergillus*, *Penicillium*, *Mucor*, *Curvularia*, *Cladosporium*, *Rhizomucor*, *Aureobasidium*, *Alternaria*, *Exserohilum*, *Cryptococcus* and *Candida* (Abdalla, 2017, Ibrahim *et al*., 2021).

The contamination of air and water used in the poultry farms, create considerable negative impact on the health and performance of the birds as well as the poultry farm workers. While contaminated air can lead to inflammation/respiratory and development of serious immune disorders (Grzinic *et al*., 2023), contaminated water can affect growth, reproduction and productivity of poultry as well as the safety of poultry products for human consumption (Mohammed, 2018). The aim of this research work is to determine the fungal contaminants of air and water samples from poultry farms that practice floor and cage systems in Anambra State, Nigeria.

Materials and Methods

Description of the poultry area

Ten farms (AK, JO, OS, CY, RA, EO, VO, TA, EM, EA), were randomly selected from six Local Government Areas (Aguata, Awka South, Idemili North, Nnewi North, Oyi,

Onitsha South) of Anambra State. Anambra State is in the South-Eastern part of Nigeria. It is situated between latitudes 5^{o} 32' and 6^{o} 45' N and longitude 6^{o} 43' and 7^{o} 22' E (Ezenwaji *et al*., 2014). Anambra State has warm humid tropical climate, with average rainfall between 1520 and 2020 mm per annum (Ifeka and Akinbobola, 2015). Mean annual temperature is 32.8^{o}C and relative humidity of 73^{o}C – 89^{o}C (Statistical yearbook, 2011).

Of the 10 poultry farms studied, six (AK, JO, OS, CY, RA, EO) practiced floor housing system while four (VO, TA, EM, EA) practiced caged housing system. The study was from October, 2022 to September, 2023.

Sample Collection

Air mycoflora was determined by modified plate exposure method described by Okiki and Ogbimi (2011). Sterile plates (100) of Sabouraud Dextrose Agar (SDA) supplemented with 0.05mg/ml of chloramphenicol, were exposed to various points for 5min. within each poultry confinement during rainy and dry seasons, and also time of sampling was kept uniform. After exposure, the plates were transported to Microbiology Laboratory of Nnamdi Azikiwe University, Awka, Anambra State. One hundred water samples (100ml) each were collected from ten poultry farms following the method described by Abdalla, 2017 at different seasons. The samples were collected from tap water and drinkers in sterile sample bottles. Sample were taken to the laboratory and processed within 3h of collection.

Isolation and enumeration of fungal organisms in the samples

Fungi from the water samples were isolated using spread plating method (Abdalla, 2017). Five hundred microliters (500µl) of samples were spread inoculated onto Sabouraud Dextrose Agar plates supplemented with 0.05mg/ml of chloramphenicol. The air mycoflora plates and water samples plates were incubated at 25^{o}C, and after 2 – 7 days, were observed for fungal growth (Parvan and Manjunath, 2014). After incubation, colonies of fungi on the medium were counted manually and colony forming unit per ml (cfu/ml) calculated.

$$\text{cfu/ml} = \frac{\text{mean colony} \times \text{volume}}{\text{inoculum size}}$$

Pure isolates were sub-cultured on SDA slant and stored at 4^{o}C.

Identification of fungal isolates

The fungal isolates were identified based on detailed studies of their morphological, microscopic and genetic features.

Morphological examination

This was carried out by observing the colonial morphology, texture, consistency, rate of growth, pigmentation, surface appearance and colour on the reverse of the SDA plate.

Microscopic examination A drop of lactophenol cotton blue solution was placed on a clean grease-free slide and a fragment of the isolate teased gently on the solution with a sterile needle and covered with a glass cover slip. This was observed under ×10 and ×40 objective lens for the presence of mycelia and fruiting bodies.

Morphological and microscopic features observed were compared to those presented in standard atlas for fungi identification (De Hoog, 2000; Diba *et al*., 2007).

Some of the organisms were identified in Macrogen laboratory, Europe, Meibergdreef Amsterdam, Netherlands, using 16S rRNA

Statistical Analysis
The results obtained were statistically analyzed by one way Analysis of Variance (ANOVA) using SPSS Version 21 Software. Values were considered significant if $p<0.05$.

Consent
Sample collection was done on the site of the farm by the assistance of the farm workers and the permission of the management of the farms.

Results
Characterization of fungal isolates from the samples
Based on the morphological, microscopic and genetic features of the isolates, a total of 14 genera of fungi were identified. These include *Aspergillus*, 506 (54.82%), Yeast 197 (21.34%), *Lichthermia corymbifera* 71 (7.69%), *Paecillomyces varioti* 44 (4.76%), *Penicillium* 29 (3.14%), *Fusarium* 20 (2.16%), *Curvularia* 20 (2.16%), *Acremonium* 9 (0.98%), *Trichoderma* 9 (0.98%), *Syncephalis* 6 (0.65%), *Chrysonelia* 3 (0.33%), *Starchybotryl* 3 (0.33%), *Cunninghamella bertholletiae* 3 (0.33%), *Scopulariopsis* 3 (0.33%).

Enumeration of fugal isolates in the air and water samples
Fig. 1 and Fig. 2, show the mean fungal load of air and water samples from six poultry farms with floor system and four poultry farms with cage system during dry and rainy seasons respectively. The fungal load for air samples ranged from 4.60×10^1 cfu/ml to 1.29×10^2 cfu/ml, while that of water samples ranged from 1.90×10^4 cfu/ml to 1.22×10^5 cfu/ml in the poultry farms with floor system during dry season (Fig. 1). During the rainy season, a fungal range of 2.23×10^1 cfu/ml to 2.80×10^2 cfu/ml was observed in the air samples and a range of 1.70×10^4 cfu/ml to 2.28×10^5 cfu/ml in the water samples.

As presented in Fig. 2, the mean fungal load for the cage system for four poultry farms during the dry season range from 9.0×10^1 cfu/ml to 1.2×10^2 cfu/ml for air samples and 5.5×10^3 cfu/ml to 1.2×10^4 cfu/ml for water samples. In rainy season, fungal load range of $1.0^5 \times 10^1$ cfu/ml to 4.0×10^1 cfu/ml for air samples and 9.50×10^3 cfu/ml to 2.30×10^4 cfu/ml for water samples were observed.

Occurrence and distribution of fungal isolates in air and water samples during rainy and dry seasons
Fig. 3 and Fig. 4 show the occurrence and distribution of fungal isolates in air and water samples during rainy and dry seasons. During the rainy season, 498 fungal isolates which include *Aspergillus* spp. 256 (51.41%), Yeast 105 (21.08%), *Lichthermia corymbifera* 59 (11.85%), *Paecillomyces varioti* 44 (8.84%) *Penicillium* sp. 13 (2.61%), *Acremonium* sp. 9 (1.81%), *Fusarium* sp. 6 (1.2%), *Syncephalis* sp. 3 (0.60%), *Chrysonelia* sp. 3 (0.60) were recovered, while 425 isolates were isolated in dry season. The different genera isolated from the samples in dry season include *Aspergillus* spp. 250 (58.82%), Yeast 92 (21.64%), *Curvularia* sp. 20 (4.71%), *Penicillium* sp. 16 (3.76%), *Fusarium* sp. 14 (3.29%), *Lichthermia corymbifera* 12 (2.82%), *Trichoderma* sp. 9 (2.12%), *Starchybotryl* sp. 3 (0.71%), *Cunninghamella bertholletiae* 3 (0.71%) *Scopulariopsis* sp. 3 (0.71%), *Syncephalis* sp. 3 (0.71%).

Discussion
Mould occurrence and growth in poultry farms is one of the major threats to poultry economy and health. The presence of these fungi in such farms could be detrimental to the health of poultry, poultry workers,

poultry consumers and residents living close to poultry farms (Chuwang *et al*., 2013).

Most of the fungal genera recovered from air and water samples in this study have been reported by many researchers (Sowiak *et al*., 2012a; Abdalla, 2017; Popescu *et al*., 2019; Ibrahim *et al*., 2021; Grzinic *et al*., 2023; Gomes *et al*., 2023). Occurrence and distribution of fungal isolates in sampled air showed the presence of different fungi with *Aspergillus* species having the highest occurrence in rainy (47.27%) and dry (52%) seasons. This finding is in line with the reports of Lonc and Plewa (2010) and Pavan and Manjunathan (2014), who also observed similar organisms from air in poultry houses. The highest prevalence of *Aspergillus* species supports the work of Girma *et al*. (2016), who reported *Aspergillus* species as the most occurring organisms in poultry farms. The result corroborates the reports of Okiki and Ogbimi (2011). They recorded the presence of *Aspergillus* species, *Trichoderma* species, *Penicillium* species, *Fusarium* species and yeast. However, they observed the presence of *Mucor, Saccharomyces cerevisiae* and *Cryptococcus neorformans*, which were not isolated in this study. Contrary to our findings, Popescu *et al*. (2019) reported the presence of *Candida*, *Cladosporium*, *Alternaria, Rhizopus, Mucor, Histoplasma* and *Cryptococcus* in air from poultry rearing houses. The variation in the organisms obtained in this study and those of other researchers may be as a result of the type of poultry house, the season of the year, wind direction and weather conditions (Guo *et al*., 2022; Rzeznik and Mielcarek-Bochenska, 2022).

Studies on mycological quality of the tap water and water from drinkers in the poultry farms studied showed the presence of 5 genera of fungi with *Aspergillus* sp.as the most prevalent fungi in both seasons. This corroborates the result of Abdalla (2017) who in the study of water-borne fungi from poultry farms, observed the presence of 9 fungal genera with *Aspergillus* species as the most prevalent. On the contrary, the researcher isolated *Mucor, Curvularia, Cladosporium, Rhizomucor, Aureobasidium*, *Alternaria* and *Exserohilum* species. This could have been as a result that the water used by the farms visited were from commercial tankers, the source of which is unknown.

The results obtained in this study on the mean fungal load of the sampled air (2.23×10^1 cfu/ml – 1.2×10^4) supports the work of Karwowska (2005), who observed a fungal load of 1.70×10^2 cfu/m^3 from air. However, contrary to our findings, higher mean fungal values of 2.20×10^5 cfu/m^3 and 5.12×10^5 cfu/m^3 were reported by Vucemilo *et al*., (2007) and Okiki and Ogbimi (2011) respectively. The differences in fungal load with that obtained in this study may be associated with the sampling methods used. Lonc and Plewa (2010) and Kostadinova *et al*. (2014), attributed this variation in fungal load to the housing system, different poultry species, capacity of buildings, density of rearing birds, age of birds and microclimate conditions. Viegas *et al*. (2012b) also noted that factors like ventilation rate, presence or absence of air cleaning technologies and manure management methods may also have contributed to these variations.

A higher mean fungal load was observed in the samples in floor housing system when compared to the value obtained in the cage housing system during dry season. The observation agrees with the report of Popescu *et al*. (2014), who also reported a high level of fungal air contaminants in farms with floor housing system and attributed it to less ground disturbances and restricted movement obtained in cage

housing system. Bruce *et al*. (2015) also accredited the high level of contaminants in farms with floor housing system to the use of litter and increased bird activities.

This study, therefore, demonstrated that the level of fungal contamination in the floor system is higher than that obtained in the cage housing system during the rainy season. This agrees with the work of Natasha *et al*. (2009), which reported that air concentration is generally lowest in cage-housed operations compared to floor-housed operations that use litter as bedding material.

In this study, water sampled from the different farms that practiced floor housing system showed high mean fungal load when compared with farms that practiced cage housing system. This observation is in line with the work of Hassan (2009), who detected the lowest fungal count in water samples from farms that practiced cage system. In farms that practiced floor housing system, water troughs or bell drinkers are usually used to provide water for the birds. Such water samples can possibly be contaminated with feed particles, litter, droppings, nasal and mouth discharge from the birds which may have influenced the results obtained.

Comparing the level of contamination in the farms between dry and rainy seasons, it was observed that the farm that practiced floor housing had the highest mean fungal load, which was statistically significant ($p<0.05$), during the rainy season than the dry season. This observation agrees with the work of Sowiak (2012a), who reported that poultry farms are usually contaminated with high levels of fungal aerosols especially in the colder season. In the case of the cage system, there was no significant difference ($p>0.05$) in the mean fungal load between the dry and rainy seasons.

The industrialization of the poultry sector poses a considerable negative impact on air and water (Gomes *et al*., 2023). It has been reported that intensive poultry farming not only create a significant risk to workers (Chmielowiec-Korzeniowska *et al*., 2018; Gorny, 2020b) but can also act as a potential public health menace (Chmielowiec-Korzeniowska *et al*., 2021; Grzinic *et al*., 2023). *Aspergillus* species, *Penicillium*, *Fusarium*, *Acremonium*, *Scopulariopsis* and yeast isolated in this study have been implicated in public health problems of poultry farm workers (Sowiak *et al*., 2012b; Ibrahim, *et al*., 2021; Grzinic *et al*., 2023) Majority of the identified fungal species are characterized as allergenic and an exposure to their spores may provoke adverse health effects, mainly of respiratory tract and skin (Chmielowiec-Korzeniowska *et al*., 2018; Ekwealor and Oyeka, 2013; Popescu *et al*., 2014) in susceptible individuals.

Conclusion:

Samples from air and water used in the poultries studied contained high load of fungi. It is, therefore, necessary that all the health threats caused by fungi in poultry farms be addressed through improvement in the poultry environment.

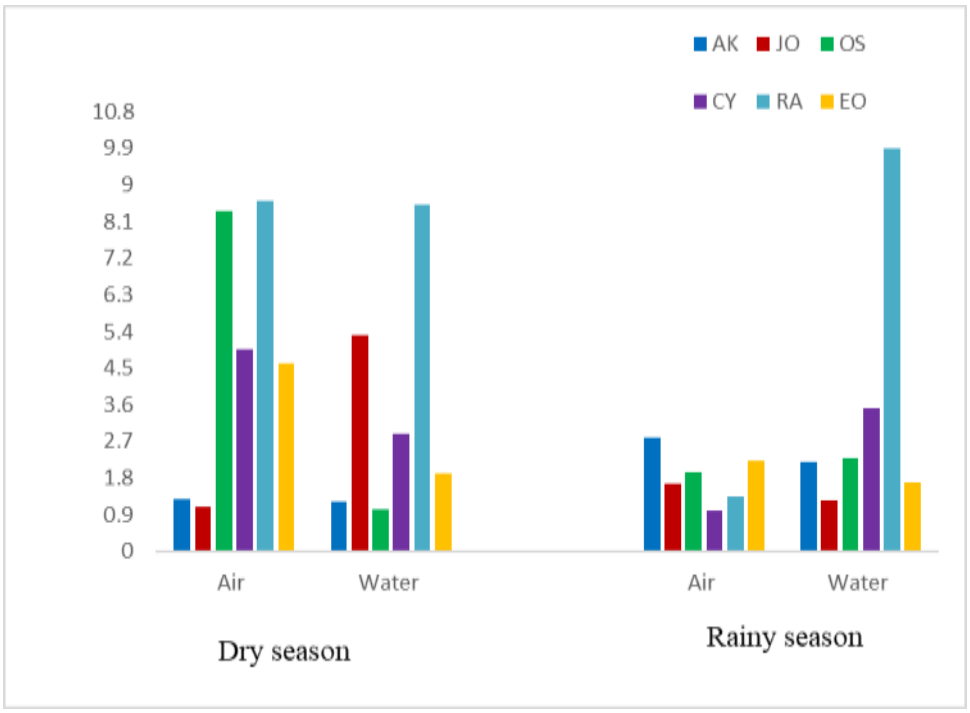


Figure 1: Mean fungal load of water and air samples from six poultry farms with floor housing system.

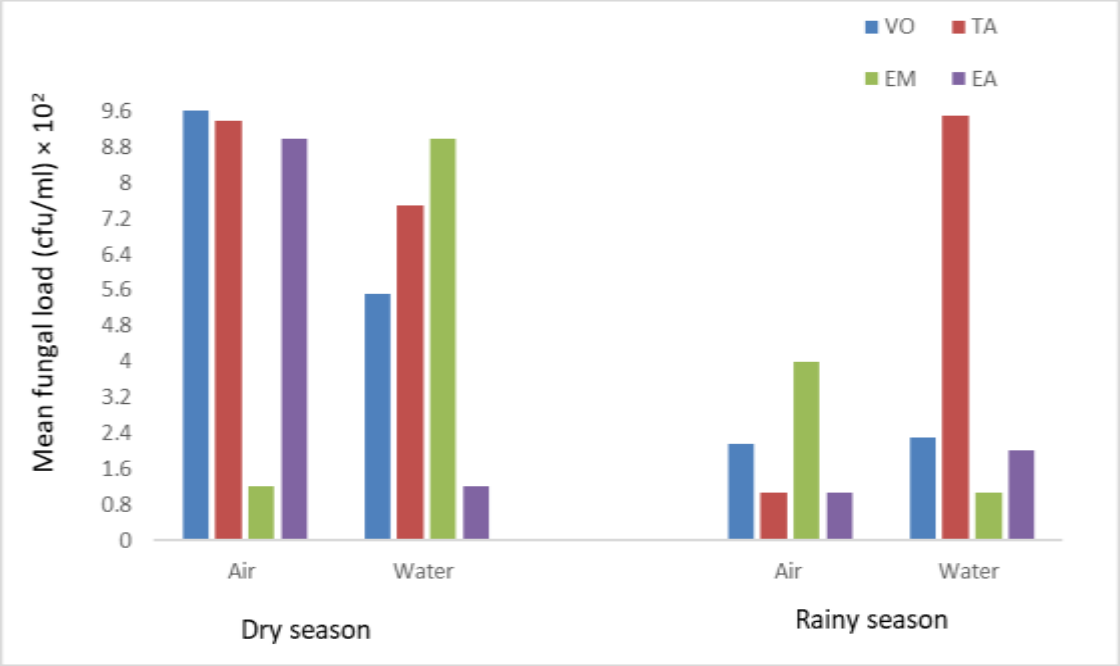


Figure 2: Mean fungal load of water and air samples from four poultry farms with cage housing system.

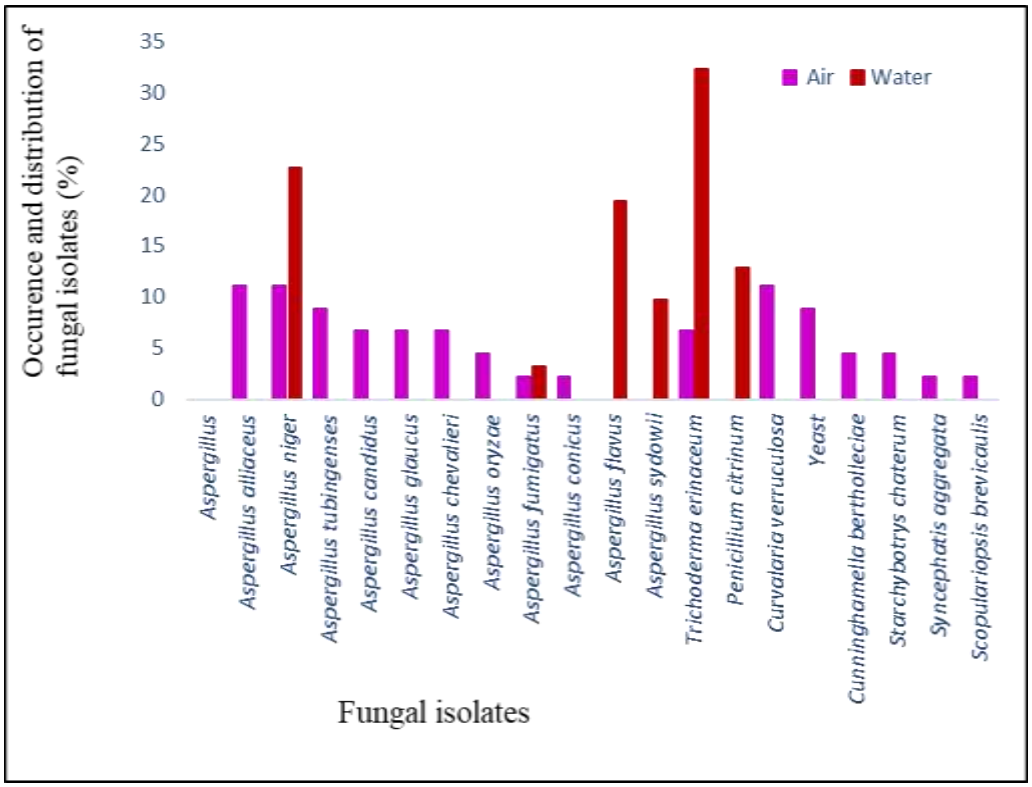


Figure 3: Occurrence and distribution of fungal isolates in air and water samples during dry season.

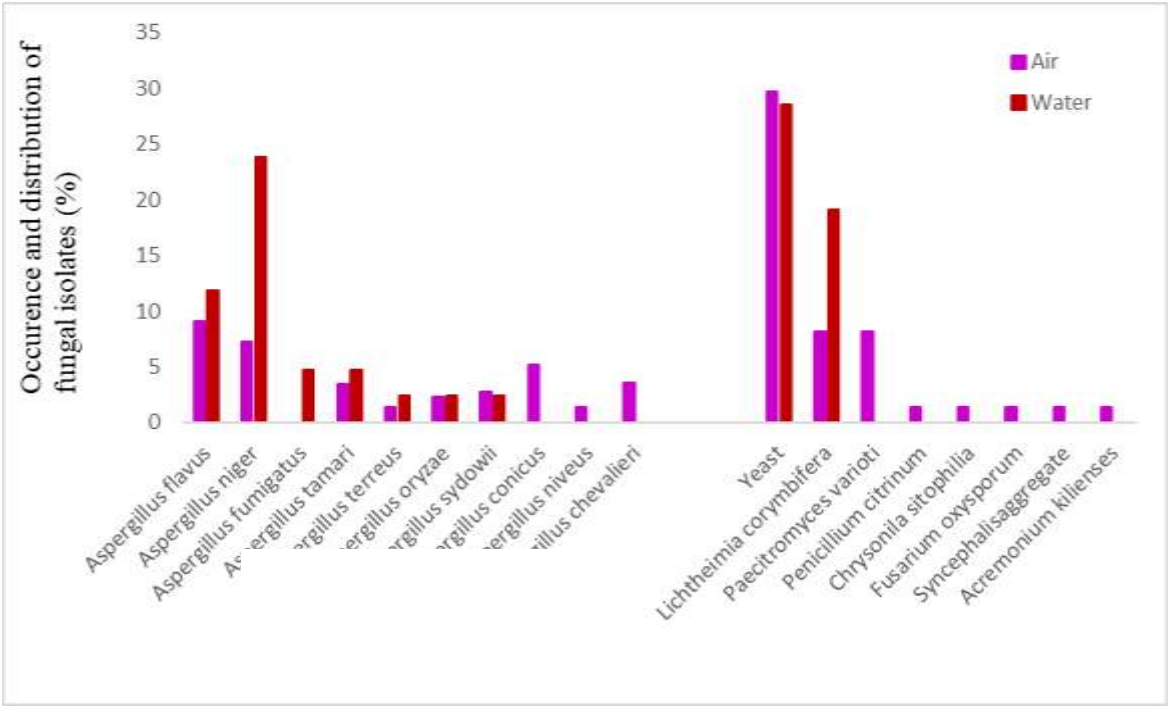


Figure 4: Occurrence and distribution of fungal isolates in air and water samples during rainy season

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