Isolation and Identification of Non Dermatophytic Moulds from Different Air and Soil Samples from Cattle Markets in Abia and Imo State, Nigeria

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Abstract: The prevalence of non-dermatophytic moulds from air and soil samples from six cattle markets in Abia and Imo state, Nigeria were carried out simultaneously. A total of 60 (sixty) samples of air and soil were analysed. The settlement plate technique, tube dilution method and hair baiting technique were evaluated for the air and soil for the presence of keratinophilic fungi. Different species of 13 non-dermatophytic moulds were identified and all occurred at different points of collection from the markets.The most frequently isolated species from air samples were *Aspergillus welwitschiae, Absidia corymbifera* (20%) respectively in Abia state and *Fusarium linchenicola, Absidia corymbifera* (20.6%) respectively in Imo state. For soil samples; *Absidia corymbifera* (29%) and *Aspergillus flavus* (21%) were frequently isolated in Abia and Imo staterespectively,while for hair bait; *Aspergillus flavus, Absidia corymbifera*(26%)respectively in Abia state and *Aspergillus welwitschiae*(20%) in Imo state. The least isolate from air samples were *Aspergillus sydowii* (3.3%) and *Cladosporium tenuissinum* (3.0%), for soil samples; *Aspergillus sydowii* (3.0%) and *Aspergillus sydowii* (3.0%), while for hair bait; *Penicillum citrinum, Aspergillus aculeatus* (6.0%)respectively and *Penicillum citrinum, Cladosporium tenuissinum* (2.2%)each in Abia and Imo state respectively. According to the study, there was a significant keratinous fungal deposition in the soil of the cattle market area as a result of mechanical activity nearby. Spores from agitated fungi may have caused a significant suspension of these spores in the atmosphere, which might constitute a serious health risk to anybody who operate in such environments.

Key word: Air, cattle market, Nigeria, non-dermatophytic moulds and soil

INTRODUCTION

Soil is known to be a mantle of weathered rock containing minerals and nutrients (Farid and Nareen, 2012). It is a major component of the ecosystem, it supports a range of microorganisms of such as fungi and other dermatophytes. It has been found or reported that one of the most complex microbial habitats in the soil is the fungi (Pahare and Shukla, 2014). The presence of these microorganisms in the soil plays a major role in determining the quality of soil and soil productivity (Ramesh and Baradwad, 2021). Reports have it that one of the group of fungi found in the soil are Keratinophilic fungus (Deshmukh and Verekar, 2006; Frac, *et al*., 2018). These group of fungi which are Keratinous in nature were also reported to be dermatophytes and non-dermatophytes and as such, they are source of infections transmitted through the soil to infect human and animals (Seyed *et al*., 2012). They are usually found in the soil and are mostly rich in creatinine, keratin (Pahare *et al*., 2018). The distribution of this microorganism in the soil are mostly affected by geographical location and activities carried out in the soil, thus making factors such as pH of the soil to be affected, when they come in close contact with animals and humans. Human occupation and activities bring them directly in contact with the soil, therefore makes the soil a reservoir for animal and human infection. A few studies have shown the availability of some dermatophytes and non-dermatophytic moulds in the soil according to Keyvan *et al*. (2013) and Subhash *et al*. (2017), but according to Deshmukh (2002), most keratinophilic fungi are soil inhabitants than dermatophytes.

MATERIALS AND METHODS

Study area: This study was carried out in Abia and Imo States and both are situated in Southeastern Nigeria. Each of the states is divided into three geopolitical zones and each of the zones possess at least one major cattle market. For Abia state, Lokpanta cattle market representing Abia north, Ahiaudele cattle market representing Abia south, while Ubakala cattle market representing Abia central were selected for the study. For Imo state, Afor Ogbe cattle market representing Owerri zone, Okigwe cattle market representing Okigwe zone and Ekeubahaeze cattle market representing Orlu zone were selected for the study.

Sample collection: Soil samples were randomly collected at depth of about 2- 4cm depth from 10 different points within the six different markets from both States. The soil samples were collected in a sterile tightly closed polythene bag. For sample collection, settlement plate technique were carried out by exposing Sabouraud dextrose agar plates supplemented with chloramphenicol (20mg) at 10 different locations for 30mins at 6ft height within the six markets. The samples were labeled based on site where it was collected and the date of collection was noted. The sample containers and exposed plates were wrapped with aluminum foil and taken to the Microbiology laboratory for analysis (Rajender and Krishma, 2017).

Dilution of soil sample: Each soil samples were diluted serially and cultured on Sabouraud dextrose agar (SDA) medium containing chloramphenicol (20 mg) according to (Sujatha and Swethalatha, 2017).

Hair bait technique for keratinophilic moulds: The hair bait approach, first described by Vanbreuseghem (1952), was employed by Rahul and Rajak (2003) to separate and identify keratinophilic fungus in soil samples. The soil samples were dampened and then placed on a sterile Petri dish. The dirt surface was then covered with a layer of sterilized, defatted human hair. For two weeks, this was kept in a dark cupboard. A portion of the hair exhibiting indications of fungal growth was placed in Sabouraud agar plates containing chloramphenicol and incubated at 27 ± 2℃ for five to seven days. The remaining hair was removed and examined under a microscope by immersing it in a drop of lactophenol cotton blue to look for hair strand peforation.

Preservation of isolates: Discrete pure colonies were preserved on Sabouraud dextrose agar slants and incubated at 37ºC for 1-2 weeks and then stored in the refrigerator until required for further studies (Cheesbrough, 2010).

Slide culture: Slide culture of the isolates were prepared and analysed using standard cultural method (Cheesbrough, 2010), and were identified using mycological atlas by David *et al*. (2007).

Statistical analysis of data: The data obtained from this study were analyzed statistically using one way analysis of variance (ANOVA) as in Martins and Igwemma (2000).

RESULTS AND DISCUSSION

A total of 8 different species of non-dermatophytic moulds were identified from the air samples within the cattle markets in Abia State Nigeria (Figure 1). The highest isolated species was *Aspergillus welwitschiae*witha total frequency of occurrenceof 12(20.0%) of which Ubakalamarket had (3), Ahiaudele market (3) and Lokpanta market (6), *Absidia corymbifera* had a total frequency of occurrence of 12(2.0%) of which Ubakala market had (2), Ahiaudele market (6) and Lokpanta market (4).This was followed by *Penicillum citrinum* that had a total frequency of occurrence 10(16,7%) of which Ubakala market had (2), Ahiaudele market (3) and Lokpanta market (5), *Aspergillus flavus* had a total frequency of occurrence of 8(13.3%) of which Ubakala market had (2), Ahiaudele market (2) and Lokpanta market (4).*Aspergillus aculeatus* had a total frequency of occurrence of 6(10.0%) of which Ubakala market had (2), Ahiaudele market (2) and Lokpanta market (2). The

Fusarium lichenicola had a total frequency of occurrence of 6(10.0%) of which Ubakala market had (2), Ahiaudele market (2) and Lokpanta market (2).*Cladosporium tenussimum* had a total frequency of occurrence of 4(6.7%)of which Ubakala market had (0), Ahiaudele market (2) and Lokpanta market (2) and *Aspergillus sydowii* had a total frequency of occurrence of 2(3.3%0 of which Ubakala market had (0), Ahiaudele market (2) and Lokpanta market (0).Statistical analysis shows that there were no significant difference between non-dermatophytic moulds isolated within cattle markets in Abia State P= 0.05. Our findings agrees with study by Ajoudanifar *et al.*(2011), who isolated similar non-dermatophytic fungal species from the indoor air and outdoor air fungi at poultry and cattle homes in Iran. The authors findings *Cladosporium* (55.3%), yeast (10.0%), and *Aspergillus* (9.4%) were the most common isolates. Pavan and Manjunath (2014), also reported similar results indoor and outdoor air borne fungi in animal rearing houses in cowshed. The authors reportedthe most dominant genera identified were *Cladosporium* sp., *Aspergillus* sp., and *Alternaria alternate*.

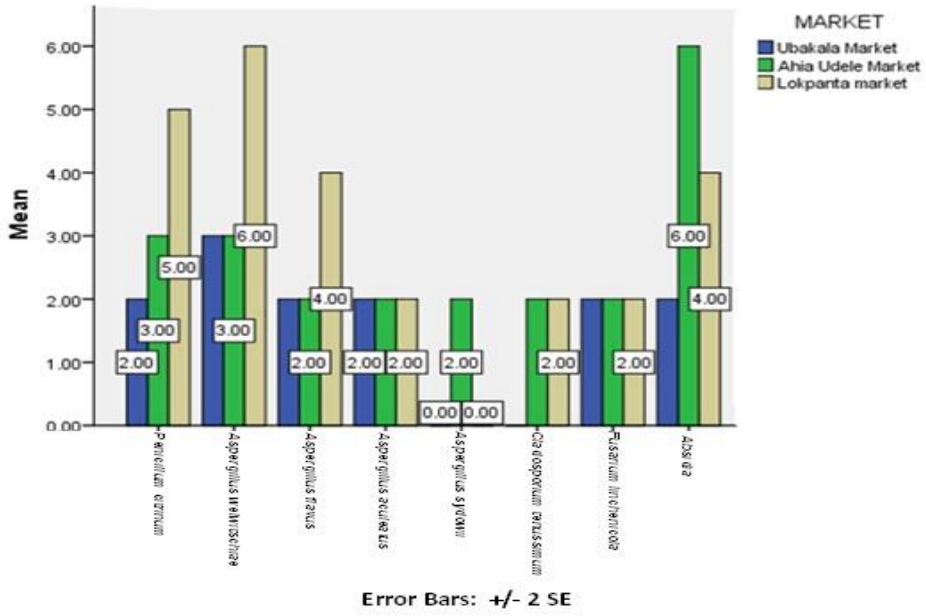

Figure 1: Frequency of occurrence of non dermatophytic moulds isolated from air within the cattle markets in Abia State Nigeria

A total of 8 species at different frequency of occurrence were identified from air samples within cattle markets in Imo State Nigeria (Figure 2). The isolated species were *Fusarium lichenicola* with a total frequency of occurrence of 14(20.6%) of which Afor Ogbe market had (5), Ekeubahaeze market (5) and Okigwe market (4). *Absidia corymbifera* had a total frequency of occurrence of 14(20.6%) of which Afor Ogbe had (5), Ekeubahaeze market (4) and Okigwe market (5) followed by *Aspergillus welwitschiae* that had a total frequency of occurrence of 12(17.6%) of which Afor Ogbe market had (5), Ekeubahaeze market (3) and Okigwe market (4). *Aspergillus flavus* had a total frequency of 8(11.8%) of which Afor Ogbe market had (5), Ekeubahaeze market (3) and Okigwe market (0). *Aspergillus aculeatus* had a total frequency of occurrence of 8(11.8%) of which Afor Ogbe market had (4), Ekeubahaeze market (1) and Okigwe market (3). *Penicillum citrinum* had a total frequency of occurrence of 5(7.4%) of whichAforOgbemarket had (3), Ekeubahaeze market (2) and Okigwe market (0). *Fusarium oxysporum* had a total

frequency of occurrence of 5(7.4%) of which Afor Ogbe had (5), Ekeubahaeze market (0) and Okigwe market (0) and *Cladosporium tenussimum* had total frequency of occurrence of 2(3.0%) of which Afor Ogbe market had (1), Ekeubahaeze market (0) and Okigwe market (1). According to statistical analysis, there was no discernible variation in the frequency of occurrence of non-dermatophytic moulds that were isolated from the air in Imo state's cattle markets (P= 0.05). This is consistent with a study by Sharm and Khade (2019), which examined the aeromyco flora of an indoor dairy cattle shed and found that *Aspergillius* spp., *Ustilago* spp., *Penicillium*, *Alternaria*, and *Cladosporium* were more frequently prevalent. In Shakil's (2007) investigation of air fungal spores in workplace settings, the results of this study are likewise in agreement. The study's conclusions may be related to the mechanical processes that occur in the market as a result of interactions between humans and animals, animals and other animals, and humans and soil. Some of these moulds may be transported from loose soils into the atmosphere by these processes. Some of these stray athrospores from the soil can also be carried into the atmosphere by wind movement. Because of the spore suspension in the air, workers, customers, and vendors are at risk for major health problems. This raises concerns about public health.

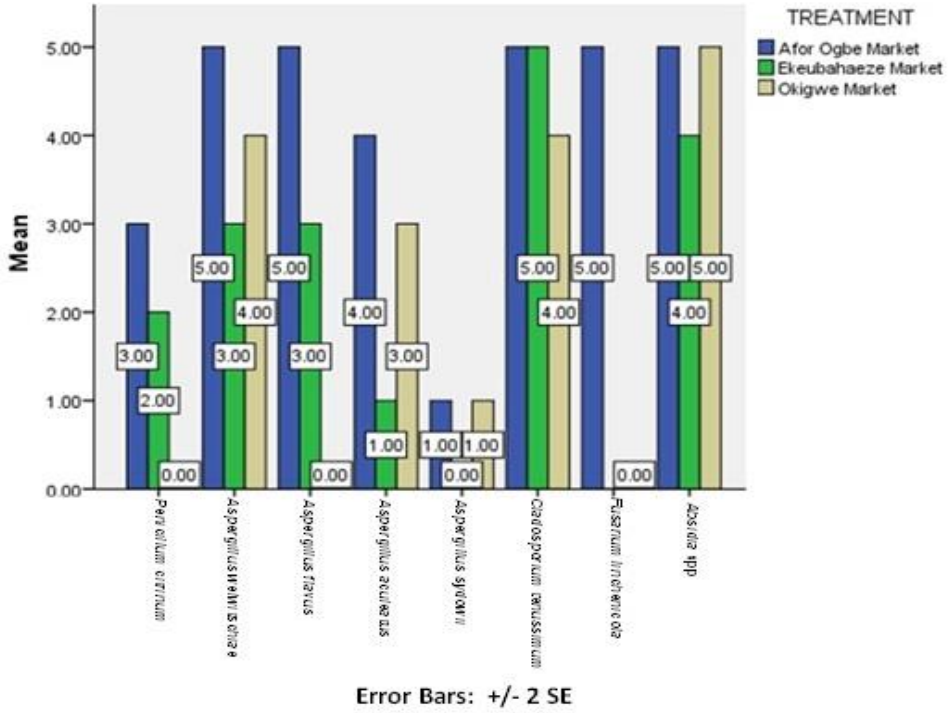

Figure 2: Frequency of occurrence of non dermatophytic moulds isolated from air samples within the cattle markets in Imo State Nigeria

Furthermore, Figure 3 illustrates frequency of occurrence of the fungal from cattle market in Abia State. A total of 6 species of non-dermatophytic moulds were identified which includes *Aspergillus welwitschiae* which had the total frequency of occurrence of 13(20.0%) of which Ubakala market had (6), Ahiaudele market (4) and Lokpanta market (3). *Aspergillus flavus* had a total frequency of occurrence of 12(18.2%) of which Ubakala market had (6), Ahiaudele market (0) and Lokpanta market (6). Also, *Aspergillus sydowii* had a total frequency of 2(3.0%) of which Ubakala market had (0), Ahiaudele market (0) and Lokpanta market (2). *Absidia corymbifera* had a total frequency of 19(29.0%) of which Ubakala market had (13), Ahiaudele market (1) and

Lokpanta market (5).*Fusarium lichenicola* had a total frequency of 14(21.2%) of which Ubakala market had (9), Ahiaudele market (1) and Lokpanta market (4).*Fusarium succisae*, had a total frequency of occurrence of 6(9.1%) of which Ubakala market had (3), Ahiaudele market (0) and Lokpanta market (3). Statistical analysis revealed that there were significant difference in frequency of occurrence of non-dermatophytic moulds isolated from the soil samples within cattle markets in Abia State, Nigeria. A total of 8 species were identified from the soil samples from the market of Imo State Nigeria with different frequency of occurrence (Figure 4) which includes *Aspergillus flavus* which had a total frequency of occurrence of 16(21.1%) of which Afor Ogbe market had (5), Ekeubahaeze market (5) and Okigwe market (6). Also, *Fusarium lichenicola* had a total frequency of 16(21.1%) of which Afor Ogbe market had (6), Ekeubahaeze market (5) and Okigwe market (5), *Absidia corymbifera* had a total frequency of occurrence of 15(20.0%) of which Afor Ogbe had (6), Ekeubahaeze market (4) and Okigwe market (5). The *Aspergillus welwitschiae* had a total frequency of occurrence of 14(18.4%) of which Afor Ogbe market had (8), Ekeubahaeze market (4) and Okigwe market (2).*Aspergillus sydowii* had a total frequency of occurrence of 2(3.0%) of which Afor Ogbe market had (0), Ekeubahaeze market (2), Okigwe market (0). *Fusarium succisae* had a total frequency of occurrence of 10(13.2%) of which Afor Ogbe market had (4), Ekeubahaeze market (3) and Okigwe market (3). *Fusarium solani* had a total frequency of occurrence of 2(3.0%) of which Afor Ogbe market had (2), Ekeubahaeze market (0) and Okigwe market (0) and *Cladosporium tenussimum* had a total frequency of occurrence of 1(1.3%) of which Afor Ogbe market had (1), Ekeubahaeze market (0) and Okigwe market (0). Statistical analysis in this study shows that there was no significant difference in the frequency of occurrence of non-dermatophytic moulds recovered from soil within cattle markets in Imo state P= 0.05. Another study by Shokohi *et al*. (2005), isolated numerous fungi and aerobic actinomycetes from surface soil in Sari with *Fusarium* species as the most prevailing saprophytic molds in South and Razavi Khorasan provinces. Malek *et al.* (2013) stated that *Aspergillus* species (15.92%) was the highest saprophytic fungi isolated when he analyzed soil samples from park soils in Gorgan, North of Iran. Nosratabadi *et al.* (2017), isolated *Chrysoporium tropicum* (18.5%) as the most prevailing species followed by *Penicillum* species (6.1%), *Aspergillus* species (6.1%), *Alternaria* species (4.6%) and *Fusarium chlamydosporum* (3.1%) when he surveyed soil samples from greater Tunb, Abu-Musa and Sirri Islands in the Persian Gulf, Iran. A separate study carried out by Rafai *et al*. (2012) and Marcelo *et al.* (2015) revealed presence of *Cladosporium* species in the soil samples analyzed. This findings justifies our result in this study from Imo State. Another study by Farid and Nareen (2012), revealed *Aspergillus* specie as the most frequently isolated fungi during the four seasons, followed by *Penicillum* specie, *Rhizopus* specie, *Emericella* specie, *Fusarium* specie, *Ulocadiumbothytis*. When they studied the seasonal distribution of soil borne fungi in different areas of Erbil Governorate. Fifteen (15) (5 from each market) soil samples were examined for the presence of keratinophilic non-dermatophytic moulds using hair baiting technique in Abia State. Figure 5 shows a total of 6 species of keratinophilic non-dermatophytic moulds were isolated which includes *Penicillium citrinum* which had a total frequency of occurrence of 9(26.0%) of which Ubakala market had (0), Ahiaudele market (0) and Lokpanta market (2) and *Aspergillus flavus* had a total frequency of occurrence of 9(26.0%) of which Ubakala market (3), Ahiaudele market (2) and Lokpanta market (4).*Absidia corymbifera* had a total frequency of occurrence of 9(26.6%) of which Ubakala market had (3), Ahiaudele market (3) and Lokpanta market (3) which was the highest occurring isolate

followed by *Aspergillus welwitschiae* which had a total frequency of occurrence of 7(20.0%) of which Ubakala market had (4), Ahiaudele market (3) and Lokpanta market (0).*Fusarium lichenicola* had a total frequency of occurrence of 6(17.1%) of which had (2), Ahiaudele market (2) and Lokpanta market (2) and *Aspergillus aculeatus* which had a total frequency of occurrence of 2(6.0%) of which Ubakala market had (0), Ahiaudele market (0) and Lokpanta market (2).

Fifteen (15) (5 from each market) soil samples were investigated for the availability of keratinophilic non-dermatophytic moulds using hair baiting technique in Imo State. Figure 6, shows a total of 10 species of keratinophilic non-dermatophytic moulds isolated which includes *Aspergillus welwitschiae* which had a total frequency of occurrence of 9(20.0%) of which Afor Ogbe market had (2), Ekeubahaeze market (4) and Okigwe market (3) which records the highest occurring specie, followed by *Aspergillus flavus* which had a total frequency of occurrence of 7(16.0%) of which Afor Ogbe market had (3), Ekeubahaeze market (2) and Okigwe market (2).The *Absidia corymbifera* had a total frequency of occurrence of 7(16.0%) of which Afor Ogbe market had (3), Ekeubahaeze market (4) and Okigwe market (0), *Fusarium lichenicola*had a total frequency of occurrence of 7(16.0%) of which Afor Ogbe market had (2), Ekeubahaeze market (3) and Okigwe market (2). *Fusarium succisae* had a total frequency of occurrence of 5(11.1%) of which A for Ogbe market had (2), Ekeubahaeze market (1) and Okigwe market (2), *Aspergillus fumigatus* had a total frequency of occurrence of 2(4.4%) of which Afor Ogbe market had (0), Ekeubahaeze market (0) and Okigwe market (2) and *Aspergillus terreus* had a total frequency of occurrence of 2(4.4%) if which Afor Ogbe market had (0), Ekeubahaeze market (0) and Okigwe market (2). *Penicillum citrinum* had a total frequency of 1(2.2%) of which Afor Ogbe market had (0), Ekeubahaeze market (0) and Okigwe market (1). *Aspergillus aculeatus* had a total frequency of occurrence of 4(9.0%) of which Afor Ogbe market had (2), Ekeubahaeze market (0), and Okigwe market (2) and *Cladosporium tenussimum* had a total frequency of occurrence of 1(2.2%) of which Afor Ogbe market had (0), Ekeubahaeze market (0) and okigwe market (1) respectively. Our findings agrees with other studies by Ganaie *et al*. (2010) and Seyed *et al*, (2012), in the study of soil samples using hair baiting technique revealed presence of similar non-dermatophytic moulds, *Penicillum* species, *Aspergillus* species, *Fusarium* species, *Candida* species, *Rhizopus* species, *Trichoderma* species and, *Alternaria* species. Moallaei *et al*. (2006) also isolated *Aspergillus* spp. as the second most populated saprophytic mold when he analyzed keratinophilic fungi from soil samples. Nwadiaro *et al.* (2015) also retrieved *Cladosporium* and *Trichoderma* species when he sampled barbers' landfill, which was in agreement with this study. Based on this study and previous studies, it is shown that most keratinophilic fungi are soil inhabitants and considering that the cattle market is an open environment where these livestocks are been kept and sold, buyers and sellers carry out alot of activities. There is a high tendency of depositing organic materials such as animal fur, human hair, dead cells on the soil which might encourage the proliferation of keratinophilic fungi. On the other hand, microscopic view of hair strands from hair bait technique in this study showed denatured hair strands (Figure 7), while some showed intact hair strands with hyphae growing around it (Figure 8). This is an indication that some of the non-dermatophytic moulds present in soil could perhaps secrete enzymes that could hydrolyse hair strands with the denatured hair strands observed.

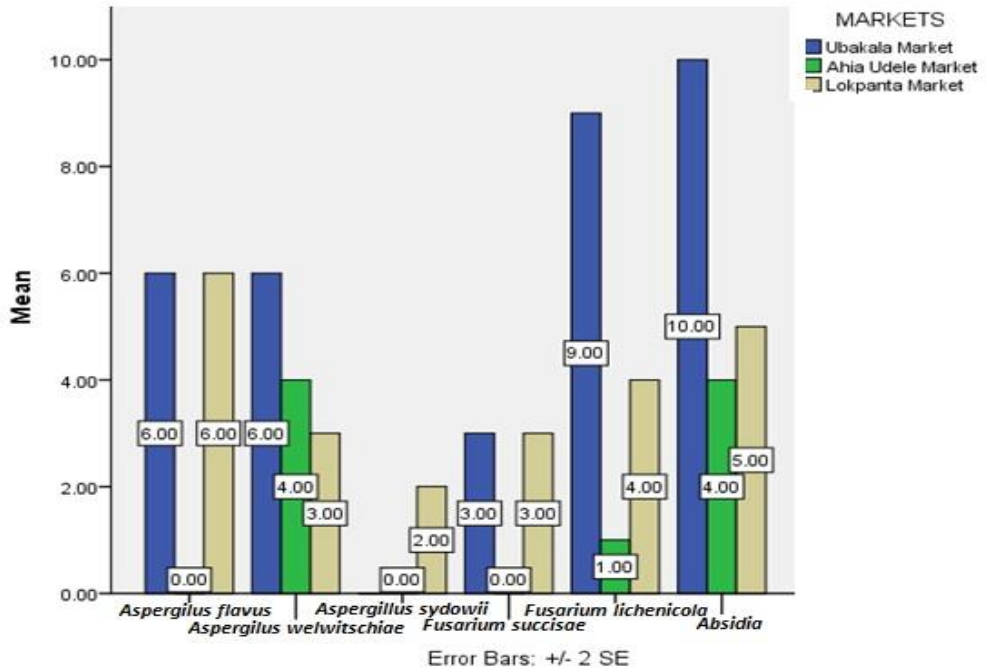

Figure 3: Frequency of occurrence of non dermatophytic moulds from soil samples within the cattle markets in Abia state, Nigeria

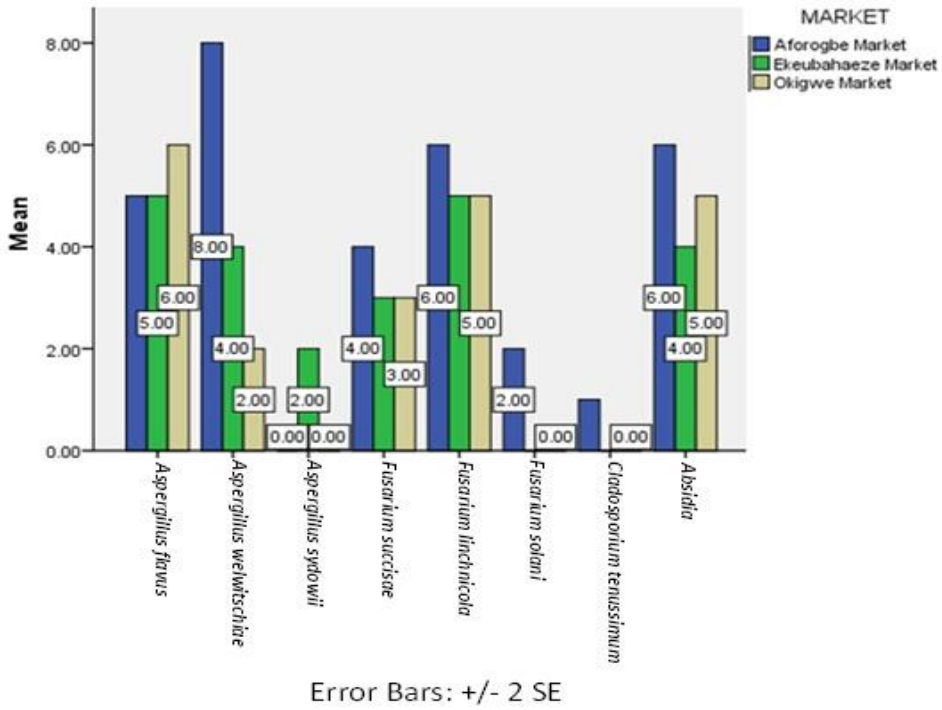

Figure 4: Frequency of occurrence of non-dermatophytic moulds from soil samples within the cattle markets in Imo State, Nigeria

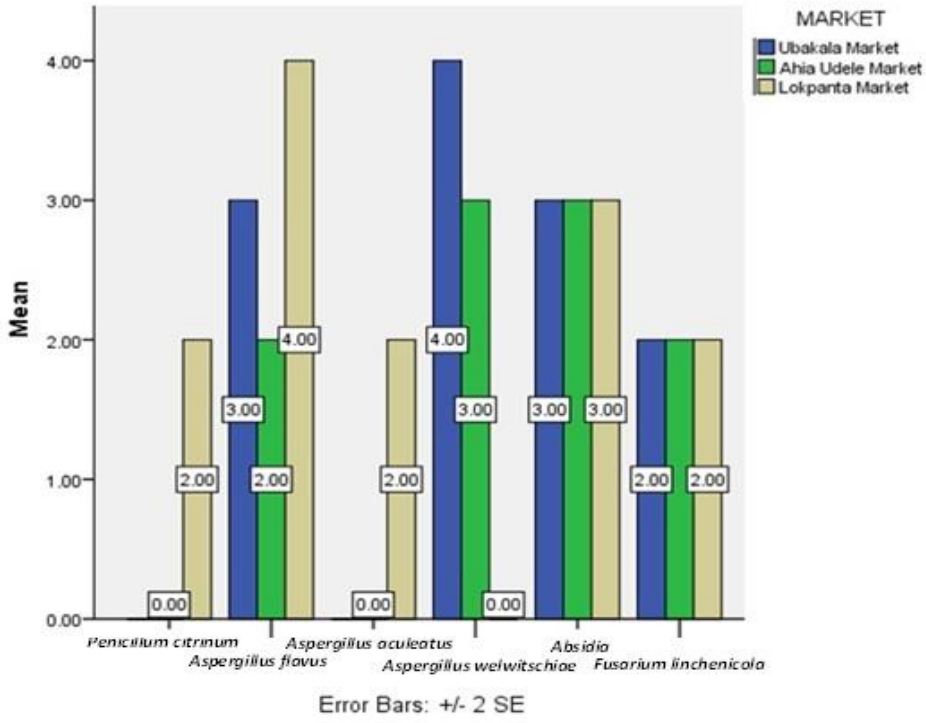

Figure 5: Frequency of occurrence of keratinophilic non-dermatophytic moulds from soil samples within cattle markets in Abia State using hair bait technique

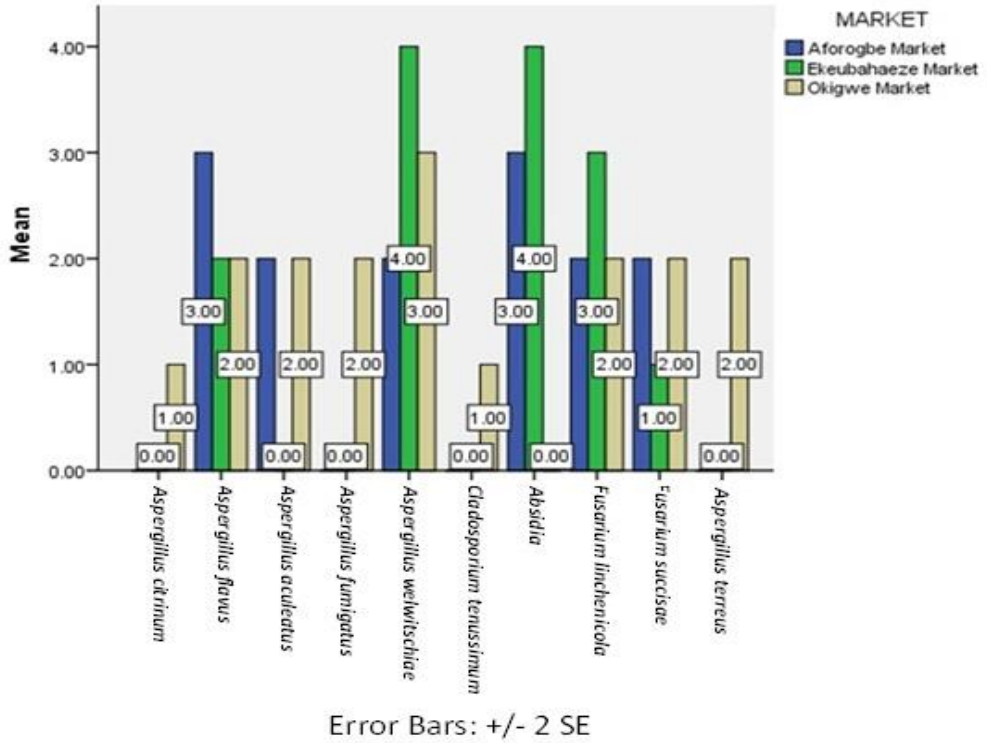

Figure 6: Frequency occurrence of keratinophilic non-dermatophytic moulds from soil samples within cattle markets in Imo State using hair bait technique

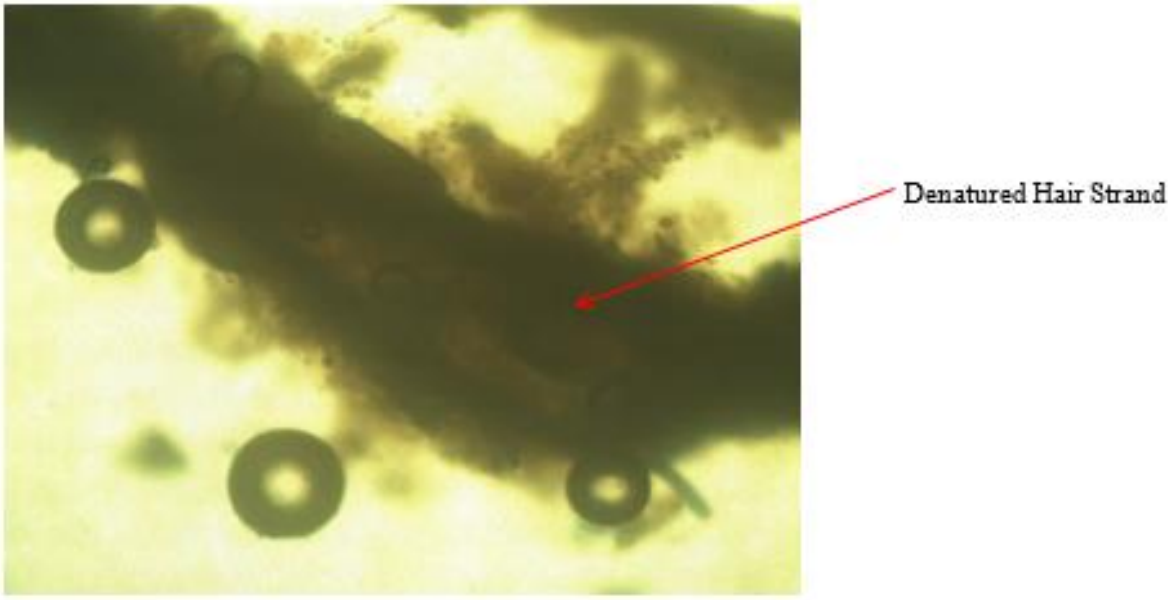

Figure 7: Denatured hair strands by fungal isolates

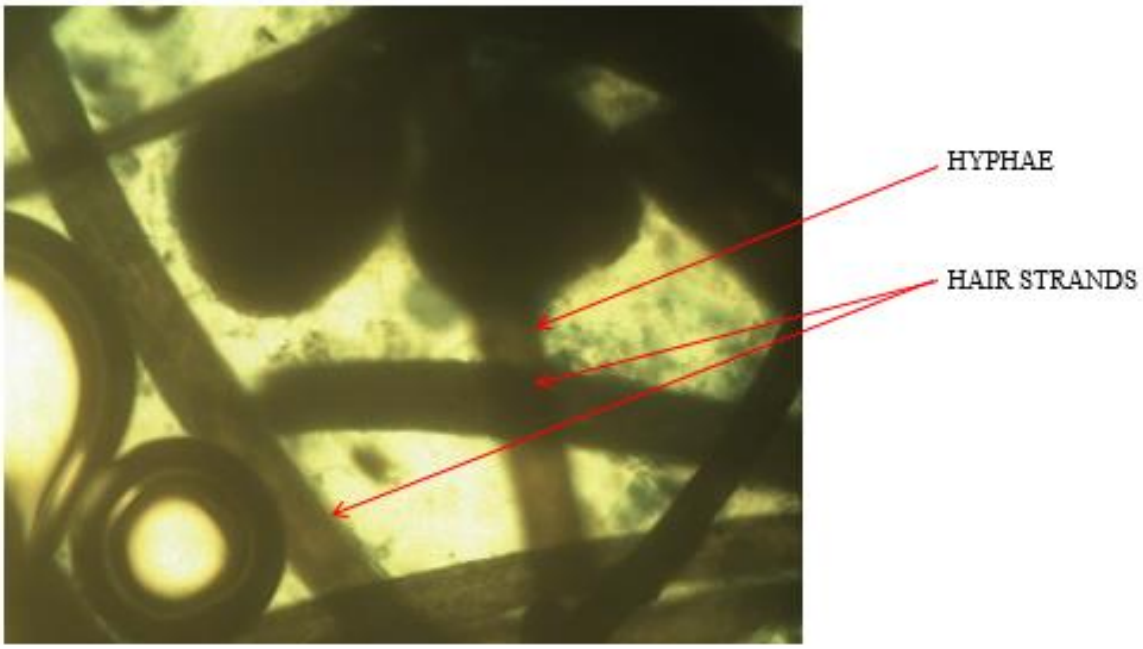

Figure 8: Intact hair strands with hyphae growing around it

CONCLUSION

This study has revealed a strong link between cattle rearing and aeromycoflora as substantial quantity of non-dermatophytic moulds were observed on agar plates exposed within the cattle market environment. This therefore implies that the air quality within the cattle market may be compromised thereby predisposing people within that environment to health challenges. Also, soil samples analyzed from the markets environment revealed presence of similar non-dermatophytic moulds and keratinophilic

fungi. This study therefore, suggests regular checks by the veterinary doctors, if possible cattle should be kept in well-constructed barns and also government should ensure that cattle markets are fumigated regularly as this will go a long way in getting rid of these pollutants in the atmosphere and also decontaminant the soil thereby getting rid of some of these moulds.

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