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The Succession and Prevalence of Pathogenic Fungi in Horse Dung

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ABSTRACT: The process of fungal succession in horse dung began with the Zygomycetes which included species of *Pilaria* occurring in the first three days of incubation. This was followed by *Pilobolus sp* from day 7 to day 21 after which *Rhizopus* started sporulating from day 14 to day 28. No Ascomycetes was recorded, probably due to hyphal antagonism by some species of *Coprinus*, a Basidiomycetes that sporulated until the dung decomposed. The prevalence of pathogenic fungi was investigated in 12 dung samples collected. A total of 8 isolates of medically important fungi were recovered from three batches. Total mean fungal counts of 165, 18 and 38 x 10^7cfu/g were obtained in batches A, B and C, respectively. Among the isolates that prevailed in horse dung in the batches were *Aspergillus fumigatus, Candida* sp , *Rhizopus* sp., *Fusarium sp*., *Penicillium digitatum*, *Penicillium chrysogenum*, *Paecilomyces* and *Aspergillus flavus*. The data analysis showed that there was significant difference at $P<0.05$ in isolation frequency of the means from each batch of dung samples collected from horse stables.

Keywords: Fungal succession, sporulation, pathogenic, medically important fungi**.**

Introduction

Dung, the faecal waste material passed by all animals, is a valuable part of the cycle of nutrients and energy in the ecosystem. In spite of being constantly produced and deposited in large quantities at convenient locations and having passed through an animal's digestive tractt still retains many nutrients. Thus, it attracts its own fauna and flora consisting of bacteria, fungi, protozoa, platyhelminths, nematodes, annelids, and arthropods. From a fungal point of view, herbivore dung is the more interesting, since bacteria are largely responsible for the breakdown of carnivore and omnivore dung.

Herbivore dung supports a wide variety of coprophilous fungi. The word coprophilous literally means "dung loving"(1) The nature of herbivore dung depends on the efficiency of the digestive tract, which, in turn, depends on the animal's digestive anatomy and its microflora (2). Dung decomposes rapidly because the macerated material has high nitrogen content, available aeration, and a high water content that is protected from fluctuations. Horses spend most of their time in pastures or stable where the majority of their excrement is deposited, collected and managed. Horse dung is about 70-80% liquid and 20-30% solids. The liquid portion is quickly retained by soil or vaporizes rapidly into the atmosphere (3).

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Although mostly microscopic, dung fungi include some zygomycetes, especially *Pilobolus*, the hat-thrower, which aims and shoots its sporangia up to 2 metres toward the light, *Pilaira* and, *Rhizopus*. There are 175 genera of ascomycetes, including many which have light-seeking (or phototropic) mechanisms built into the tips of their asci, or the necks of their perithecial ascomata, so that their spores can be shot away from the dung, examples of which are: *Saccobolus, Podospora, and Ascobolus*. There are also a number of basidiomycetes, mostly of the agaric genus *Coprinus*, which conduct chemical warfare against the hyphae of other fungi, and after vanquishing them, produce wave after wave of delicate and beautiful but ephemeral mushrooms that rain dark spores into the air (4). Succession involves a change in the species composition and species abundance values over a period of time. The succession phenomenon in nature can be ascribed to the influence of a number of factors which can be ecological or of a genetic nature and could possibly play a role in determining the species composition and species diversity of a substrate at any time (3).

The significance that these diverse fungal habitats have on the increasing number of immunosuppressed individuals is just beginning to be known. The accidental or permanent presence of fungi in animals, plants, soils and watercourses should not be taken too lightly because they constitute the source where potential pathogens will be contracted. Pathogenic fungi secrete various lytic enzymes, such as proteases and lipases, which may play a part in invading and parasitizing the host tissue. These enzymes help the fungi by altering the immediate environment or act directly by digesting host proteins and lipids, thus providing a source of energy (5). In various degrees, stable operatives come in contact with this dung which is also used as manure and often washed into water bodies making them susceptible to disease (6)

The objectives of this study, therefore include : i)To record fungal succession on decomposing horse dung ; ii)To isolate and identify fungi from horse dung in a University Community and iii)To study the health implication of the dung on the immediate community and its subsequent effect on the human population at large.

Materials and Methods

Study site

The study site was located in the stable of Bells University of Technology Ota, Ogun State, Nigeria where the horses deposit their dung. This dung site is 30.8 meters away from the cafeteria and clinic respectively.

Methods

Samples were collected in triplicate randomly from 10 horses residing in the stable at Bells University of Technology Ota. Method of collection was based on scooping the upper most layer of the dung with a sterile disposable plastic spoon into sterile plastic bags immediately the horses defecate. Upon return to the laboratory, the dung samples were processed immediately.

Fungal Succession: Fresh dung samples were collected in sterile plastic bags. Layers of sterile filter papers dampened with sterile distilled water were placed at the btom of the jar and a lump of dung was placed on the moist filter paper. The jar was placed close to a light source and parafilm was used as lid to permit for aerobic condition at room temperature. The succession was then examined frequently using a magnifying lens for weeks. Fruiting bodies were removed and mounted in water for examination and identification at higher magnification (x100).

Sample Processing

Each horse dung (1 gram) sample was suspended in 9mls of sterile distilled water. This was homogenized and allowed to stand for 15 minutes. A tenfold serial dilution was carried out after which 0.1 ml of each of the diluents was used as inoculum. Sterile Petri dishes containing potato dextrose agar supplemented with chloramphenicol ($0.5gl^{-1}$) was inoculated with the supernatant and incubated at room temperature ($28^{o}C$) for 4 days. Fungal counts were done for every plate and the mean recorded.

After fungal count, different growth on the previous Petri-dishes was further sub cultured onto potato dextrose agar for 4 days to attain pure cultures which were then used for identification (7).

Identification of Fungal Isolates

Identification was based on the morphology and physiological characteristics (8); Tests for ascospores and ballistospores were performed following the procedures of Obire *et al*. (9).

Statistical Analysis: Each sample was analysed in triplicate and data were analysed using Student t-test. Statistical level of significance at $P < 0.05$ was determined (10).

Results

Mean fungal counts of 165, 18 and 38 x 10^7cfu/g were obtained in the three batches of horse dung collected from the stable. Out of the 12 dung samples collected from Bells University stable, a total of 8 isolates were recovered from the plating technique. For fungal succession on the dung, a total of 4 genera were recorded comprising of 3 Zygomycetes, 1 Basidiomycetes and no Ascomycetes (Table 1).

Table 1: Fungal succession on horse dung

Fungi	Days of incubation on plates				
	3days	7days	14days	21days	28days
Zygomycetes:					
Pilaria species	*	*	-	-	-
Pilobulus species	-	*	*	*	-
Rhizopus species	-	-	*	*	*
Basidiomycetes:					
Coprinus species	-	-	-	*	*
Ascomycetes: (No isolates)	-	-	-	-	-

(An asterisk indicates presence of at least one fruit-body)

The Zygomycetes included species of *Pilobolus, Pilaria* (Figs. 1&2) *and Rhizopus.* The Basidiomycetes was represented by species of *Coprinus* (Fig. 3). *Pilaira and Pilobolus* spp. were the earliest with most *Pilaria* being observed within the first three days (Fig. 2), and *Pilobolus* taking an average of three days to develop (Fig.1). The Basidiocarp of *Coprinus,* the only Basidiomycetes to sporulate occurred after about three weeks (Fig. 3). The isolate from the plating technique were of the species; *Aspergillus fumigatus, Aspergillus flavus, Penicillium digitatum, Penicillium chrysogenum, Fusarium, Candida, Rhizopus, Paecilomyces* (Figs. 4- 6).

From the results (Figs. 4-6), the isolates varied in their frequency of occurrences. *Candida* recorded the highest frequency of occurrence in all 3 batches of samples collected, followed by *Aspergillus fumigatus* (56%) and the least was *Rhizopus* (16%). *Rhizopus* and *Fusarium* were second in abundance in batch two with a percentage occurrence of 33.30%, respectively. *Penicillium digitatum, Penicillium chrysogenum* and *Paecilomyces* had the least percentage occurrences of 5.56%, respectively (Fig. 5). *Fusarium* and *Aspergillus flavus* were second in abundance with percentage occurrences of 5.3%, respectively while *Rhizopus* and *Penicillium digitatum* had the least occurrences of 2.6%, respectively in the third batch (Fig. 6).

The study revealed that pathogenic fungi occurred in all three batches of samples collected with *Candida* having the highest frequency of occurrence in all three batches (Figs.4-6), while, *Fusarium* sp. was second in two batches of dung samples(Figs. 5 & 6) and *Aspergillus fumigatus* had the second highest occurrence in batch one (Fig. 4).

1. BLACK SPORES OF *Pilobolus* SPORANGIUM OF *Pilobolus*

Fig. 1 : *Pilobolus* from horse dung sample collected from Bells University of Technology stables

ii. SPORANGIUM OF *Pilaira* sporulating toward light source.

Fig. 2: *Pilaira* occurring on horse dung sample collected from Bells University of Technology stables.

Fig. 3 : *Coprinus* species occurring on horse dung sample collected from Bells University of Technology stables.

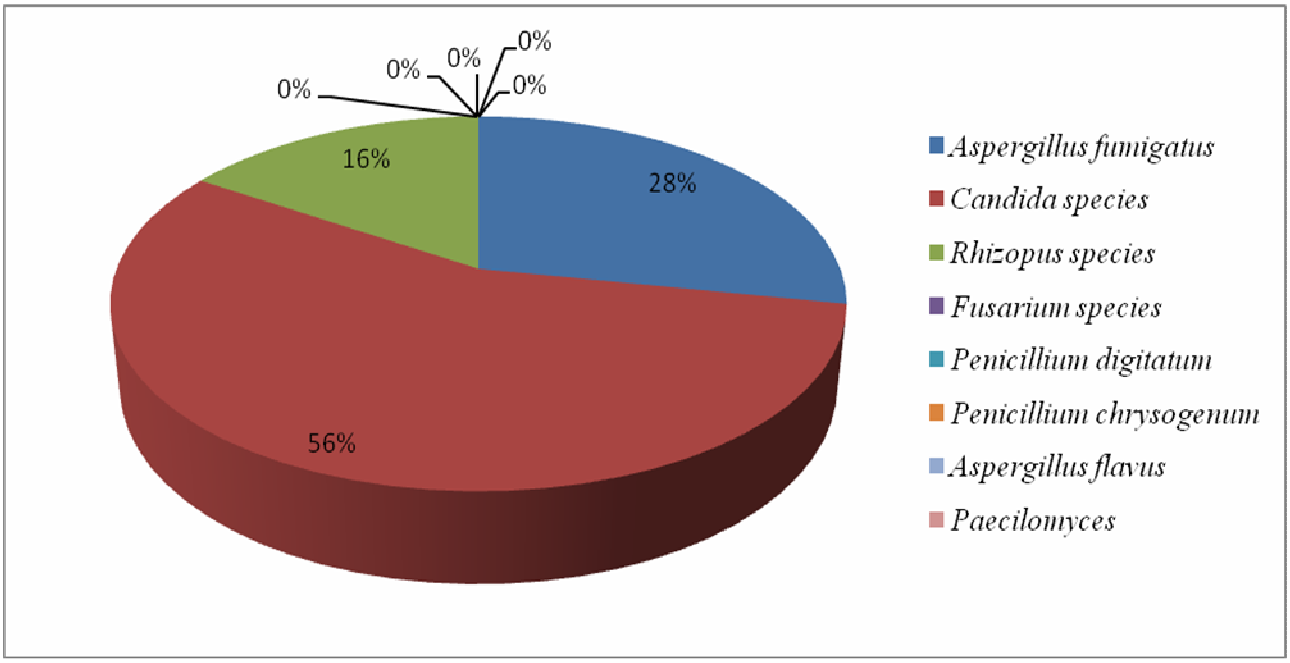

Figure 4: First set of isolates recovered from batch one horse dung samples collected from Bells University of Technology stables.

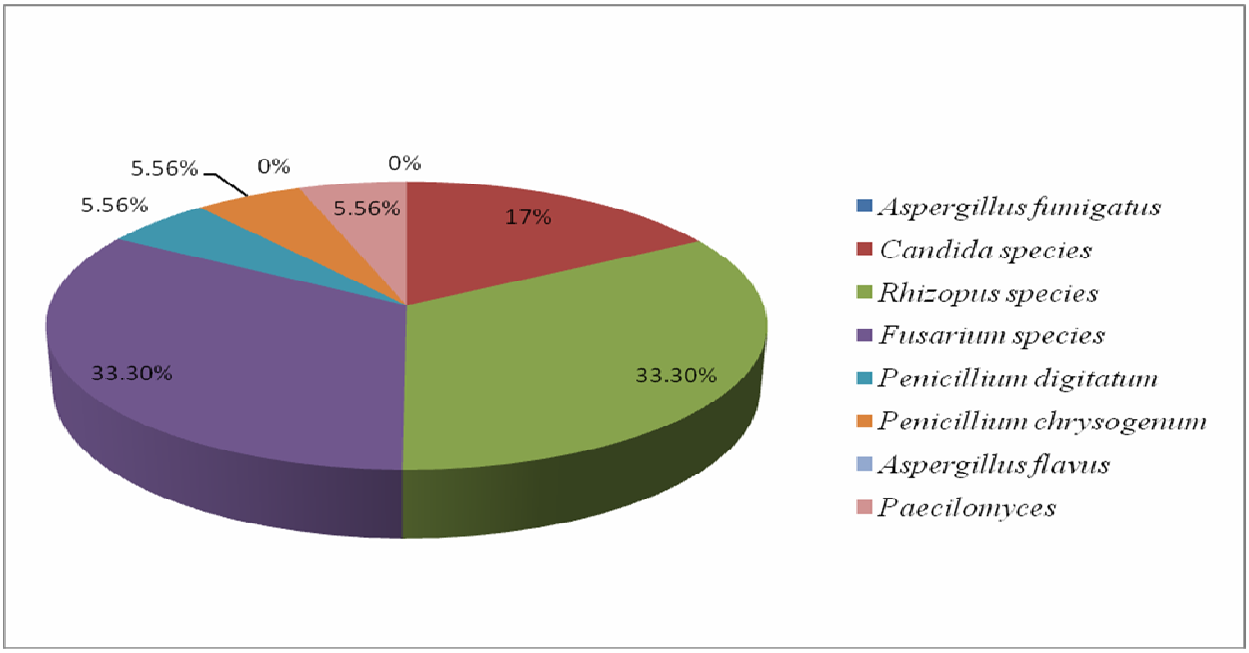

Figure 5: Second set of isolates recovered from batch two horse dung samples collected from Bells University of Technology stables.

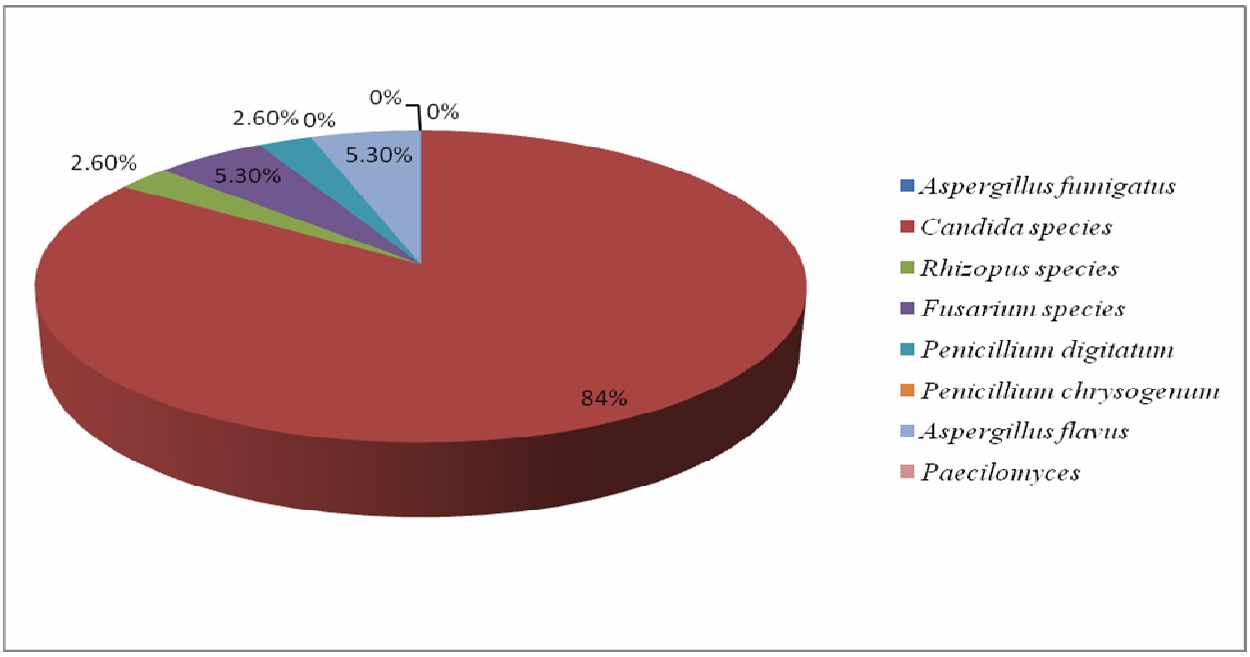

Figure 6 : Third set of isolates recovered from batch three horse dung samples collected from Bells University of Technology stables.

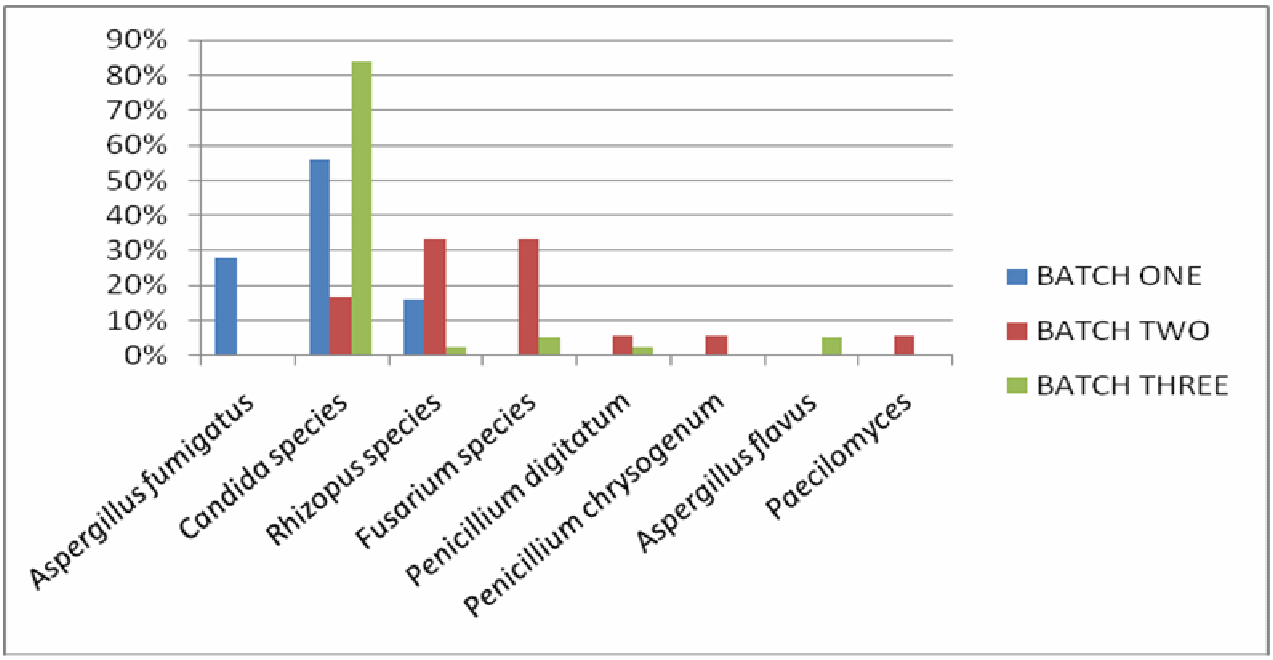

Figure 7 : Comparison of the occurrence of the isolates from the three batches of horse dung samples .

Discussion

The results revealed that horse dung served as an important reservoir for a few medically important fungi in the environment. This can be attributed to the fact that this dung contains important nutrients for the growth of these pathogenic fungi. In the process of dung succession, *Pilaria* (Fig. 2)observed first was not surprising, given the more complex nature of *Pilobolus* sporangiophore (fig. 1) and the possibility that more investment is needed for its production than the less sophisticated *Pilaira* sporangiophore,these organisms are Zygomycetes and pathogens of Mucorales (11). The inability of ascomycetes to fruit could be due to hyphal antagonism by some *Coprinus* species affecting especially early succession species as reported by Harper and Webster, (12), which may explain the early fruiting of *Coprinus*. Also environmental factors such as temperature fluctuations, photoperiodicities, water potential of substrates, the role of other dung inhabiting organisms, and interspecific fungal species competition, will definitely influence the species composition of any substrate all of which could have affected the sporulation of ascomycetes (13).

Although much work has not being done on horses, the set of fungi isolated from the horse dung samples collected from Bells University stables can be related to the work of Chukwura *et al* (14) in which the prevalent pathogenic fungi in cow and goat droppings were *Aspergillus fumigatus, Aspergillus flavus, Paecilomyces, Candida, Fusarium and Penicillium chrysogenum*.

One of the most important pathogenic fungus isolated is *Candida*, occurring on a large scale (56%, 17%, 84%, respectively in the three batches) of the samples collected (figs 4 -7).. Candidiasis is a primary or secondary infection involving members of the genus *Candida* mainly *Candida albicans* (15). The clinical manifestations of this disease are varied, ranging from acute, sub acute to chronic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, and gastrointestinal tract or become systemic. Since *Candida albicans* is an endogenous species, the disease represents an opportunistic infection, it is also involved in several allergic conditions such as eczema, and hence, horse dung could pose a great health risk to immune-compromised individuals.

Another important pathogenic fungus isolated is *Aspergillus fumigatus* (Fig. 4). Other species of the *Aspergillus* were also recovered and include *Aspergillus flavus* (fig.6). Bronchial and pulmonary diseases have been traced to *Aspergillus fumigatus*. The type of disease caused by *Aspergillus* is varied, ranging from an allergy type of illness to life threatening generalized infections. The severity of Aspergillosis is determined by various factors but the most important is the state of the immune system of the individual, which could be as a result of debilitating disease, chemotherapy, disruption of normal flora or use of antimicrobial agents. Hence, these microorganisms also cause opportunistic infections (16). Three different species in pure culture (*Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus nodulans*) have been isolated from different lesions in the lungs and heart in a single case (11). *Aspergillus flavus* is the cause of many primary infections of the nasal sinuses.

Aflatoxicosis is poisoning that result from ingestion of aflatoxins in contaminated feeds. The aflatoxins are a group of structurally related toxic compounds produced by certain strains of the fungus *Aspergillus flavus.* The frequency of *Aspergillus fumigatus* isolated from the samples is average. This implies that horse dung could be an important source of Aspergillosis for members residing in community especially those handling this dung with bare hands (14).

Zygomycosis is an opportunistic infection caused by saprophytic fungi, notably species of *Rhizopus* with high percentage frequencies of 16%, 33%, 2.6%, respectively in the samples collected (Figures 4-6). This organism closely followed *Candida* sp when comparing the occurrence of isolates in the three batches of dung (Fig. 7). *Rhizopus* sp are common saprophytes of soil and manure. The major predisposing factors are acute diabetes mellitus, debilitating diseases and widespread use of broad spectrum antibiotics. Zygomycosis may lead to rhinocerebral, pulmonary, gastrointestinal, disseminated, dermal, subcutaneous Zygomycosis (17) therefore horse dung should be handled with care especially when used as manure to grow vegetables.

Fusarium species occurring in Figs. 5 and 6, a known plant pathogen exist in nature and encroaches on humans and manifest as onychomycosis which usually affects the great toe nail with the pathology occurring after trauma (18). At present, several *Fusarium* species have been increasingly reported causing colonization and localized infections (19). Infections have also reported in cancer patients undergoing chemotherapy or bone marrow transplantation causing Fusariosis apart from onychomycosis. *Fusarium* also causes serious morbidity and mortality, and may mimic Aspergillosis. *Fusarium* and *Aspergillus* are similar not only in tissue morphology but in their propensity for vascular invasion which causes thrombosis and tissue necrosis (20). *Paecilomyces* occurring in a minimal percentage frequency of 5.56% (Fig. 5), in humans causes infection known as paecilomycosis. *Paecilomyces* is among the emerging causative agent of opportunistic mycoses in immunocompromised hosts. *Paecilomyces* can also cause allergic disorders (21). *Penicillium* (Figs. 5and 6) also occurring minimally (5.56%) is known to cause penicilliosis (22) and many of them produce mycotoxins, including penicillic acid. Potential

ingestion of mouldy feed and food are significant hazards. Inhalation of spores contaminating mycotoxins is implicated as a contributory factor for organic dust syndrome. Penicillium is also a proven causal agent of hay fever, allergy, and asthma and hypersensitivity pneumonitis.

Conclusion and Recommendation

The results have showed that medically important fungi abound in horse dung. This indicates that horse dung pose an important health risk problem to members in the community particularly immune-compromised patients visiting the clinic opposite the stables due to the fact that most of the fungi of medical importance isolated are opportunistic. The use of bare hands to handle this dung is unadvisable to those working in the stables therefore, protective coverings should be worn to avoid direct contact with skin. Preparation of foods in the cafeteria should be done properly to eliminate potential spores on food surfaces and vegetables obtained from around that area should be properly washed before consumption as this could serve as an important source of infection. Most individuals residing in the University often pass the stables thereby transporting spores from the stable region to other parts of the University. During the rainy season, most of the dung would be washed away into water bodies and around the University area, thereby enhancing a widespread dissemination of fungal spores. The horses and their stables should therefore be moved some distance away from these places.

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