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Occurrence of molds (fungi) in indoor tissue culture laboratories

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Abstract

Laboratory indoor environments play important roles in human health and laboratory experiments. Contamination wastes time and resources as well as posing serious risks to human health due to toxicity. Fungi (molds), like other microorganisms are ubiquitous in distribution and in complexity towards causing human diseases. However, the awareness of the number and nature of molds in any giving laboratory per time will help to better strategize their control mechanisms. The aim of this study was to evaluate some tissue culture laboratory surfaces and indoor air for fungal contamination in some selected Laboratories in the Federal Capital territory (FCT), Abuja, Nigeria. Using swab and air sampling procedures, fungi were isolated from plantlets, tables, wall and the air of the laboratory rooms of fifteen tissue culture laboratories and cultured on Streptomycin-supplemented Potato Dextrose Agar (PDA). The discrete colonies that developed were identified using morphological and microscopical characterization. The fungal species isolated from these laboratories include; *Aspergillus niger, Alterneria sp., Aspergillus terreus, Aspergillus flavus, Cladosporium sp., Rhizopus sp., Penicillium sp., Fusarium sp. and Geotrichum sp.* The results shown that most of the laboratories were highly contaminated and that Aspergillus was the predominant fungal genera and A. niger recorded the highest overal occurence. Since using plant tissue culture techniques holds promise in substantially augmenting the number of novel cultivars and genotypes of many fruit crops, adhering to standard operating procedures is highly recommended in the studied areas and Nigeria as a whole.

Keywords: Tissue Culture; Contamination; Molds; Aspergillus; Indoor and Human Health

1. Introduction

Microbiology and Biotechnology laboratory indoor environments play important roles in human health and laboratory experiments. Obvious research development takes place in most of these laboratories (Chew *et al.*, 2016). Contamination of cultures and experimental procedures often wastes time and resources as well as posing serious risks to human health due to toxicity. Many cases, there are often mold growth where there is high moisture due to water damage or constant high humidity. From these places, invisible spores can be transported indoors on clothing, shoes, or pets, or they may blow in through open doors, windows, or ventilation systems. It is recorded that spores can survive on wood, paper, carpet, soil, plants, fabrics, and other surfaces. When they are right conditions, such as humidity and temperature, mold spores begin to germinate, gradually producing an increasingly expanding network of hyphae (Larenas *et al*., 2016). One of the symtoms of the presence of mould is that they may be perceived by the nose because of this, the pungent organic compounds molds release into the air. The presence of musty odor raises suspicion that a major mold contamination is present before they are seen physically. Also, an initial indoor environmental assessment involves a visual inspection of the floor, walls, windows, and air handling system. Any damp areas in the laboratory often ought to be monitored for the presence of molds from where they the spores can move to other parts of the laboratory where contamination of laboratory procedures do take place (Sothern *et al*., 2022).

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1.1. Statement of Problem

Fungi (molds), like other microorganisms are ubiquitous in distribution and in complexity towards causing human diseases. Mold is ubiquitous outdoors, and it is readily introduced or physically transported into the workplaces and laboratory procedures (Mendell and Adams, 2022). Report has shown that there are approximately 100 indoor molds that have been identified as potentially hazardous to human health. Some of them are found both in the air and dust in indoor experimental laboratory areas.

1.2. Justification

In most laboratory, the awareness of the number and nature of molds in any giving laboratory per time will help to better strategize their control mechanisms. In indoor mold investigations, air sampling is performed to detect and quantify airborne mold spores and other ill-health associated with their presence such as allergens to assess the level and severity of their health effects due to the human exposure. Understanding their presence and prevalence will also help to monitor the effectiveness of control measures during remediation, and for post-remediation clearance testing as well as other control plans that might be required. Air samples analysis of cultured media has been reported as an effective method of studying airborne fungi in an enclosed area (Aktas *et al*., 2018). The aim of this study was to evaluate some tissue culture laboratory surfaces and indoor air for fungal contamination in some selected Laboratories in the Federal Capital territory (FCT), Abuja, Nigeria.

2. Materials and Methods

Using swab and air sampling procedures, fungi were isolated from plantlets, tables, wall and the air of the laboratory rooms of a tissue culture laboratory and cultured on Streptomycin-supplemented Potato Dextrose Agar (PDA) for 3-5 days at 28^0C. Using the method of Kator et al., 2015, the discrete colonies that developed were studied for the identification of the fungi, both from the surface and the reverse views of the respective culture on the media plates. For the macroscopic identification, colony characteristics including the appearance, change in medium colour (reverse) and growth rate were observed on the Petri plates. For the microscopic identification, a thin smear of fungi isolates from 5–7-day-old cultures were inoculated aseptically on a clean glass slide using a sterile inoculating loop. A drop of lactophenol cotton blue was added and the mixture was covered with a cover slip and viewed under 40x objective of the light microscope. The respective shapes of the conidia, the conidiophores and all other microscopic details were observed and taken note of, and the features were matched according to the method of Kator et al. (2018).

3. Results and Discussion

The fungi contamination has been reported as a constant problem, which often compromise development of all *in vitro* techniques. This study aimed at investigating the sources of microbial contamination in tissue culture laboratories in FCT, Abuja, Nigeria. Nineteen microbial contaminants (consisting of eleven bacteria and eight fungi) were found associated with the tissue culture plants and the laboratory environments. This result agrees with early experiments by Miller *et al*., (2020).

The fungal species and the frequency of occurrence is as shown on Table 1. They include; *Aspergillus niger, Alterneria sp., Aspergillus terreus, Aspergillus flavus, Cladosporium sp., Rhizopus sp., Penicillium sp., Fusarium sp.* and *Geotrichum sp.* The results shown that most of the laboratories were highly contaminated and that Aspergillus was the predominant fungal genera and *A. niger* recorded the highest occurrence. Nine species of fungi were found associated with the tissue culture laboratory contamination. High incidence of Aspergillus has been reported in several literatures as a major contaminating mold (Shafiq *et al*., 2015).

The rate of occurrence of Aspergillus isolates was higher than that of other fungal isolates in the plant tissue cultures studied (Table 1).

Table 1 Occurrence of fungi (mold) in the tissue culture Laboratory studied

Fungi Isolate	Sample source/ Frequency of Occurrence (%)			
	Plantlets	Table	Wall	Air
Fusarium sp.	7.3	12.5	-	21.7
Rhizopus sp.	17.3	-	22.8	-
Asp. terreus	27.4	-	-	16.9
Alternaria sp.	2.7	-	-	12.0
Asp. niger	45.3	39.2	36.6	-
Penicilium sp.	-	28.5	26.1	15.8
Cladosporium sp.	-	19.8	-	-
Geotricum sp.	-	-	14.5	-
Asp. flavus	-	-	-	33.6

Plantlet had the highest presence of *A. niger* (45.3). The laboratory walls and tables also harbored fungal contaminants. The laboratory indoor air was found associated with all the contaminating fungi isolated in the work.

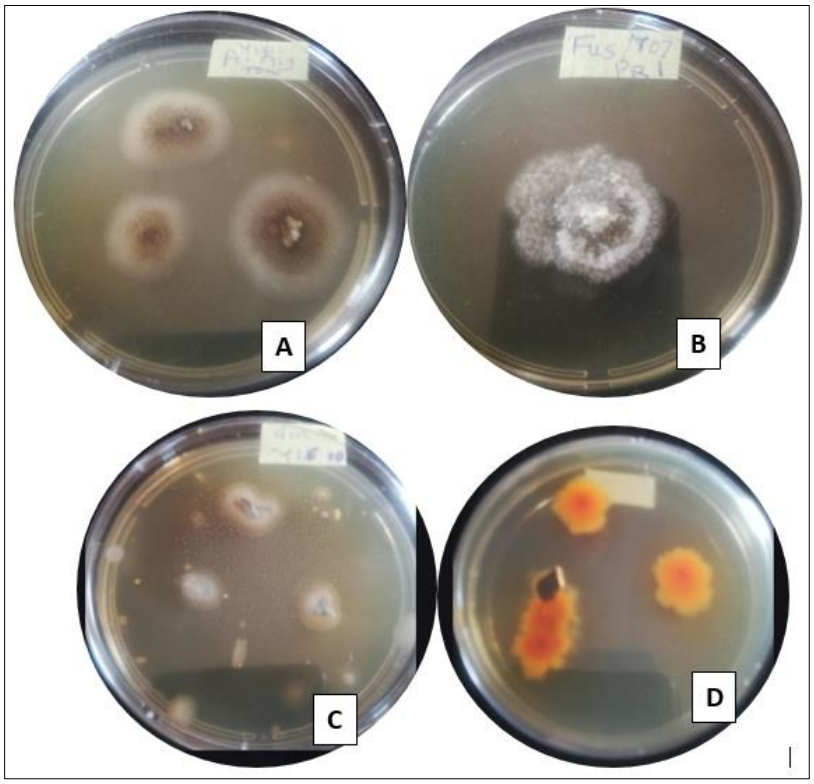

Figure 1 Colonial Morphologies of Some of the Fungal Isolated; A = *Aspergillus niger; Fusarium sp.* (Matured) and C & D = Front and Reverse view of Fusarium sp. (Young)

Saglani *et al.*, 2021 has earlier reported severe contamination of laboratory air space with fungi. Fungal contaminants of plant tissue cultures have also been reported by Risslegger *et al.,* (2017). Most of these fungal contaminants have been reported to increase culture inferiority, the presence of latent infections can result in variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting (Lackner *et al.,* 2017). Although some of these contaminants might be endogenously embedded in the plant tissues, some might also have emanated from contaminated tools, which were not investigated. Ullmann *et al.,* (2017) reported that the spread of fungal contamination was caused by insufficient flaming of contaminated tools and by survival of fungal spores in 96% ethanol for a few hours. While flaming for 5s or more (till the inoculating tools become red hot) did eliminate the spread of bacterial contamination at transfers. The use of Bacti-Cinerator during 12s, by inserting inoculating tools in the middle of the heating element, not on the edges has also proved effective (Jukic *et al*., 2017). Tissue culture vessels are always closed with loose-fitting caps to allow gaseous exchange with the external environment. However, Mites and thrips carrying spores of fungi might enter thereby contaminating it. Therefore, there is need for proper observation of procedures to reduce the incidence of fungi in the sensitive laboratories.

Aspergillus complex refers to the Aspergillus species represented by common groups as the *Aspergillus fumigatus* species complex, *Aspergillus flavus* species complex, *Aspergillus terreus* species complex, and *Aspergillus niger* species complex (Arendrup *et al.,* 2012). The occurrence of these species in the laboratory is as shown in Fig. I.

3.1. The Aspergillus complex

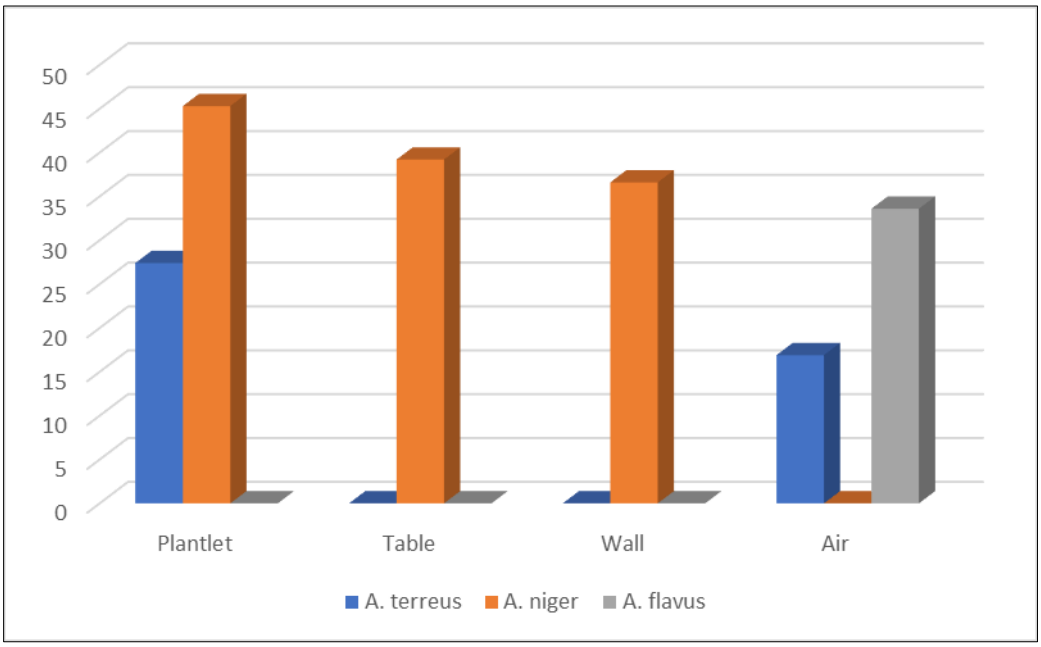

Figure 2 Occurrence of Aspergillus complex in the tissue culture Laboratory studied

Aspergillus niger is associated with many crops and plant parts that are moist. While it is less likely to cause disease in humans and animals compared to other common species of Aspergillus, it can still cause opportunistic invasive pulmonary aspergillosis especially in individuals with a compromised immune system (Chowdhary *et al*., 2017). This can occur on inhalation of spores from a damaged epithelial lining and respiratory tract system, leading to severe lung disease. *Aspergillus niger* can also cause aspergillosis in horticulture and tissue culture workers who are frequently exposed to inhalation of peat dust and decaying explants, which are rich in Aspergillus spores. In addition, *Aspergillus niger* is a common cause of otomycosis, a type of fungal ear infection (Risslegger *et al.,* 2018). Otomycosis is associated with temporary impaired hearing, pain, and severe cases that can damage the ear canal and the tympanic membrane. It is typically seen in tropical and subtropical regions and is more prevalent in individuals with a history of exposure to contaminated water, ear canal trauma, or the use of hearing aids. Thus, the need to document the occurrence and status of the organisms in the air of laboratory cannot be over emphasized to understand the nature of their compositions and how they can affect the laboratory procedures and the health risks of the technicians (Steinbach *et al*., 2012).

Although, not all *Alternaria* species are pests and pathogens; some have shown promise as biocontrol agents against invasive plant species. Some species have also been reported as endophytic microorganisms with highly bioactive

metabolites. However, their presence as contaminants in tissue culture laboratories is of much concern. They are normal agents of decay and decomposition and this explains why twigs and other explant materials should not be left undisposed from the tissue culture environments (Blatzer *et al*., 2015). The spores of this fungus are airborne and are found in the soil and water, as well as indoors and on objects such as laboratory benches.

Aspergillus terreus is a filamentous fungi species that is commonly found in soil, decaying plant material, and indoor environments such as tissue culture laboratories. Although it is typically considered to be of low pathogenicity, reports have shown that it can still cause a range of health conditions, particularly in individuals with compromised immune systems. Infections associated with *A. terreus* have been reported increasingly as the cause of acute and chronic aspergillosis (Lewis *et al.* 2012). Invasive diseases are associated with a high rate of dissemination of spores through the air and may show poor sanitary conditions. It is an opportunistic pathogen that can cause both systemic and superficial infections (Blum *et al*., 2013). It is responsible for illnesses related to aspergillosis, affecting the respiratory system. Hey can also cause other illnesses such as allergic bronchopulmonary aspergillosis, aspergilloma, and invasive diseases.

Aspergillus flavus is a saprotrophic and pathogenic fungus with a highly well-distributed status. It is best known to colonizing plants such as cereal grains, legumes and tree nuts (Salas *et al.*, 2013). It is unique in that it is a thermotolerant fungus, as such can survive at temperatures that other fungi cannot. *A. flavus* can contribute to the storage rots, especially when the plant material is stored at high moisture levels. This might be accounted for the occurrence of the fungus in tissue culture environment because, twigs of plant parts left from explants untidy might be responsible for hosting the spores by providing the necessary conditions for colonization (Binder *et al*, 2015).

A. flavus grows and thrives in hot and humid climate, which thus explain their high incidence in Africa. The pathogen is also recorded to causing liver cancer through consumption of contaminated feed or aspergillosis through invasive growth. (). After A. fumigatus, A. flavus is reported to be the second-leading cause of aspergillosis through the inhalation of their spores (). Two allergens have been characterized in *A. flavus*: Asp fl 13 and Asp fl 18. In tropical and warm climates, *A. flavus* has been shown to cause keratitis in about 80% of infections.

4. Conclusion

Since the use of plant tissue culture techniques have several biotechnology advantages, and promise in substantially adding values to the number of novel cultivars and genotypes of many fruit crops, it is noteworthy to adhere to basic standard operating procedures during the laboratory operation as well as designing efficient cleanliness of those laboratories. It is also recommended here that this type of study be extended to other laboratories in Nigeria in order to have more knowledge of the status of contaminating fungi in such laboratories. The knowledge gleaned in this study should be communicated to health care givers for their knowledge and planning purposes. Comparison of levels of contamination of tissue culture laboratories with other non-tissue laboratories should also be carried out in order to have better understanding of the possible sources and movement of contaminating agents. Lastly, reduction of humidity by increasing ventilation, covering cold surfaces such as water pipes with insulation, and increasing the air temperature to reduce surface humidity should be encouraged in the laboratory.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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