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Isolation and Identification of Fungal Pathogens Contaminating Some Coffee Powder Marketed in the City of Abuja

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ABSTRACT

Introduction: Coffee is the most important commercial crop in the economy of many countries in the world. The processing of raw coffee bean to coffee powder is subject to various operations of contamination by microorganisms during growth, after harvesting (when the beans are de-hulled, washed and stored) and during storing. This study was conducted to isolate and identify the contaminating fungi in some coffee powder marketed in the city of Abuja.

Materials and Methods: In this study, four different Coffee samples (Gorilla's Coffee, Nescafe 3 in 1, Café Najjar and Alcafe) were examined for fungi growth using potato dextrose agar.

Results: The samples were contaminated by two fungi Aspergillus fumigatus from Gorilla's Coffee and Candida albicans from Café Najjar with their occurrence frequencies of 4.16%.

Conclusion: The isolated species were environmental contaminants, indicating poor hygienic practices during postharvest handling and processing. Aspergillus fumigatus is a ubiquitous saprophytic mold that forms airborne spores (conidia). Therefore, strict Good manufacturing practice (GMP) and hygienic practices should be followed to reduce fungal contamination to ensure the products quality and safety.

Key words: Fungal pathogens, Coffee, Isolation, Identification

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INTRODUCTION

Coffee belongs to the family *Rubiaceae*, which is widely disseminated throughout the tropical regions of the world [1]. It is one of the most important agricultural products of world trade which is exceeded only by oil [2]. Several legends exist on the discovery of this plant [3]. Due to the high demand and increase of sales of coffee beverages worldwide, most coffee producing countries aim to obtain a good quality and safe product by applying food control systems side the production and trading chains [2]. However, some factors are out of control such as environmental condition and undesirable changes in coffee bean characteristics because of logistic infra-structures that lead to contamination of fungal in coffee beans [4]. The contamination can occur at different stages of growing, harvesting, processing, transport, and storage. The high density of this contaminant generally corresponds to poor cleaning practices or the use of unhygienic techniques in using of equipment [5].

Besides, fungal contamination could occur during transportation. Measurements of relative humidity in containers during shipping showed that condensation can occur and cause re-wetting of the beans [4]. Therefore, the aim of this study was to assess possible fungal pathogens contaminating some coffee powders marketed in Abuja, Nigeria.

MATERIALS AND METHODS

Samples collection areas

Ten varieties of Coffee Powder were collected from different locations of the Federal Capital

Territory of Nigeria: Jabi Mall (904 '35 °N 7°25'30°E); Wuse Market (9.07° N, 7.46° E) Tipper Garage Market, Gwarimpa Estate (9°06' 33°N 7°24' 08E); Kado Bimko Market (9°05'03N 7°25'04E); Mabuchi Market (9,09° 33°N 7,44° E) and SAHAD Store Ltd (9,05°N 7,49°E) to isolate and identify fungal pathogens responsible for Commercial Coffee Powder. But only four samples of the collected Coffee Powder were randomly used for this experiment.

Methods

Total of Ten different coffee powder samples from various places were purchased across the local markets and generally in various settlement covering particularly Abuja with different brands. The samples were kept in sterile polyethylene bag during the transportation to the laboratory. All the samples were kept place till ready for use. The samples were classified into four categories (A) Gorilla's Coffee, (B) 3 in1 Coffee, (C) Café Najjar and (D) ALCAFE. Each sample was properly ready for analysis.

Agar preparation

Potato Dextrose Agar (PDA) was used in this study and was prepared according to the manufacture's guideline. Agar was added to allow the quick solidification of the media. Bacterial contamination was inhibited by adding 500mg of tetracycline into 500 ml of the agar solution prior to autoclaving and pouring into Petri dishes.

Sterilization of agar

Media was placed in the autoclave to allow for homogenization and sterilization. Reliable sterilization with moist heat requires temperatures above that of boiling water. These temperatures which are high is achieved through the principle of steam under pressure done in an autoclave. The Steam under pressure in an autoclave is about 15min with the temperature of 121℃. This steam in an autoclave can kill microorganism and their endospore in about 15 minutes. The temperature of water in autoclave increase above the standard water boiling point because of the chemistry principle which suggests that increase in gas lead to increase in temperature.

Sterilization of working bench

The working area was sterilized using alcohol 70° swap to prevent contamination.

Fill plates

The Petri dishes were labeled according to the Coffee sample that will be inoculated onto them and arranged on the working bench. The conical flask containing the autoclaved media was handled using a heat resistant glove and allow to cool handle with bare hand but not too cool to prevent solidification of media in the container. Then swirl without introduction bubbles before pouring into plates. The agar plates can solidify then turn upside down.

Chemicals

Sodium Chloride, Potato Dextrose Agar (TM MEDIA, India), Lacto phenol Cotton Blue reagent and Tetracycline were used.

Isolation of fungi

20 g of coffee powder sample was properly weighed and mixed with 100 mL of NaCl (Sodium Chloride) using beaker and allow to mix properly for about 2-3 min 1 ml of the mixture of suspension coffee was added to 9 mL of NaCl solution (MRD) 10-1 and mix using Vortex for 1min. A serial dilution of 10-1; 10-2; 10-3; 10-4; 10-5; 10-6; 10-7; 10-8 and 10-9 were respectably prepared. Each of the dilution 10-4; 10-5 and 10-6 was separated on plate of Potato Dextrose Agar. A total of (24) Plates were incubated at 30°C for 14 days and the colonies were observed macroscopically.

Identification of fungi

After incubation examination of culture was made. The fungal diversity present in the coffee samples was characterized and identified based on their morphological features under macroscope using a Scanning Electron Microscope (SEM).

RESULTS

Fungi isolates

Two contaminating fungi (*Aspergillus fumigatus* in Gorilla's Coffee and Candida albicans in Café Najjar) were isolated and identified). However, no growth was observed in Alcafé Coffee and Nescafe 3 in 1.

Aspergillus fumigatus appeared in a single petri dish of Agar containing Najjar Coffee and *Candida albicans* appeared in a single Petri dish of Agar inoculate by Gorilla's Coffee. No growth was observed on the agar inoculated with Alcafé

Table 1: Total fungal count in different coffee powder.

Coffee infected	Fungi isolated	Number isolated	Percentage of Contaminated Coffee
Gorilla's Coffee	*A. fumigatus*	1	25%
Café Najjar	*C. albicans*	1	25%
Alcafé coffee	Negative	0	0
Nescafe 3 in 1	Negative	0	0

Coffee plate and Nescafe 3 in 1 after 14 days; however, bacteria of the coliform species were observed (Table 1).

Therefore, in this study, we strongly believe that the pollution of the final coffee product may be as a result of poor personal hygiene, improper cleaning of storage (with uncontrolled temperature) of the final coffee products (coffee powders), preparation areas (kitchens or tea rooms) and unclean utensils (cups or glasses). Although, mishandling of raw and roasted coffee beans from the production factory could allow in fungal pathogens which, enhance the coffee contamination.

DISCUSSION

One of the fungi identified in this study is *Aspergillus fumigatus* which is among the most common fungi present in the environment, it can tolerate growth in different substrates and environmental conditions, and their complete elimination is difficult. *Aspergillus* was observed after the incubation of plated coffee sample from Gorillas coffee, which is in correlation with the result reported by Ayob, et al. [6]. Many studies revealed that *Aspergillus* species is a natural coffee contaminant and carried over from the field to storage. However, conidia or spore of *Aspergillus* is not heat resistant and usually destroyed by heat processes [7]. The heat stress contributed to the relatively rapid death of fungal spores [8]. Thus, the appearance of green colony of *Aspergillus* species in the coffee powders studied is a clear indication of air contamination.

The contamination of this fungi in coffee products may be associated with insufficient heat treatment during roasting or after heat treatment, during packaging, storage, or transportation due to the unhygienic environments [9]. However, in this study, we strongly believe that the pollution of the final coffee product may be as a result of poor personal hygiene, improper cleaning of storage (with uncontrolled temperature) of the final coffee products (coffee powders), preparation areas (kitchens or tea rooms) and unclean utensils (cups or glasses). Although, mishandling of raw and roasted coffee beans from the production factory could allow in fungal pathogens which, enhance the coffee contamination. The presence of *Aspergillus* species confirms the widespread natural contamination of coffee with these fungi [10]. But the presence of *Saccharomycetes* was surprising as this group of fungi is not a common environmental contaminant. *Candida albicans* was also found in café Najjar (sample C) after incubation for 14days. *Candida albicans* are pathogenic fungi and are rarely found in edible food product but other beneficial fungi have been reported as seen in the research report by [11]. According to Yadav et al. [12], *Candida albicans* is normally a harmless commensal of human beings, but it can cause superficial infections of the mucosa (oral/vaginal thrush) in healthy individuals and (rarely) infections of the skin or nails. It can also become invasive, causing life-threatening systemic and bloodstream infections in immunocompromised hosts, where the mortality rate can be as high as 50 % [13]. It is the most common cause of serious fungal infection and is a common cause of nosocomial infections in hospitals [14]. Some strains have been recognized that are resistant to azoles or echinocandins, which are the first-line antifungals for treatment of *C. albicans* infections [15].

CONCLUSION

This study was based on the isolation and identification of fungal pathogens contaminating some Coffee Powder marketed in the city of Abuja. Coffee is one of the world's most popular beverages. Thanks to its high levels of antioxidants and beneficial nutrients, it also quite healthy. In this study two contaminating fungi were isolated and identified as follows: *Aspergillus fumigatus* at 25% which is a ubiquitous saprophytic mold that forms airborne spores (conidia). Humans inhale, on average, hundreds of these infectious propagules daily. The fungal pathogen *Candida albicans* at 25% which is a highly specialized inhabitant of warm-blooded animals (mammals and birds). It preferentially colonizes mucosal surfaces and the skin but can also invade deeper-

lying tissues and cause systemic infections that are difficult to treat and frequently lethal. Therefore, strict Good manufacturing practice (GMP) and hygienic practices should be followed to reduce fungal contamination to ensure the products quality and safety.

RECOMMENDATIONS

Future studies need to be developed to understand how different roasting conditions contribute to reduce fungal pathogens contaminating coffee powders. Appropriate precaution measures should therefore be taken by farmers, Industries and coffee handlers during the harvesting, postharvest transportation, storage, and packaging of coffee to reduce the risk of contamination.

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