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African Journal of Microbiology Research

August 2022 ISSN 1996-0808
DOI: 10.5897/AJMR
www.academicjournals.org

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Vol. 16(8), pp. 273-282, August 2022
DOI: 10.5897/AJMR2022.9626
Article Number: F12DF1369601
ISSN: 1996-0808

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African Journal of Microbiology Research

Full Length Research Paper

Microbiological characterization of traditionally fermented food in southern Mozambique

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Received 22 February, 2022; Accepted 10 August, 2022

Traditionally fermented foods are source of income and improve quality of diets in rural communities. In Mozambique there are several locally fermented foods, however little is known about fermentation technology and microbial composition. This study aimed to determine physico-chemical parameters and the microbial diversity of traditionally fermented foods in Mozambique. Samples of *ucanhi*, *maheu*, *massi* and *rhali* were analyzed in regard to pH and titratable acidity; lactic acid bacteria (LAB), yeasts and microorganisms of food safety concern (*Echerichia coli* and molds). Purified isolates were identified at the species level using identification kits. The results show that in fermented foods, the pH ranged from 3.33±0.17 to 4.42±0.12 and the titratable acidity from 2.74±0.92 to 9.75±2.87. Counts ranged from 2.54±0.57 to 4.23±1.09 Log CFU/ml for LAB and 2.24±0.43 to 3.63±0.55 Log CFU/ml for yeast. Apart from *ucanhi*, molds were present in almost all products in quantities that reached 3.59±0.42 to 4.29±0.45 Log CFU/ml. The isolated species from the fermented products were *Lactobacillus plantarum*, *L. fermentum,* for LAB, and *Candida albicans, C. famata, Cryptococcus humicola, Rhodotorula mucilaninosa* 2, *Saccharomyces cerevisiae*, for yeasts. In general, fermented foods in Mozambique are quite acidic; LAB and yeast counts were low; microorganisms with public health importance were isolated in these products.

Key words: Fermented foods, food safety, lactic acid bacteria, Mozambique, yeasts.

INTRODUCTION

Food fermentation is one of the oldest processing technologies where the growth of spoilage and pathogenic organisms are suppressed to promote the extension of the shelf life of perishable products (Terefe, 2016). Fermented foods play an important role in food security, sustainable development, and economic growth in Africa through the provision of employment facilities, contribution to empowerment initiatives for unemployed women, opportunities for scaling up of traditional food processing techniques and, distribution of resultant products (Obafemi et al., 2022).

Fermentation can contribute to preservation of food, actively participate in the development of their texture, flavor and aroma, help to eliminate pathogens, allergens

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and toxic substances, improve digestibility, create new products for new markets and increase nutrient value (Voidarou et al., 2021; Obafemi et al., 2022). The associated microbiomes of fermentations can have health-promoting properties, with some probiotic strains (Terefe, 2016; Voidarou et al., 2021).

Lactic acid bacteria (LAB) and yeasts belong to this group, and the predominant genus and species in foods vary according to the climatic conditions of each region (Akabanda et al., 2010). The intake of probiotics has been reported to be efficient in the prevention of several types of diarrheas and colitis in children and adults, as well as in the treatment of other gastrointestinal disorders (Syal and Vohra, 2013).

In Mozambique, a dual public health concern has risen in recent years. The occurrence of chronic diarrhea in both children and adults and the actual high levels of chronic malnutrition (44%) gives a picture of the current nutritional status of the national population, although, micronutrient deficiency is transversal between the urban and rural population (UNICEF, 2013). Regarding this matter, there is a need to identify natural sources of probiotics from traditional foods in southern Mozambique, which could be an aspect to consider introducing in future food fortification strategies, especially at the rural level, where high rates of chronic malnutrition have been widely reported.

Some authors have described the fermentation technology and microbiology of some traditionally fermented foods produced in African countries, as well as the introduction of the use of microorganisms involved in fermentation and their products in food fortification programs. Hjortmo et al. (2008), observed high levels of folate during the fermentation of *togwa* (maize-based fermented beverage produced in Tanzania). In *amasi* (food produced in South Africa and Zimbabwe, by spontaneous fermentation of milk) bacteria of the genus *Lactobacillus* with probiotic effect, such as *L. helvetieus, L. plantarum, L. delbrueekii* subsp. *Lactis* and *Lactis e L. casei* subsp*. casei* were isolated (Beukes et al., 2001).

Achi (2005) isolated *Lactobacillus rhamnosus*, *L. reuteri* and *Saccharomyces cerevisiae* in *Ogi*, a porridge produced from the fermentation of corn (*Zea mays*), *sorghum* (*Sorghum bicolor*) or *millet* (*Peninselum americarum*), intended for feeding babies. The same author also isolated several species of *Lactobacillus* and *Saccharomyces cerevisiae* in alcoholic beverages produced from the fermentation of sorghum called *bukuruto* and *pito* in Nigeria, and sorghum beverage in South Africa. Akabanda and collaborators (2010) isolated species of *Lactobacillus* (*L. acidophilus* and *L.bulgaris*), *Lactococcus species* (*L. cremoni* and *L. lactis*), *Streptococcus thermophilus*, *Leuconostoc* sp and *Saccharomyces* sp in *nunu*, traditionally fermented dairy beverage in Ghana. However, for the microorganisms present in fermented foods to exert their effect efficiently, their ingestion must be continuous so that they colonize the gastro-intestinal tract (Acurcio, 2011).

In Mozambique there are several locally fermented foods, such as *maheu* (a refreshing drink made from corn flour), *massi* (spontaneously fermented milk), fermented fruit drinks (marula or *ucanhi* and cashew), among others. However, little has been described about the technology of production, microbial diversity, and the potential use of these products as natural probiotics. The present work aimed at determining the physicochemical parameters, identifying, and quantifying the micro-organisms present during the fermentation process, as well as assessing to what extent is it safe to consume traditional fermented foods, considering the microbiological quality of the final product.

MATHERIALS AND METHODS

Description of study site

The study was carried out in, Maputo City (25°57'13.4064"S, 32°35'19.3596"E), Gaza (25°02'60.00"S, 33°38'59.99") and Inhambane (23°51′53″S, 35°22′59″E) provinces, located in Southern Mozambique. The provinces were selected according to their history of production of traditionally fermented foods. Twenty samples of *ucanhi* were acquired in Marracuene, Magude, Manhiça and Goba districts (Maputo City province), 19 samples of *maheu* in Chamanculo and Kamubukwana district (Maputo City province), 18 samples of *massi* in Chokwé and Guijá districts (Gaza province), and 20 samples of *rhali* in Maxixe and Inharrime districts (Inhambane province).

Preparation of the fermented foods

The *ucanhi* is a beverage consumed in traditional occasions in southern Mozambique. The processing of preparation starts with the manual pressing of the pulp of the marula fruit (*Sclerocarya birrea*) to remove the juice, followed by the placement of the liquid in plastic containers, where the juice is kept in a cool place for 2 to 3 days, to allow the fermentation to take place using the natural microflora. During the fermentation, the juice is separated into two layers, a foamy supernatant that is discarded and the liquid that is the proper *ucanhi*. The fermentation is the main stage and the critical point of control.

Maheu is a traditional beverage made by a mixture of corn flour and water in a proportion of 2:6 to obtain a porridge that is boiled for about an hour. Afterwards, the porridge is cooled at room temperature, and after cooling down the porridge is placed in a container, where sugar is added to stimulate the natural microflora. To allow the fermentation to take place, the porridge is stored at room temperature for 3 to 7 days. Maheu's processing technology has two stages that are the critical points of control: the porridge boiling and the natural fermentation.

The *masi* production is performed with the deposition of raw cow milk is plastic buckets, that are sealed and stored indoors for 2 to 3 days to allow the fermentation. The fermentation is a critical control point and is carried out relying both on the natural microflora of the milk and the container used for keeping the raw milk. During the fermentation, two layers are formed, a liquid layer (whey) that is decanted and a thick clot (massi) that is kept in the bucket for consumption.

The processing of *rhali* starts with the peeling of cassava (*Manihot esculenta*), followed by grating it into a fine pulp using a metal grater. After that the fine pulp is placed in bags and pressed

with stones or wood to remove excess of moisture, and the bags are placed at room temperature for 6 days to allow the fermentation process to occur. After 6 days the fermented pulp is mixed with cassava flour, then roasted in metal containers or clay pots heated over in open fire and after that the roasted flour is sieved according with the size of the granules. The *rhali* processing technology has the roasting as a main of critical control point.

Sampling procedure of the fermented foods and performed analysis

For *ucanhi, maheu* and *massi* approximately 1000ml of each product were collected per sample. For the *rhali* samples 1000g were collected per sample. All liquid samples (*ucanhi*, *massi* and *maheu*) were placed in sterile plastic bottles, properly marked, stored in coolers containing icepacks, and kept in cold conditions before transportation (-40°C). The solid samples (*rhali*) were placed in sterile plastic bags, marked accordingly and thereafter stored at room temperature (+25°C), before transportation. After the sampling process (approximately 4 h), all the collected samples were transported immediately to the Food Hygiene and Technology Laboratory at the Veterinary Medicine Faculty, at Eduardo Mondlane University (FAVET-UEM), for further analysis.

The experimental design followed a chronological order which includes physico-chemical tests (determination of pH and titratable acidity); isolation, identification, and quantification of lactic acid bacteria (LAB) and yeast; isolation, quantification and identification of *E. coli* and molds. All samples were analyzed in triplicate and plated in duplicate.

pH and titratable acidity determination

The pH was determined using the electrometric method (using a digital potentiometer) (Instituto Adolfo Lutz, 2008).
The determination of the titratable acidity was carried out using the volumetric titration method with an indicator (Instituto Adolfo Lutz, 2008). Based on the results obtained, the acidity was calculated based on the following formula:

$$\text{Acidity in molar solution percent v/m} = \frac{(\text{Vx}f\text{x}100)}{\text{Pxc}}$$

where V = number of ml of 0.1 or 0.01 M sodium hydroxide solution used in the titration, f = 0.1 or 0.01 M sodium hydroxide solution factor, P = nr of grams of the sample used in the titration, c = correction factor for 1M NaOH solution, 10 for 0.1 M NaOH solution and 100 for 0.01 M NaOH solution.

The titratable acidity was expressed in ml of the 1N NaOH solution/100 g of sample.

Isolation, identification, and quantification of microorganisms

LAB and yeasts

The isolation and identification of LAB and yeasts in *ucanhi*, *maheu*, *massi* and *rhali* was carried out using the methodology described by Hellström et al. (2010) and Greppi et al. (2017). Two serial dilutions (10^{-1} and 10^{-2}) were performed using peptone water, following the methodology described by Akabanda et al. (2010). From each of the dilutions obtained, an aliquot of 0.1 ml was pipetted, and surface plated on agar Petri dishes containing the culture media MRS Agar (Sigma - Aldrich) supplemented with 0. 31µg/ml Griseofulvin (Griseofulvin, from *Penicillium griseofulvum*, 97.0 - 102.0%; Sigma-Aldrich), to prevent yeast growth in the case of LAB; and YPD Agar (Sigma-Aldrich) supplemented with 25 mg/ml Chloramphenicol (Chloramphenicol ≥ 98% HPLC, Sigma-Aldrich) to prevent bacterial growth in the case of yeast. All agar plates were placed inside glass jars with screw caps containing anaerobic generating sachets (Sigma - Aldrich) and inclubated at 30ºC for 48 h in the case of LAB, and at 30ºC for 72 h in the case of yeast (Hellström et al., 2010 and Greppi et al., 2017). After the incubation, the morphological characterization of the colonies (visual appearance eg. colour, size, shape, type) was performed, and the total number of the colonies per dilution was estimated using the colony count method. Dilution factor was considered, and results were expressed in CFU/g, and after that transformed to logarithm units.

For all plates with different morphological aspect, purification was performed by surface plating in Petri dishes containing YPD Agar (Sigma-Aldrich), supplemented with Chloramphenicol (0.25 mg/ml) [Chloramphenicol ≥ 98% (HPLC), Sigma - Aldrich] and incubated at 30°C for 24 h for yeast; and MRS Agar (Sigma-Aldrich) supplemented with Griseofulvin (0.31 µg/ml) (Griseofulvin, from Penicillium griseofulvum, 97.0 - 102.0%; Sigma-Aldrich) and incubated in an oven in screw-top glass jars containing generator sachets of anaerobiosis (Sigma-Aldrich) at 30°C for 24 h for LAB. For LAB, the catalase test was then performed, which consisted of making smears of catalase negative colonies on slides, followed by staining by the Gram method and observation under an optical microscope using 100X resolution.

LAB and yeast colonies were cryopreserved following the methodology described by Nordvall (2007) and Greppi et al. (2017). All pure LAB colonies that were catalase negative and Gram positive (Gram +) were cryopreserved. Pure colonies were inoculated in cryo-tubes containing 40% glycerol (Glycerol, ≥ 99.0%, Sigma - Aldrich) in MRS broth (Sigma - Aldrich) for the case of LAB, and YPD broth (Sigma - Aldrich) for the case of yeasts, and preserved at temperature of -32°C. For the confirmatory tests, bioMérieux's API identification products that consist of test kits for identification of Gram-positive and Gram-negative bacteria and yeast were used.

The API method is a quick and well-established system for manual microorganism identification to the species level. This system offers a large and robust database available online (APIWEB™ service).

For the identification of yeats was used to test the API® 20 C AUX (bioMérieux's), that is a system for the precise identification of the most frequently encountered yeasts. The API® 50 CH test (bioMérieux's) were used as a system for the precise identification of the most frequently encountered Lactobacillus and related genera. The API identification tests were performed following the manufacturer's instructions (bioMérieux's)

Microorganisms of food safety concern

The microorganisms of food safety concern selected for analysis were *E. coli* and contaminating molds. The microorganisms studied were chosen due to their better resistance to the acidic conditions of fermented foods. Detection of *E. coli* was performed in accordance with the International Commission on Microbiological Specifications for Foods (ICMSF, 2012). About 0.1 ml volume of the 10^{-1} dilution was pipetted, plated by spread on the surface of Eosin Methylene Blue Agar (EMB Agar, Sigma - Aldrich). Duplicate plates were incubated at 37°C for 24 h. For confirmation of *E. coli* colonies, the indol test was performed.

Mold isolation was carried out according to the method established by the ICMSF (2012). For this purpose, about 1 ml aliquot of the serial dilutions was taken into Petri dishes, containing about 15 ml of molten Sabouraud Dextrose Agar (SDA), followed by incubation at 30°C for 72 h. Plates were analyzed in duplicate. The colonies were estimated using a colony counter. Dilution factor was considered for the estimation of the final results, and results were expressed in CFU/g, and after that transformed to logarithm units.

Table 1. pH and titratable acidity of Traditional fermented fool.

Samples	Parameters	
	pH	Titratable acidity (1N/100g)
Rhali	4.42 ± 0.12	5.30 ± 0.72
Massi	4.19 ± 0.27	6.49 ± 0.92
Maheu	3.33 ± 0.17	2.74 ± 0.92
Ucanhi	3.43 ± 0.34	9.75 ± 2.87

Source: Authors

RESULTS AND DISCUSSION

Physico-chemical parameters

Table 1 illustrates the mean values of pH and titratable acidity (expressed in ml of the 1N NaOH solution/100g of sample) in the traditionally fermented foods under study. In general, the results obtained show that traditionally fermented foods in southern Mozambique are very acidic, with 3.33 ± 0.17 to 4.42 ± 0.12, where *rhali* and *massi* stood out as more acidic foods. Titratable acidity ranged from 2.74 ± 0.92 to 9.75 ± 2.87, with the highest values reported in *ucanhi* and *massi*.

Similar results to present the study were reported by Penidoa et al. (2018) during the evaluation of the selection of starter cultures to produce cassava starch through the fermentation of cassava flour. The authors obtained pH values ranging from 3.29 to 5.69 and titratable acidity in the range of 0.14 to 0.71% in the fermented cassava flour, and the titratable acidity values referenced by them are below those observed in the present study for the case of *rhali.* Oyeyinka et al. (2020) during the characterization of physical, chemical, and sensory properties of flakes (*gari*) prepared from refrigerated cassava roots, obtained pH values ranging from 4.30 to 5.40. *Gari* is a fermented product with pH found to vary between 3.42 and 4.88 depending on processing methods. The acid pH contribute largely to the flavor and consequently the acceptability of *rhali* by consumers (Oyeyinka et al., 2020).

The pH ranges obtained in the present study for *maheu* are similar to those obtained by Mwale (2014), which reported pH values in the order of 3.5 and titratable acidity in the range of 0.4 - 0.5% for the food in reference, presenting a range that is relatively lower than that reported in the present study. Simatende (2016) during the characterization of the microflora diversity present in *emahewu* produced in Swaziland, obtained 3.62 as an average pH value similarly with the present study, and titratable acidity values in the order of 0.43%, relatively lower compared to the present study. Mashau *et al.* (2020) during the evaluation of the shelf-life extension and sensory properties of *mahewu* – a non-alcoholic fermented beverage, by adding *Aloe vera* powder, obtained pH value ranging from 2.4 to 3.3 and titratable acidity in the order of 0.2 to 1.8%.

Exceptionally, among the four traditionally fermented foods under study, *maheu* stood out for being the least acidic food than the others (pH 3.33 ± 0.17) and for having relatively lower titratable acidity values than the other foods (2.74 ± 0.92). The low pH values obtained in some traditionally fermented foods analyzed in this study may be associated with the long fermentation time, since the cold system is not applied to stop the fermentation process. Factors such as the production of high levels of organic acids and, consequently, the accentuated sour flavor enhancement were notorious, corroborating to the results described by Nyambane et al. (2014). The low pH values obtained in the present study are crucial, since most bacteria, including the pathogenic microorganisms, struggle to grow at low pH values, and this condition provides the microbial safety, as well as the extension of the shelf life of *mahewu* samples.

The pH results obtained for *massi* in the present study are similar to those reported by Yu et al. (2015) also found that the mean pH of fermented cow's milk ranges from 4.12 ± 0.35 to 4.31 ± 0.39. Regarding titratable acidity, the values obtained in this study are above those mentioned by Simatende (2016), who obtained in his study a titratable acidity of 0.89% in samples of cow's milk traditionally fermented in Swaziland. The origin of these differences may be associated with the microbiological composition of the *massi*, the production of organic acids and the fermentation time.

In relation to *ucanhi*, the results of the study in reference are similar to those reported by Naeem et al. (2012), which reported pH mean values ranged from 2.0 to 4.5. In contrast, results reported by Motlhanka et al. (2018), showed that the pH of marula wine produced in Sub-Saharan Africa reached 4.10, a relatively higher value compared to the ones obtained in the present study (3.43 ± 0.34). The differences in results may be associated with variations in the processing technology used to obtain the fermented product, where in the present study the producers of this drink usually add water after fermentation to increase the amount of *ucanhi* produced.

The low acidity of the fermented products tested in the present study is a desirable characteristic in terms of food safety and sensory for the adult consumer, however, for children consumption, it can be a denial factor.

Quantification and identification of LAB

Figure 1 illustrates the quantification of LAB by each fermented food. All traditionally fermented foods under study had low LAB counts, ranging from 2.54 to 4.23 Log CFU/g or ml. Specifically, the counts were 2.54 ± 0.57; 3.82 ± 0.73; 4.07 ± 0.41; 4.23 ± 1.09 Log CFU/ml or g, for *ucanhi*, *rhali*, *maheu* and *massi*, respectively.

The results regarding the LAB counts obtained in the

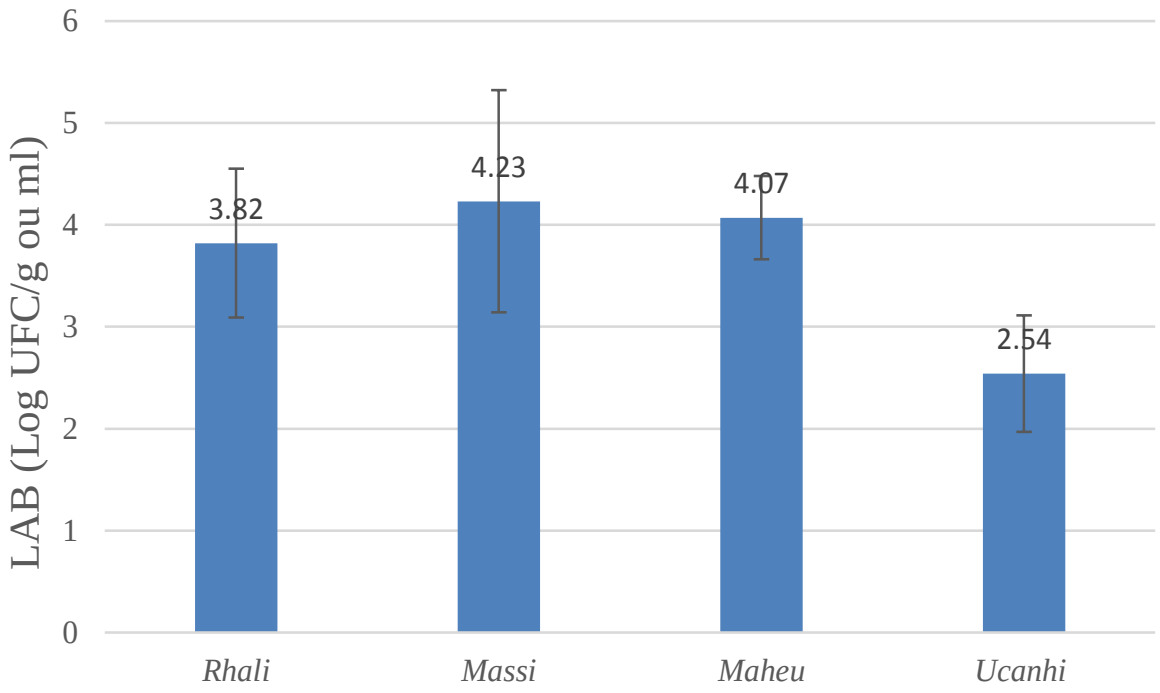

Figure 1. Quantification of LAB by each fermented food.
Source: Authors

present study for *rhali* are below those reported by Huch et al. (2008) who obtained LAB counts variable from 2 × 10^2 to 6 × 10^3 CFU/g during the evaluation of the use of species of *Lactobacillus* for initiation of cassava fermentation in *Gari* production. In turn, Penidoa et al. (2018) reported LAB counts above those observed in the present study, ranging from 5.8 to 7.9 Log CFU/g, in samples of fermented cassava flour using natural microflora for 56 days. The difference in results between the two studies may be associated with the technology used to process this product in Mozambique, where the fermentation process usually takes 3 to 6 days, followed by the roasting step of the fermented pulp.

Similarly to the *rhali*, the results of the LAB counts obtained in the *massi* are below the results reported by Beukes et al. (2001) where they observed that LAB counts ranged from 5.7 to 9.1 Log CFU/ml in *amasi* produced in South Africa.

Nyambane et al. (2014) obtained LAB mean counts higher than those reported in the present study, varied from 7.86 Log CFU/ml to 8.32 Log CFU/ml in *massi* samples processed in gourds and plastic containers, respectively. This fact can be explained by the existing variations in the processing technologies used in the two studies in reference for this traditionally fermented food, as is the case of time (which varies from 1-3 days) for the present study and 4 days for the study of Nyambane et al. (2014), the environment fermentation temperature of the present study and the environment temperature (18 to 32ºC) in the study by Nyambane et al. (2014).

The LAB counts obtained for the *maheu* samples are below the results reported by Simatende (2016), which found that LAB count varies from 8 - 10 Log CFU/ml, during the characterization and diversity of the microflora present in the *emahewu* produced in Swaziland, results that approximate twice the LAB counts obtained in the present study. In the same order of ideas, Mashau et al. (2020), when assessing the evaluation of the shelf-life extension and sensory properties of *mahewu* - a non-alcoholic fermented beverage, by adding *Aloe vera* powder obtained LAB counts ranging from 3.0086 to 7.7559 Log CFU/g.

This fact may be explained because of the addition of warm water after fermentation of the *maheu* and the reduction of the fermentation period (from 3days to 1 day) during the process of preparation of the samples used in this study. This statement differs from the ones reported by Simatende (2016), whose technology of production does not include the addition of water and long fermentation (2-3 days in summer and up to 5 days in winter). The low pH of *mahewu* contributed for the increase of lactic acid microfola during fermentation allowing the growth of LAB, which resulted in competing microorganisms being inhibited.

The results obtained in *ucanhi* are similar to the results reported by Nyanga et al. (2007), who verified that the LAB count in the marula pulp (*Sclerocarya birrea*) ranges from 2.91 to 2.99 Log UFC/g. Phiri (2018) found that the

Table 2. Morphological characteristics of LAB.

Food	Morphological characteristics of microorganisms microscopic	Identification
Massi	Curl-shaped bacilli, Gram positive, catalase negative	*Lactobacillus plantarum* *Lactobacillus fermentum*
Maheu	Curl-shaped bacilli, Gram positive, catalase negative	*Lactobacillus collinoides* *Lactobacillus plantarum*
	Cocos ou cocobacilli, dispostos em cadeias curtas, Gram positive, catalase negative	*Leuconostoc mesenteroides ssp mesenteroides/ dextranicum 2*
Ucanhi	Cocos, Gram positive, catalase negative	*Pedicoccus pentosaceus*
	Curl-shaped bacilli, Gram positive, catalase negative	*Lactobacillus plantarum*
Rhali	Curl-shaped bacilli, Gram positive, catalase negative	*Lactobacillus plantarum*

Source: Authors

LAB count ranges from 2.27×10^3 to 1.57×10^5 CFU/ml. The similar results can be justified by the similarity in the processing technology described in the literature by the authors (Nyanga et al., 2007; Phiri, 2018) and those mentioned in the present study. Although, LAB have a beneficial effect and some strains have a probiotic effect, where a high concentration of these in food must be guaranteed.

From the isolated pure colonies, the following LAB species were identified: *Lactobacillus plantarum*, *L. fermentum*, *L. collinoides*, *Leuconostoc mesenteroides* subsp *mesenteroides/dextranicum* 2 and *Pedicoccus pentosaceus*. The summary of the morphological characteristics of each identified LAB is shown in Table 2. However, it was still observed the growth of molds of the genus *Fusarium* in *rhali* and *Mucor* in *massi*.

LAB species with probiotic potential described in the literature on similar products in different African countries (Beukes et al., 2001; Jans et al., 2017; Kayitesi et al., 2017) are *L. plantarum, L. fermentum, Leuconostoc mesenteroides ssp. mesenteroids and Lactococcus. Lactobacillus plantarum* as a probiotic has been described by some authors (Nyanga et al., 2007; Nyambane et al., 2014). This LAB is homo-fermentative and ferments lactose to produce lactic acid as its main metabolic product.

Simatende (2016), describes *L. plantarum* as a typical biota of non-alcoholic beverages spontaneously fermented with corn and soy, playing a key role in defining the attributes of these products. *Leuconostoc mesenteroides ssp. mesenteroids* during spontaneous corn fermentation can inhibit the growth of *Aspergillus flavus* (Rahmawati et al., 2013).

Yeast quantification and identification

All traditionally fermented foods under study had low yeast counts, around 2.24 to 3.63 Log CFU/g or ml, ranging from 2.24 ± 0.43; 2.92 ± 0.37; 3.22 ± 0.87 and 3.63 ± 0.55 Log CFU/g or ml for *ucanhi, massi, maheu* and *rhali*, respectively. It was also observed the growth of molds of the genus *Fusarium* in a sample of *rhali*. Figure 2 illustrates the morphology and quantification of yeasts by fermented food.

The obtained results referring to yeast counts for dough samples are below the results reported by Nyambane et al. (2014), obtained yeast counts that ranged from 6.65 to 7.62 Log CFU/ml and from 5.50 to 6.65 Log CFU/ml in dough samples processed in gourds and plastic containers, respectively. The high acidity, allied to the high environment, temperature verified in the sampling area, can constitute inhibitory factors to the multiplication of yeasts in these products.

For the case of *maheu*, the results obtained from the yeast counts in the present study were similar to those reported by Rahmawati et al. (2013), who obtained variable values of 3-5.5 Log CFU/g in *maheu*. The results obtained in *ucanhi* are similar to the results reported by Nyanga et al. (2007) and Phiri (2018) which verified that the yeast count varies from 2 to 6 Log CFU/ml.

Regarding *rhali*, the results obtained are below those reported by Penidoa et al. (2018) which reported yeast counts from 1.7 to 7.8 Log CFU/g in fermented cassava flour. This factor may be associated with the fact that the samples used in this study undergo the process of removing excess moisture, a long fermentation time of 3 to 6 days and heat treatment (roasting over an open fire).

The identification of yeasts at the species level confirmed that the most prevalent are: *Candida albicans, Cryptococcus humicola, Rhodotorula mucilaninosa 2, Saccaromyces cerevisiae, Candida tropicalis, Stephanoascus ciferri, Trichosporan mucoides, Candida dubliniensis, and Candida famata*. The summary of the morphological characteristics of each identified yeast is illustrated in Table 3.

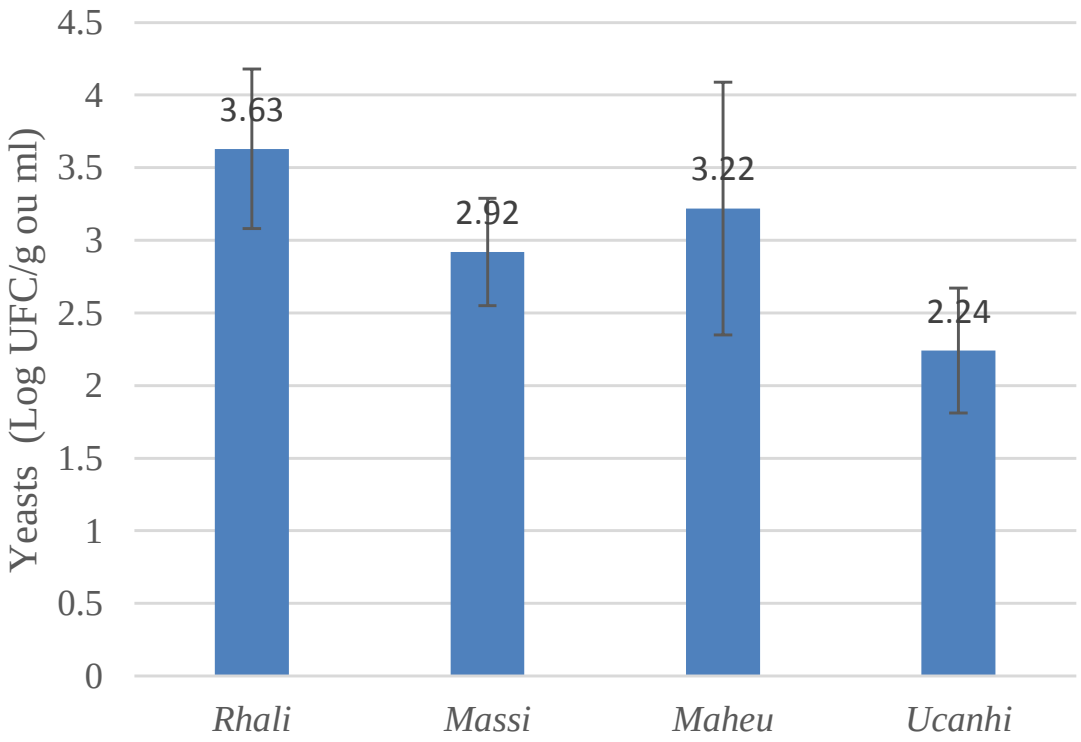

Figure 2. Quantification of yeasts by fermented food.
Source: Authors

Table 3. Morphological characteristics of yeast.

Food	Morphological characteristics of microorganisms	Identification
	1 cm in diameter, creamy, white, concave, round	*Candida albicans*
	2 a 7 mm in diameter, creamy, yellowish, concave, round	*Cryptococcus humicola*
Massi	1 mm a 1 cm in diameter, shiny, mucous, smooth, red, round	*Rhodotorula mucilaninosa 2*
	1 cm in diameter, flat, smooth, shiny, cream	*Saccharomyces cerevisiae*
	1 mm a 1 cm in diameter, opaque, smooth, cream, irregular edges	*Candida tropicalis*
		Stephanoascus ciferri
	1 a 2 mm in diameter, cream, shiny, mucous, round, concaves	*Trichosporan mucoides*
Maheu	2 a 7 mm in diameter, creamy, yellowish, concaves, round	*Cryptococcus humicola*
	1 a 2 mm in diameter, white, shiny, smooth, creamy, round	*Candida dubliniensis*
	1 a 2 mm in diameter, yellowish, opaque, smooth, round	*Candida famata*
	2 a 7 mm in diameter, creamy, yellowish, concaves, round	*Cryptococcus humicola*
Ucanhi	1 a 2 mm in diameter, yellowish, opaque, smooth, round	*Candida famata*
	1 mm a 1 cm in diameter, shiny, mucous, smooth, red, round	*Rhodotorula mucilaninosa*
	1 a 2 mm in diameter, cream, shiny, mucous, round, concaves	*Trichosporon mucoides*
	2 a 7 mm in diameter, creamy, yellowish, concaves, round	*Crytococcus humicola*
Rhali		*Stephanoascus ciferri*
	1 cm in diameter, flat, smooth, moist, shiny, cream	*Saccharomyces cerevisiae*
	1 cm in diameter, creamy, with, concaves, round	*Candida albicans*

Source: Authors

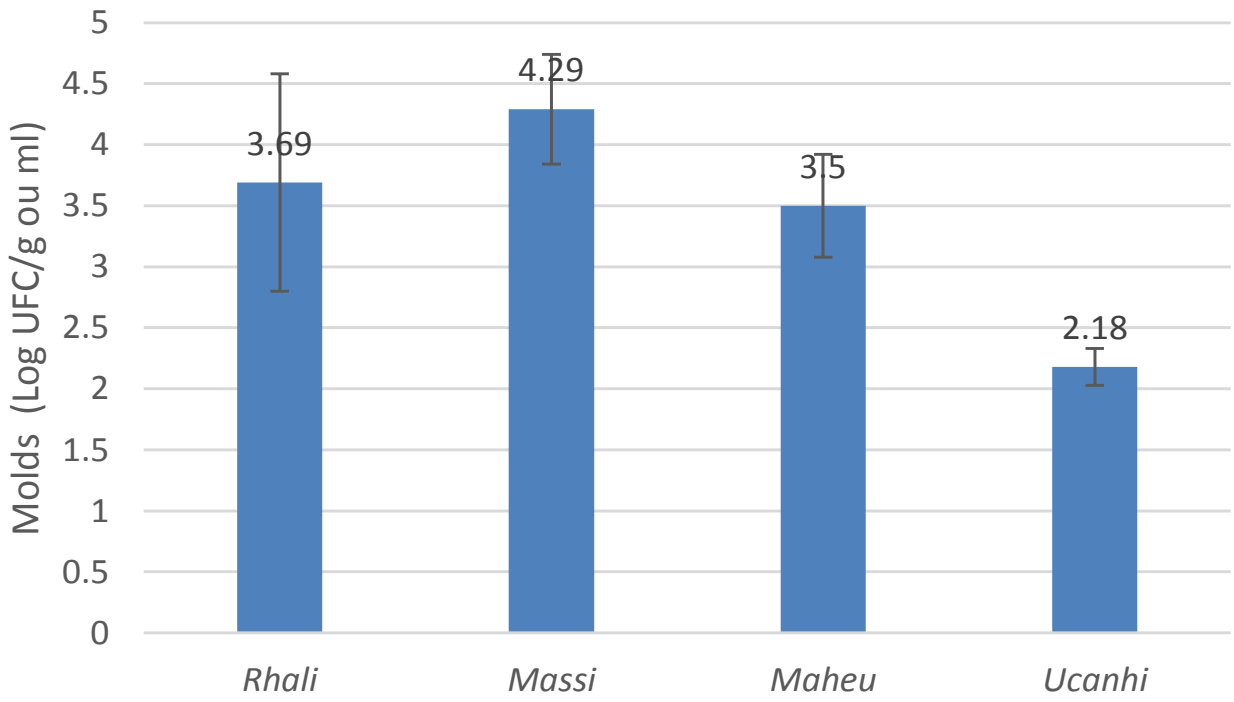

Figure 3. Quantification of molds by fermented food.
Source: Authors

Yeast species with probiotic potential described in the literature for traditionally fermented products evaluated in different African countries similar those obtained in the present study include: *Candida albicans, Candida famata, S. cerevisiae, Rhodotorula mucilaninosa* (Nyanga et al., 2007; Rahmawati et al., 2013; Nyambane et al., 2014). According to Nyambane et al. (2014), *S. cerevisiae* has been associated with the production of alcohols and other aromatic compounds, stimulation of LAB, improvement in nutritional value and inhibition of undesirable microorganisms.

Rahmawati and collaborators (2013), by isolating and identifying microorganisms during spontaneous corn fermentation (*maheu*), described *Candida famata* as having high glucoamylase activity, producing biomass and exhibiting lipolytic and proteolytic activity. Nyanga et al. (2007) reported that *Rhodotorula mucilaninosa* occurs as a natural flora in marula fruits, when the fruit matures, fermentation occurs naturally as a result of its presence, fermenting sugars into alcohol.

Contrary to expectations, the opportunistic pathogen *C. albicans* was isolated in some samples of *massi* and *rhali*. Nyambane and collaborators (2014) stated that the presence of *C. albicans* is of concern, as it can cause superficial, localized and/or systemic infections in humans. The presence of this species in the samples evaluated in this study requires additional and in-depth investigations and may be is an indicator of deficient hygienic processing and/or commercialization practices.

Microbiological safety of traditionally fermented foods

The growth of green colonies with metallic shine on EMB Agar was verified in 4 samples of *rhali*, 17 samples in *massi* and 7 samples in *maheu*. These samples were also positive to the Indole test, confirming the presence of *E. coli* in the products under analysis.

As described by Mwale (2014) and Kayitesi et al. (2017), the presence of pathogenic microorganisms occurs due to the use of primitive methods of production of fermented foods, as well as non-compliance with good hygiene and processing practices. Nyambane et al. (2014) related the high prevalence of Enterobacteriaceae to the presence of acid-resistant *E. coli* strains and coliforms.

Molds and yeasts were observed in some samples of traditionally fermented foods, 5 in *rhali*, 18 in *maheu* and *ucanhi*, and 15 in the *massi*. Traditionally fermented foods presented mean count of mold colonies around 2.18 to 4.29 Log CFU/ml or g, ranging from 2.18 ± 0.15; 3.50 ± 0.42; 3.69 ± 0.89; 4.29 ± 0.45 Log CFU/ml or g for *ucanhi, maheu, rhali* and *massi* respectively. The growth of molds was verified in samples of *massi* cultivated in MRS having 3.09 ± 0.60 Log CFU/ml and *ucanhi* samles grown on YPD agar having 1.40 ± 0.15 Log CFU/ml. Figure 3 illustrates the quantification of molds by fermented food.

In samples that showed growth of molds and yeasts, some yeasts were identified. Mold colonies such as

Table 4. Morphological characteristics of yeast.

Food	Morphological characteristics	Identification
Rhali	Thin macroconidia, septate (3 – 5 septa), straight, spindle-shaped with elongated and curved apical cell, pedicled basal cell	*Fusarium spp*
Massi	Cenocytic mycelia, hyaline, without stolons and rhizoids, smooth spherical to elliptical sporangiospores, dark and equinulate zygospores	*Mucor*
Maheu	Conidia are unicellular, dark, smooth, ovoid, form long chains	*Paecilomyces fumosoroseus*
Ucanhi	Hyaline hyphae, septate, branched, unicellular, support chlamydospores	*Geotrichium candidum*
	Septate hyphae, oval conidia, colorless, flower-shaped	*Sporotrichum*

Source: Authors

Fusarium spp on *rhali* have also been identified; *Mucor* in *massi*; *Paecilomyces fumosoroseus* in the *maheu*; *Geotrichium candidum* and *Sporotrichum* in *b. canhú*. The summary of the morphological characteristics of each identified yeast is illustrated in Table 4.

The presence of these microorganisms may be due to non-compliance with good hygiene practices in processing and especially in marketing. It is well known that fungi generally withstand extreme conditions better than bacteria and are found in foods with a low pH (with acidity up to 3.5). Some authors (Simatende, 2016; Phiri, 2018) have identified *Geotrichum capitatum* as part of the normal microflora of the marula.

As *rhali* is a product with low water activity and often exposed to environmental contaminants during fermentation, a certain growth of molds of the *Fusarium* species was expected, which is generally associated with inadequate storage of the final product. This fact is alarming in terms of public health, since *rhali* is a product for direct human consumption.

Conclusion

According to the results obtained, it can be concluded that traditionally fermented foods under this study, are quite acidic and have relatively low LAB and yeast counts. In the four products studied, new LAB species and yeasts with probiotic potential were identified. In *maheu*, *Lactobacillus collinoides, Stephanoascus ciferri, Trichosporan mucoides, Cryptococcus humicola and Candida dubliniensis*; in *ucanhi*, *Pedicoccus pentosaceus, Cryptococcus humicola and Candida famata;* in *rhali*, *Trichosporon mucoides, Cryptococcus humicola, Stephanoascus ciferri, Saccharomyces cerevisiae* and *Candida albicans*; yeasts in *massi, Cryptococcus humicola, Rhodotorula mucilaninosa 2 and Candida tropicalis*. For all traditionally fermented foods, microorganisms of food safety concern were isolated, namely *E. coli, Fusarium sp, Mucor, Paecilomyces fumosoroseus, Geotrichium candidum* and *Sporotrichum*, showing a risk to the health of the consumer.

ACKNOWLEDGMENTS

The authors would like to thank the National Research Fund (FNI) for funding the PROBIO project that contributed to this work and to Chalmers University of Technology for their cooperation in identifying LAB and yeast isolates.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 16(8), pp. 283-295, August 2022
DOI: 10.5897/AJMR2021.9486
Article Number: 323B1E669609
ISSN: 1996-0808

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Fungal colonization of air-conditioning systems and indoor cultivated plants and its relation to human health

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Received 14 January, 2021; Accepted 7 July, 2022

Fungi have been implicated as quantitatively the most important bio-aerosol component of indoor air associated with contaminated air-conditioning systems and soil of indoor cultivated plants. The objectives of this study were not only to assess the level of fungal contamination in the filter dust of air conditioning systems and soil of indoor cultivated potted plants present inside homes, offices and hospitals for one year, but also fungal identification and examination of their potentiality to produce extracellular hydrolytic enzymes. A total of 5740 fungal colony-forming units (CFU) were collected belonging to 57 fungal species. The predominant molds isolated were Aspergillus spp., Penicillium spp., Cladosporium spp. and Fusarium spp. Enzymatic activity test of the isolated fungi revealed that many isolates showed cellulolytic and keratinolytic activity. In addition, some isolates showed lipolytic, proteolytic and hemolytic activity and could grow at 37oC, which indicate their pathogenic potentiality as human opportunistic pathogens. The results of this surveillance study indicated that in the case of CF, the abundance of fungal colonies was much higher in homes than offices and hospitals while in case of CP, it was much higher in hospitals followed by offices and homes. It is important to stress that fungal colonization of air-conditioning systems and soil of potted plants should not be ignored and to educate homes, offices and hospitals about the need of routine cleaning and disinfection of gadgets like air-conditioning systems and soil of cultivated plants for minimizing the chances of proliferation and dispersal of potentially pathogenic fung.

Key words: Contamination, degrading enzymes, diseases, filter dust, genus diversity, micromycetes, soil.

INTRODUCTION

Contemporary lifestyles dictate that people spend between 60 and 90% of their daily lives indoors. For those living in warm climates, air conditioning is thus considered a necessity. Air conditioners function by removing hot and humid air from the building interior and replacing it with cooler air. Microorganisms are

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considered among the most important sources of poor quality of indoor air, and contamination of this air by microbial pollutants is being increasingly recognized as a public health problem and a probable cause of the so-called sick building syndrome. However, recent research has demonstrated that certain microorganisms can colonize panel filter surfaces, particularly fungi can colonize the materials used in heating, ventilation, and air-conditioning systems (Jaakkola et al., 1991; Božić et al., 2019; Al-abdalall et al., 2019).

Indoor environment affects health and indoor air quality (IAQ) is an important issue for occupational and public health. Microbial incidences and the concentrations of fungi are usually higher indoors than outdoors (Božić et al., 2019). IAQ is important in all buildings, especially in hospitals. A wide range of factors affect IAQ; the quality of the outdoor air, building construction and materials (McCunney, 1987), heating, ventilating and air conditioning systems, temperature, humidity, contaminant sources, occupants and possible pollutant pathways are the basic factors that influence indoor air quality (Jaakkola et al., 1991).

Many studies have focused on the sources of fungal contamination in indoor spaces. Pathogenic fungi have been detected in the potting mix of indoor plants; however, it is unclear if plants in indoor work spaces make qualitative or quantitative contributions to the aeromycota within buildings (Torpy et al., 2013). Since soil is one of the most important biotopes for fungi, relatively high concentrations of fungal propagules are to be expected (Haas et al., 2016). Indoor plants could act as a significant source of pathogenic fungal inocula. Relative humidity of indoor conditions is thought to be the leading cause of fungal amplification (Adan and Samson, 2011). Indoor air may vary in humidity due to numerous factors such as seasonal variability and building design, while indoor plants tend to require watering and contain dead organic matter in the potting mix (Torpy et al., 2013).

Fungi are known to elaborate extracellular enzymes based on the substrate they utilize for growth. Cellulases are a group of hydrolytic enzymes, which are capable of degrading cellulose to smaller glucose units. These enzymes are produced mainly by fungi (Hussain et al., 2012; Parveen et al., 2017). In addition, fungi are capable of producing lipase, a principal enzyme involved in the hydrolysis of lipids to free fatty acids and glycerol (El-Diasty and Salem, 2007; Negedu et al., 2012). Keratins are insoluble proteins found in wool, hooves, scales, hair and nails. Due to the strength and stability of keratin, very few organisms can break it down and utilize it. Some fungal strains can produce keratin proteases, which have keratinolytic activity (Ramakrishnaiah et al., 2013; Kumawat et al., 2013). Hemolytic activity of many fungi was previously reported by Taira et al. (2011) and Aboul-Nasr et al. (2013).

Production and secretion of hydrolytic enzymes are very important virulence factors. These enzymes play a role in nutrition, tissue damage, fungal dissemination within the human body. Thus, they affect fungal pathogenicity. Also, these enzymes could enable tissue invasion easier by impairing some mechanisms of the immune system and causing various injuries to the host (Hass et al., 2016; Al-abdalall et al., 2019; Golofit-Szymczak et al., 2019).

The aim of this study was not only to assess the level of fungal contamination in the filter dust of air conditioning systems and soil of indoor cultivated potted plants present inside homes, offices and hospitals for one year but also fungal identification and examination of their potentiality to produce extracellular hydrolytic enzymes, which are important virulence factors involved in fungal pathogenicity and influence people health.

MATERIALS AND METHODS

Samples collection

Thirty-six dust samples were collected from different air-conditioning filters (CF) from homes, lecturer's offices of Ain Shams University and hospitals at Shoubra, Cairo, Egypt for one year (one sample per month from each site). None of the analyzed filters from these sites had been removed or cleaned for at least one year (Figure 1). Sampling was carried out by removing the filter and collecting its dust. Dust was sampled via manual wiping according to ACV hygienic specification (MOH, 2012; Liu et al., 2021). For each filter, sampling included three sampling points (that is, top surface, bottom surface and side surface) and the area of each sampling point was 100 cm^2 . The manual wiping was conducted with the use of non-woven fabric and dust sampling frame to wipe all dust accumulated at sampling points. A specific procedure was to wear disposable plastic gloves, take 100 mm × 100 mm non-woven and presterilized fabric by tweezers to collect dust. Afterwards, dust samples were sealed in a sterile wild-mouth bottle (Zhou and Gao, 2000; Xu, 2013) and stored at room temperature in dark. Meanwhile, other 36 samples from the soil of different indoor potted cultivated plants (CP) (one sample per month from each site) were collected from the same sites. The samples from potted plants were collected manually from the surface to 2 to 5 cm below the soil surface. Sampling was conducted at monthly intervals from April 2017- March 2018.

Isolation and identification of fungal isolates from CF and CP

For detection of fungi, samples of air-conditioning filters dust and soils from potted plants were suspended and plated onto several culture media. Culturable fungal spores are presented in terms of CFU/g of the dust of air-conditioning filter (CF) and soil of potted cultivated plants (CP). Sub-samples (0.5 g) were taken from each dust sample and suspended in distilled water (0.0425 g/l KH_2PO_4, 0.25 g/L $MgSO_4$, 0.008 g/L NaOH, 0.02% Tween 80 detergent). Dilution series were prepared and three successive dilutions were plated in triplicate according to Pasanen et al. (1997) with some modification using the following media instead of malt agar medium:(i) Sabouraud's dextrose agar (SDA) (20 g dextrose, 10 g peptone, 5 g yeast extract and 20 g agar in 1 Lwater); (ii) potato dextrose agar (PDA) (10 g dextrose, 200 g sliced potato and 15 g

Figure 1. Photograph showing a grossly contaminated filter of the window mounted air-conditioning unit.
Source: Author

agar in 1L water) and (iii) Czapek's agar (10 g dextrose, 3 g sodium nitrate, 1 g KH_2PO_4, 1 g KCl_2, 0.5 g $MgSO_4$, 0.01 g ferrous and ferric sulphate, 20 g agar in 1 L water) with the antibiotic streptomycin to prevent bacterial growth. On the other hand, mold fungi of potted plants soils were estimated using the soil dilution plate method (Johnson and Curl, 1972). The plates were incubated at 28 ± 2°C and observed after 5 to 7 days. Fungal colonies formed on the medium were identified based on both cultural and microscopic characteristics of each isolated colony using various identification keys (Ainsworth et al., 1973; Arx, 1981; Ellis and Ellis, 1985; Pitt, 1979; Samson et al., 2006; Booth, 1971; Carmichael et al., 1980).

Colony counting and microbiological studies

Isolated fungi from different isolation sites were encountered during the four seasons: fall (from October to December), winter (from January to March), spring (from April to June) and summer (from July to September). The following microbiological parameters were estimated: (i) species count, (ii) species and genus richness = the number of species and genera recorded and (iii) species dominance = percentage of each species about the total count of all species. The objective of this methodological stage is to give an idea about fungal species and genus richness and diversity of CF and CP in all isolation sites throughout the different seasons.

Screening of fungal isolates for extracellular enzymes production

From the various isolates, screening for cellulolytic fungi was made using PDA medium supplemented with 5% carboxymethyl cellulose (CMC). Cellulolytic fungi create a clearing zone around the colony on the agar (Gautam et al., 2010). Keratinolytic activity was tested by culturing the isolated fungi on keratin agar medium (gm/250 ml) containing keratin - 2.5, $MgSO_4$ - 0.25, KH_2PO_4 - 0.115, K_2HPO_4 - 0.25 and Agar- 5. Streptomycin 1% was mixed with the medium. Plates were incubated at 28ºC for 5 days. Keratinolytic activity was detected as a clear zone around the colony (Mini et al., 2012).

Protease activity was determined using a casein hydrolysis medium in which skim milk gives an opaque final appearance and hydrolysis of the casein resulted in a clear zone around the fungal colony (Paterson and Bridge, 1994). Lipase activity was measured using the method of Ullmann and Blasius (1974) with some modification using Tween 80 instead of Tween 20. The lipolytic producing ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. Hemolytic activity of fungal isolates was measured using human blood agar medium (Ronald, 2000).

Statistical analysis

Data collected were subjected to one-way ANOVA using the statistical analysis software Minitab V19 of least significant difference test (LSD) at 5% probability level was used to compare the difference among the treatment means. Data are mean of three replicates.

RESULTS AND DISCUSSION

Isolation, identification and microbiological analysis of fungal isolates

The total count of fungal isolates of the investigated air-conditioning systems filter dust (CF) and indoor soil of cultivated plants (CP) from homes, offices and hospitals during the year of isolation was 5740 colony forming units (CFU) (Table 1). Regarding the isolation source, the higher total CFU count from all isolation sites was detected in CP while that of CF was lower. It has been proposed that indoor plants could act as a significant source of fungal inocula. As most fungi require moisture and are saprophytic, and indoor plants tend to require watering and contain dead organic matter in the potting mix, as a result, it harbors large numbers of spores from fungal taxa to the indoor environment (Torpy et al., 2013). Also, indoor relative humidity (RH) levels above 80% are thought to be the leading cause of fungal amplification (Adan and Samson, 2011) and the relative humidity levels around the air-conditioning filters are not always more than 80%, which depends on many factors such as

Table 1. Mean total count in CFU of fungi isolated from the dust of indoor conditioning systems filter (CF) and soil of indoor cultivated plants (CP) from different isolation sites during a year

Isolation site	Isolation source	
	CF	CP
Homes	1278±70.7[a]	750±14.1[c]
Offices	832±7.07[b]	853±4.24[b]
Hospitals	685±2.83[c]	1342±3.54[a]
Significance Level	*	*
LSD (0.05)	41.06	8.7

Values followed by the same letter within the column do not differ statistically (P> 0.05). * = Significant (P≤ 0.05).
Source: Author

environmental conditions (Ljaljevic et al., 2008).

Concerning various isolation sites, in the case of CF, results indicated that the abundance of fungal colonies was much higher in homes (46%) than offices and hospitals (30 and 24%, respectively). In this study, none of the investigated sites was following a maintenance program for the air-conditioning (AC) units. The abundance of fungal species isolated from different air-conditioning systems from different isolation sites can be attributed to (i) different ways of maintaining the systems themselves. Unfortunately, bad maintenance of AC systems or their low efficiency can often lead to unintentional contamination of indoor spaces (Gołofit-Szymczak and Górny, 2010). Window mounted AC unit draws in atmospheric air from an air vent and the chances of filters acquiring a higher volume of dust and fungal spores from the atmosphere are therefore high and variable. The filters utilized in these units if left unattended can act as a suitable nidus for the growth and proliferation of fungi (Kelkar and Kulkarni, 2011). Also, the study of Kalwasińska et al. (2012) emphasizes the fact that rooms with efficient ventilation or air-conditioning systems and guaranteed air-tightness are less contaminated than rooms where air-conditioning was not installed. However, (ii) the differences in relative humidity (RH) levels around the air-conditioning filters depend on many factors such as environmental conditions (Ljaljevic et al., 2008). Moreover, (iii) ventilation air-conditioning systems moderate heat and moisture in buildings produce environmental conditions such that indoor RH is generally different (between 60 and 80%) (Torpy et al., 2013). Finally, (iv) it was reported that air-conditioning systems are highly linked with fungal pollution of indoor air, and the infiltration of outdoor air into the building envelope air through its filters can be the major mechanism responsible for fungal contamination (Gołofit-Szymczak and Górny, 2010).

On the other hand, results of the fungal concentration of CP in various isolation sites indicated that the abundance of fungal colonies was much higher in hospitals (46%) followed by offices and homes (29 and 25%, respectively). Generally, the highest CFU total count from CF and CP was detected in homes and hospitals which was near equals, while that of offices was lower (Table 1). The high fungal concentration of homes can be discussed as homes contain the toilet and kitchen. The toilet contains the toilet-bowl, washbasin and humidifier and apart from the people who produce large amounts of microorganisms in the air. However, in the kitchen, there are tiny particles that may form a suspension in bio aerosols are released into the air during food preparation. On the other hand, hospitals are characterized by a large circulation of people and many visitors who discuss the appearance of a new significant microbiological contamination source. Finally, a strong relationship between occupant density, human activity and microorganisms concentration in the indoor air was previously reported (Fleischer et al., 2006; Stryjakowska-Sekulska et al., 2007). It was also reported that the fungal spectrum in potted soils may also be affected by factors such as the cultivated plant, substrate, ambient temperatures, or watering habits (Haas et al., 2016).

Generally, the variability of total fungal concentration in CF and CP and in various isolation sites may be because indoor air may vary in humidity due to numerous physical, chemical and biological factors, microbial pollutants reservoirs (people, plants, animals, to some extent soil and water as well as human-made materials), seasonal variability and building design (Skowroń et al., 2004; Torpy et al., 2013).

Concerning the diversity and concentration of fungal isolates, results indicated that 57 species belonging to 20 genera were obtained (Table 2). Quality characteristics of fungal flora isolated from CF and CP showed dominating contributions of the genera: *Aspergillus* (37.3%), *Penicillium* (9.3%), *Cladosporium* (7.2%), *Fusarium* (5.9%) and *Scopulariopsis* (5.5%) in which *A. flavus, P. spinulosum, C. herbarum, F. solani* and *S. brevicaulis* were the most dominant species of these genera. In terms of number of species isolated (species richness),

Table 2. Fungal genera, total count (CFU), species count and most dominant species isolated from CF and CP from different isolation sites

Fungal genus	Most dominant species	No. of species	Total count (CFU)	Abundance (%)
Aspergillus	*A. flavus*	17	2145±7.07[a]	37.3
Penicillium	*P. spinulosum*	6	537±9.8[b]	9.3
Cladosporium	*C. herbarum*	3	417±4.24[c]	7.2
Fusarium	*F. solani*	6	340±14.1[d]	5.9
Scopulariopsis	*S. brevicaulis*	2	318±2.7[e]	5.5
Alternaria	*Al. alternata*	2	286±8.49[f]	4.9
Curvularia	*Cu. Lunata*	2	276±8.5[f]	4.8
Rhizopus	*R. stolonifera*	1	240±7.2[g]	4.1
Chrysosporium	*Ch. Tropicum*	2	203±6.96[h]	3.5
Acremonium	*Ac. Curtips*	2	185±7.07[i]	3.2
Geotrichum	*G. candidum*	1	167±9.90[j]	2.9
Trichoderma	*T. viride*	2	160±2.9[j]	2.7
Mucor	*M. circinilloides*	2	99±5.66[k]	1.7
Phialophora	*Ph. Bubakii*	2	70±1.41[l]	1.2
Bipolaris	*B. specifera*	1	67±2.83[l]	1.15
Cunninghamella	*c.* sp.	1	65±7.1[l]	1.1
Syncephalastrum	*Sy. Racemosum*	1	50±2.6[m]	0.87
Ulocladium	*U. atrum*	2	42±2.83[mn]	0.73
Trichothecium	*Tr. Roseum*	1	38±2.8[mn]	0.66
Nigrospora	*N. spherica*	1	35±4.5[n]	0.6
Total		**57**	**5740***	**100**

Values followed by the same letter within the column do not differ significantly (P>0.05); * = Significant (P≤0.05).
Note: Sr. No. = Serial number**.**
Source: Author

the genus *Aspergillus* was the highest and represented by 17 species followed by the genera *Penicillium* and *Fusarium* (6 species for each) and *Cladosporium* (3 species) (Table 2). Similar results were obtained by Torpy et al. (2013) and Mousavi et al. (2016). Moreover, many of *Aspergillus* species and members of the order Mucorales proliferate in the air-conditioning units (Ljaljevic et al., 2008; Kelkar and Kulkarni, 2011).

The main reason for the dominance of *Aspergillus, Penicillium* and *Cladosporium* is that they produce numerous small (2-3.5 µm) and light spores that generally remain in the air, whereas *Alternaria* and some other fungal genera produce fewer, larger and heavier spores that tend to have faster settling (Gołofit-Szymczak and Górny, 2010).

Seasonal variation of fungal concentrations

Results of this study revealed that fungal concentrations in the CF and CP vary not only throughout various isolation sites but also in the course of the season. The average number of fungi present in indoor CF and CP in different isolation sites during the year of study are represented in Figure 2, which showed that winter represented the highest fungal concentration followed by the fall and spring, whereas summer represented the lowest CFU count. Similar results were reported by Hariri et al. (1978) and Bunnag et al. (1982). The possible explanation of these results is that, during fall and winter the relative humidity often rises to levels of 80-90%. Moisture gets dehumidified (converted to water) when it comes in an air-conditioned environment. These conditions could create a suitable nidus for the proliferation of fungi (Kelkar and Kulkarni, 2011).

Looking at the seasonal variations of fungal concentrations in homes, offices and hospitals investigated (Figure 2), it is easy to notice a sharp difference in the different seasons. In winter, the concentration of fungal isolates in homes was the highest followed by offices then hospitals. This sequence of fungal concentration (homes > offices > hospitals) was recorded in fall, spring and summer. These results were confirmed by Mentese et al. (2009) who found that the highest fungal counts obtained in high-humidity indoor spaces such as home kitchens and bathrooms and also

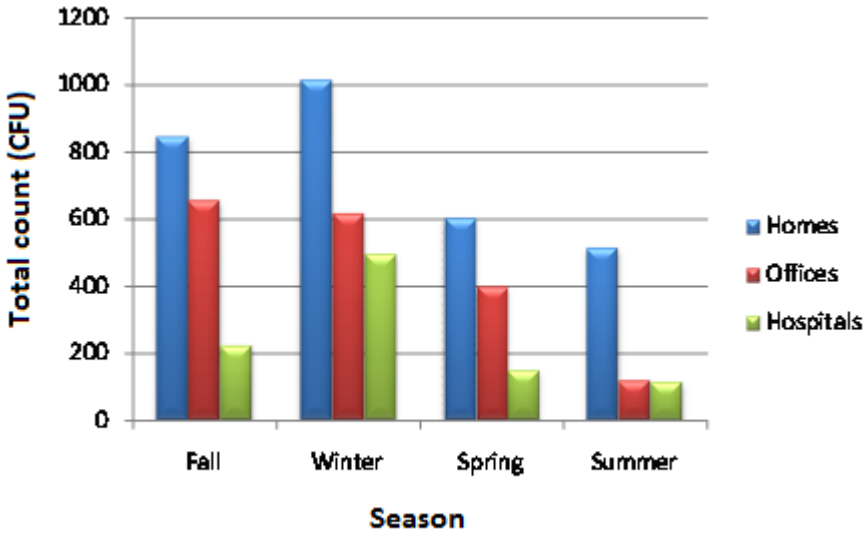

Figure 2. Mean seasonal total count (CFU) of fungi from CF and CP of homes, offices and hospitals.
Source: Author

agreed with those of Božić et al. (2019) who stated that there was a positive correlation between the concentrations of fungi and relative humidity.

Fungal diversity of CF and CP in all isolation sites throughout the different seasons are represented in Table 3. In fall, thirty-three fungal species belonging to 15 genera were isolated in which *Aspergillus* was the most dominant genus (10 species) followed by the genus *Penicillium* (5 species). On the other hand, forty-five fungal species belonging to 17 genera were isolated in winter. *Aspergillus niger*, *Cl. herbarum*, *A. flavus*, *A. fumigatus*, *A. terreus*, *F. solani*, *P. citrinum*, *P. spinulosum*, *R. stolonifer*, *S. brevicaulis* and *S. candida* were the most dominant species. *Aspergillus* was the most dominant genus followed by the genera *Fusarium* and *Penicillium* (Table 3).

Forty-four fungal species belonging to 19 genera were isolated in spring in which *A. niger, Al. alternata*, *Curvularia lunata, A. flavus, A. wentii, C. tropicum, M. circinelloides* and *P. spinulosum* were the most dominant species. On the other hand, *Aspergillus* was the most dominant genus followed by *Penicillium.* Concerning summer, 29 fungal species belonging to 16 genera were isolated and *A. niger*, *C. tropicum*, *C. lunata*, *P. chrysogenum, A. flavus* and *S. brevicaulis* were the most dominant species. However, *Aspergillus* was the most dominant genus followed by *Penicillium.* On the other hand, *A. niger* was the most dominant species isolated from CF and CP from all sites of isolation in all seasons, while *Aspergillus* and *Penicillium* were the most dominant genera found in all seasons with high frequency (Table 3). In contrast to this, Gonçalves et al. (2010) found *Penicillium* and *Aspergillus* species to be dominant across all seasons both indoors and outdoors, the results which confirm the present results.

Generally, winter was the highest in species richness (45 species) followed by spring (44 species), while fall and summer represented lower species richness (33 and 29 species, respectively). However, spring represented the highest genus richness (19 genera) followed by winter, summer and fall (17, 16 and 15 genera, respectively). According to earlier studies, the microbiological quality of indoor air is formed by two main factors: the microbiological composition of outdoor air and indoor air microbial sources (Stryjakowska-Sekulska et al., 2007). Outdoor air is very much influenced by environment, season, the weather and even daytime. Some pathogenic *Aspergillus* and *Fusarium* spp. isolated from CF and CP throughout the isolation period are presented in Figure 3.

Screening of fungal isolates for extracellular enzymes production

Fungal isolates obtained from CF and CP from various isolation sites were tested for their ability to produce extracellular enzymes on solid media. Data presented in Table 4 indicated that 65.4% of tested isolates could produce cellulase. The genus *Aspergillus* represented the highest percentage of cellulase production (27.9%) followed by *Penicillium* (7.5%), *Cladosporium* (5.0%), *Scopulariopsis* (4.2%) and *Fusarium* (3.6%). Other isolates were less in their cellulose activity (Table 4).

Table 3. Fungi isolated from the dust of conditioning systems filters and soil of indoor cultivated plants from different isolation sites during different seasons

Ge. S.N.	Sp. S.N.	Fungal isolate	Season																							
			Fall						Winter						Spring						Summer					
			Home		Offices		Hospitals		Home		Offices		Hospitals		Home		Offices		Hospitals		Home		Offices		Hospitals	
			CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP	CF	CP
1	1	***Acremonium***																								
		Ac. alternatum	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-
	2	*Ac. Curtips*	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-
2	3	***Alternaria***																								
		Al. alternata	+	-	-	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-	-	-	-
	4	*Al. solani*	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+	+	-	-
3	5	***Aspergillus***																								
		A. avenaeus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
	6	*A. awamori*	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
	7	*A. carneus*	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-
	8	*A. clavatus*	+	-	-	+	-	-	+		+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	9	*A. duricaulis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
	10	*A. effusus*	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	+	-		+	-	-
	11	*A. flavus*	+		+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+
	12	*A. fumigatus*	+	+	-	+	-	-	+	+	+	+	-	+	+	-	+	-	+	-	+	-	-	-	+	-
	13	*A. nidulans*	+	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-
	14	*A. niger*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15	*A. oryzae*	-	-	-	+	-	-	+	+	+	-	+	-	+	+	+	-	+	-	+	-	+	-	+	-
	16	*A. sulphureus*	-	-	-	-	-	-	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
	17	*A. tamarii*	-	-	+	+	-	-	+	-	+	+	+	-	+	+	-	-	+	-	+	+	-	-	-	-
	18	*A. terreus*	+	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-
	19	*A. ustus*	-	-	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
	20	*A. versicolor*	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	21	*A. wentii*	+	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	-	+	-
4	22	***Bipolaris***																								
		B. spicifera	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+	+	-
5	23	***Chrysosporium***																								
		C. queenslandicum	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	+	-	+	-	+	+
	24	*C. tropicum*	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+
6	25	***Cladosporium***																								
		Cl. herbarum	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+		+	-	+	-	-	-	-	+
	26	*Cl. oxysporum*	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	27	*Cl. sphaerospermum*	-	-	+	-	+	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	28	***Cunninghamella***																								
		Cunninghamella sp.	-	-	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-
8	29	***Curvularia***																								
		Cu. brachyspora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+
	30	*Cu. lunata*	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3. Contd.

9	31	***Fusarium***																								
		F. chlamydosporum	-	-	-	-	-	-	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
	32	*F. moniliforme*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
	33	*F. oxysporum*	+		+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	34	*F. solani*	+	+	+	-	+	-	+	-	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-
	35	*F. sulphureum*	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
10	36	***Geotrichum***																								
		G. candidum	+	-	+	+	+	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-
11	37	***Mucor***																								
		M. circinelloides	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
	38	*M. racemosus*	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-
12	39	***Nigrospora***																								
		N. sphaerica	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
13	40	***Penicillium***																								
		P. chrysogenum	+	+	+	-	-	-	+	-	-	+	-	-	+	-	+	-	+		+	+	+	+	+	+
	41	*P. citrinum*	-	-	-	-	-	+	+	+	+		+	+	+	-	-	-	-	-	-	-	-	-	-	-
	42	*P. madriti*	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	-	+	
	43	*P. rugulosum*	+		+	-	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+
	44	*P. restrictum*	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-
	45	*P. spinulosum*	+	-	+	-	-	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	-	-	-	-
14	46	***Phialophora***																								
		Ph. bubakii	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-
	47	*Ph. lagerbergii*	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	48	***Rhizopus***																								
		R. stolonifer	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-
16	49	***Scopulariopsis***																								
		S. brevicaulis	+	+	+	+	-	-	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
	50	*S. candida*	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	-	-	+	+	-	+	+	+	-
17	51	***Syncephalastrum***																								
		Syncephalastrum sp.	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	52	*S. racemosum*	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-
18	53	***Trichoderma***																								
		T. koningii	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	54	*T. viride*	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	-	+	+	+	-
19	55	***Trichothecium***																								
		Trichothecium sp.	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
20	56	***Ulocladium***																								
		U. atrum	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	57	*U. chartarum*	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-

Note: Ge. S.N. = Genus serial number. Sp. S. N. = Species serial number. Positive (+) = Isolated. Negative (-) = Not isolated.
Source: Author

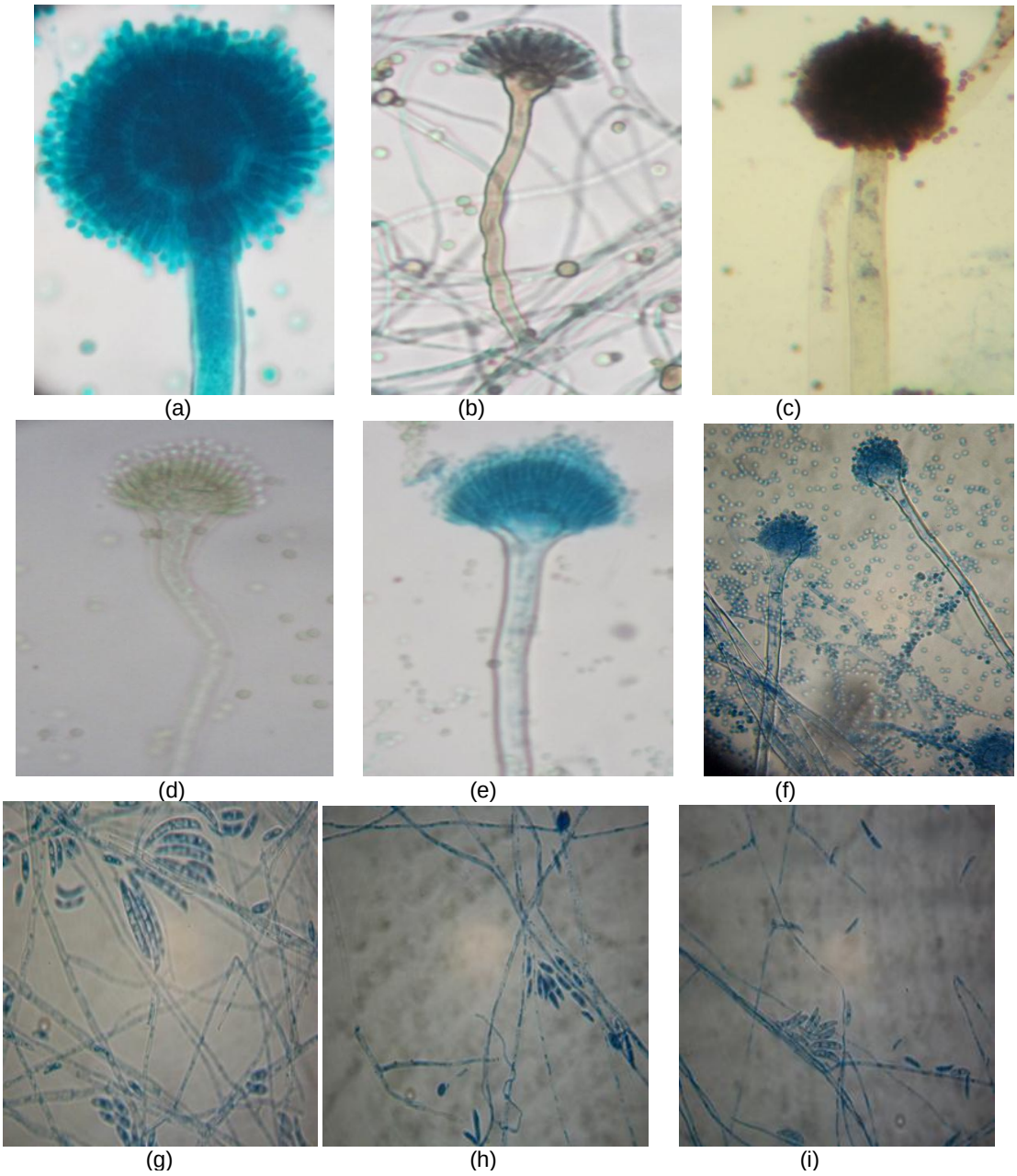

Figure 3. Some pathogenic species of the genera *Aspergillus* and *Fusarium* isolated from CF and CP: (a) *Aspergillus flavus*; (b) *A. nidulans*; (c) *A. niger*; (d) *A. terreus;* (e) *A. carneus* (f) *A. fumigatus;*(g) *Fusarium solani*; (h) *F. moniliforme* and (i) *F. oxysporum.*
Source: Author

Fungi are well-known agents for the decomposition of organic matter in general and of cellulosic substrate in particular (Gautam et al., 2010; Raveendran et al., 2018; Barone et al., 2019). Many studies reported that the most common and potent cellulase producers were *Aspergillus, Penicillium* and *Fusarium* species and that there were some differences in the cellulase activity of different members of fungal genera (Rana and Kaur, 2012; Coronado-Ruiz et al., 2018). This finding indicates that the cellulase system of these fungal forms contains enzymes complexes for the effective hydrolysis of cellulose (Gautam et al., 2010; Hussain et al., 2012).

Concerning keratinase activity, about 42.3% of the tested isolates could produce keratinase. The genus

Table 4. Extracellular cellulolytic and keratinolytic activity of the tested fungal genera grown at 28°C and collected from CF and CP from all sites

Fungal genera	Cellu. A.	Kera. A.
Acremonium	12±2.83^{ij}	7±1.41^{gh}
Alternaria	25±1.41^{gh}	13±2.83^{g}
Aspergillus	340±5.66^{a}	265±5.66^{a}
Bipolaris	9±1.39^{jk}	4±1.41^{hi}
Chrysosporium	30±2.83^{g}	35±1.41^{d}
Cladosporium	63±2.83^{c}	30±2.83^{e}
Cunninghamella	4±1.4^{kl}	1^{hi}
Curvularia	38±4.24^{f}	17±4.24^{f}
Fusarium	47±2.7^{e}	42±2.83^{c}
Geotichum	9±1.41^{jk}	4±1.41^{hi}
Mucor	23±1.41^{h}	5±1.41^{hi}
Nigrospora	10±2.83^{jk}	0^{i}
Penicillium	90±4.25^{b}	60±7.07^{b}
Phialophora	11±1.41^{ij}	0^{i}
Rhizopus	17±1.41^{i}	1±1.41^{hi}
Scopulariopsis	50±2.83^{d}	40±2.83^{c}
Syncephalastrum	8±1.4^{jk}	0^{i}
Trichoderma	30±2.7^{g}	9±1.41^{g}
Trichothecium	1^{l}	1^{hi}
Ulocladium	5±1.39^{kl}	0^{i}
Significance Level	*	*
LSD (0.05)	2.72	2.70

Values followed by the same letter within the column do not differ significantly (P>0.05); * = Significant (P≤0.05).
Note: S.N. = serial number, Cellu. A. = Cellulolytic activity and Kera. A. = Keratinolytic activity.
Source: Author

Aspergillus represented the highest percentage of keratinase production (21.2%) followed by *Penicillium* (4.5%), *Scopulariopsis* (3.4%), *Fusarium* (3.2%) and *Chrysosporium* (2.8%) (Table 4). The data are coincident with those reported by Singh et al. (2009), who isolated keratinophilic fungi from soil of planted pots in indoor environments. However, *Chrysosporium, Alternaria, Cladosporium*, *Scopulariopsis*, *Curvularia lunata* and *Fusarium solani* has been reported for their keratinolytic activity (Franca and Caretta, 1984; Mukesh and Meenakshi, 2010). It is recorded that the organic matter content of soils is one of the major factors affecting the presence of keratinophilic fungi in them (Chmel et al., 1972). Evidence of keratinolysis lies in the ability of fungi to release soluble sulphur-containing amino acids and polypeptides into the medium (Mini et al., 2012).

In this study, all fungal isolates have been tested for growth at 37°C and a percentage of 1.14% of the total tested fungi were recorded as lipase producers and belonging to the genus *Aspergillus* (Figure 4a,b), the results were supported by Negedu et al. (2012) and Raveendran et al. (2018). On the other hand, a percentage of 13.6% had proteolytic activity and also belonging to the genus *Aspergillus.* Several species of *Aspergillus* are known to secrete protease as reported by Aboul-Nasr et al. (2013) and Raveendran et al. (2018). Results also indicated that 16.9% of the screened fungal isolates exhibited lysis activity (hemolysis) on human blood (Figure 4c). Several studies have reported fungal hemolytic activity and characterized fungal hemolysins (Greenhill et al., 2010; Nayak et al., 2013; Aboul-Nasr et al., 2013).

The isolated Aspergillus spp., Penicillium spp. and Cladosporium spp. were identified as well-known agents of mycosis, acting as opportunistic pathogens in immunocompromised hosts. They are known to contain glucan, a compound with inflammatory properties and they contain allergens and chemicals that have toxic properties (Ljaljevic et al., 2008; Mousavi et al., 2016; Haas et al., 2016).

With different species of Aspergillus isolated, A. fumigatus is a primary concern as it can cause a range of saprophytic, severe invasive diseases with high mortality (O'Gorman, 2011). Aspergillus fumigatus can also cause

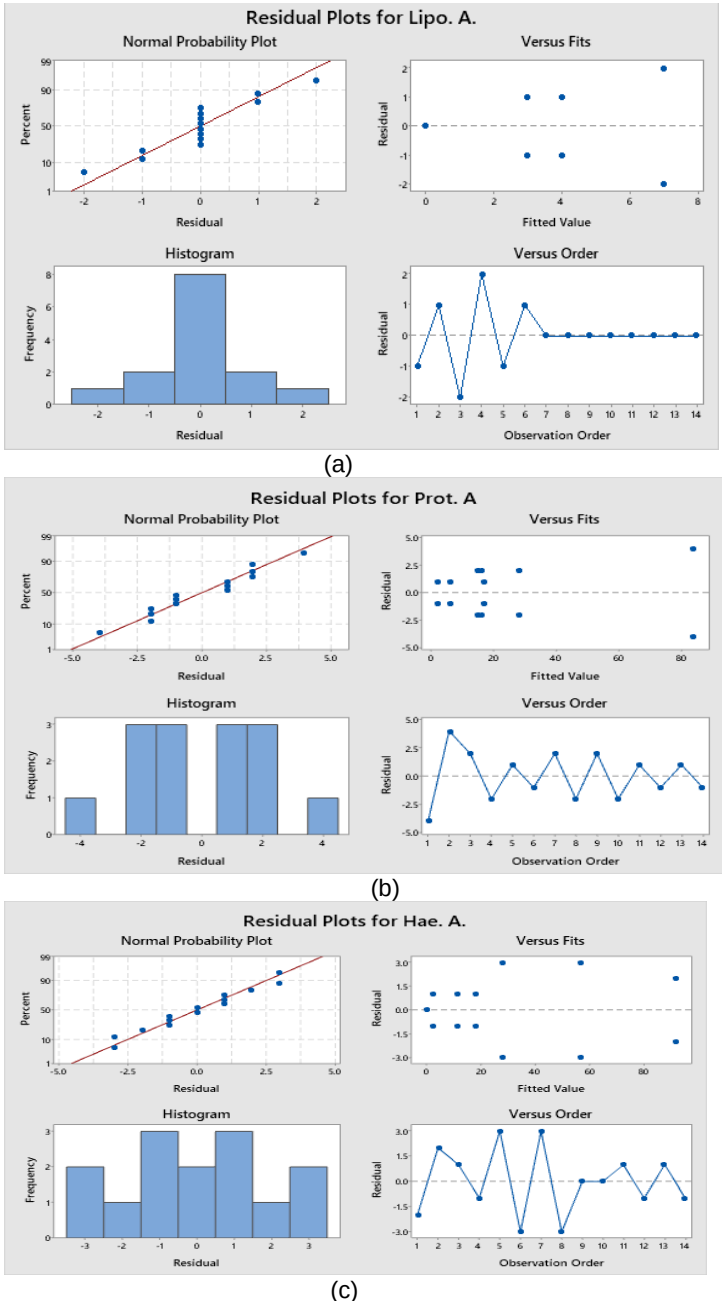

Figure 4. Extracellular lipolytic, proteolytic and hemolytic activity of *Aspergillus* spp. grown at 37°C and collected from CF and CP from all sites (a, b and c, respectively).
Source: Author

opportunistic infection in immunocompromised and healthy individuals and severe allergic diseases (Knutsen and Slavin, 2011). On the other hand, Aspergillus flavus, Fusarium moniliforme, *F. oxysporum*, *Chrysosporium* sp. and others were among the isolated species. Most of these saprophytic isolates are potential pathogens causing skin mycosis (Bernardo et al., 2005; Avasn et al., 2015). Also, exposure to some species of the genus Penicillium, which were isolated in this study, has been associated with a variety of adverse health outcomes including respiratory, hematological, immunological, and neurological system disorders and diseases (Gołofit-

Szymczak and Górny, 2010), while exposure to species of the genus Acremonium causing fungemia (Mattei et al., 2003).

Dematiaceous fungi as Alternaria, Cladosporium and Curvularia were isolated in the present study. Some species have been reported as causing human infections such as subcutaneous mycosis by *Alternaria* spp. (Taira et al., 2011). However, it was reported that cladosporin was produced by Cladosporium and that some species of this genus can cause skin lesions, keratitis and pulmonary infections. On the other hand, Curvularia species may cause infections in humans and has been described as a pathogen that causes respiratory tract, cerebral, cutaneous and corneal infections (Aboul-Nasr et al., 2013).

Virulence factors are properties that increase the survival, growth, and propagation of fungi in human and animal tissue. Some factors are well known, such as the ability of the organism to grow at 37°C and to excrete enzymes (Taira et al., 2011; Aboul-Nasr et al., 2013). It was reported that microbial cells secrete hydrolytic enzymes that are considered the most important virulence factors influencing the pathogenicity of opportunistic fungal infections and destroy the constituents of host cell membranes leading to membrane dysfunction and the invasion of host tissues (Aboul-Nasr et al., 2013) and have immunomodulating activity in humans (Ljaljevic et al., 2008).

Conclusion

Most fungi isolated in this study were considered saprobionts, but depending on the situation they might have the potential to become opportunistic pathogens. Fungal flora can be hazardous for health, particularly in rooms with heating, ventilation and air-conditioning systems and indoor potted plants as potential sources of human diseases. To avoid and reduce potential fungal pollution (infections) in homes, offices and hospitals, the air-conditioning systems must be subjected to regular maintenance. Potted plants, on the other hand, have to subjected to regular cleaning of their soil and elimination eradication of the dead and infected plant parts with the treatment of the soil with a suitable fungicide with a controlled cultivation system. The results show that potentially pathogenic fungi are present in soils. Immunocompromised individuals should avoid handling soils or potted plants in their immediate vicinity.

This surveillance study recommends homes, offices and hospitals about the need for routine cleaning and disinfection of gadgets like air-conditioning systems and soil of cultivated plants for minimizing the chances of proliferation and dispersal of potentially pathogenic fungi. Also, immunocompromised individuals should avoid handling soils of potted plants in their immediate vicinity. Finally, exposure to bioaerosols through air-conditioning systems filter dust can cause various adverse health effects, including infectious and respiratory diseases and hypersensitivity. Consequently, controlling the exposure to bioaerosols constitutes an important aspect of disease control and prevention.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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