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AN EVALUATION OF SOME CHEMICAL AND NATURAL AGENTS FOR THEIR ANTIFUNGAL ACTIVITIES AGAINST *ASPERGILLUS NIGER* AS A CONTAMINATION FUNGAL IN INDOOR ENVIRONMENT

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Abstract

Fungal contamination in indoor environments has been linked with adverse health effects for the inhabitants. Remediation of fungal pollution requires removal of the fungi present and modifying the indoor environment to become less favourable to growth. These steps may include treatment of indoor environments with an antifungal agent to prevent future growth. This study aims to detect the effects of some chemical and natural agents against *A. niger* as a reason for the indoor environment contaminations. The highest effect caused by the essential oil of white musk, which completely inhibited the growth of fungi.

Furthermore, the MIC of essential oil of white musk as well had inhibited the growth of *A. niger* by 100% at the concentrations between 10% and 1%, but at 0.5% of essential oil of white musk, *A. niger* had inhibited by 92.8%. Nevertheless, all the chemical agents had generally high effects against the fungi. Also, the evaporations of both of *Boswellia carterii* and *Pistacia lentiscus* had reduced the growth of *A niger*. While *Cinnamomum camphora*, *Boswellia carterii* and *Cocos nucifera* oil did not show any inhibitory effects on the growth of *A. niger*.

***Keywords*:** Indoor Contamination, Natural products, chemical products, *Aspergillus niger,* Musk.

Introduction

In recent years, the issue of indoor air quality enticed more concern compared to the case several decades ago. Environmental pollution control microbiology is concerned with solving a vast spectrum of environmental pollution problems that affect individuals around the world. Microbiological scientists and engineers are contributing with their knowledge and experience to meet the challenges of environmental pollution control in the world. Pan American Health Organization (PAHO) has reported that the predominant form of pollution in developing countries is indoor air pollution.

Centres for Disease Control (CDC) suggested that the most important approaches are the gravity settle plate cultures, represented in the periodic review of mycology. This approach followed by the utilising of adequate procedures such as the chemical disinfection used in busy hospitals, clinics and houses. The fungicidal proportion of disinfectants is classified based on the Association of Official Analytical Chemists (AOAC) suspension test. Despite the claims of manufacturers of disinfectants about the recommendations of dose and time, disinfectants need to be tested with environmental and clinical isolated fungi in order to determine a better judgment on their fungicidal activity. In contrast, there are inadequate data on the fungicidal activity of disinfectants.

Several authors reported the efficiency of the common disinfectants on certain species of microorganisms included bacteria, fungi and yeasts (Tsai & Lin, 1999). Disinfection against specific environmental pathogens has long been tried and established. A lot of disinfecting agents had been proved to have significant germicidal action against certain bacterial, fungal and yeast species. Many of the previous trials were conducted on a single type of microorganisms or disinfectants (Huang *et al*., 1997).

Medicinal plants are considered as rich sources of antimicrobial agents (Mahesh & Satish, 2008). Plants generally produce many secondary metabolites which constitute an essential source of microbicides, pesticides and many pharmaceutical drugs. Plant products remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997 and Ogundipe *et al*., 1998).

This study purposed to test the efficacy of some common chemical disinfectants and alternative medical chemicals against *A. niger* as a contamination agent in indoor environments. Then, examine some aqueous or evaporation of some plant extracts to compare their results with the chemical agents and white musk for their effects against the tested fungi.

Material and Methods

Media preparation

The media which used in this study was Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB). It was prepared, as mentioned in Frankland *et al*. (1995), to isolate *Aspergillus niger*. An appropriate amount of media powder was suspended in a respective amount of distilled water. In order to dissolve the medium completely, it was heated till boiling. The media was autoclaved at 121°C and 15 lbs pressure.

Isolation and identification

Environmental air samples were collected from different samples of air from various locations in Umluj city by using Sabouraud Dextrose Agar (SDA) Petri dishes (Robertson and Egger, 2010). The Petri dishes have been incubated at 28° C temperature for five days, as mentioned by Cruz Sánchez *et al*., 2014. Morphological characteristics of all different colonies were observed by Gupta, (2006) and have been detected by a light microscope to identify the fungi isolates (Atlas Ronald, 1984).

Preparation of plant extract

For testing, the effect of five aqueous plants extracts P*runus mahaleb* (seeds), *Commiphora myrrha* (gum), *Boswellia carterii* (gum), *Frangula alnus* (leaves) and *Pistacia lentiscus* (gum). Also, *Cinnamomum camphora* and

Cocos nucifera were used as essential oils on *A. niger* by a concentration of 10% from each extract was prepared.

Rodino *et al*. 2014 proposed that The aqueous plant extracts were acquired by maceration and used in the antifungal assay, for this process was used a quantity of 5g of dried, fine powdered plant to 50 ml of distilled water. Rodino *et al*., 2015 informed that the mixture was left for 1 hour in sealed glass recipients, at room temperature, with occasional stirring. The extract obtained was filtrated under vacuum through filter paper (Whatman No.1).

Disinfectant formulations

The examined disinfectants in this study were including Formaldehyde (3.7%), Methanol (10%), Ethanol (70%), Dettol (100%), Clorox (10%), Tide (100%), Liquid soap (100%), Flash (NaClO) (50%), Acetic acid (10%), Vikus (100%), Sodium Bicarbonate (NaHCO3) (100%), Alum (AB(SO4)2.12H2O) (100%) and sea salt (NaCl) (100%) which had been prepared with distilled water except the sea salt. Initially, 10% concentration of mentioned disinfectants were examined for evaluation of antifungal activities, and then concentrations of disinfectants were regularly decreased until the margin of effective dilution for control organisms were obtained.

White Musk Samples

Two selected samples of musk, which were used for this study, were the concentrated essential oil of white musk (from Abdulsamad Alqurashi shop), and the other sample was the white musk powder which is sold in the aromatherapy shops.

A fast test of all tested samples against *Aspergillus niger* to determine their antifungal activities under the light microscope:

All samples were used for inactivation of disinfectants after timed exposure (10 minutes) with 0.5×10 four fungal cells of *Aspergillus niger* on a slide together and covered by a glass cover. After that, all the slides were checked under light microscopes.

Assay of Antifungal evaluation of all tested samples against *Aspergillus niger* on SDB by dry weight method:

All tested samples were serially added by 10 ml to 90 ml of (SDB) in each flask (250 ml. Then, disks (with 0.5 mm) of *Aspergillus niger* colonies were added to each flask which was incubated at 25°C for five days when control samples were completely grown. The dry weight was detected, and the percentage of inhibition growth was recorded as well (Banerjee *et al*., 1933).

The MIC of white musk essential oil against *Aspergillus niger* on SDB by dry weight method:

White musk essential oil was added by 10%, 7.5%, 5%, 2.5% 1% and 0.5% to flasks with Saboroud dextrose broth (SDB) and disks (with 0.5 mm) of *A. Niger* colonies were added to each flask before incubated at 25°C for five days. The dry weight was calculated (Banerjee *et al*., 1933).

Results

The results in table (1) show the various impacts of all tested samples on both mycelium and spores of *A. niger.* These results had individually separated into two aspects which were chemical agents and natural agents. Figure (1) clarifies that all tested chemical agents affected both mycelium and spores of *A. niger* except ethanol.

Table 1 : The effects of some chemical disinfectants, some natural agents on the mycelium and spores of *A. niger.*

Type	Material	The effect on	
		Mycelium	Spores
Chemical Disinfectants	Dettol	+	+
	Clorox	+	+
	Tide	+	+
	Liquid soap	+	+
	Ethanol	-	-
	Methanol	+	+
	Formaldehyde	+	+
	Flash	+	+
	Alum	+	+
	Sodium bicarbonate	+	+
	Vikus	+	+
	Acetic acid	+	+
Aqueous Plant Extracts	*Commiphora myrrha*	+	+
	Prunus mahaleb	+	+
	Boswellia carterii	-	-
	Frangula alnus	-	-
	Cinnamomum camphora	-	-
	Cocos nucifera oil	-	-
Evaporation	*Boswellia carterii*	-	-
	Pistacia lentiscus	-	-
White Musk	Essential oil	+	+
	Powder	+	+

Key: (+) There is an effect of the tested agent on the fungi mycelium or spores.

(-) There is no effect of the tested agent on the fungi mycelium or spores.

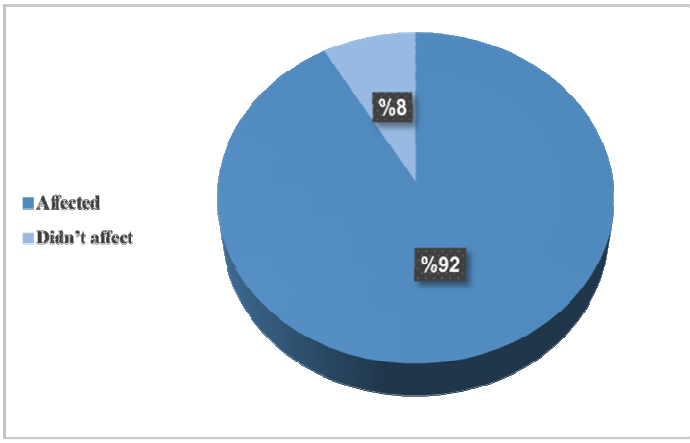

Fig. 1 : The effects of some chemical disinfectants on the mycelium and spores of *A. niger.*

Furthermore, the natural agents were divided into three groups which were the aqueous plant extracts, the evaporated plant gums and white musk samples. The results in figure (2) indicate that all the evaporated plant gums almost all aqueous plant extracts except *Commiphora myrrha* and *Prunus mahaleb* did not cause any effect of the mycelium or spores of *A. niger,* While both essential oil of white musk and the white musk powder affected *A. niger's mycelium and spores.*

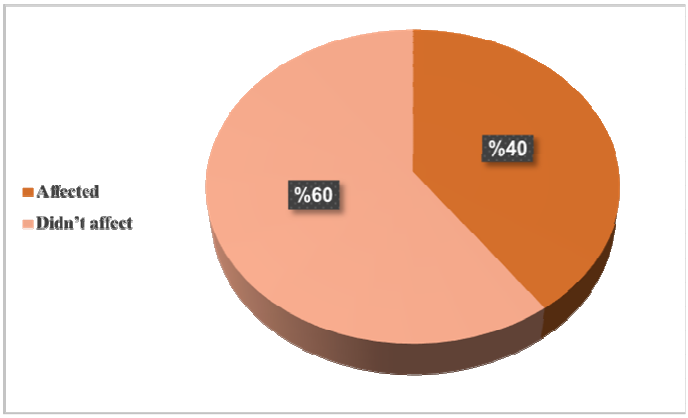

Fig. 2 : The effects of some natural agents on the mycelium and spores of *A. niger*.

Results in table (2) and figure (3) illustrate the impact of some chemical and natural agents which used to inhibit the growth of *A. niger* as a contaminated factor in indoor environments. *A. niger* completely inhibited by white musk essential oil. Both of Dettol and the evaporation of *Boswellia carterii* had inhibited the growth of *A. niger* by 94.1% and 93.7% respectively. While Clorox and Acetic acid had the same effect on *A. niger* growth by 92.2 %. Moreover, some chemical agents had close results to the previous agents such as Methanol, Flash, Alum and Sodium Bicarbonate by 91%, 90.8, 90.6% and 90.5% respectively. Ethanol, the evaporation of *Pistacia lentiscus* and Formaldehyde had an impact on the inhibition growth of *A. niger* by 89.9%, 89.4% and

Table 2 : The effects of some chemical disinfectants, some natural agents on the inhibition percentage of the dry weight of *A. niger*.

Type	Material	Percentage of dry weight inhibition of *A. niger* growth
Chemical Disinfectants	Dettol	94.1
	Clorox	92.2
	Tide	63.1
	Liquid soap	82.4
	Ethanol	89.9
	Methanol	91
	Formaldehyde	88.5
	Flash	90.8
	Alum	90.6
	Sodium Bicarbonate	90.5
	Vikus	68.3
	Acetic acid	92.2
Aqueous Plant Extracts	*Commiphora myrrha*	78.6
	Prunus mahaleb	14.0
	Boswellia carterii	0
	Frangula alnus	17.6
	Cinnamomum camphora	0
	Cocos nucifera oil	0
Evaporation	*Boswellia carterii*	93.7
	Pistacia lentiscus	89.4
White Musk	Essential oil	100
	Powder	48.6

88.5% respectively. Nevertheless, *Cinnamomum camphora*, *Cocos nucifera* oil and *Boswellia carterii* had no inhibition effect on *A. niger* growth.

The results in Table (3) and figure (4) reveal that essential oil of white musk had massive inhibition activities with low concentrations against *A. niger*. All the concentrations between 1% and 10% had inhibited the growth of *A. niger* while the inhibition effect had declined with 0.5% by 92.8%.

Table 3 : The MIC of the white musk essential oil effects on the inhibition percentage of the dry weight of *A. niger*.

Concentration %	Inhibition Percentage of *A. niger* growth %
10	100
7.5	100
5	100
2.5	100
1	100
0.5	92.8

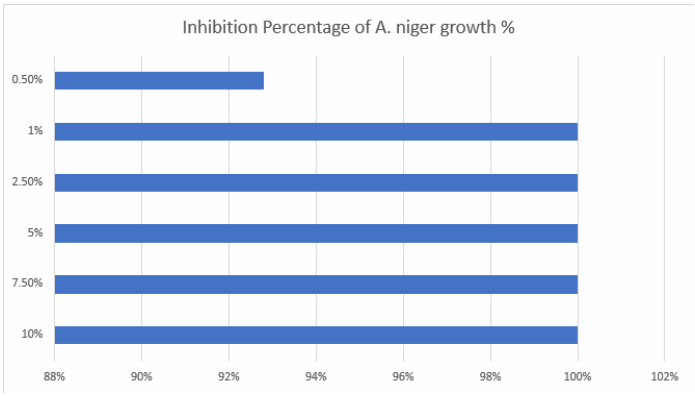

Fig. 4 : The MIC of the white musk essential oil effects on the inhibition percentage of the dry weight of *A. niger*.

Discussion

The most common fungus isolated from cases of otomycosis in our study was *A. niger* which was also the most common isolate recorded in other studies (Kumar, 2005 and Sarvan *et al.*, 2012). *A. niger* is commonly regarded as a pathogenic allergen generally associate with lung infections in individuals with a weak immune system (Kierownik, 1990). Results indicated that white musk essential oil is more effective on the tested fungi all concentrations (Saddiq, 2007). The Australian Mold Guidelines recommended the use of vinegar or alcohol for the removal of mould from contaminated surfaces (Kemp and Neumeister, 2005). Vinegar was found to be an antimicrobial agent, and there are some evidences which suggest that it possesses antifungal properties (Sholberg *et al.*, 2000) Dettol 2.5 % solution was the most effective disinfectant against *A. niger* as well as Benzalkonium chloride 6% solution was in the second level among four disinfectants from aspect of antifungal activity (Thèraud *et al.*, 2004). *Commiphora myrrha* revealed antifungal activity against *A. niger*. That result agrees with (Batool, 2007; Bhanu *et al.*, 2012; Hamedi *et al.*, 2015 and Abd-Ulgadir *et al.*, 2015).

Conclusion

From this study, it is deduced that a total of twelve chemical agents, six aqueous plant extracts, two evaporated gums from plant sources and two samples of musk had various antifungal activities against *A. niger*. Initially, the growth of *A. niger* was entirely inhibited by 10 % of white musk essential oil. Then 10% of Dettol (Spitera) and

evaporation of *Boswellia carterii* gum were effective in inhibiting 94% and 93.7 % of *A. niger* growth respectively.

Recommendations

We recommend using the white musk essential oil and evaporation of *Boswellia carterii* gum as a natural solution for the internal contamination of buildings caused by *A. niger*. Further studies are recommended as well to purify the active compounds in *Boswellia carterii* gum. We suggest analysing the components of the white musk oil which are needed to evaluate the effectiveness of each of them against *A. niger* and its application for other pathogenic fungi and other purposes.

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