

Spread of Sporulating Fungi Inside Shisha (Hookah or Waterpipe) and a Recommended way to Reduce Risks

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Nowadays, smoking shisha has been steadily spreading among people around the world. This study shed light on the presence of sporulating fungi as a second dimension to the risks of smoking shisha. *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purporogenum* were isolated from inner parts of shishas'. These three taxa have a bad reputation in the excretion of mycotoxins and causing similar symptoms of pneumonia. *Penicillium purporogenum*, *Paecilomyces lilacinus*, *Paecilomyces variotii*, *Aspergillus niger* and *Ulocladium utrum* were also isolated from air of the tested smoking places. Results showed that the laden air smell of mixture of menthol, eucalyptus oil and methyl salicylate (commercially, Abou Faz), inhibited by 100% growth of fungi isolated from inner structures of shishas'. The laden air smell of mint oil completely inhibited mycelia growth of *Paecilomyces lilacinus* while it did not affect others. Laden air smell of clove oil reduced amount of conidia and the red secretion of *Penicillium purporogenum* while it did not affect the rest. Air laden with each of anise, cardamom, eucalyptus and ginger had no effect on the three tested fungi. Application of a mixture of menthol and eucalyptus oils with methyl salicylate reduces risks of fungi circulating within the internal structures of the shisha and not reached by cleaning methods.

Key words: *Paecilomyces lilacinus*, *Paecilomyces variotii*, *Penicillium purporogenum*, shisha (hookah & waterpipe), Volatile oils.

Shisha is one of the most popular methods of smoking, and it knows among billion people in the world, dating to four hundred years. This smoking meets a strange silence, most encyclopedias ignored completely, while discussed in the media in terms of social problems that are considered "serious" and impressive. But that cannot be denied that this tool is used daily for long hours by more than one million people from the men and women in Asia, Africa and Europe¹.

Shisha originated in the east, and spread with the discovery of tobacco and the spread of cafes, accompanied the cup of tea and coffee

regularly. It is not a simple device, it has a length of two meters in height, and there is an installation of water which makes the smoke to cool and purified by water, and ends with a hose looks like a warped live pulls him smoker breaths, which may also reach several meters in length.

Modern scientific studies show² that smoking a shisha (WP) is still spreading globally among young people and is the second global epidemic after epidemic of smoking cigarette. It indicates the available evidence (Maziak, 2001) that euphoric smoking shisha among young people in the middle east region rate was 34% in August 2010, and reached 17% among American teenagers, and the ratio in scary steady increase in the whole world².

In addition of course to what it is known that hookah hose that contains within it a stable of

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microorganisms that thrive in moisture and warmth, which multiply and then spread across breathtaking clenched to where wreak havoc and a corruption in the health of smokers. It is known that chest physicians attribute the spread of tuberculosis, fungal pneumonia and lung diseases to smoking shisha³.

Interestingly, also of wonder that smokers apathetic believe that smoke shisha after the water is the purification and sterilization and disinfection of the smoke, making them feel safe healthy false, while it adds water moisture to passing the smoke stream which is - with warmth - and compromise is ideal for the growth of fungi.

It had previously thought, until recently that fungi do not cause fatal diseases to humans, but cause disease mostly superficial skin diseases or infections heal quickly, in most cases. It has been newly appeared, on the surface of pathology that many fungi cause diseases of the lung, the heart, the brain and the eye, especially for people with weakened immune systems. Employees and some workers are exposed to poultry farms lung diseases caused by infection of *Aspergillus* sp. This fungus produces huge amounts of conidia that spread in the air of farms where inhalation of this infested air may cause serious diseases of the lung called Aspergillosis, and this type of fungus is widespread strongly in shisha⁴.

Smokers of shisha can change the water in the water tank, but they cannot clean tubes from the inside where developing fungi multiply on the remains of humid nicotine and tobacco burn products. It is worth mentioning that when the fungi thrive and multiply enormous amounts of spores and conidia which starts with the entry into force of the smoke to settle in the lungs of smokers.

The majority of studies on smoking shisha concentrated on the effect of nicotine and carbon oxides on the health⁵. Unfortunately, these studies do not address to another dimension of the threat of the spread of dangerous fungi within the chests of smokers.

Volatile essential oils were used in the treatment of many fungi by direct touching⁶. In a previous study, the effect of 12 kinds of essential oils of cloves, rosemary, cinnamon, Alemramip, pine, orange blossom, mint, anise, caraway, lavender and thyme on 12 types of pathogenic fungi was done. The experiment found that all of

these oils damper mycelial growth of the fungi under study, except oily orange blossom and pine and to varying degrees⁶. In a recent study, the effect of three types of essential oils derived from and *Thymus Kotschyanus*, *Thymus vulgaris* and *Zataria multiflora* on 4 types of pathogenic fungi revealed that all those oils inhibited the fungal mycelial growth⁷. It is worth mentioning that most of the previous studies revealed the positive effect of many volatile oils on fungal growth.

The aim of this study is to isolate and identify fungi located within the structures of the number 125 shisha distributed in cafe and lounge in Sakaka city, Saudi Arabia as well as testing the effect of a number of volatile plant oils in reducing spread of these fungi.

MATERIALS AND METHODS

Sites and sampling

Isolation of fungi from shisha in smoking places

Twenty-five cafes and resting-places located in Sakaka city, Aljouf, Saudi Arabia, were chosen for sampling during April, 2014. Samples were collected from five shisha sets in each of 25 cafes and resting-places by pushing 200 ml sterilized distilled water inside the hose of shishas' and then received the water in another empty sterilized bottle. Fungi were isolated on rose-benal potato dextrose agar (rose-bengal-PDA) by putting 5 ml of each sample on the rose-bengal PDA medium⁸. Plates were incubated at 27°C for 7 days or until emergence of colonies and then purified on PDA without rose-bengal. The number of purified fungi were identified and counted.

Isolation of fungi from the air of the same places of smoking shisha

Trapping of fungal spores and conidia by deposition using the 'exposed plate' method was implemented to trap indoor air fungi at cafes and rest-places⁹. Five plates (9 cm diam.) containing rose-bengal-PDA were used to catch in each place¹⁰. Sampling time was set for 30 minutes at an approximate height of 50 cm from the floor. After trapping reproductive units, plates were sealed with parafilm and then incubated at 27°C for 7 days. Growing fungi were counted, purified and identified. Fungi may fail to sporulate with slow growing on the medium was transferred to other medium (PDA without rose-bengal). Counts

were expressed as colony forming units (CFUs) “ 5 plates in individual exposures.

Identification of fungi

It was performed using morphological characteristics of taxonomic keys¹¹⁻¹⁶. Fungi were examined and photographed using Motic microscope (Carolina Biological, Supply Company, Burlington, N.C.).

Study the effect of seven kinds of volatile oils [anise, camphor, cardamom, cloves, ginger, mint and a mixture of menthol, eucalyptus and methyl salicylate (20, 15, 15%)] on growth of *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purporogenum*:

Conidial suspension of each of the three tested fungi were prepared by putting a piece (2x2 cm) of fungal growth on PDA medium in 25 ml sterilized distilled H₂O in 100 ml Erlenmeyer flasks and leave for 5 hours at 27°C prior use.

Five ml of spore suspension of each fungus were placed in a sterile Petri dish (9 cm diameter dish) and then supplemented with 15 ml PDA (just before solidification) and leaves until the mixture solidifies. Each 9 cm Petri-dish was placed in another platter of 15 cm diam. The small dish (9 cm) of each treatment was placed inside the platter (15 cm diam.). One ml of each tested volatile oil was added inside the platter and outside the small dish. The cover of the small dish was removed and all the set was enclosed only with the cover of the platter and finally sealed with parafilm. Dishes were incubated at 27°C for 7 days

Table 1. Total counts of fungi recovered from 125 shisha in 25 cafes and resting-places in Sakaka city, Aljouf governorate, Saudi Arabia, number of cases of isolation (NCl; out of 125 cases), occurrence remarks (OR), and percentage of total counts (TC%) on PDA agar at 27°C

Fungi	Isolation remarks		
	NCl	OR	TC (%)
<i>Paecilomyces lilacinus</i>	378	113H	38.8
<i>Paecilomyces variotii</i>	321	99 H	33.0
<i>Penicillium purporogenum</i>	139	62 M	14.3
Gross total counts	973		
No. of genera	2		
No. of species	3		

OR = Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0% , L = 20 - 32%, and R = 7 – 19%.

in dark. Growth noted then samples were visualized. Five replications were performed for each treatment.

Statistical analysis

Number of cases of isolation of each obtained fungus, fungal occurrence remarks and percentage of total counts were analyzed through this study. Experiments of the effect of volatile oils on fungal growth were analyzed using mean of 5 replications of each trial.

RESULTS

Data in Table (1) show that 3 species belonging to 2 genera were isolated from 125 shisha in 25 cafes and rest-places in Sakaka city, Aljouf governorate, Saudi Arabia. *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purporogenum* were obtained from inner parts of the shisha (hoses). *Paecilomyces lilacinus* was steadily the most frequent fungus (113 out of 125 samples) and contributed 38.8% of total count. *Paecilomyces variotii* was recovered from 99 samples matching 33% of total count fungal isolates while *Penicillium purporogenum* was recovered from 62 samples matching 14.3% of total count.

Data in Table (2) reveal that *Aspergillus niger*, *Ulocladium atrum* together *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium*

Table 2. Total counts of fungi recovered from from 25 air samples from cafes and rest-places in Sakaka city, Aljouf governorate, Saudi Arabia, number of cases of isolation (NCl; out of 25 cases), occurrence remarks (OR), and percentage of total counts (TC%) on PDA agar at 27°C.

Fungi	Isolation remarks		
	NCl	OR	TC (%)
<i>Aspergillus niger</i>	132	25 H	31.9
<i>Ulocladium atrum</i>	115	12 M	27.8
<i>Paecilomyces lilacinus</i>	60	7 L	14.5
<i>Paecilomyces variotii</i>	61	7 L	14.7
<i>Penicillium purporogenum</i>	46	6 L	11.1
Gross total counts	414		
No. of genera	4		
No. of species	5		

OR = Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0% , L = 20 - 32%, and R = 7 – 19%.

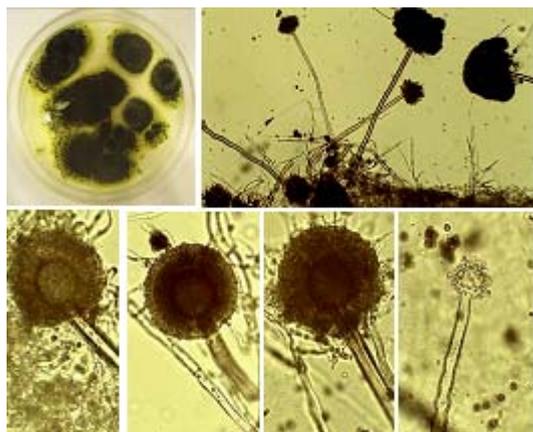


Plate 1. Morphology of *Aspergillus niger* isolated from the air of the studied cafes and rest-places. (a) colony morphology on PDA, (b) conidiophores and heads heavily possess conidia, (c, d, e) radiate heads with biserial strigmata, (f) a bare head showing a globose vesicle. Bar on photo (b) is equal 20 μm for this photo only while bar on photo (d) is equal 20 μm and is the same for all the rest of the images

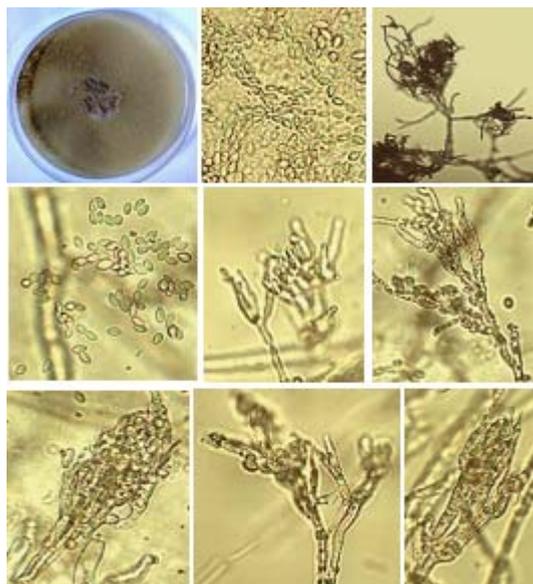


Plate 2. Morphology of *Paecilomyces lilacinus* isolated from shishas' and the air of the studied cafes and rest-places. (a) colony morphology on PDA, (b) conidia in chains, (c) chains of conidia on strigmata (low magnification), (d) conidia (high magnification), (e – i) conidiophore and spear shaped strigmata. Bar on photo (b) is equal 5 μm for this photo only while bar on photo (c) is equal 5 μm for this photo only and bar on photo (e) is equal 5 μm and is the same for all the rest of the images

purporogenum were isolated from the from air of the tested smoking places. Five species belonging to 2 genera were isolated from 25 air samples in 25 cafes and rest-places in Sakaka city. *Aspergillus niger* was the maximum frequent fungus (100% of places) contributing 31.9% of total count of the isolated fungi. *Ulocladium atrum* came in second with 27.8% of total count. *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purporogenum* represent a few isolated rates of 7, 7 and 6, respectively, cases out of 25 cases.

Effect of seven types of volatile oils [anise, camphor, cardamom, cloves, ginger, mint and a mixture of menthol, eucalyptus and methyl salicylate (20, 15, 15%)] on growth of *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purpurogenum*:

Results show that air saturated with the mixture of menthol, eucalyptus and methyl salicylate (20, 15, 15%) oil (Abu Fez) inhibited growth of the three tested fungi by 100% as compared to the control (Fig. 1).

Air saturated with mint oil smell completely inhibited growth of *Paecilomyces lilacinus* by 100% as compared to the control

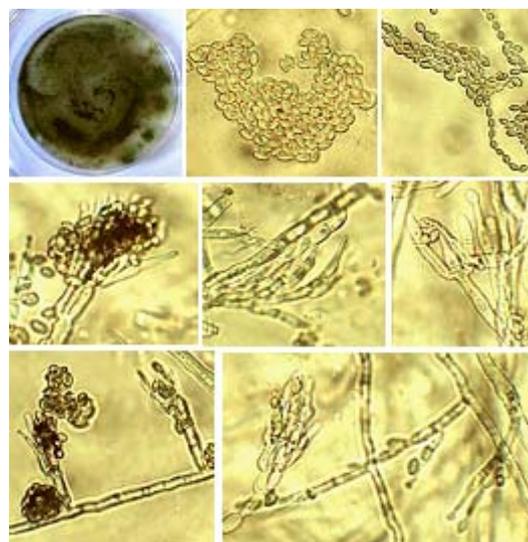


Plate 3. Morphology of *Paecilomyces variotii* isolated from shishas' sets and the air of the studied cafes and rest-places. (a) colony morphology on PDA, (b, c) conidia in chains, (d – h) conidiophore and spear shaped strigmata. Bar on photo (c) is equal 5 μm for this photo only while bar on photo (b) is equal 5 μm for this photo only and bar on photo (e) is equal 5 μm and is the same for all the rest of the images

sample while not affecting other two fungi (Fig. 2).

Air saturated with clove oil smell reduced sporulation and red secretion of *Penicillium purpurogenum* compared to the control sample while not affecting other two fungi (Fig. 3).

On the other hand, air saturated with anise, cardamom, eucalyptus and ginger oil smell have not appeared any effect on growth and sporulation of the tested fungi.

DISCUSSION

Results here showed the presence of *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purpurogenum* in water-laden with precipitation of outputs burning and smoking tobacco inside structures of the inner parts of shisha such as hoses in which cleaning ways not reached them in various cafes and resting-places in Sakaka, Aljouf, Saudi Arabia. It can be said that conidia of these three fungi isolated from shisha is puffing with smokers' exhalation. Literature review revealed the absence of explicit studies on the presence of fungus inside structures of

shishas' especially parts that could not be reach by the normal cleaning ways. Generally, earlier studies on smoking shisha focused on the effect of nicotine and carbon oxides on smokers' health². In this study, we added a second dimension to the seriousness of smoking shisha of existed and grew up fungi located inside structures of shisha.

Fungi isolated from internal parts of shisha have a bad reputation which causes a risk to the health of smokers. Scientific references of fungal pathology shows the seriousness of those fungi in many human and animal diseases¹⁷⁻²².

Paecilomyces lilacinus, *Paecilomyces variotii* and *Penicillium purpurogenum* which were obtained from inside shisha beside two other fungi of *Aspergillus niger* and *Ulocladium atrum* were also isolated from the air cafes and resting-places. Presence of *Aspergillus niger* and *Ulocladium atrum* in the air of smoking places and their absence within the shisha is probably due to enable them to withstand the amount of nicotine contained within the structures of shisha. Environment inside shisha hoses and internal parts with high humidity and adsorbed tobacco smokes is suitable for the presence of *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purpurogenum*.

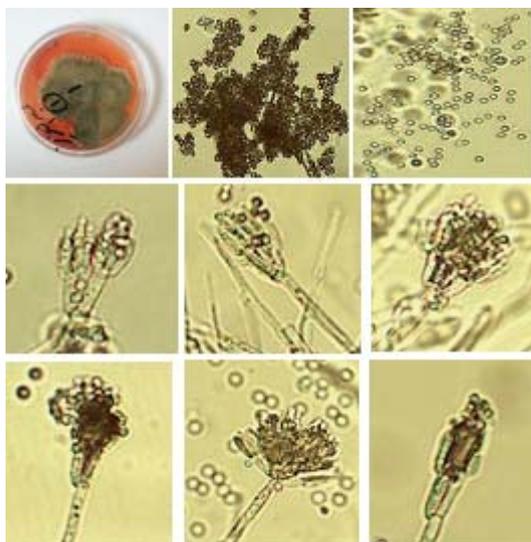


Plate 4. Morphology of *Penicillium purporogenum* isolated from shishas' and the air of the studied cafes and rest-places, (a) colony morphology on PDA, (b, c) clusters of conidia, (d – i) conidiophore and strigmata (biverticillata symmetrical). Bar on photo (b) is equal 5 μm for this photo only while bar on photo (e) is equal 5 μm for this photo only and bar on photo (e) is equal 5 μm and is the same for all the rest of the images

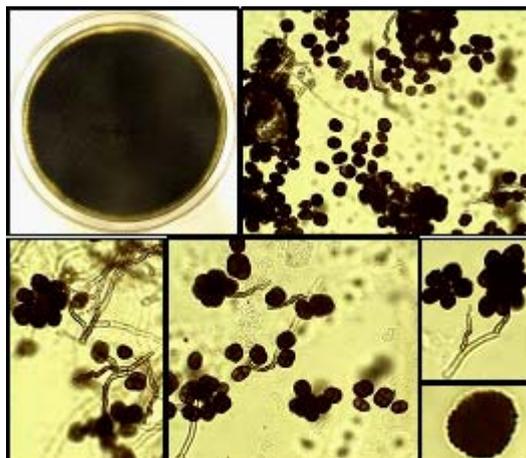


Plate 5. Morphology of *Ulocladium atrum* isolated from the air of the studied cafes and rest-places, (a) colony morphology on PDA, (b - e) conidia and conidiophores, (f) spiny conidia. Bar on photo (b) is equal 20 μm for this photo only while bar on photo (f) is equal 20 μm for this photo only and bar on photo (c) is 20 μm and is the same for all the rest of the images

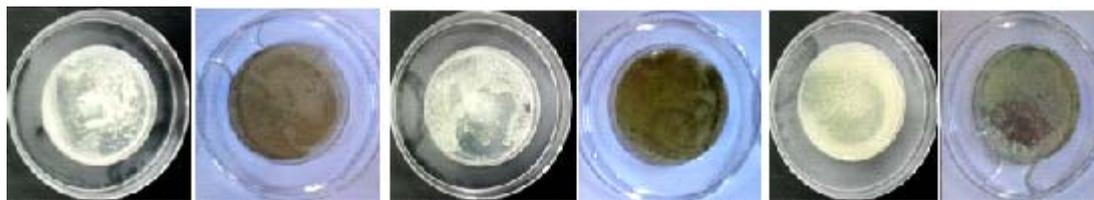


Fig. 1. Inhibition of fungal growth of *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purpurogenum* after exposure to the vapor of the mixture of menthol, eucalyptus and methyl salicylate (20, 15, 15%) oil (Abu Fez) and then incubation for one week at 27°C. For each fungus, the left photos show the treatment (1 ml oil) and the right photos show the control (1 ml H₂O)

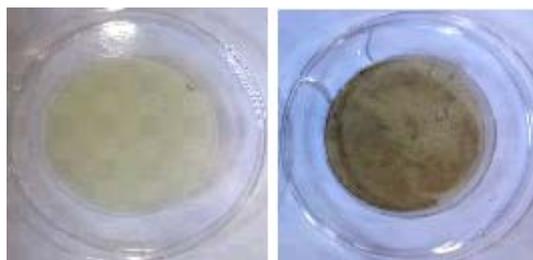


Fig. 2. Inhibition of fungal growth of *Paecilomyces lilacinus* after exposure to the vapor of mint oil and then incubation for one week at 27°C. The left photos show the treatment (1 ml oil) and the right photos show the control (1 ml H₂O)



Fig. 3. Inhibition of sporulation and production of red pigmentation of *Penicillium purpurogenum* after exposure to the vapor of mint oil and then incubation for one week at 27°C. The left photos show the treatment (1 ml oil) and the right photos show the control (1 ml H₂O)

With the sequential use and daily smoking shisha containing huge amount of fungal conidia, and exposed its users to inhale these germs which may cause a lot of diseases especially for the elderly and impaired immune and children. Therefore, it was necessary for us to find a safe natural way to minimize risks of smoking shisha.

Plant volatile oils were commonly used in the control of some fungal diseases²³. Results obtained in this study show that the use of air saturated and laden vapor of the commercial brand of "Abu Faz" oil (menthol + camphor oil + methyl salicylate) 100% inhibited the growth of *Paecilomyces lilacinus*, *Paecilomyces variotii* and *Penicillium purpurogenum*. Mint oil proved its inhibitory effect on growth of *Paecilomyces lilacinus* only, but it did not affect the other two tested fungi. Clove oil reduced production of conidia and red secretions of *Penicillium purpurogenum*, but it did not affect the other two tested fungi. On the other hand, anise, camphor, cardamom ginger and did not show any effect towards the three tested fungi. Causes of these

effects can not be given at present and need further studies.

Therefore, we recommend the reality of the results of this research using and inhaling any commercial compound that contains a mixture of menthol and eucalyptus oil and methyl salicylate in order to assist in alleviate the risk of inhalation of such fungal spores. Drinks containing mint and cloves can soften and lessen fungal spores carried on tobacco smoke. Further studies on the presence of fungi inside structures of shisha in many places all over the world should be done. Consolidation of innovative ways to eliminate these fungi, in addition to educate drug abusers second dimension to the risk of smoking shisha are required.

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