Egypt. Acad. J. Biolog. Sci., 11(1): 59-70 (2019)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

Possibility of Reducing Presence of Harmful Fungi in Air-Conditioner Windows Using A Transcendental Antifungal Chemical

Shaima M. N. Moustafa
Department of Botany and Microbiology, Faculty of Science, Minia University, El-Minia, 61511, Egypt
E.Mail: **moustafa.shaima@yahoo.com**

ARTICLE INFO
Article History
Received: 6/10/2019
Accepted:28/10/2019

***Keywords*:**
Air-conditions, Fungal mycelial growth, Hemolysis, Naphthalene, Sakaka city.

ABSTRACT

Fungi are usually spread in humid areas, even if they are scarce in nutrients, which can capture a small amount of them from the surrounding air. Fungi in air-conditioner windows were studied in 13 air conditioners in houses located in Sakaka city, Jouf, Saudi Arabia, in the spring of 2018. Air passages inside these air conditioners are suitable environments for the occurrence of fungi. This research was designed to reveal the presence of dangerous fungi in air passages of these conditioners and to find suitable practical ways to eliminate these harmful taxa. Isolated fungi were identified using morphological and ultrastructure criteria and were Aspergillus niger, *Chaetomium globosum*, *Penicillium frequentans*, *Penicillium italicum*, *Paecilomyces lilacinus, and Xanthophyllomyces dendrorhous*. Results showed that *Aspergillus niger*, *Paecilomyces lilacinus,* and *Penicillium italicum* caused severe decomposition of in human blood red cells, the strongest of which was the fungus *Paecilomyces*. The aromatic pungent smell of transcendental naphthalene has been shown to have an inhibitory effect on three of the isolated fungal species. Transcendental naphthalene inhibited mycelium growth of *Aspergillus niger*, *Penicillium frequentans* and *Penicillium italicum* by 100% while never affecting *Paecilomyces lilacinus*. The study recommends further studies on the presence of fungi in the air - driven corridors of air conditioners in different countries and devising new ways to reduce the presence of fungi.

INTRODUCTION

Fungi spread everywhere on the globe, especially in moist, shady places. In the past years, fungi have been a vital contaminant of many unhealthy indoor spaces (Flannigan & Miller, 1994) Typically, indoor fungi include species of *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium* and *Penicillium*. These fungi were usually obtained from wood, roof-roofing, house dust, pets, and ornamental plants, however, some taxa were associated with heating devices, suctions, and air conditioners (Ahearn *et al.*, 1992,1996; Simmon & Crow 1995)

Occasionally, indoor fungal spores caused human diseases such as allergies ranging from pneumonia to asthma-like symptoms (Morpeth *et al.,* 1995) Compounds produced by these fungi may be toxic or adversely affect immune activity, contributing to responses to anaphylaxis and making those exposed to those fungi more susceptible to other microorganisms (Flannigan & Miller, 1994). In addition, many fungi were known to produce much harmful volatiles (Crow & Ahearn 1997).

The indoor environment of buildings is likely to be a source of health problems, often caused by allergies due to fungi circulating in the air of these rooms, similar to those found in poorly ventilated and ivory contaminated places. (Kumar *et al.,* 1981, 1984) attributed allergic rhinitis and pneumonia to hypersensitivity due to *Aspergillus*, *Cladosporium* and *Penicillium* emanating from air conditioning vents in cars.

Insufficient studies have been recorded on the prevalence of fungi associated with closed rooms with air conditioning systems, especially for people who have complained about the unpleasant odors from those places.

Fungi found in the corridors of air conditioners are sources of inconvenience and pollution to the places where they are located since it is hardly without those devices, which the majority of the population can not live comfortably because of the extreme temperature in summer.

The hypothesis of this study is: Are these devices scattered in houses of Sakaka, Jouf, Saudi Arabia represent a source of contamination and spread of fungal spores and growth units to the houses? Is it possible to devise a way to solve this problem and reduce the spread of those fungi?

This research was designed to identify the fungi found in the air push corridors in household air conditioners scattered in many houses in Sakaka, Jouf, Saudi Arabia. Therefore, the light was shed on a serious source of fungal diseases and to identify the seriousness of fungi isolated from these places in order to find ways to reduce and eliminate those harmful fungi.

MATERIALS AND METHODS

Collecting Samples from Air Passages of Air Conditioners in Sakaka And Isolation of Fungi:

In 300 ml sterilized drinking water bottles put cotton sticks (earmuffs) that have already been wiped on the air conditioning corridors (taking into account, wearing gloves and not touching the samples). Bottles were closed and listed with data collection. Place 5 ml of water containing each sample separately in a sterile Petri dish and pour 15 ml of rose Bengal Potato dextrose agar (PDA) on it and leave to solidify (Maghazy *et al.,* 2013). Plates were placed inverted, after sealing them with parafilm, in an incubator at 28 ° C, for 7 days.

After appearance of various fungal colonies in the dishes, each colony (containing one fungal species) was transferred in a process called purification, in a petri dish containing PDA (without rose bengal), then incubated at 28 °C, for 7 days, or until the emergence of fungal colonies, giving the opportunity for slow-growing species not inconsistent with the overlap of colonies.

Identification of Isolated Fungi:

Species were identified with the help of Ainsworth & Bisby, 1971; Ainsworth *et al.,* 2001, using the digital camera Motic microscope as well as the scanning electron microscope in the electronic microscope unit, Department of Zoology, King Saud University, Saudi Arabia.

Hemolytic Activity Of The Isolated Fungi:

Red blood cells of humans were used by experimenting with hemolytic activity according to (Tasca & Adecarrli 1999; Mukherjee & Rajasekaran 2010). To study the hemolytic effect, solutions of different fungi spores or conidia (spores) were prepared using a 0.9% NaCl solution per ml. One hundred µl of human blood was incubated (after washing with saline three times) with 900 µl of fungal spore suspension or sodium chloride solution representing

negative control sample and distilled water (positive control sample) for comparison. After 24 hours, the blood solution was separated in the tubes using a centrifuge and measured the color of the floating fluid at the top of the tube using the UV visdspectrophotometer (spectro uv-2505) at a wavelength of 540 nm. The percentage of hemolysis was calculated according to the following equation and the experiment was repeated 3 times.

%Hemolytic activity= absorbance of the sample - absorbance of saline/absorbance of dist. water X 100.

Study the effect of naphthalene on mycelium growth and spore production in *Aspergillus niger, Penicillium frequentans, Penicillium italicum,* and *Paecilomyces lilacinus*

The experiment was done according to the following method:

A - Each PDA-Petri dish (9 cm diameter) was inoculated with each tested fungus.

B- The pre-prepared inoculated dishes (9 cm diameter) were placed in another 15 cm dish and then the small dish was opened and 3 pieces (each 5 g) of naphthalene were placed inside the big one.

C - Dishes were incubated at 28 ° C for 7 days in the dark. Control dishes of each fungus was performed without naphthalene, and this method is innovative from us. Growth is observed and samples are visualized.

Statistical Analysis:

ANOVA was used to evaluate data following the Minitab software (version 12).

RESULTS

Fungi appeared in all of the collected samples. The following table shows an isolated species (Table 1, Figure 1):

Identification of Fungi:

Isolated fungi were identified using keys (see Materials and Methods), and obtained taxa were fell under the following groups:

1.Unicellular ascomycetes of the yeast of *Xanthophyllomyces dendrorhous* (Figure 6).

2. Multicellular ascomycetes of the genera *Aspergillus*, *Penicillium* and *Chaetomium* and they were *Aspergillus niger*, *Penicillium frequentans*, *Penicillium italicum* and *Chaetomium globosum* (Figs. 2-5).

3.Deuteromycetes of the genus *Paecilomyces* which was *Paecilomyces lilacinus* (Figures 7).

Hemolytic Activity of Tested Fungi:

Results showed that fungi of *Paeciolomyces lilacinus* (sample No. 4), *Penicillium italicum* (sample No. 5) and *Aspergillus niger* (sample No. 2) demonstrated the ability to analyze and break red blood cells as shown in figures (8). *Paeciolomyces lilacinus* appeared the highest percentage of the analytical activity, followed by *Penicillium italicum* and *Aspergillus niger* compared to the rest of fungi.

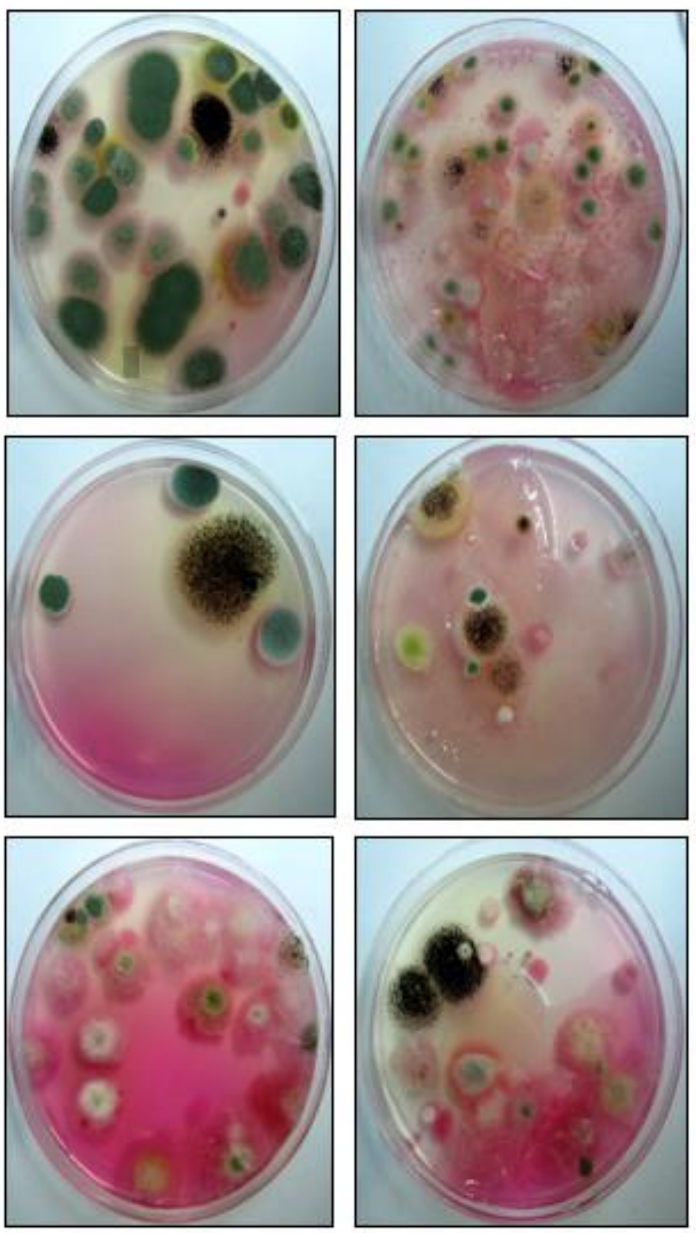
Effect of Naphthalene on Mycelial Growth and Spore (Conidial) Production in *Aspergillus Niger, Penicillium Frequentans, Penicillium Italicum and Paecilomyces Lilacinus*:

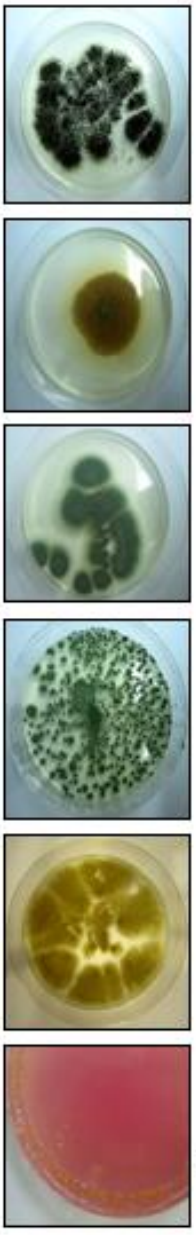
Results showed that the air infested with naphthalene inhibited the mycelial growth of the three fungi of *Aspergillus niger*, *Penicillium frequentans* and *Penicillium italicum* by 100%, while it did not affect *Paecilomyces lilacinus* in comparison with the control sample (Figure 9). It is worth noting that all results have 5 replicates and all experiments are repeated twice to confirm these important results, as in the following forms:

.

Table 1. Fungal species from 13 air conditioners in Sakaka city, Jouf, Saudi Arabia

Isolated Fungi	Sample No.
Aspergillus niger *Chaetomium globosum* *Penicillium frequentans* *Penicillium italicum* *Xanthophyllomyces dendrorhous* (yeast)	1
Aspergillus niger *Chaetomium globosum* *Penicillium frequentans* *Penicillium italicum* *Xanthophyllomyces dendrorhous*	2
Aspergillus niger *Chaetomium globosum* *Penicillium frequentans* *Penicillium italicum*	3
Aspergillus niger *Penicillium frequentans* *Penicillium italicum*	4
Aspergillus niger *Penicillium italicum* *Xanthophyllomyces dendrorhous*	5
Aspergillus niger *Chaetomium globosum* *Penicillium frequentans* *Penicillium italicum*	6
Aspergillus niger *Chaetomium globosum* *Paecilomyces lilacinus* *Penicillium frequentans*	7
Aspergillus niger *Paecilomyces lilacinus* *Penicillium italicum*	8
Aspergillus niger *Paecilomyces lilacinus* *Penicillium italicum* *Xanthophyllomyces dendrorhous*	9
Aspergillus niger *Paecilomyces lilacinus* *Penicillium frequentans*	10
Aspergillus niger *Chaetomium globosum* *Penicillium frequentans*	11
Aspergillus niger *Penicillium frequentans*	12
Aspergillus niger *Chaetomium globosum* *Penicillium frequentans*	13





Colony morphology of *Aspergillus niger*

Colony morphology of *Chaetomium globosum*

Colony morphology of *Penicillium italicum*

Colony morphology of *Penicillium frequentans*

Colony morphology of *Paecilomyces lilacinus*

Colony morphology of *Xanthophyllomyces dendrorhous*

Fig. 1. Total fungal colonies from air-conditioner air flow windows, cultured on Rose-bengal PDA after 7 days at 28 ° C, in the dark .

Pure cultures

.

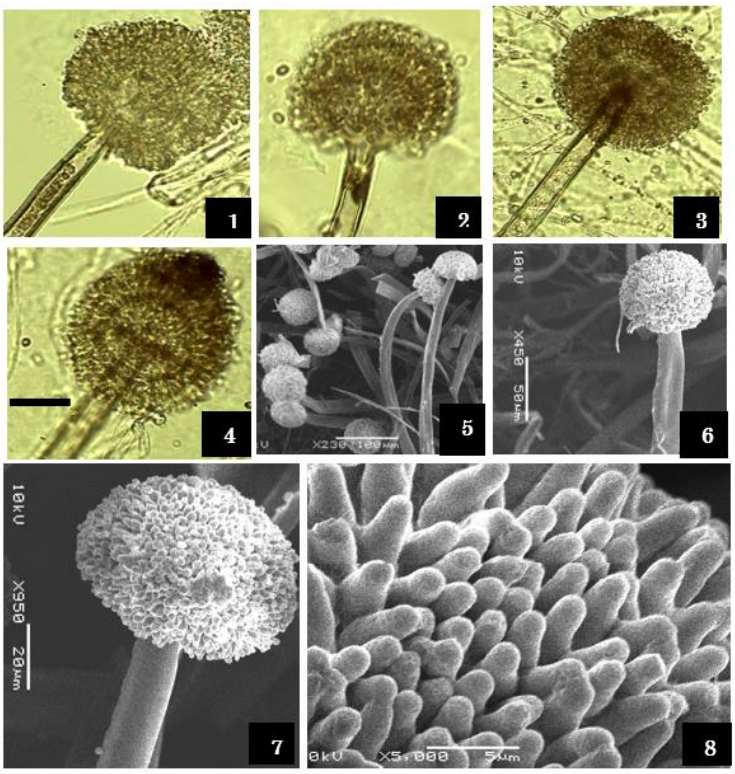


Fig.2: Mycelial growth of *Aspergillus niger* on PDA, under compound microscope. 1-4. A Thick long conidiophore carrying biseriate strerigmata. 5-8. Growths under SEM Electron Microscopy. Bar 10 µm in images (4) is the same in images 2-4, and in electron microscope images, each magnification is showed on each image, separately

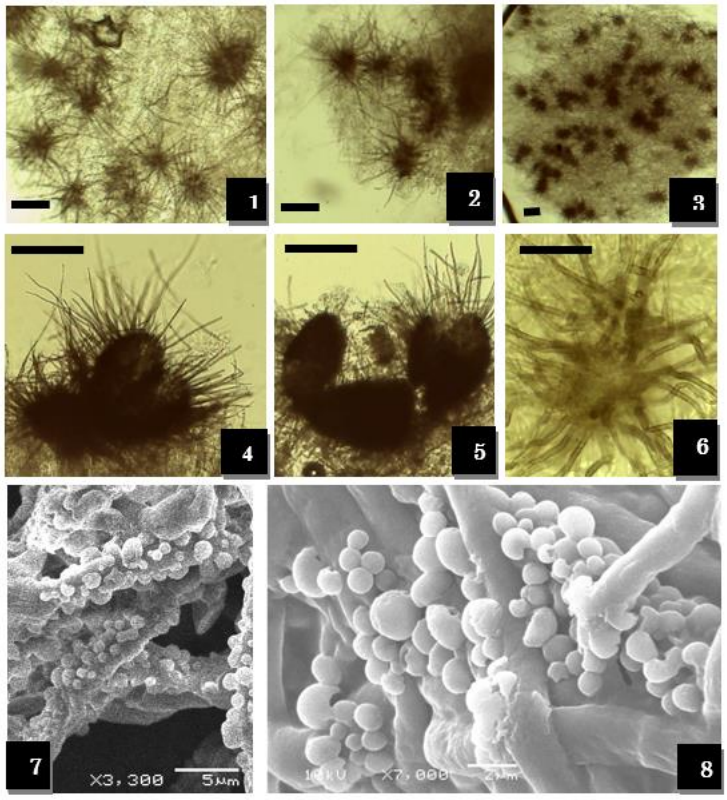


Fig.3: Mycelium growth of *Chaetomium globosum* on PDA. 1-6. growth under a compound microscope. 1-6. Cleistothecium with long appendages. 7, 8 Under SEM electron microscope. Bar 10 µm in pictures (1-6) and in electron microscope images, each enlargement is listed on each image separately.

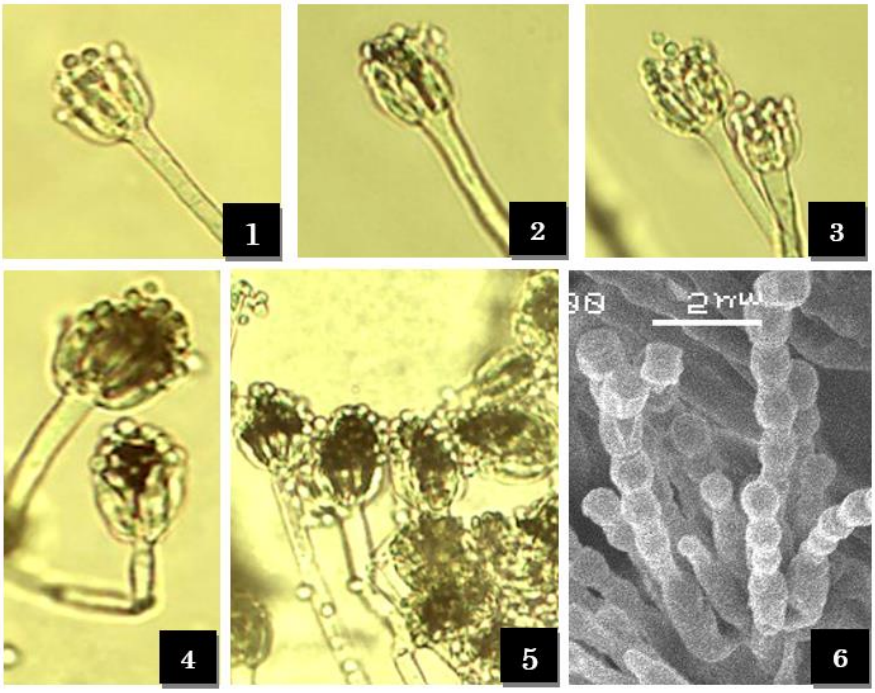


Fig. 4: Mycelial growth of *Penicillium frequentans* on PDA. 1-5. Growth under a compound microscope. 6. Growth under SEM electron microscope. Bar 10 µm is in the image (1) the same for all the images 2-5, and in the electron microscope (No. 6) Bar is indicated in the scale. 1-5. Septate conidiophore with monoseriate sterigmata.

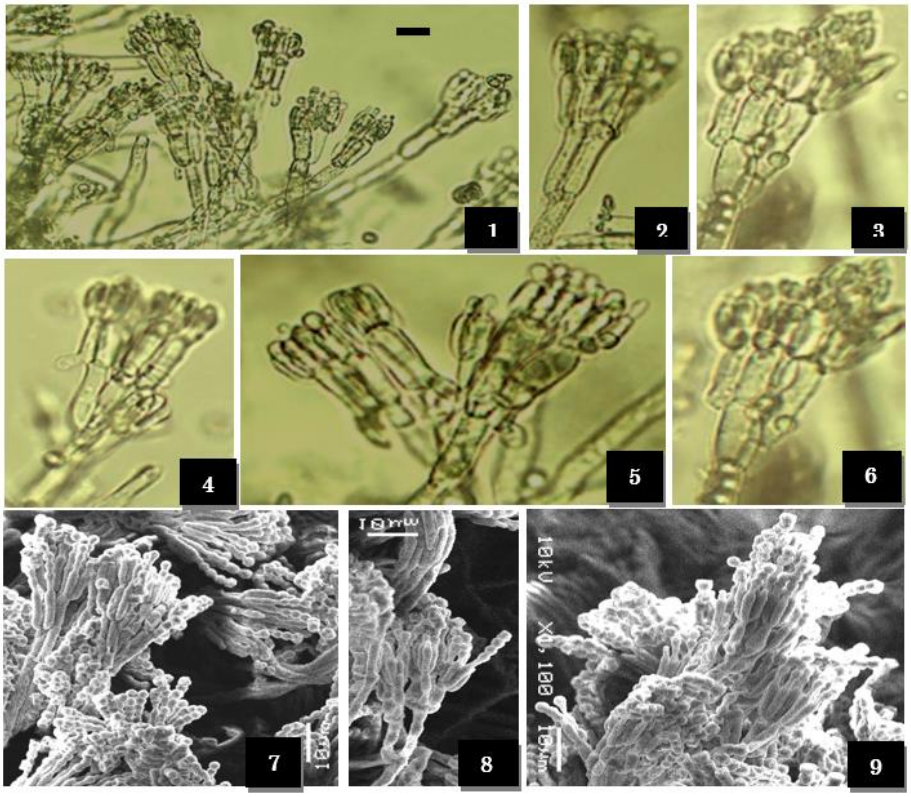


Fig. 5: Myceloom growth of *Penicillium italicum* on the PDA. 1-6. Growth under a compound microscope. 7-9. Growths under SEM electron microscope. Bar 10 µm in picture (1) is the same for all of 2-6 pictures, and in electron microscope images (7-9) each has its own scale. (1-6) Septate conidiophore with asymmetrical biseriate sterigmata

.

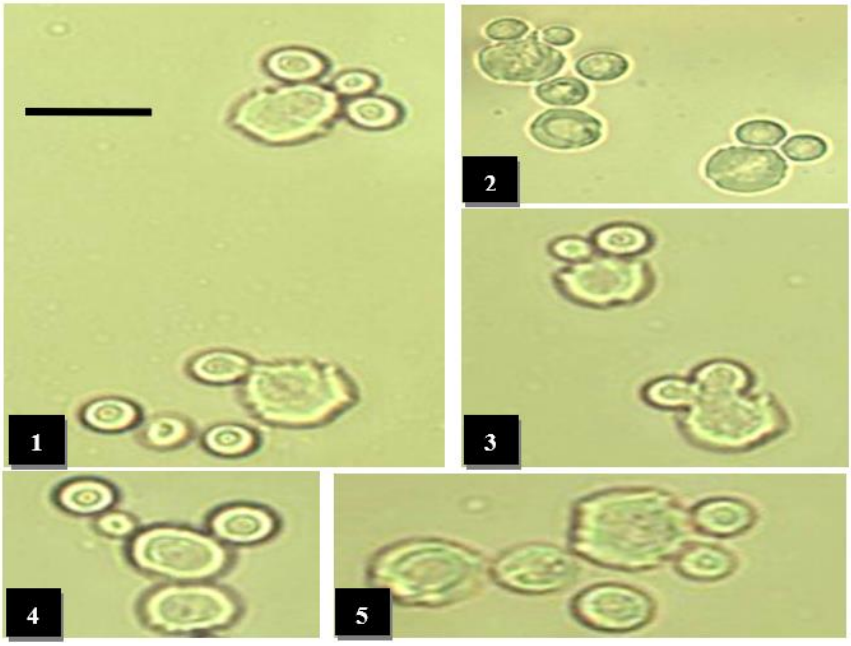


Fig. 6: Mycelial growth of *xanthophyllomyces dendrorhous* on PDA. 1-5 Under a compound microscope. Single-cell yeast cells are characterized by multibudding. Bar 5 µm in image 1 is the same for all pictures.

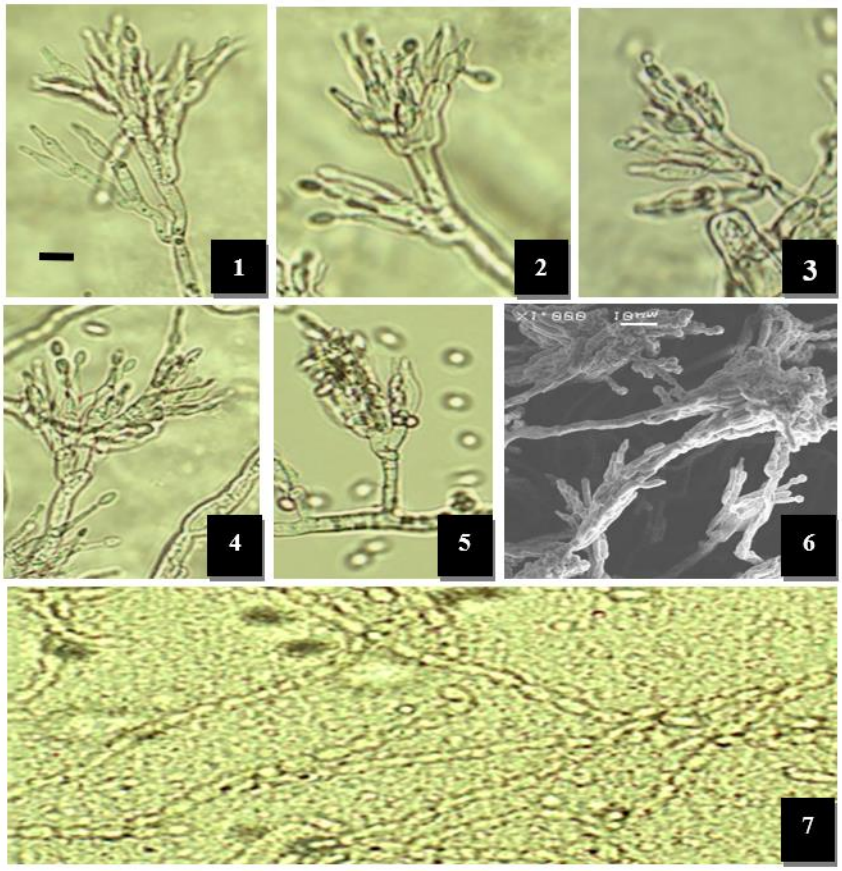


Fig. 7: Mycelium growth of *Paecilomyces lilacinus* on a PDA. 1-5, 7. Growth under a compound microscope. 6. Growth under SEM electron microscope. Bar 10 µm in image (1) is the same for all pictures except for image 6 which is an electronic microscope in which one has its scale. 1-6. Septate conidiophores with spear-shaped asymetrical biseriate sterigmata. (7) Conidia in chains.

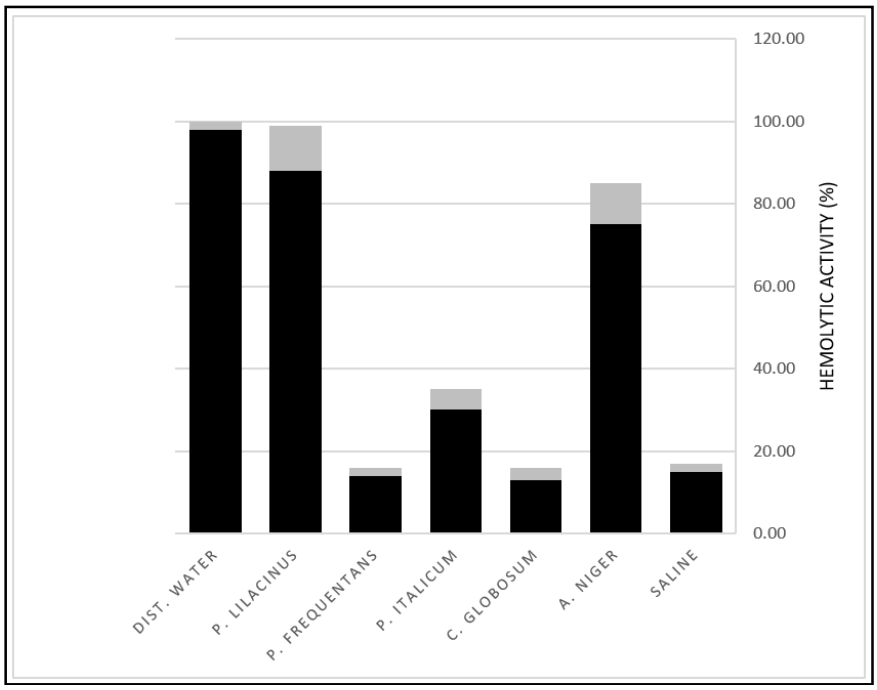


Fig. 8: Effect of fungal spore suspension on red blood cell analysis of a human sample. The grey bars above each plot represents the standard error of the data mean of 3 replicates and reflects the average difference between the averages of the samples and controls.

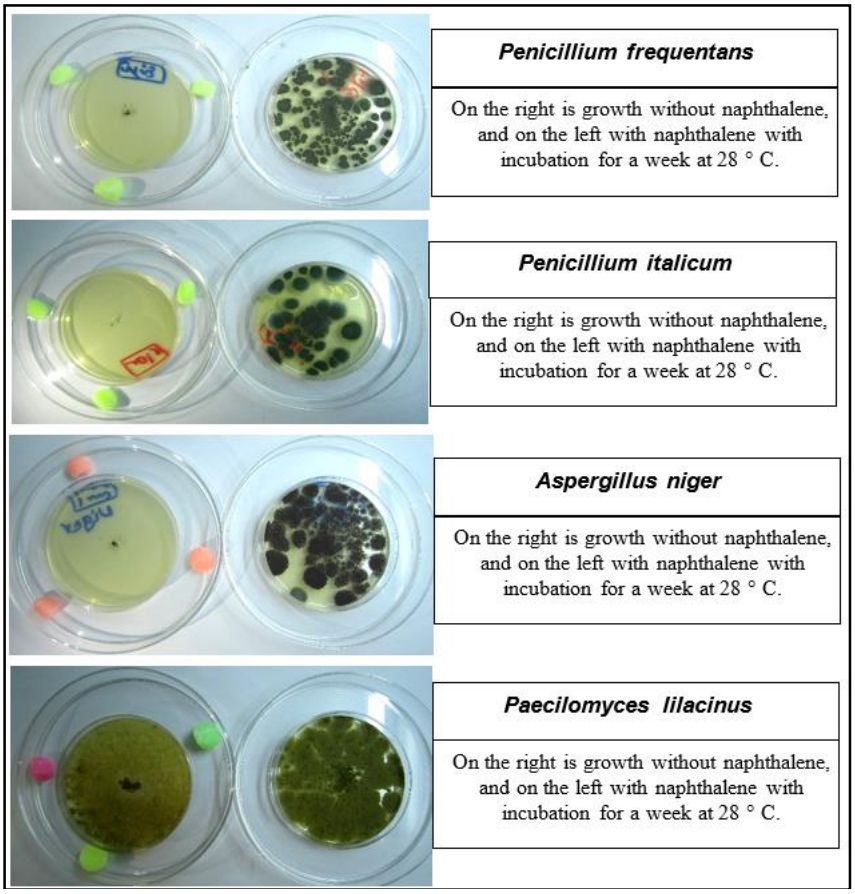


Fig. 9: Effect of naphthalene-infused air on the growth of *Aspergillus niger, Penicillium frequentans, Penicillium italicum* and *Paecilomyces lilacinus*

DISCUSSION

Results showed that fungi of *Aspergillus niger*, *Chaetomium globosum*, *Penicillium frequentans*, *Penicillium italicum*, *Paecilomyces lilacinus*, and *Xanthophyllomyces dendrorhos* were present inside the airway corridors in 13 air conditioners in houses located in Sakaka City, Jouf, Saudi Arabia. Air passages of these air conditioners constitute an appropriate environment for the growth of fungi where condensed moisture is available on their walls as a result of the cold air emitted.

Isolated fungi in this study were known to have precedents in causing serious diseases of humans and animals (Karam & Griffin, 1986; Bryden , 1988; Mohamed & Abdelghafour 1989; Paivi, 2007). Some previous studies confirm the cause of fungi of the genus *Paecilomyces* in many diseases, such as diseases of opportunistic clinical infections, cornea, skin, endocarditis, and peritoneum, lung diseases similar to pneumonia and allergies, as well as diseases of animals such as dogs, cattle, insects, and plants, as well as Proven to produce dangerous toxins http://www.envirocheckonline.com/paecilomycessp.html.

For many *Penicillium* fungi, it is clear that the involvement of *Penicillium fequentans* and *Penicillium italicum* may be attributed to the pathogenesis of pneumonia, hypersensitivity, allergic alveolitis (alveoli), skin sensitivity and emphysema, as well as the production of dangerous fungal toxins in the health of living organisms (Pitt *et al.,* 2000; Kirk *et al.,* 2008). *Aspergillus niger*, which was isolated from all the samples here and producing huge quantities of conidia, is considered one of the biggest causes of very serious tuberculosis-like diseases called Aspergillosis, which spreads in poorly ventilated places. (Ainsworth *et al.,* 2011). As for the isolated fungus *Chaetomium globosum* in this study, previous research indicated that it was responsible for serious diseases such as brain abscess, peritonitis. In addition, *Chaetomium globosum* secreted encroachment dangerous type of fungal toxins called Chaetoglobosin (Paivi *et al.,* 2007). In this study, the isolated *Xanthophyllomyces* was well known yeasts that may cause diseases of the mouth, throat, and folds of the skin in the exposed human (Ainsworth *et al.,* 2011).

Results of this study showed that *Paecilomyces lilacinus*, *Aspergillus niger*, *Penicillium italicum* caused severe decomposition of human red blood cells, and the most powerful of these fungi was *Paecilomyces*, which gives a strong warning about exposure to those fungi and emitted constantly from the air conditioners under study and that, logically, are found in most air conditioners. This is consistent with previous studies documenting the relationship of these fungi to human diseases Atagazli, *et al.,* 2010; Novak, *et al.,* 2014).

Data obtained in this research showed that the use of naphthalene has had an inhibitory effect on three out of the 4 fungal species. *Aspergillus niger*, *Penicillium frequentans*, and *Penicillium italicum* was 100% inhibited by the naphthalene, while it did not affect *Paecilomyces lilacinus*. No reason has been studied in this research for the effect of *Paecilomyces lilacinum* not being affected by the air infused with naphthalene, which needs more future studies. Therefore, we recommend from the results of this research, the use of naphthalene, which is sold in markets in many ways, by placing it inside the airway passages with air conditioners and closing the covers of these passages for hours each period of non-use of air conditioners.

REFERENCES

Flannigan, B. and Miller, J. D. Health implications of fungi in indoor environments-an overview In Health Implications of Fungi in Indoor Environments, 1994; **2**, pp 3-28, Elsevier, Amsterdam.

Ahearn, D. G., Price, D. L., Simmon, R. B., and CROW, S. A. Colonization studies of various HVAC insulation materials. In Environments for people IAQ92 American Society of Heating,

Ventilation, and Air Conditioning Engineers (ASHRAE). Atlanta. GA., 1992; Pp 101-105.

Ahearn, D. G., Crow, S. A., Simmon, R. B., Price, D. L., Noble, J. A., Mishra, S. K. and Pierson, D. L. Fungal colonization of fiberglass insulation in the air distribution system of a multi-story office bulding VOC production and possible relationship to sick bulding syndrome. *J Ind Microbiol*., 1996;16: 280-285.

Simmon, R. B. and Crow, S. A. Fungal colonization of air filters for use in heating, ventilating, and air conditioning (HVAC) systems. *J Ind Microbiol*., 1995; 14: 41-45.

Morpeth, J. F., Rupp, N. T., Dolen, W. k., Bent, J. P. and Kuhn, F. A. Fungal sinusitis an update. *Ann Alergy Asthma*. Imunol., 1996; 76: 128-140.

Crow, S. A. and Ahearn, D. G. Fungal colonization of solid surfaces and the sick building syndrome. In Biotechnology International (Connor TH and CF Fox, eds), 1997; pp 216-220, Universal Medical Press, San Francisco.

Kumar, P., Marier, R. and Leech, S. H. Hypersensitivity pneumonitis due to a car air conditioner. N Engl J Med., 1981; 305, 1619.

Kumar, P., Marier, R. and Leech, S. H. Respiratory allergies related to automobile air conditioner. N Engl J Med, 1984; 311, 1531.

Maghazy, S. M. N., Abdelzaher, H. M. A. and El-Gendy, Z. K. Studies on mycobiota of monumental elements in Minia governorate. *El-Minia Science Bulletin*, 2013; 24 (1): 83-115.

Ainsworth, G. C., and Bisby, G. R. Ainsworth & Bisby's dictionary of the fungi. Farnham Royal: Commonwealth Agricultural Bureaux [for the] Commonwealth Mycological Institute, 1971.

Ainsworth, G. C., Bisby, G. R., Kirk, P. M., and CABI Bioscience. Ainsworth & Bisby's dictionary of the fungi / by P.M. Kirk ... [et al.] ; with the assistance of A. Aptroot ... [et al.]. 2001; Wallingford, Oxon, UK: CABI Pub.

Tasca, T. and A decarrli, G. Hemolytic activity of fresh isolates and clones of *Trichomonas gallinae*. *Parasitol. Día*, 1999; 23 n.3-4 Santiago jul.

Mukherjee, A. and Rajasekaran, C. In-vitro hemolytic activity of *Allium stracheyi* Baker. *Journal of Pharmacy Research*, 2010; 3(5), 1160-1162.

Karam, G. H. and Griffin, F. M. Jr. Invasive pulmonary aspergillosis in nonimmuno compromiscd, nonneutropenic hosts. *Rev Infect Dis.*, 1986; 357.

Bryden , W. L. Chronic effects of mycotoxins in animals . mycotoxin symposium. 1988; University of Sydney, N.S.W, Australia. pp11.

Mohamed, F. and Abdelghafour, T. Production of toxic metabolities by *Penicillium italicum* and *P. digitatum* isolated from citrus fruits. *Journal of food production*, 1989; **3**: 194-197.

Paivi, M. S., Samuel, A. Jr., Michelle, S., Renee, J., Richard, D. C., Stephanie, J. L. and Darryl, C. Z. Exposure to *Alternaria alternata* in US homes is associated with asthma symptoms. *J Allergy Clin Immunol*., 2007; 118 (4): 892-898.

Pitt, J. I., Basílico, J. C., Abarca, M. L. and López, C. "Mycotoxins and toxigenic fungi". *Medical Mycology*, 2000; 38 (Suppl 1): 41–46.

Kirk, P.M., Cannon, P.F., Minter, D.W. and Stalpers, J. A. Dictionary of the Fungi (10th ed.). 2008; Wallingford, UK: CABI. p. 505.

Atagazli, L., Greenhill, A. R., Melrose, W., Pue, A. G. and Warner, J. M. Is *Penicillium citrinum* implicated in sago hemolytic disease? *The Southeast Asian journal of tropical medicine and public health*, 2010; 41(3): 641-646.

Novak, M., Sepčić, K., Kraševec, N., Križaj, I., Maček, P., Anderluh, G., Guella, G. and Mancini, I. Targeted lipid analysis of haemolytic mycelial extracts of *Aspergillus niger*. Molecules, 2014; 19 (7): 9051-9069.