Fungal Contamination of Air-conditioned Air Flow with Special Reference to Antifungal Activity of Eight Plant Oil Vapor Against Aspergillus Niger

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Abstract. Modern buildings without air conditioning are unimaginable. This made them closely related to indoor air quality, especially microbial content. Filters and ducts of AC are colonized by microorganisms, and the air flowing from the AC will carry fungal structures that affect human health. Forty indoor air samples were collected by the plate exposure method from the air current of conditioners in classrooms/secondary school. Samples were collected at the start of operation (S1) and two hours after (S2). This study aimed to isolate and identify of fungi from AC air current, and analysis of the fungal community, as well as in vitro antifungal exam of eight oil vapor against Aspergillus niger. Oils of Hacinathus sp., Cymbopogon citratus, Myrtus communis, Eucalyptus sp., Laurus nobilis, Cinnamon sp., Rosemary officinalis, and Cyperus rotundus were used. Eight fungal genera were listed from a total of 355 fungal colonies: Aspergillus (4 sp.), Penicillium sp, Cladosporium sp, Mucor sp, Alternaria, Geotrichum, Candida, Rhodotorula, besides sterile mycelia. From S1 and S2 samples, 189 colonies (8 genera), and 83 colonies (5 genera) were recognized respectively. Aspergilli showed the highest occurrence of 100% and frequency76.4%. All examined vapor reduced growth of A. niger, C. citratus, Eucalyptus sp., and Cinnamon sp. oils gave the highest significant antifungal activity in comparison with control. It was realized that ACs are an effective source of indoor airborne fungi, and eco-friendly materials have shown significant antifungal activity. They can be the choice of the future.

Kew words. Indoor air, Bio-fungicide, A. Niger, C. citratus, vapor phase.

1. Introduction
Air conditioners (ACs) have been one of the common essential requirements of modern buildings, they were used around the year to provide a comfortable indoor environment. As ACs provide a suitable temperature and humidity, they cause increasing of indoor airborne microorganisms (IABM) which can benefit from the availability of nutrients and lack of direct sunlight to grow and multiply quickly. Therefore, ACs may be a potential source of air contamination [1, 2]. The effect of the indoor environment on human health refers to as sick building syndrome (SBS), and no doubt, IABMs play an important role in this subject [3, 4, 5].
The IABF was studied thoroughly in several types of buildings such as hospitals, classrooms, offices, malls, and residents [2, 6, 7, 8, 9], and the relation between IABF and ACs were carefully discussed. The ACs may act as a reservoir and spreader of indoor biological air pollutants [10], at the same time several technical applications were taking place to minimize the air conditioners contamination, as using of chemical compounds and ultraviolet as germicidal agents [11, 12, 13]. Even though chemical compounds have a high effect on fungi associated with ACs, but they cause air pollution (Myatt 2008). Looking for active and safety treatments was a goal of several previous studies, and the current study was conducted to explain the indoor airborne fungal community concerning ACs operation time. The study aimed also to evaluate the effect of vapor state of eight plant oils on the commonest fungus isolating here Aspergillus niger.

2. Materials and Methods

2.1. Samples collection

Air samples were taken by plate exposure method (passive sampling method), Plates with Typha polLens agar medium [15] were uncovered for 5 minutes in two appointments (start of operation and two hours after). The AC was continuously work during these period (2 hours). Plates were fixed 15 cm far from the AC air outlet, then they were covered, and were sealed by paraffin tape, and were kept in clean nylon bag at room temperature.

2.2. Fungal identification

The fungal colonies have been counted after four days, and fungal identification lasted for three weeks, isolates were identified according to morphological characteristics base on [16, 17]. Occurrence%, and frequency% for fungal isolates were collected as in [1]. Richness index (R) was calculated as the formula below:

\[
O\% = \frac{\text{no.of fungal appear} \times 100}{\text{no.of collected samples}}
\]

\[
F\% = \frac{\text{no.of isolated species} \times 100}{\text{no.of total isolates}}
\]

\[
R = \frac{\text{no.of colonies}}{\text{no.of samp}}
\]

2.3. Test of oils vapor affect

The Crude oils of Hacinathus sp., C. citratus, M. communis, Eucalptus sp., L. nobilis, Cinnamon sp., R. officinalis and C. rotundus were extracted from plant materials which were purchased from a specific privat traditional medicine center in Erbil city. A simple steam distillation apparatus was fitted for this purpose. Evaluation of the vapor phase of oils against A. niger carried out firstly in sterile vials with screw cap to avoid vapor losing. A 15 ml of TPA slant medium were prepared, and then was inoculated by single needle prick of a 10 days A. niger pure culture. 0.1 ml of each oil were placed in vial cap, and incubated in upside down vertical direction. Test was performed by three replicates inside the isolation chamber. The growth of A. niger was checked and carefully observed after seven days. According to their higher antifungal activity, oil of C. citratus, Eucalyptus sp. and Cinnamon sp. were examined by disc evaporation method [18].

3. Results and Discussion

Due to their close relationship to human daily life, the indoor airborne fungi have been deeply studied, and researchers discussed there taxonomic, ecologic, and hygienic impact [19, 20, 21]. In the current study, all forty collected samples were positive, 272 colonies related to 8 genera were recorded. Aspergillus (4 sp.), Penicillium, Cladosporium, Alterna ria, and Geotrichum, beside one genus related to zygomycetes, ascomycetes, and basidiomycetes, they were Mucor, Candida, and Rhodotorula respectively (Table-1). The isolated fungi are common as indoor airborne, and highly related to outdoor environment. [22].
Table 1. Isolated fungi from air samples with their occurrence% (O%), frequency% (F%), and taxonomic groups (TG).

| S | Fungi       | O%  | F%  | TG          | S1 | S2 |
|---|-------------|-----|-----|-------------|----|----|
| 1 | *Alternaria*| 5%  | 0.7%| Hyphomycetes| +  | +  |
| 2 | *Aspergillus*(4sp.) | 100% | 76.4% | Hyphomycetes | +  | +  |
| 3 | *Candida*   | 10% | 5.8%| Ascomycetes | +  | +  |
| 4 | *Cladosporium* | 10%  | 2.9% | Hyphomycetes | +  | +  |
| 5 | *Geotricum* | 5%  | 0.7%| Hyphomycetes | +  | -  |
| 6 | *Mucor*     | 10% | 0.7%| Zygomyctes  | +  | -  |
| 7 | *Penicillium* | 10% | 5.8% | Hyphomycetes | +  | +  |
| 8 | *Rhodotorula* | 5%   | 0.7% | Basidiomycetes | +  | -  |
| 9 | Sterile mycelia | 20%  | 4.4%| Unknown     | +  | +  |

TCC 189 colonies related to 8 genera were counted in S1 samples, while they were 89 colonies and 5 genera in S2 (table-1). The high colony count of S1 air samples indicate the role of ACs machines as a source of fungal fragments, which were hold /puffed by the air current to hall space. The total colonies count inversely proportional to the operating time. The richness index (R) of S1 = 4.725, it was about twice of S2 = 2.075. The spores formation and dryness during shutdown period (14-16 h.) may cause the increasing of fungal structures and made them easily to separate. Hamada and Fujita [23] mentioned the role of ACs as a source and spreader of IABM and the air current at the beginning of AC operation hold more fungal structures than afterward.

Most previous studies for indoor airborne fungi recorded Aspergillus, Penicillium, Cladosporium as common isolates. [24]. Aspergillus niger showed the highest prevalence in the current study, thus it was used as an indicator to evaluate the antifungal activity of tested oil vapors. The eight oils of Hacinathus sp., *C. citratus*, *M. communis*, Eucalptus sp., *L. nobilis*, Cinnamon sp., R. officinalis and C. rotundus were selected according to their aromatic characteristics and antifungal activity which was mentioned previously [25, 26, 27]. The results of the primary checking of antifungal activity of oil vapor explained that all treatments decreased the growth of A. niger in comparison with control (Figure 1.).

*C. citratus* oil showed the highest activity followed by Eucalyptus sp. and Cinnamon sp (fig-1/2,4,7 respectively). The antifungal activity of these oils was mentioned by several workers [27, 28]. Results of vapor state antifungal activity of the three oils against *A. niger* radial growth showed significant difference (fig. 2 and 3), the highest reduction in fungal radial growth caused by *C. citratus* treatment.
Figure 2. Antifungal activity of three oil vapors on radial growth (mm) of A. niger (mean of 3 replicates).

Eucalyptus sp., and Cinnamon sp. showed full inhibition to spore formation and effect on the morphology of fungal mycelia growth (fig.3).

Figure 3. Antifungal activity of C. citratus (1), Eucalyptus sp. (2), and Cinnamon sp. (3) vapor oil on A. niger, control (4)

The antifungal activity of C. citratus oil as well as its chemical ingredients were carefully discussed, the plant essential oil was suggested as a successful alternative antifungal agent. [18, 29]. Sites of effect and the mechanism of inhibition were explained, and there is a semi-agreement that essential oils damage the functions of cell membranes and disturbed cell transport systems [30].

Although Eucalyptus oil vapor has widely used in traditional medicine its antifungal activity attracts less attention, the volatile compounds showed strong activity against several phytopatho gens [31]. The antifungal activity of Cinnamon oil and its effective compounds were reported by several studies, the effectiveness of oil vapor has also been demonstrated against many bacterial and fungal isolates from various sources, [32, 33].

4. Conclusion
The current study gave hopeful results to use vapor state of essential oils as antifungal agents against indoor aerobiological pollution, and air conditioner contamination. Farther studies should be conducted to increase the fungicidal effect threw test of oil combination (C. citratus, Eucalyptus sp., and Cinnamon sp.), duration of evaporation and vapor concentration. Due to their activity, quick degradation, low toxicity, and aromatic characteristics, the plant essential oils and/or their components highly expected to be the expectable treatment of indoor airborne fungi.

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