Antifungal Activity of Essential Oils Vapors Against Fungi Isolated from Car Air- Conditioner Filters

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Abstract

Background/A car air conditioner AC filter is a suitable place for microbial growth. Fungal structures or/ and their by-products are pollutants of car air space. They are transmitted via air current forward passengers. Most isolates were reported as allergens and mycotoxins producers. Chemical and less nonchemical treatments were followed to solve this problem. The current study aims to isolate and identify fungi associated with CAC filters and discuss the relationship between the fungal community with car age and the types of seat coats. Also, we inspected the antifungal activity of the vapor and liquid phases of five essential oils *in vitro*. Methods/ A cross-section study was followed, a swap samples were collected during one week. They were cultured on Sabouroad's dextrose agar and Typha pollens agar. Plates were incubated at (25^{o}C ±2). The highest occurrence% were *Penicillium sp*. > *Alternaria sp*. > *Aspergillus* (3*sp*.), while *Acremonium sp.,Rhizopus sp*., *Rodotorula sp*. and *Stachybotrys sp*., showed low O% as well as yellow yeast and sterile mycelia. The crude oils of *Hacinathus sp*., *Cymbopogon citratus*, *Myrtus communis*, *Eucalyptus sp*., and *Cyperus rotundus* were extracted and examined. ANOVA test showed that *C.citratus* oil vapor phase significantly affects the dominant isolate. **Conclusion/** Car AC filter is a source of fungal structures with health impact. Liquid and vapor phases of EOs are probably practical alternatives to control fungal AC contamination.

Kew words: *Car, Antifungal, Air Conditioner Filter, Essential Oil, Vapor*

Introduction

Contemporary lifestyle force humans to spend about 80% of the daily time within closed environments as residents, offices, and vehicles [(1).] Thus the indoor airborne fungi are more effective on humans health than outdoor airborne fungi according to the fact "Risk increase synchronously with a time of exposure". The indoor airborne fungi take a lot of attention, and their relation with building characteristics was deeply discussed [(2,3,4)]. On the other hand, the closed environment of land transport modes did not receive enough attention and needed further investigations. AC systems are closely related to the indoor air quality in different closed spaces, and they may act as a reservoir or a transmitter of bio-air pollutants [(6)]. AC system is one of the main factors causing a remarkable increase of indoor airborne fungi. Their filters and ducts are a favorable setting for the growth and sporulation of airborne fungi [(7)].

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The AC system of land transporter modes -as in building- provides a suitable condition conducive to the growth and development of fungi and consequently raises their effect on air quality and the hygienic impact [8]. Individuals traveling in road transport daily for several hours will be more exposed to bio-air pollutants related to transport mode [9].

Due to the high humidity and availability of nutritional sources inside the car driver chamber, filters of car AC will act as an incubator for fungal fragments as well as for other air pollutants [10,11]. Using chemical and natural antifungal agents was applicable to reduce fungal filter contamination[1,12]. In 1960, the first mention was reported to use essential oils vapors as antimicrobial agents [13]. The current study aimed to distinguish fungi associated with air conditioner filters of private cars in Erbil city and test the vapor phase activity of five crude plant oils as antifungal agents. It is worth mentioning that the current study, regarded as the first in the Kurdistan region, focuses on this point.

Materials & Methods

Collection of samples/ During one week, a hundred swab samples were collected randomly from (AC) filters of cars interred (Hyundai service center) in Erbil city for periodical maintenance. The traveling distanced, and the type of seat coating for each car was documented. Samples were immediately brought to the mycological lab. They were cultured by streaking method on Sabouraud's dextrose agar and Typha pollens agar [14] supplemented by 150mg/L chloramphenicol. Plates were incubated at 25^{o} C±2 and were checked periodically from the 4th day to the 21st. The developing fungi were identified as genera except for *Aspergillus* spp. Detection of fungi had been done base on taxonomic keys [15,16].

Analysis of fungal community/The occurrence% of recorded genera was calculated following the formula:

TO% = (no. of times fungal appear *100) /no. of collected samples.

Similarity% between isolates of (4-7 years old cars) and (1- <4 years old cars) was calculated following the formula of Jaccard's index:

S%= (no. of genera in both sets)/(no. of genera in either set)* 100.

Pure cultures for the higher occurrence fungi were prepared for the farther test.

Plants and oil extraction/The plant materials were purchased from a specialized pharmacy for folk and alternative medicine in Erbil city (Iraq). The crude oils of *Hacinathus sp*., *Cymbopogon citratus*, *Myrtus communis, Eucalptus sp*., and *Cyperus rotundus* were extracted by steam distillation [17]. Antifungal activity test/ According to [18,19], the agar absorption assay method and the evaporative disc method was followed . the inhibition% of oil liquid and vapor was calculated. Inhibition%= 1- (diameter of colony growth with treatment/ diameter of colony growth without treatment) X100.

The richness index was calculated according to the formula: R=no. of colony/no of samples X100

The experiment was done in replicate for all tests.

Results and Discussion

From the hundred swab samples, 782 fungal colonies were counted. They related to seven fungal genera besides undefined yellowish yeast and sterile mycelium. The isolated fungi showed various occurrence% (table-1), *Penicillium sp*, *Alternaria* sp. , and *Aspergillus* (3 sp.) had 85%, 65%, and 60%, respectively The rest isolates were less than 20%. All these genera are well-known airborne fungi. They had been recorded previously as agents of several human infections and are implicated in human respiratory disorders [16,20]. Because of the continental climate (a significant variation between the temperature of summer/winter and night/daytime) of Erbil city, the use of car AC was on the most days during the year. The risks of microbial AC pollutants are increase, as well as a remarkable decrease of inside car air quality. There is a clear relation between filter age and total airborne fungi and bacteria inside the car space [21],

making the maintenance of (AC) devices necessary for a healthy human requirement. In the current samples, the richness index showed no significant differences between the oldest cars group (4-7 years old) and the newest group (1-<4 years old), the number (R=0.375 and R=0.363, respectively). These values may be related to the periodical change of filter AC for both age groups. Jaccard's index of similarity showed a moderate similarity between the two groups (0.555%).

(Table-1) The isolated fungal genera and their occurrence%

	Fungi	**Occurrence%**
1	Penicillium sp.	85%
2	Alternaria sp.	65%
3	Aspergillus (3 sp.)	60%
4	Rhizopus sp.	18%
5	Stachybotrys sp.	16%
6	Acremonium sp.	12%
7	Rhodotorula sp.	4%

The preliminary results of the agar absorption assay showed a distinguishable difference between the total colony count of treated and non-treated plates. Simultaneously, the tested oils showed a variable activity against the mycelia growth and sporulation (fig-1).

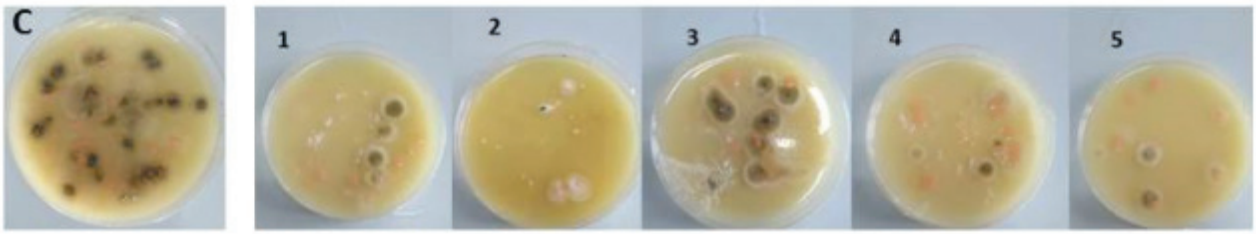

Fig.1- The antifungal activity of five oils against total colony count. 1-*Hycinthus sp*.

2-*Cymbogon citratus* 3- *Myrtus communis* 4-*Eucalyptus sp*. 5-*Cyperus rotundus*

The variability is related to the specific active ingredients of each oil, as well as to the different interference mechanisms of oil/fungal isolates [22]. Oils of *Cymbopogon citratus*, *Cyperus rotundus*, and *Eucalyptus sp*. exhibited a notable effect. These oils were mentioned by several workers and were used for numerous applications. [23.24.25]

Based on their high activities against most filamentous fungi (plate1-no.2,4,5), the vapor phase of *Cymbogon citratus* , *Eucalyptus sp*., and *Cyperus rotandus* was separately tested against the dominant isolates *Penicillium sp*, *Alternaria sp*., and *Aspergillus fumigatus*. They are among the most related fungi with human respiratory disorders. The agar absorption assay test presented different effects on vegetative growth and sporulation (table-2).

Table-2/ The vapor phase effect of the three oils on the radial growth of common fungi (mean of replicates)

Fungi		C.citratus	Eucalyptus sp.	C. rotandus	Control
Penicillium sp.	M	H*(9mm)64%	H*(22mm)12%	H(10mm)60%	25mm
	S	L	H	H	
Alternaria sp.	M	H*(14mm)68%	L(38mm)13.6%	Mo(30mm)31.8%	44mm
	S	L	Mo	L	
Asp. fumigatus	M	H*(24mm)31.4%	L(29mm)17.1%	L (28mm)20%	35mm
	S	Mo	Mo	H	

(H=high) (Mo=moderate) (L=low) (M=mycelia growth- mm diameter) (S=sporulation)

ANOVA test throw SSPS 20 showed that vapor of *C.citratus* and *Eucalyptus sp*. had a significant effect against the three tested isolates, while *C. rotandus* oil vapor significantly affected the growth of *Penicillium sp. and Alternaria sp.* but not on *Aspergillus fumigatus*. The insignificant results may be related to the concentration of effective ingredients of vapor oil.

Penicillium *sp*. was the most susceptible fungus, followed by *A.fumigatus* and *Alternaria sp.* (Fig.2)

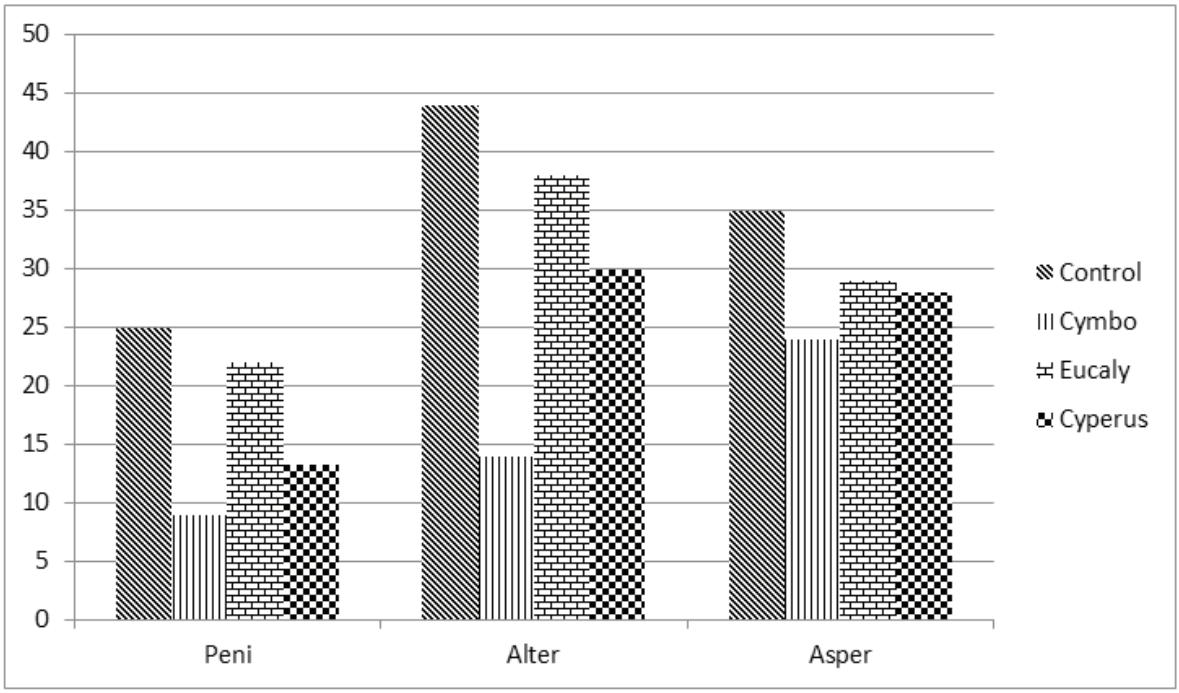

Fig.2-Effect of three vapor oils on radial growth (mm) of *Penicillium sp, Alternaria sp,* **and** *Aspergillus fumigatus.*

Cymbogon citratuus oil was more effective on mycelia growth, while *Cyperus rotandus* was more effective on sporulation.

Eucalyptus sp. oil also showed more activity against sporulation rather than mycelia growth. Plant extracts include oils that represented antifungal activity, inhibit or disturbed germination, growth, spores formation, and enzymatic activity, and the essential oils showed numerous effects according to their chemical ingredients [26,27]. The oil of *C. citratus* showed remarkable activity against *Fusarium* growth [28]. The GC analysis showed that its oil is rich in citral and other phenolic compounds, which has been identified as a compound showing antifungal properties [17]. *Eucalyptus* essential oil exhibits potent antifungal agents against *Candida albicans*. It affects cell growth and morphology [29,30]. The antifungal activity of Eucalyptus oil may cause

by several components. The volatile monoterpenoid citronellal, as an example, exhibits a well-known antifungal activity [31].

Conclusion

Results indicated the role of an inside car environment as a probable source of fungi which were documented as respiratory disorders causes, especially to atopic individuals. Filters of car air conditioners increase the risk when they are old or during the warm/ humidity climate. Eco-friendly products from several plant oils can successfully use to prevent or reduce fungal contamination. A periodical changing or cleaning by air blower is required to reduce fungal filter contamination. Looking for a mixture of oils with high vapor effectiveness should be the goal of further researchers.

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Ethical Clearance: Non

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