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Research Article

Fungal Contamination of Automobile Air Conditioner System with Reference to Aqueous Extract Effect of *Dodonaea viscosa* on *Aspergillus fumigatus*

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ABSTRACT

One of the indoor environments where people spend a lot of time these days is public and private transportation, and there is more and more attention to explaining the correlation between human health and indoor air quality in transportation. The present investigation aims to identify the fungus species in car cooling dispensers and quantify the antifungal efficacy of an aqueous crude extract derived from *Dodonaea viscosa* leaves and seeds against *Aspergillus flavus*. Fifty swab samples were obtained in 8 h and then they were cultivated on Sabourad's dextrose agar medium. The emerging fungi were *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Ulocladum* spp., *Alternaria* spp., and white and black mycelia. The occurrence, frequency, and importance value index were counted. The predominant isolate was *A. fumigatus* (importance value index = 36.2). *In vitro*, fungal growth suppression was evaluated using the hot water extract of *D. viscosa* seeds and leaves. 20% and 40% (w/v) concentrations were made. The results showed that seed extract (40%) presented the highest activity against the predominant isolate *Aspergillus fumigatus*.

Keywords: Automobile, Air conditioner, antifungal activity, *Dodonaea viscosa*, water extract

INTRODUCTION

Automobiles are one of the most environmentally polluting machines and the toxic compounds released by cars and other transportation types are well highlighted. On the other hand, the closed environment of land transport modes did not receive enough attention and needed further investigations. As probable sources of bio-pollution, there were 947 million passenger automobiles and 335 million commercial vehicles on the planet Just in Europe, there are 256 million registered passenger cars.

(https://theicct.org/wp-content/uploads/2021/06/ICCT_Pocketbook). The poor interior air quality of cabs, buses, lorries, and private vehicles poses a risk to both drivers and passengers, particularly for professional drivers who spend long hours on the road.[1-3]

Actually, because of the surface bioaerosols and increasing automobile indoor air humidity car air conditioning systems frequently operate as active sources of hazardous biological agent emissions (mostly fungi and bacteria) and microorganisms develop ducts and depressors as a result. Certain microorganisms can readily form a biofilm in the air conditioning ducts, which allows them to spread throughout the other installation components and the automobile interior.[4,5]

Fungi attached to car's air conditioners (AC) filters were recorded by Al-Bader *et al*.,[7] genera were recorded *Penicillium* spp., *Alternaria* spp., and *Aspergillus* spp. the predominant, while *Acremonium, Rhizopus, Stachybotrys,* and *Rhodotorula* represented low O%.[6]

Aquino examined fungal contamination in air conditioning filters from 21 automobiles. The study discovered that 17 fungal genera were present in all samples (100%), including toxic fungi, such as *Fusarium, Aspergillus,* and *Penicillium.* This suggests that indoor air quality can harm certain groups, including professional drivers.[7]

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Jo and Lee examined the overall colony-forming unit both before and after turning on the car's air conditioning. They discovered that the levels of bioaerosols in cars before they used air conditioning were either lower or comparable to those in the outside air. Elevated amounts often happened 5–15 min after driving and over time, these concentrations dropped.[2]

Numerous physical and chemical methods have been used to clean air conditioning systems,[1] and as a result, a significant impact on the quality of air inside cars. Unfortunately, the eco-friendly application had less attention in this field. Antifungal activity of five essential oils in liquid and vapor phases against fungi isolated from automobile air conditioner filters was examined in Erbil city.[6]

The present study was conducted to investigate fungi that inhabit ducts of automobile AC, as well as estimate the antifungal efficacy of the aqueous extract of *Dodonaea viscosa* seeds and leaves on *Aspergillus fumigatus*.

MATERIALS AND METHODS

Samples Collection

Fifty swab samples were collected from the air conditioner duct of 50 privet cars. After that swabs were cultured by streaking method on Sabouraud's Dextrose Agar (SDA) supplemented by Chloramphenicol (15 mg/L), then plates were incubated at (25 ± 2°C), and the cultures were checked daily from the 3rd day over 2 weeks. To create a pure culture for further steps, the growing fungi were immediately transferred to SDA plates. The identification was done through morphological characteristics based on.[8,9]

Analysis of Fungal Community

After the identification of isolated fungi, the frequency% (F%), occurrence% (O%), and important value index (IVI) for distinguishable genera and species were determined. The following equations were used for this purpose: [10,11]

$$TO\% = \frac{No.\,of\ times\ appear}{No.\,of\ collected\ samples} \times 100 \quad (1)$$

$$TF\% = \frac{No.\,of\ \ fungal\ isolates}{No.\,of\ total\ fungal\ isolates} \times 100 \quad (2)$$

$$IVI = \frac{TO + TF}{2} [11] \quad (3)$$

Aqueous Extract Preparation

D. viscosa materials were collected from the public garden in Erbil city including fresh/green leaves, and dry flowers with seeds, they were washed thoroughly by tape then sterile distilled water SDW and left to dray at laboratory temperature until no change in their weight were occurred. An electric grinder machine was used to ground the leaves and seeds into a powder. 20 mL of SDW was added to five grams of powdered plant material to reach a concentration of 40% and the mixture was shaken for 24 h. The mixture was filtered using a metallic mesh (50 microns), and the resulting filtrate was stored at 4°C in a sterile conical flask as a stock solution (40%).[12] From this stock extract, a diluted extract (20%) was prepared for the antifungal test.

Antifungal Activity Test

The modified agar disc diffusion method was used[13] and sterilized filter paper (no. 1) disks with a diameter of 10 mm were prepared. Conversely, a water spore suspension of active *A. fumigatus* was prepared at a concentration of 1×10^{-6}, and 0.250 mL of the suspension was spread on the SDA surface. The filter paper disks were carefully saturated by lemongrass oil extracts and were fixed 1 cm from the Petri dish edge. Disk with SDW was done as control. All work was completed inside a laminar flow cabinet, with three replicas. An average of two perpendicular diameters of each oil was calculated to determine the antifungal activity. Finally, paraffin tape was used to seal the Petri dishes, and they were then incubated at 25°C. Four days later, the results were recorded.

RESULTS AND DISCUSSION

Fungal community structure

101 isolates related to 5 fungal genera were recorded beside white and black mycelium [Table 1 and Figure 1]. They included 4 anamorphic *Aspergillus* (3 spp.), *Cladosporium, Penicillium,* and *Stemphylium* beside the zygomycotic *Rhizopus*.

Aspergillus species, which are cosmopolitan fungi that can grow on a variety of substrates in a wide range of temperatures, exhibited the highest level of O%, F%, and IVI%. As airborne fungi, Aspergilli are the most common isolate worldwide.[14,15]

Previous studies for airborne fungi in Erbil City exhibited the predominance of *Aspergillus*.[16,17]

The indoor air fungal community is affected by its external counterpart, as well as internal environmental factors, one of the most important factors that interact with the indoor environment is the air conditioning systems. Fungal formations can spread into indoor air environments through air conditioning systems. The indoor air environment of automobiles is no exception to this. Fungi that were recorded

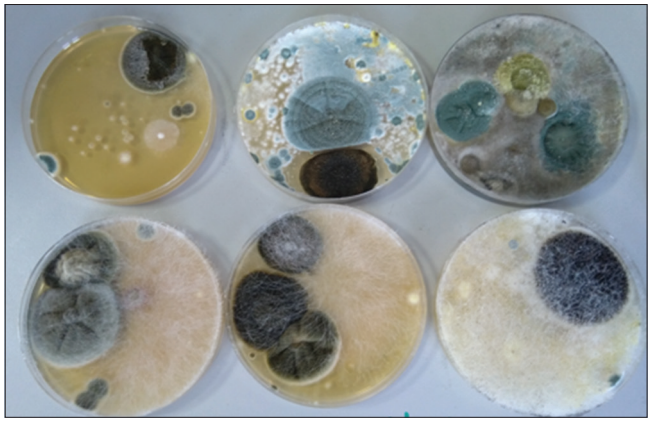

Figure 1: Fungal colonies developed on Sabouraud's Dextrose Agar medium

Table 1: Occurrence%, Frequency%, and important value index of isolated fungi (IVI)

S. No.	Fungi	No. of colonies	Occurrence%	Frequency%	IVI
1.	*Aspergillus fumigatus*	82	32	40.5	36.2
2.	*Aspergillus niger*	54	17	26.7	21.8
3.	*Aspergillus flavus*	28	11	13.8	12.4
4.	*Cladosporium* spp.	16	6	7.9	6.95
5.	*Penicillium* spp.	10	3	4.9	3.95
6.	*Stemphilium* spp.	4	2	1.9	1.95
7.	*Rhizopus* spp.	2	1	0.99	0.99
8.	White mycelia	4	1	1.9	1.45
9.	Black mycelia	2	1	0.99	1.45

IVI: Important value index

Table 2: Inhibition ratio of *Dodonaea* extracts

Concentration	Seed's extraction	Leave's extraction
40%	11 mm	6 mm
20%	2 mm	0

in the present study [Table 1] were commonly isolated from outdoor air samples; moreover, they were identified in air conditioning systems of types of buildings such as hospitals and schools.[18,19]

The Antifungal Test

The aqueous extract of *D. viscosa* seeds and leaves was scanned [Table 2 and Figure 2] it was found that the 40% seed extract exhibited the highest inhibitory effect (11 mm). This was followed by the 40% leaf extract and 20% seed extract, which showed an inhibitory effect of 6 mm and 2 mm, respectively while, the 20% leaf extract showed no activity.

In addition to its antimicrobial activity, different types of extracted tissues contain a variety of compounds with diverse insecticidal and pharmaceutical properties.[20,21]

Our results were consistent with previous studies that were conducted to use different solvents to extract the bioactive compound in *D. viscosa*, different tissues of the plant represented antifungal compounds against *Aspergillus niger, Aspergillus fumigatus, Fusarium,* and *Rhizopus.*[22,23]

CONCLUSION

Based on the results, it is clear that there was a clear relationship between outdoor air-borne fungi and interior automobile air fungi. The car air conditioner system may be a source of allergy since *Aspergillus, Cladosporium,* and *Penicillium,* the most fungal allergens are predominant in AC dispensers. An allergy may arise due to a car air conditioner depending on the time of exposure and tidiness of the AC system. Given that *D. viscosa* shrubs are plentiful and common in the area, have a pleasant scent, and are highly effective as antifungal, they could be the source of successful natural antiseptics of the future.

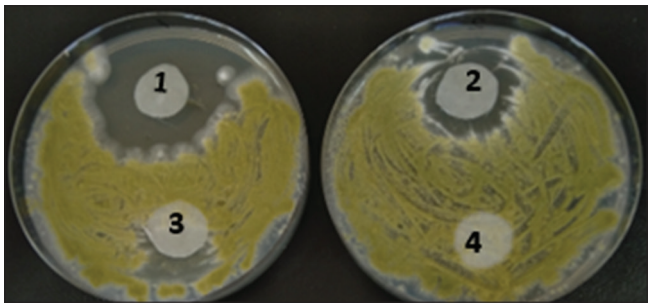

Figure 2: Inhibition zones (I H) of aqueous seeds and leaves extracts of Dodonaea viscosa 1-seeds (concentration [conc.] = 40%, I H = 11 mm), 2-Leaves (conc. = 40%, I H= 6 mm), 3-seeds (conc. = 20%, 2 mm), 4- leaves (conc.=20%, I H = 0)

REFERENCES

1. M. Gołofit-Szymczak, A. Stobnicka-Kupiec and R. L. Górny. Impact of air-conditioning system disinfection on microbial contamination of passenger cars. *Air Quality, Atmosphere & Health*, vol. 12, pp. 1127-1135, 2019.
2. W. K. Jo and J. H. Lee. Airborne fungal and bacterial levels associated with the use of automobile air conditioners or heaters, room air conditioners, and humidifiers. *Archives of Environmental & Occupational Health*, vol. 63, no. 3, pp. 101-107, 2008.
3. R. P. Vonberg, P. Gastmeier, B. Kenneweg, H. Holdack-Janssen, D. Sohr and I. F. Chaberny. The microbiological quality of air improves when using air conditioning systems in cars. *BMC Infectious Diseases*, vol. 10, p. 146, 2010.
4. R. B. Simmons, J. A. Noble, L. Rose, D. L. Price and D. G. Ahearn. Fungal colonization of automobile air conditioning systems. *Journal of Industrial Microbiology and Biotechnology*, vol. 19, pp. 150-153, 1997.
5. R. B. Simmons, L. J. Rose, S. A. Crow and D. G. Ahearn. The occurrence and persistence of mixed biofilms in automobile air conditioning systems. *Current Microbiology*, vol. 39, pp. 141-145, 1999.
6. S. M. Al-Bader, L. Q. Ismael, H. S. Ali, A. S. Al-Tami and A. A. Saleh. Antifungal activity of essential oils vapors against fungi isolated from car air-conditioner filters. *Medico-Legal Update*, vol. 21, no. 4, p. 301, 2021.
7. S. Aquino, J. E. A. de Lima, A. P. B. do Nascimento and F. C. Reis. Analysis of fungal contamination in vehicle air filters and their impact as a bioaccumulator on indoor air quality. *Air Quality, Atmosphere & Health*, vol. 11, no. 10, pp. 1143-1153, 2018.

8. K. H. Domsch, W. Gams and T. H. Anderson. *Compendium of Soil Fungi*. Vol. 1. Academic Press, London, 1980.
9. G. D. Hoog, J. Gene, J. F. M. J. Guarro and M. J. Figueras. *Atlas of Clinical Fungi*. 2nd ed. American Society for Microbiology, Washington, DC, p. 1126, 2000.
10. S. M. Al-Bader, A. Q. Aleryani and A. A. Almutaa. A study on soil thermophilic and thermotolerant fungi in Thamar-Yemen. *Journal of Agricultural Environmental and Veterinary Sciences*, vol. 1, 1-44, 2017.
11. R. Prayudyaningsih, C. A. Prasetyawati and H. Suryanto. Diversity and association of arbuscular mycorrhizae fungi (AMF) in landslide-impacted area. *IOP Conference Series: Earth and Environmental Science*. vol. 807, no. 2, p. 022046, 2021.
12. A. Q. Al Eryani and S. M. Al-Bader. The antimicrobial effect of aqueous and ethanol extracts of stems, roots and leaves of *Salvadora persica* on oral microbes. *Assiut Journal of Agricultural Sciences*, vol. 43, no. 5, pp. 97-104, 2012.
13. A. Gahlaut, R. Dabur and A. K. Chhillar. Anti-*Aspergillus* activity of selected medicinal plants. *Journal of Pharmacy Research*, vol. 6, no. 4, pp. 419-422, 2013.
14. D. M. C. E. Silva. Incidence of the genus *Aspergillus* and its species in the atmosphere of Sao Paulo, Brazil and its relations with the environment. *Journal of Pollution*, vol. 1, 1-5, 2020.
15. F. Al Salameen, N. Habibi, S. Uddin, K. Al Mataqi, V. Kumar, B. Al Doaij, S. Al Amad, E. Al Ali and F. Shirshikhar. Spatio-temporal variations in bacterial and fungal community associated with dust aerosol in Kuwait. *PLoS One*, vol. 15, no. 11, p. e0241283, 2020.
16. S. M. Al-Bader, A. D. A. Aljoborey, A. Q. Ahmed and A. Yahia. A cross-sectional study on the 24-hour variation of fungal pollution in the atmosphere of Erbil City, Iraq. *International Journal of Membrane Science and Technology*, vol. 10, no. 1, pp. 1170-1176, 2023.
17. S. M. Saleem and Z. Zefenkey. The airborne mycobiota of a dust storm in comparison with a calm climate in Erbil city-Iraq. *Science Journal of University of Zakho*, vol. 11, no. 1, pp. 45-49, 2023.
18. S. M. Al-Bader, L. Q. Ismael and A. A. Ahmood. Fungal contamination of airconditioner units in five hospitals of Erbil province-Kurdistan region/Iraq. *Science Journal of University of Zakho*, vol. 6, no. 4, pp. 146-149, 2018.
19. S. M. Al-Bader, A. A. Osman and S. Hussainnagad. Fungal contamination of air-conditioned air flow with special reference to antifungal activity of eight plant oil vapor against *Aspergillus niger*. *Journal of Physics: Conference Series*. vol. 1660, no. 1, p. 012001, 2020.
20. S. Venkatesh, Y. S. R. Reddy, M. Ramesh, M. M. Swamy, N. Mahadevan and B. Suresh. Pharmacognostical studies on *Dodonaea viscosa* leaves. *African Journal of Pharmacy and Pharmacology*, vol. 2, no. 4, pp. 83-88, 2008.
21. M. Pkashatel and M. M. Coogan. Antifungal activity of the plant *Dodonaea viscosa* var. *Angustifolia* on *Candida albicans* from HIV-infected patients. *Journal of Ethnopharmacology*, vol. 118, no. 1, pp. 173-176, 2008.
22. T. Riaz, A. M. Abbasi, T. Shahzadi, M. Ajaib and M. K. Khan. Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscosa*. *Journal of the Serbian Chemical Society*, vol. 77, no. 4, pp. 423-435, 2012.
23. M. N. Malik, I. U. Haq, H. Fatima, M. Ahmad, I. Naz, B. Mirza and N. Kanwal. Bioprospecting *Dodonaea viscosa* Jacq.; A traditional medicinal plant for antioxidant, cytotoxic, antidiabetic and antimicrobial potential. *Arabian Journal of Chemistry*, vol. 15, no. 3, p. 103688, 2022.