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Vehicle Indoor Air pollution with Fungi Generated by Air Conditioning Systems (AC) and Treatment by Using Aqueous Extracts Mushroom (*Ganoderma lucidum*)

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Abstract

Vehicle Air Conditioning (AC) systems are used in countries that suffer high temperature degrees during the summer season, one of these countries is Iraq. Most passengers suffer manifestations which may lead to Allergic or Respiratory diseases as they inhale polluted air produced by AC systems.

In this study 10 vehicle were selected randomly on the basis of noxious odors emitted from AC systems. Indoor air samples were collected by using impaction method and using Sabouraud's dextrose agar to grow and isolate dominant fungi species accompanied with AC polluted air. Dust particle diameters aggregated in AC filters were measured because the particles work as transporters to carry fungi then get inhaled by the passengers. After isolating and identifying air fungi, frequency, occurrences percentage calculated. The *Ganoderma lucidum* extract was used as inhibitor agent and antifungal growth by using different concentrations: (10, 15, 20, 25) mg/ml. The results showed that the dominant species air fungi related to AC systems are: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* and *Rhizopus*. The highest occurrences percent 80%, and highest frequency percent 33.3% was recorded to *Aspergillus niger*, while the lowest occurrences percent 30%, and lowest frequency percent 12.5% was records to *Rhizopus*. Microscopic examination for dust particles showed that the particles size ranged between (1 μm to 10 μm), and the highest distribution percentage was 43% for particles of less than 5 μm in size which are respirable and can cause allergic and respiratory diseases. The *Ganoderma lucidum* in concentration of 25mg/ml have highly inhibition zone than other concentrations.

Keywords: indoor air pollution, fungi, Air Conditioning systems (AC)

تلوث الهواء الداخلي للسيارات بالفطريات المتولده في انظمه تكييف الهواء ومعالجته باستخدام

المستخلص المائي للفطر *Ganoderma lucidum*

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الخلاصة

تستخدم انظمه تكييف هواء السيارات بنسبه عاليه في البلدان التي تعاني من ارتفاع درجات الحراره صيفا ومنها العراق. ويعاني العديد من ركاب السيارات اعراض قد تقود الى امراض تحسبيه او تنفسيه بسبب استنشاقها للهواء الملوث المنبعث من تلك المكيفات. في هذه الدراسه البحثيه تم اختيار 10 سيارات بصوره عشوائيه بالاعتماد على ما تطلقه من رائحه هواء غير مرغوبه بعد تشغيل مكيف الهواء، تمت نمذجه الهواء الداخلي للسياره باستخدام وسط ال Sabouraud's dextrose agar باستخدام طريقه الطمر لاقتناص وتنميه

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وعزل انواع الفطريات السائده في هواء التبريد. تم قياس اقطار دقائق الغبار المتجمع في مرشح المكيف على اعتبار ان هذه الدقائق تعمل كنواقل للفطريات والتي تستنشق من قبل الركاب. بعد عزل وتشخيص الانواع السائده وحساب نسبة الظهور والتزداد لكل نوع استخدم مستخلص فطر الـ *Ganoderma lucidum* و الذي يمتلك فاعلية معروفه ضد الامراض المختلفه كماده مثبطه لنمو الانواع السائده من الفطريات المعزوله باستخدام تراكيز مختلفه من ذلك المستخلص وهي: (10,15,20,25) ملغم/مل. اظهرت النتائج سياده واعلى نسبة تردد 33.3% للنوع *Aspergillus niger* وكانت اقل نسبه ظهور 30% واقل نسبه تردد 12.5% هي للنوع *Rhizopus* وقد وجد من خلال الفحص المجهرى بأن احجام دقائق الغبار المفحوصه تتراوح بين (10 μm - 1 μm) ، كما كان اعلى توزيع نسبي هو 43% لاقطار الدقائق الاقل من 5 μm وهي دقائق قابله للاستنشاق لتحدث امراضاً تنفسية وتحسسية. وقد بينت النتائج بان مستخلص الفطر *Ganoderma lucidum* عندما يكون تركيزه 25ملغم/مل يكون ذو فاعلية تثبيطيه عاليه مقارنة بالتراكيز الاخرى .

Introduction

Air conditioning (AC) system in vehicle is highly used these days in Iraq, due to very high temperature during summer season. AC systems provide drivers and passengers moderate cooling and comfort especially in hot days. Most AC systems include filters which are of combination of cotton fibers for traps dust particles and layers of activated charcoal to eliminate bad odors from outside air. AC filter protects passengers from different sources of air pollutants that come from industry, traffic and variable biological sources (Fungi, moulds and bacteria) [1].

Previous studies refer that AC filters must be changed every two years at least, to prevent the aggregation of particles and growth of biological organisms through the filters membrane [2] previous study refers that fungal colonization were formed in insulation materials of AC systems after absorbed moisture and volatile organic . also high humidity and airborne fungal populations are major factors to emit noxious odors or products that lead to sensitizing the occupant of vehicle[3]. Air born particles and microorganisms spread through the cabin of vehicle by operating AC system and produce allergic reaction of the respiratory tract for the exposed passengers [4] indoor fungi may cause human infection due to the relative small size of vehicle cabin ,passengers will be able to inhale fungi through inhaling airborne particles ,this problem considered as one of indoor pollution type [5, 6].

Ganoderma lucidum, is one of the most famous traditional Chinese medicinal herb Bio-products of mushroom has a multi beneficial effects for human welfare. Medicinal mushrooms are widely used as traditional medicinal ingredient for the treatment of various diseases related to health problems. Most of the medicinal extracts from mushroom are different forms of polysaccharides which strengthens the immune system with little or no side effects. Medicinal mushroom research has focused on the discovery of compounds that can modulate positively or negatively the biological response of immune cells. The mushroom extracts has an interesting antifungal performance that it contains enzymes inhibit fungal growth [7]. Mushrooms contain a wide variety of bioactive molecules, such as terpenoids, steroids, phenols, nucleotides and their derivatives, glycoproteins, and polysaccharides. Mushroom proteins contain all the essential amino acids and are especially rich in lysine and leucine. The low total fat content and high proportion of polyunsaturated fatty acids relative to the total fatty acids of mushrooms are considered significant contributors to the health value of mushrooms [8].

The aim of current study to biological treatment of air pollution was applied to inhibit the effects of some indoor air fungi by using a mushroom *Ganoderma lucidum* extract.

Materials and Methods

Sampling and fungi isolation

The number of experimental vehicles was 10 vehicles Model (2000-2006) which selected randomly on the basis of noxious odors emitted from the Air Conditioning system (AC). Five different brand new vehicles Model 2015 which have new AC filters were used as control to compare with elder Models. Air inside vehicles was sampled using impaction method as mentioned in reference [9]. Microorganisms of air flow were impacted and directly collected on Sabouraud's dextrose agar by putting plates in front of the conditioning vents of the dashboard after the system had been running for

2min [10][11] the distance between air outlets of AC and plate was 15 cm . Each sample took 1min [2].

To isolation of fungi Plates were incubated for 5 to 7 days at 25°C; the fungi were grown and identified morphologically under light microscope according to simplified key by [12-14].

Particle size measurement

Particles size distribution was done by collecting dust particles that aggregate in AC filters, then partials size were measured by using light microscope with oculometer using the power 40x. The aim behind this examination is to determine the dust particles diameters which considered as one of the most important physical properties for deposition particles along respiratory tract and dust particles can adsorb fungus and so help them to enter the organs [15].

Mushrooms culturing

Ganoderma lucidum was used throughout this study. It was maintained on malt extract agar plate at 4°C and was subculture every four weeks. Fully grown culture of *Ganoderma lucidum* was used for spawn production in a wide mouth bottle using wheat grains. Wheat grains were sterilized first then mixed thoroughly with 1% and 2% of calcium carbonate and calcium sulphate respectively by the grain weight under dry condition. The mixture was filled in a wide mouthed bottle, plugged tightly and sterilized for two consecutive days at 15 psi, 121 °C for 90 min and inoculated with *Ganoderma lucidum*. Bottle was incubated at 25 °C for 15 days under dark condition to give an optimum time for spawn production [16]. Figure-1 showed morphological characters for *Ganoderma lucidum* on malt extract agar in 25 ° for two weeks.



Figure 1- *Ganoderma lucidum* on malt extract agar tweeks.

Extraction of bioactive compounds from aqueous extract of *G. lucidum*

For cold-water extraction, a sub sample 10 g was extracted by stirring with cold water 100 ml at 4 °C for 24 h and filtering through Whatman Number 1 filter paper. The residue was further extracted with two additional volumes of 100 ml cold water as described above. The combined cold-water extracts were rotary evaporated at 40 °C to dryness and the resulting filtrate was freeze-dried [17].

Antifungal activity

Disc diffusion assay

Filter paper discs (0.6 mm) after being sterilized by autoclave were soaked in aqueous extract for 5 minute and control soaked in distal water, filter paper discs with extracts were placed on the surface of Sabroud dextrose agar after 7 days measured by ruler clear inhibition zone [18]

Results and discussion

In experimental Vehicle the microscopic examination for dust particles showed that particles have amorphous shape and their diameters ranged between (1 µm -10 µm) as well as for control vehicle Table-1.

Table 1- Dust particle size distribution

particles diameters (µm)	particles size distribution%	
	experimental Vehicle	control vehicle
< 5	43%	2%
5-10	32%	5%
>10	25%	4%

Previous studies refers that coarse particles with diameters ranged between (2.5–10 Micrometers) deposited in the upper respiratory tract and large airways, while fine particles with diameters (< 2.5Micrometres) may reach terminal bronchioles and alveoli [19].

The results of current study referred that control vehicle showed low percentage in all dust particles diameters while the experimental vehicles showed that 43% of particles have diameter <5 μ m that mean most of these particles considered as fine particles and deposit along bronchioles and sensitive alveolar region causing damages with a possibility for allergic reactions. The deposition of airborne particles and microorganisms in passenger respiratory tract belongs to the small space available in cars cabin and frequent changes of the direction of air flow support particles deposition within airways. [4, 5]. Other study reports that exposed persons to aerosols of contaminated AC caused increased prevalence of IgG induce sensitization and hypersensitivity pneumoitis [20]. It has been observed that hyperventilation of cold dry air contaminated with particles what we inhale while using AC's causes bronchoconstriction in asthmatic patients [21,22] hence alteration in pulmonary functions may also be simulated in AC users.

Isolation of fungi

Four species from fungi isolated from air which represents as abundant fungi in vehicle AC, these species are *Aspergillus niger*, *Aspergillus flavus*, *Penicillium sp.* and *Rhizopus sp.* identified by morphological characters on Sabroud dextrose agar and microscopic feature.

Colony characters

Figure-3 showed Surface color for *Aspergillus niger*, white with typical black spores and reverse color, yellow. *Aspergillus flavus* color is green and reverse color is orange as it showed in Figure-4. Surface color for *Penicillium* olivaceous green with sterile white margin and reverse color is orange to red as it showed in Figure-5. Figure-6 showed *Rhizopus* Colonies which have very fast growing at 25°C, about 5 - 8 mm high, with some tendency to collapse, white cottony initially becoming brownish grey to blackish - grey depending on the amount of sporulation[23].

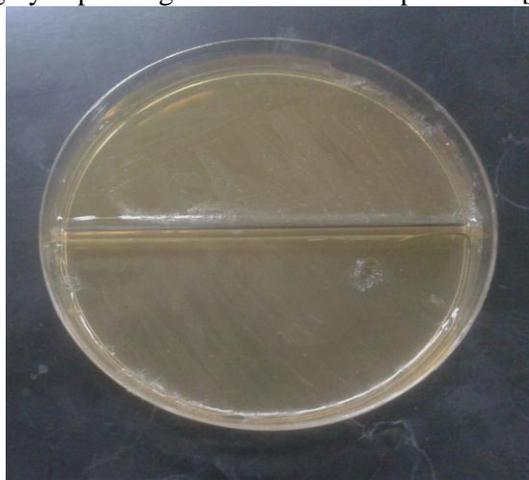


Figure 2- Control



Figure 3- *Aspergillus niger* on Sabroud dextrose agar



Figure 4- *Aspergillus flavus* on Sabroud dextrose agar

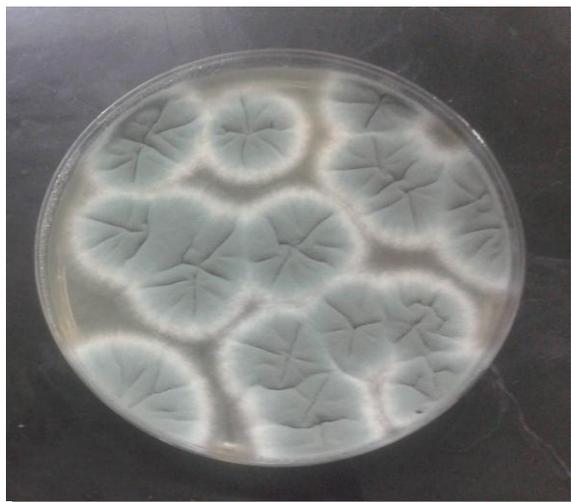


Figure 5- *Penicillium* on Sabroud dextrose agar



Figure 6- *Rhizopus* on Sabroud dextrose agar

Microscopic morphology

The microscopic morphology of *Aspergillus niger* showed large, globose, dark brown conidial heads, which become radiate, tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biserial with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose, dark brown to black and rough-walled, while the microscopic morphology of *Aspergillus flavus* appear Conidial heads typically radiate, later splitting to form loose columns, biserial but having some heads with phialides borne directly on the vesicle. Conidiophores are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose, pale green and conspicuously echinulate. Some strains produce brownish sclerotia.

The results showed *Penicillium* Hyphae septate, hyaline. Conidiophores simple or branched. Phialides grouped in brush-like clusters (penicilli) at the ends of the conidiophores; conidia unicellular, round to ovoid, hyaline or pigmented, rough walled or smooth, in chains.

Rhizopus sporangiophores appeared non - septate, simple or branched arises from stolons opposite rhizoids usually in groups of 3 or more. Sporangia are globose, often with a flattened base .the microscopic morphology that mentioned previously comes agree with reference [24].

Frequency and occurrence percentage for fungi isolated from vehicle air

The occurrence of Fungi derived from vehicle AC media presented in Table-2. The fungus *Aspergillus niger* have the maximum occurrence percentage and highly in frequency percentage than other fungi isolated from air Table-3, it is known that fungi have the ability to grow in the different environmental conditions especially *Aspergillus niger* , it could resist difficult condition because it has ability to secrete enzymes used for degradation [25]. Previous study done by [26] Refers that more than 80 genera of fungi such as *Aspergillus* , *Penicillium*, *Cladosporium*, and *Alternaria*, have been associated with symptoms of respiratory tract allergies with amongst the most common allergenic genera and caused adverse health effects in indoor environment including Headache , allergy, asthma, irritant effects, respiratory problems, mycoses (fungal diseases) [27]. *Rhizopus* have the minimum percentage , frequency percentage and occurrence percentage lower than other fungi may be this fungus not found the suitable condition as temperature , humidity and other conditions [7].

Table 2- Occurrence percentage for fungi isolated from air

Fungi isolated from air	%	Samples number have growth	Frequency percentage	Occurrence percentage
1- <i>Aspergillus niger</i>	%80	8	%33.3	%27.6
2- <i>Aspergillus flavus</i>	%70	7	%29.1	%24.1
3- <i>Penicillium</i>	%50	5	%25	%20.6
4- <i>Rhizopus</i>	%30	3	%12.5	%10.3
Total number		23	%99.9	%82.6

Antifungal activity

The antifungal activity of different concentrations of *Ganoderma lucidum* suppresses the growth of fungi differently. The antifungal activities were found to be concentration dependent. Table-3 showed that concentration 25 mg per ml for *Ganoderma lucidum* have highly inhibition zone than other concentrations. Many pharmaceutical substances as phenols ,triterpenoids etc.with potent and unique health-enhancing properties have been isolated from medicinal mushrooms and distributed worldwide [28]. Previous study achieved by Nitha *et al* referred that Mushroom based products either from the mycelia or fruiting bodies are consumed in the form of capsules, tablets or extracts as in present study and the selected macro fungus showed antifungal activity in high level which was screened against the selected fungi [29]. The antifungal inhibitory impact of these extracts can be related to the bioactivity of these compounds as several phenolic compounds including tannin are potent inhibitors of microbial enzymes like protease [30].

Table 3- Antifungal activity by inhibition zone (mm) of different concentration for *Ganoderma lucidum*

Fungi isolated from air	Control	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml
<i>Aspergillus niger</i>	-	8.83±0.26	10.5±0.45	13±0	14±0.89
<i>Aspergillus flavus</i>	-	9±0	12±0	12.6±0.52	12.7±0.52
<i>Penicillium</i>	-	8±0.26	10±0	12.3 ±0.52	13±0.45
<i>Rhizopus</i>	-	9±0	10±0	13±0	14±0

The results of the present study supported the usage *Ganoderma lucidum* as an ideal biopharmaceutics and suggested that *Ganoderma lucidum* can be used as antimicrobial agent in the development of new drug for the different fungal pathogenesis in humans [31] and it's recommended to use the *Ganoderma lucidum* extraction as spry or solution to wipe AC system components to avoid fungal growth that cause adverse health effects.

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