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Full Length Article

Effect of Ten Essential Oils in Vapor Phase on Airborne Fungal Isolates from Sawdust of Evaporative Air Coolers

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Abstract

Evaporative air cooler (EAC) is a natural cooling principle that is widely used in warm/dry climates. Airborne fungi contaminate the sawdust that are used in EAC and several techniques are followed to control their growth. This study aimed to evaluate *in vitro* antifungal effect of oil vapors of *Hyacinthus* sp., *Cymbopogon citratus, Myrtus communis, Eucalyptus* sp., *Laurus noblis*, *Rosemary officinalis, Cinnamon* sp., *Pistacia lentiscus, Thymus vulgaris* and *Syzygium aromaticum*. The reverse Petri plate (fumigation) method was followed and the effective concentrations of oils in relation to air space above the culture medium were reported. The tested isolates named *Pithomyces* sp., *Rhizopus* sp., *Stachybotrys* sp., *Trichoderma* sp., *Actinomucor* sp., *Drechslera* sp. and *Phoma* sp. The oil vapor of *C. citratus*, *Eucalptus* sp. and *Cinamon* sp. displayed strong antifungal activity with minimum inhibition concentration (MIC) of 0.5 μL for the susceptible isolates. It is worth mentioning that *M. communis* and *Cinnamons* sp. oil vapors clearly influenced spore's formation of examined isolates.

Keywords: Airborne fungi; Antifungal activity; Evaporative aircooler; Vapor phase

Introduction

Evaporative coolers (swamp coolers) are virtual air conditioner devices that are widely used in dry/warm environments such as the Middle East. Direct evaporative coolers (DEC) and indirect evaporative coolers (IEC) are the two methods by which they function, and they can either be stationary or mobile. The device induces cooling by passing air through an evaporative cooling wet cellulosic material such as wood shaving or straw. The DEC type draws the outside air, water droplets and aerobiological particles into occupied space. A known disadvantage of ECs devices is that they are liable to microbial contamination. An early note by Macher and Girman (1990) explained a relationship between the microbial contamination of water tanks and indoor air quality. Macher *et al.* (1995) used *Micrococcus luteus* as a tracer to determine the number of microorganisms detected in the flowed air based on the microbial air volume. Previous studies suggested the evaporative cooler increases personal exposure to particulate matter along with the rise of respiratory illnesses (Paschold *et al*. 2003; Lemons *et al*. 2017). These particles contribute to many respiratory disorders such as asthma, allergic sensitization, and hypersensitivity pneumonitis (Green *et al.* 2006; Mendell *et al.* 2011; Sio *et al.* 2021). Improving indoor air quality needs to reduce the aerobiological particles including fungal agents. A preferred anti-microbial catalyst material as titanium dioxide was used to eliminate microbial growth in EC devices (Kim *et al*. 2018). Also, The ultraviolet source was used by natural sunlight, or by electrical source (Gómez *et al*. 2010). Essential oils (EOs) have antimicrobial effects, they were used widely in traditional medicine and food preservation, and they were found to be safe, effective, and environmentally friendly factors (Aleryani and Al-Bader 2012; Ma *et al*. 2019). The effect of EOs was successfully examined against phytopathogen and saprophytic fungi that are part of causative agents of human opportunistic infections (Othman *et al.* 2020).

The past decades have witnessed an increasing interest in the applications of essential oils as antimicrobial agents (Alotibi and Rizwana 2019). Essential of different plant species are known to possess antimicrobial propeties (Naz *et al.* 2014; Ferdosi *et al.* 2020, 2021, 2022). However, the use of their vapors did not get enough attention. Inouye *et al.* (2000) reported that essential oils (EOs) have higher potency in a vapor state than in a solution. The vapor phase of EOs showed a significant effect on clinical and environmental fungal isolates. The lack of information about fungi associated with evaporative air coolers in Iraq has also led to a lack of studies on methods of getting rid of them. Al-Bader and Mohhamed (2023) examined the effect

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of three EO vapors on the predominant contaminant *Aspergillus niger* isolated from women's shoulder handbags. The current study was conducted to evaluate the antifungal activity of ten EOs in the vapor phase on fungi inhabiting the sawdusts of evaporative air coolers, and detection of the MIC of the highest effective vapors.

Materials and Methods

Preparation of essential oils

Hacinathus sp. (dry flowers), *Laurus noblis* (dry leaves), *Cinnamon* sp. (bark), *Pistacia lentiscus*, and *Syzygium aromaticum* (flowers) were purchased from a specific traditional medicine center in Erbil City, while leaves of *Cympogon citratus, Myrtus communis, Eucalyptus* sp., *Rosemary officinalis* and *Thymus vulgaris* were collected from the field and public gardens in Erbil city. They were cleaned and dried in a laboratory environment. The dried leaves and flowers were ground into a fine powder using an electric grinder. The Crude oils of the ten plant materials (25 g/200mL sterile distilled water) were prepared by simple steam distillation *via* the Clevenger apparatus as explained by Harbone (Harborne 1998). Distillation was done for 3 h and the collected oil was dried by Na_2SO_4 (Ayoola *et al.* 2008).

Antifungal activity

Petri dishes containing 15 mL of Sabouraud's dextrose agar supplemented by the antibiotic were inoculated from 7-day-old fungal cultures. A filter paper disc (2 cm in diameter) saturated with 150 μL oil was placed on the inner surface of the Petri dish lid, and all the plates were sealed with parafilm tape and incubated at 25 ± 2^{o}C .The antifungal activity is determined by measuring the growth diameter after four days. A replicate test for each oil was performed, in addition to the negative control. To distinguish between fungistatic and fungicidal effects, treatments that showed complete fungal inhibition at the end of day four were kept for an additional three days.

Minimum inhibition concentration (MIC)

Essential oils with anti-fungal activity were tested to determine MIC. The filter paper discs were used to add 150, 100, 50, 25, 12.5 and 6.25 μL from each EO to determine the MIC, as previously mentioned. Fungal growth was observed after four days, and the IJC was determined by the lowest concentration of EOs that inhibited the visible growth of the fungus, the test was performed in triplicate.

Statistical analysis

The difference between the diameters was determined by statistical analysis of variance (ANOVA) for each essential oil. The Vassar Stats statistical software (http://vassarstats.net/) displayed, and P value lower than 0.05 to indicate statistical significance.

Results

Antifungal effect of EOs by vapor phase

The oil's vapors showed variable antifungal actions. The stronger antifungal activity displayed by *C. citratus*, *Eucalyptus* sp. and *Cinnamon* sp. with statistically significant differences ($P \leq 0.05$) in comparison to the control (Fig. 1). The results indicated a fungicidal effect of *C. citratus* and *Cinnamon* sp. in addition to a fungistatic effect of *Eucalyptus* sp. where a tiny growth appeared after seven days. *M. communis* and *R. officinalis* have a considered effect on the formation of the spores without a similar effect on the mycelium growth as shown in Table 1.

Determination of minimum inhibitory concentration (MIC)

MIC level for effective oil vapors was 0.5 μL cm^{-3} for most of the isolates. *Phoma* sp. indicated a resistance state for the three oil vapors. The inhibition was observed in 2 μL cm^{-3}. *Rhizopus* sp. resists Eucalyptus oil vapor only (MIC = 2 μL cm^{-3}) while *Drachslera* sp. resists cinnamon oil vapor (MIC = 1 μL cm^{-3}) (Table 2).

Discussion

Three essential oils *viz., C. citratus, Eucalyptus* sp. and *Cinnamon* sp. showed strong antifungal effects. The oil of *C. citratus* has significant components, including monoterpenes compounds and citral at levels of about 65–85%. It possesses antibacterial and antifungal properties (Silva *et al.* 2008). The oil was also used commercially, and it was a component of perfumes, flavors, cosmetics, detergents and medicines (Oladeji *et al.* 2019). *Eucalyptus* sp. essential oils possess several biological properties, including antifungal antibacterial, antitumor, and insecticidal (Baptista *et al.* 2015). The chemical ingredients of leaves oil vary according to the species. The most abundant compounds in the oil were 1,8-cineole, α-pinene, α-phellandrene, and p-cymene (Gakuubi *et al.* 2017). Several species of eucalyptus have been commercially used in the pharmaceutical and cosmetic industries. Oil drives from cinnamon showed a significant inhibition to several microorganisms, and its oil had noticeable sensitivity against albicans and non-albicans spp. of Candida (Goel *et al.* 2016). It was suggested as a helpful treatment for opportunistic and dermatophytes (Maness and Zubov 2019). The contents of benzaldehyde and trans-cinnamaldehyde were significantly higher than other components in cinnamon oil (Wang *et al.* 2019).

Table 1: Growth diameter (mm) of isolated fungi treated by EO's vapor

Essential oils		*Pitho.*		*Rhizo.*		*Stachy.*		*Tricho.*		*Actino.*		*Drech.*		*Phoma*			*A. niger*	P value
		MD	SD	MD	SD	MD	SD	MD	SD	MD	SD	MD	SD	MD	SD	MD	SD	
1	*Hacinathus* sp.	28	28	55	0	6	0	40	15	55	30	32	20	32	20	26	22	*0.053*
2	*C. citratus*	0	0	50	0	0	0	0	0	2	0	0	0	0	0	0	0	*0.0004*
3	*M. communis*	27	10	85	0	14	12	40	0	62	36	39	12	39	12	30	26	*0.36*
4	*Eucalyptus* sp.	0	0	50	0	2	0	12	0	30	0	2	0	2	0	18	12	*0.0003*
5	*L. noblis*	26	24	90	80	7	7	64	25	55	30	38	20	38	20	30	24	*0.28*
6	*Cinnamon* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*0.001*
7	*R.officinalis*	18	11	90	0	6	5	30	25	56	35	28	2	28	2	28	22	*0.051*
8	*P. lantiscus*	25	23	90	60	11	10	67	27	58	40	42	28	42	28	15	0	*0.5*
9	*T. vulgaris*	24	12	90	50	12	10	55	30	44	20	37	32	37	32	32	20	*0.12*
10	*Z. aromaticum*	22	20	90	75	11	10	62	33	55	28	32	20	32	20	45	43	*0.2*
11	Control	21	20	90	80	13	12	70	40	58	33	36	26	36	26	47	43	

MD = Mycelium diameter; SD = Spore diameter

Pitho. = *Pithomyces* sp., Rhizo. = *Rhizopus* sp., Stachy. = *Stachybotrys* sp., Trico. = *Tricoderma viride,* Actino. = *Actinomyces* sp., Drech. = *Drechslera* sp., *Phoma* sp., *A.nger* = *Aspergillus niger. niger*

Pitho. = *Pithomyces* sp., Rhizo. = *Rhizopus* sp., Stachy Stach. = *Stachybotrys* sp., Trico. = *Tricoderma viride*

Actino. = *Actinomyces* sp., Drech. = *Drechclera* sp., *Poma* sp., *A. niger* = *Aspergillus niger*

Table 2: MIC of the effective oils against selected fungi (μL cm^{-3})

	Pitho.	*Rhizo.*	*Stachy.*	*Tricho.*	*Actino.*	*Drech.*	*Phoma*	*A. niger*
C. citratus	0.5	0.5	0.5	0.5	0.5	0.5	2	1
Eucalyptus sp.	0.5	2	0.5	0.5	0.5	0.5	2	0.5
Cinnamon sp.	0.5	0.5	0.5	0.5	0.5	1	2	1

Pitho. = *Pithomyces* sp., Rhizo. = *Rhizopus* sp., Stachy. = *Stachybotrys* sp.,Tricho. = *Trichoderma viride,* Actino. = *Actinomyces* sp., Drech. = *Drechclera* sp

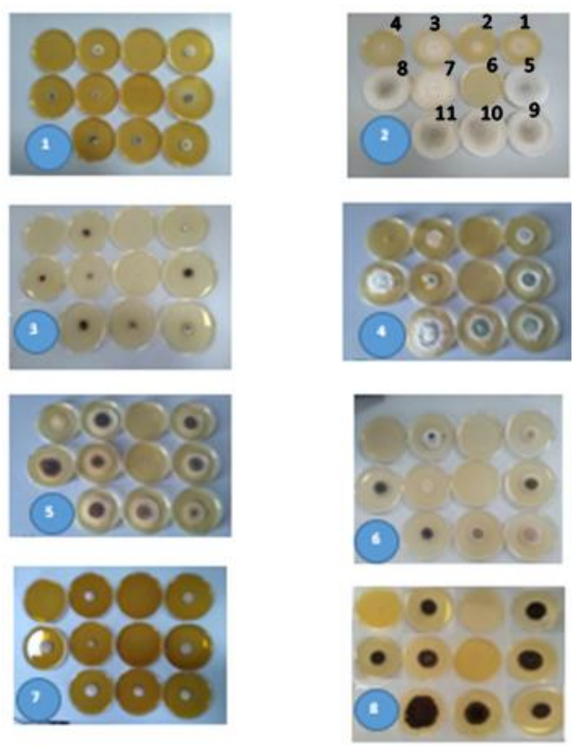

Fig. 1: Effect of eight essential oils in vapor phase against fungi isolated from sawdust

Oils: 1- *Hacinathus* sp., 2- *C. citratus,* 3- *M. communis,* 4- *Eucalyptus* sp., 5- *L. noblis,* 6- *Cinnamon* sp., 7- *R.officinalis,* 8- *P. lantiscus,* 9- *T. vulgaris,* 10- *Z. aromaticum,* 11- control

Fungi inside the rectangles: 1-*Pithomyces* sp., 2- *Rhizopus* sp., 3- *Stachybotrys* sp., 4- *Trichoderma* sp., 5- *Actinomucor* sp., 6- *Drechslera* sp., 7- *Phoma* sp

Essential oils act on the fungal structure in different ways, they may cause significant changes in the shape and structure of the hyphal cell wall. Billerbeck *et al.* (2001) reported that the active ingredients of EOs interact with cell wall synthesis enzymes leading to abnormal glucan, chitin, and glycoproteins. Helal *et al.* (2006) mentioned plasma membrane disruption and mitochondrial structure disorganization after EO treatment. Most Studies about the effect of oil vapor suggested that direct contact (vapor/hyphae) or indirect contact (vapor-medium/hyphae) disturbed cell wall integrity. The synergism of both mechanisms was also suggested (Cavanagh 2007). However, the main act resulted from the oil vapor accumulation on mycelia more than the agar. The targets of action of several oils and their vapor were confirmed by Scan electron microscope and transmission electron investigation (Shao *et al.* 2013; Wang *et al.* 2019). The high activity of the oil gas phase, as well as the easy use, made it more applicable for several purposes such as treatment of otitis, aromatherapy, controling on fungal spoilage of bread, and cereales (Kristinsson *et al.* 2005; Ali *et al.* 2015; Císarová *et al.* 2020; Střelková *et al.* 2021) The results showed different MIC values, as well as oil vapor, showed variable effects. Indeed, inhibition is the result of the interaction between the properties of fungal strain and the vapor effect (Střelková *et al.* 2021). The rsistance that showed by *Phoma* isolate and its high MCQ may be related to the morphology of pycnidia, which protects the spores and minimized direct contact with oil vapor. The resistance of *Phoma* sp. represented by a high MIC may be related to the morphology of pycnidia, which protects the spores and minimized direct contact with oil vapor.

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Conclusion

The essential oils were considard by the United States Food and Drug Administration as a"Generally recognized as safe", and their vapors were successfully used against phytopathology and seed-borne diseases .The variable level of antifungal effects that obseved here related to the interaction between oil vapor properties and endogenous fungal characteristics. This subject needs farther studies to evaluate the antifungal activity of EOs vapor on aero-mycobiota and the opportunistic fungi. According to variable inhibition properties and MIC levels, it is clear that the inhibitory action is a result of the interaction between oil vapor properties and endogenous fungal characteristics. Although EOs were classified by the United States Food and Drug Administration as "Generally recognized as safe", and the vapor phase was successfully used against phytopathology and seed-borne diseases, there is a shortage of information on their effects on saprophytic and opportunistic fungi including the indoor aero-mycobiota.

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Author Contributions

SMA did the conceptualization, ZZ and ASMJ did the data curation, SMA and ASMJ did the formal analysis, SMA and ZZ wrote the original draft and SMA wrote the review and did editing.

Conflicts of Interest

The authors declare no conflict of interest

Data Availability

Available upon request

Ethics Approval

Not applicable

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