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SCREENING OF ESSENTIAL OILS OF ANGIOSPERMIC PLANTS FOR THEIR FUNGITOXICITY AGAINST *ASPERGILLUS FLAVUS* AND *ASPERGILLUS NIGER*

ATUL SRIVASTAVA*[1], PRADEEP KUMAR SHUKLA[1], ASHWINI KUMAR MISHRA[2], SUBHASHINI[3] AND SUCHIT JOHN[1]

[1] *Department of Biological Sciences, Sam Higginbottom Institute of Agricultural, Technology & Sciences, Allahabad-211008*
[2] *Department of Botany, Kashi Naresh Government Post Graduate College, Gyanpur-221304*
[3] *Department of Zoology, Mahila Mahavidalya, Banaras Hindu University, Varanasi-221005*

ABSTRACT

Essential oils obtained from plants have recently gained a great scientific interest as a potent source of antimicrobials of natural origin. The present study highlights the anti-fungal activity of essential oils against *Aspergillus flavus* and *Aspergillus niger*. During screening of essential oils of six selected Angiospermic plants at 2000 ppm (mg/l) against the test fungus, *Mentha arvensis and Citrus aurantifolia,* being most effective was selected for further study. The selected oils were subsequently standardized through physico-chemical and fungitoxic properties. The MIC values of *Mentha arvensis* and *Citrus aurantifolia* were found to be 1000 and 2000 ppm (mg/l) respectively. Fungitoxicity of both oils represents fungicidal, as well as fungistatic behavior at their respective MIC(s). Study also revealed that oil of *Mentha arvensis and Citrus aurantifoila* are highly thermo stable (up to 80ºC) and had the potency to withstand high inoculum density. The fungitoxicity of both the oils remained unaltered up to 180-210 days at room temperature. The antifungal potency of oils was found greater when compared with some prevalent synthetic commercial fungicides. Therefore oils could be recommended as a potential source of eco-friendly herbal fungicide and might have role as pharmaceutical and preservatives.

KEYWORDS: Essential oil, Fungitoxic, *Aspergillus flavus*, *Aspergillus niger*.

ATUL SRIVASTAVA
Department of Biological Sciences, Sam Higginbottom Institute of Agricultural, Technology & Sciences, Allahabad-211008

***Corresponding author**

INTRODUCTION

Microorganisms are very diverse and include bacteria, fungi, viruses and protozoans. These being crucial in ecosystem are also beneficial to human subjects. Small proportions of microorganisms are pathogenic to humans, animals and plants and even lead to deaths which have raised a great safety concern to public health. In several geographic regions a large proportion of bacteria and fungi diseases have been confirmed by hazardous pathogens [1, 2] affecting human health and economically important plants [3, 4]. Environment comprises several species of fungi which are known to be pathogenic in plants and humans leading to mycoses. Among these the most common and important opportunistic fungal species which cause disease in plants and humans is *Aspergillus*. The genus *Aspergillus* comprising many species has a great impact in various field of research and is important as human and plant pathogens. Species of *Aspergillus* as *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus clavatus*, *Aspergillus niger* is known to cause aspergillosis, allergic reactions, lung infections, and infection in other organs in humans. In world, crop production pre-harvest loss due to fungal diseases amount to 12% in developing countries [5]. *Aspergillus flavus (A. flavus)* and *Aspergillus niger* (*A. niger)* are well known agricultural pathogen and destroys the grain crops especially maize. *A. niger* known as black mold and considered as “Weed of laboratory”, causes black mould and root stalk rot diseases in several crop. *A. flavus* and *A. niger* produces highly carcinogenic mycotoxins as Aflotoxin and Ochratoxin during the decay of food [6].

Pathogenic or infectious fungus can be killed or controlled by various biological and non-biological agents, commonly referred as antifungal agent. In the past few decades, the repeated and continuous use of the synthetic antifungal drugs has led to the development of the resistant strain which remains unaffected by these drugs. The increasing incidences of drug resistance and emergence and re-emergence of deadly microorganisms have raised question on their use. Also, fungicides primarily used for controlling the growth have recently come under special scrutiny as posing a potential oncogenic risk. The disease cause of fungi as an agent of plant human diseases and decomposers has spurred scientist world wide for safer alternative products to control these pathogen [7]. Naturally occurring biologically active compounds from plants and there extracts including Essential oil are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents with no adverse side-effect on humans [8, 9, 10, 11]. Therefore the present work was designed to explore the fungitoxic activity of essential oil of some Angiospermic plants against *A. flavus* and *A. niger.*

MATERIALS AND METHODS

Potato Dextrose Agar medium, Czapek Dox Agar Medium and streptomycin were purchased from Himedia Laboratories Pvt. Ltd., India. Anhydrous sodium sulphate, acetone, chromic acid, Potassium hydroxide and hydrochloric acid were purchased from Loba Chemine Pvt. Ltd., India and were of analytical grades. All the synthetic fumigates were purchased commercially.

(1) Collection of plant material

Higher plants of different angiospermic taxa of three families (Zingiberaceae, Rutaceae, and Lamiaceae) were randomly selected from different areas of Botanical garden of Banaras Hindu University, Varanasi, and college campus of Kashi Naresh Government Post Graduate College, Gyanpur, Bhadohi and Sam Higginbottom Institute of Agricultural, Technology & Sciences, Allahabad. These were further identified with the help of different floras [12, 13] as *Amomum subulatum Robx* (Black cardomom), *Citrus aurantifolia* (kaghzi-nimbu), *Citrus reticulate Blanco* (Mausami), *Citrus sinensis Osbeek* (Sweet orange), *Mentha arvensis* (Menthol Mint) *and Murraya koenigii* (Curry leaves) (Fig 1). The healthy, fresh plant parts specially the young leaves and peels free from any visible contamination were used for the extraction purpose.

Plants used for the extraction of essential oil

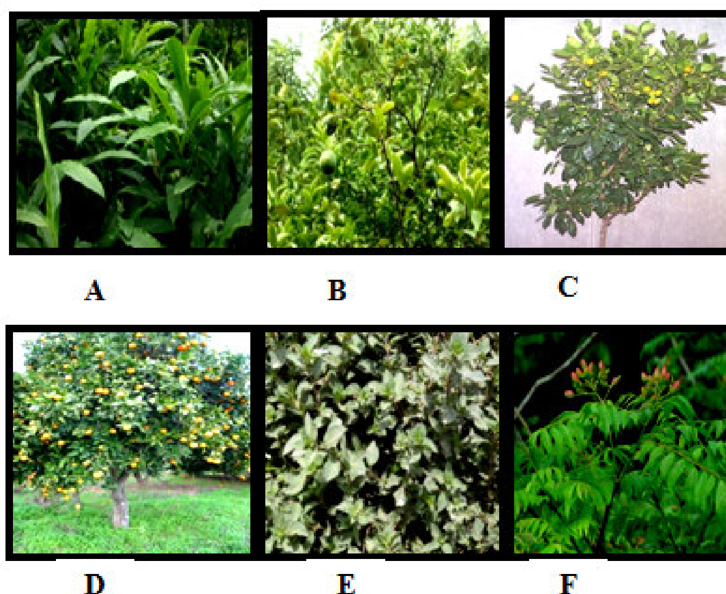

Figure 1
(A) Amomum subulatum Robx (Black cardomom), (B) Citrus aurantifolia (kaghzi-nimbu), (C) Citrus reticulate Blanco (Mausami), (D) Citrus sinensis Osbeek (Sweet orange), (E) Mentha arvensis (Menthol Mint), and (F) Murraya koenigii (Curry leaves)

(2) ISOLATION OF ESSENTIAL OIL FROM THE DIFFERENT PARTS OF THE PLANT

The essential oils of these plants were isolated by hydrodistillation through Clevengers apparatus. 500gms of fresh parts (leaves and peel) of each plant were cut separately into small pieces and then thoroughly washed with sterilized water. The plant material along with water was then placed in the round-bottom flask of the Clevengers apparatus. Water was heated to produce steam that carried the most volatile fractions (essential oils) with it. The steam was then chilled (in a condenser) and the resulting distillate was collected in the collecting funnel of the apparatus. The obtained chilled suspension showed two distinct layers- the lower aqueous layer and an- upper oily layer consisting the essential oil which was found to float on the top of the hydrosol (the distilled water component). Both the layers were separated and the essential oils were dehydrated by the addition of anhydrous sodium sulphate, followed by shaking and standing for 6–8 h and filtration. The extracted oils were stored in clean glass vials after removing water traces with the help of capillary tubes. The percent recovery (W/V) of oil was determined [14] and calculated as following:

$$Percentage\ recovery\ of\ oil = \frac{Volume\ of\ essential\ oil}{Weight\ of\ plant\ part(g)} \times 100$$

(3) MAINTENANCE OF FUNGAL CULTURES OF A. flavus and A. niger

A. flavus and A. niger were cultured and grown in the laboratory on Potato Dextrose Agar medium (PDA; 42 gm in 1000 ml of distilled water) and Czapek Dox agar (CZ) medium. PDA medium was prepared, autoclaved and cooled down to 40^0C before use. CZ media was used mainly for the selective identification of *A. flavus and A. niger* [15]. The cultures were incubated at 26±2^0 C. Fungal discs (4 mm diameter) were taken from the periphery of a seven day old culture of *A. flavus* and *A. niger* for the study.

Culture of Aspergillus flavus and Aspergillus niger

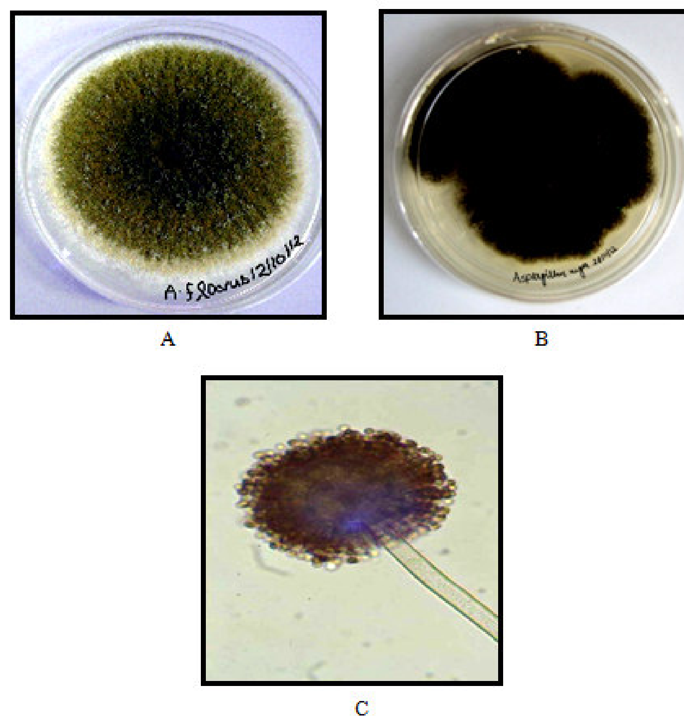

Figure 2

The fungus were grown on petri plates containing Potato Dextrose Agar medium and incubated at a temp. of 26^{0}C ± 2 for seven days. (A) Shows the conidial formation in Aspergillus flavus (B) shows the conidial formation in Aspergillus niger (C) Spore of Aspergillus niger.

(4) SCREENING OF THE ESSENTIAL OIL AGAINST THE TEST FUNGUS

Fungitoxic activity of the oils was tested by the poisoned food technique [16] using PDA medium for the growth of test fungus. 10 mg of streptomycin was added thoroughly to prevent any bacterial contamination. Seven days old cultures of fungus were used for the experiment. A concentration of 2000ppm of all the oils was prepared by dissolving requisite amounts of the oil separately in 0.5 ml of acetone in pre sterilized petriplates (9.5mm diam). PDA medium of 9.5ml was pipette to each petri plate and mixed thoroughly to make up the volume of 10 ml so as to obtain 2000ppm concentration. The plates were swiveled thoroughly in order to obtain homogenous medium. For control sets same amount of water has been used in place of oil. Fungal discs (4 mm diameter) retrieved from the periphery of a seven day old culture of *A. flavus* and *A niger* and were placed aseptically with the help of sterilized cork borer into the centre of each Petri plate of treated and control sets separately. The Petri plates were further incubated at 26 ± 2^{0}C for five days in incubation chamber and observed on the sixth day. Diameters of fungal colony of treated and control sets were observed and measured in mutually perpendicular directions on the sixth day. The percentage mycelial inhibition was calculated by the mean value of colony diameters by the following formula:

$$Percentage\ mycelia\ inhibition = \frac{dc - dt}{dt} \times 100$$

Where,

dc = Average diameter of fungal colony in control sets

dt = Average diameter of fungal colony in treatment sets

Due to the strong fungitoxicity (100% growth inhibition) and maximum percentage recovery of *M. arvensis and C. aurantifolia*, these were selected for further investigation including minimum inhibitory concentration (MIC) at different ppm, fungistatic and fungicidal property along with their physicochemical property.

(5) PHYSICOCHEMICAL PROPERTIES OF M. arvensis AND C. aurantifolia

Since, it is well reported that the activity of the herbal product is due to the presence of different constituents in it which represents its property. Hence, further oils of *M arvensis and C. aurantifolia* were studied for its various physicochemical properties, [17, 18] which included specific gravity, Optical rotation, Refractive index, Solubility in different organic solvents, Acid number, Saponification value, Ester value, Phenolic content, pH value.

(6) MINIMUM INHIBITORY CONCENTRATION (MIC) AND NATURE OF TOXICITY OF OILS AGAINST TEST FUNGUS

The screening of all the oils was performed at a concentration of 2000ppm so further the aim was to scrutinize the minimum inhibitory concentration below 2000ppm. To find out the minimum inhibitory concentration at which the oils showed absolute fungitoxicity, experiments were carried out by the above-mentioned poisoned food technique using graded concentration of essential oils below 2000 ppm. Fungal discs (seven days old culture) were incubated with different concentrations of the oils (2000, 1500, 1000 and 500ppm) for five days and observed on 6th day. Diameters of fungal colony of treated and control sets were noted and the percentage mycelia inhibition was calculated by the above formula. Both the oil was tested for the toxicity test (fungistatic/fungicidal) on the fungus [19, 20]. Briefly, requisite amounts of the oils dissolved in acetone were mixed with PDA medium to get final concentration of 1000, 1500, 2000, 2500 and 3000ppm. The plates were inoculated aseptically with fungal disc (4mm diameter taken from the periphery of a seven day old culture of the test fungi) and were incubated for five days at 26 ± 2^0C. On sixth day the incubated discs were taken out from the plates, washed with sterilized water and re-inoculated aseptically to plates containing fresh PDA medium to observe revival of their growth. Further the observation was recorded on the sixth day and percentage mycelial inhibition was calculated as above.

(6) EFFECT OF INCREASED INOCULUMS ON TOXICITY OF THE OIL

The antifungal effect of *M arvensis* and *C. aurantifolia* on increased inoculums density of both test fungi was observed [21] at their respective MICs as 1000 and 2000 ppm respectively. Briefly, MICs of dissolved in acetone and mixed with Potato dextrose broth medium were divided into six sets and inoculated separately with the assay discs (4mm) of the test fungi in geometrical progression of two, i.e., 1, 2, 4, 8, 16 and 32. All flasks were incubated for five days at 26 ± 2^0C. Observation was recorded on the sixth day and calculated as above.

(7) EFFECT OF STORAGE TIME AND TEMPERATURE ON FUNGITOXICITY OF THE OILS

To test the effect of long term storage on the fungitoxic property of the oil, these were air tightened and kept separately at room temperature for eight months and then further tested at regular interval of one month at their respective minimum inhibitory concentrations following poisoned food technique. To study the effects of temperature on antifungal property of *M. arvensis* and *C. aurantifolia* oils, 3 lots of oils each containing 2 ml were kept in air tight at different temperature's of 40, 60 and 80^0C. The oils were cooled to room temperature and tested for fungitoxic activity by the usual poisoned food technique at their MIC.

(8) COMPARATIVE EFFICACY OF SELECTED OIL WITH SOME SYNTHETIC FUNGICIDES

The antifungal efficacy of *M. arvensisa* and *C. aurantifolia* were compared with some commercially available standard synthetic fungicides *viz.* Celphos, Sulphex, Agrozim, Bavistin. Emison, Capton, Dithane M-45, Benlate and Dhanuka M-45. Experiments were followed by the above poisoned food technique to investigate their MICs against the test fungus.

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RESULTS

(I) *M. arvensis AND C. aurantifolia SHOWED THE ABSOLUTE TOXICITY WITH GOOD RECOVERY OF OIL*

The percent recovery of oil varied between 14% to 51% with highest of 51 % in *A. subulatum* and 45% *in C. reticulate* while *C. aurantifolia and M. arvensis* were found to extort 36% of oil as figured in table 1. It is also evident from the Table 1 that all the seven essential oils including *C. aurantifolia and M. arvensis* tested against the test fungus (*A. flavus and Aspergillus niger)* at 2000 ppm exhibited significantly diverse and broad fungitoxic spectrum by inhibiting the mycelial growth of test fungus. *M. arvensis* and *C. aurantifolia* illustrated the best outcome and inhibited the mycelial growth of both the test fungus completely with absolute toxicity (100% growth inhibition) at 2000 ppm (mg/l). However, it was interesting to observe that none of the essential oil was found to accelerate the growth of the test fungus. Also the essential oil of *A. subulatum* was absolutely effective against *A. niger* only. Since absolute inhibition was observed in *M. arvensis* and *C. aurantifolia* against the both test fungus, these two were further selected for the elaborative study.

Table 1
Screening of essential oil of angiospermic plant parts for their recovery and antifungal activity against Aspergillus flavus & Aspergillus niger at 2000 ppm.

Name of the plants	Family	Plant part used	Percent recovery of oil (Mean ± SD)	Percent mycelia inhibition of test fungi (Mean ± SD)	
				A. flavus	*A. niger*
Amomum subulatum Robx.	Zingiberaceae	Leaf	0.51 ± .019	82.13 ± 8.30	100
Citrus reticulate Blanco	Rutaceae	Peel	0.45±.041	51.33 ± 6.6	58 ± 1.63
Citrus sinensis (L) Osbeek	Rutaceae	Leaf	0.14 ± .017	82.67 ± 3.77	84.67 ± 3.4
Citrus sinensis (L) Osbeek	Rutaceae	Peel	0.30 ± .021	25.33 ± 4.12	56.67 ± 3.4
Mentha arvensis	Lamiaceae	Leaf	0.36 ± .021	100	100
Murraya koenigii (L) Spreng	Rutaceae	Leaf	0.24 ± .032	34.67 ± 3.4	44 ± 4.32
Citrus aurantifolia	Rutaceae	Leaf	0.36 ± 0.13	100	100

(II) PHYSICOCHEMICAL PROPERTIES OF M.arvensis AND C.aurantifolia

The yield of oil of *M. arvensis* and *C. aurantifolia* was found 36%. The test for the physicochemical properties of both the essential oi*ls* indicates their acidic nature (pH 4.0) with aromatic pleasant and pungent smell and whitish yellow and pale yellow in appearance respectively. The oils were completely miscible with all the tested organic solvent viz. acetone, absolute and 90% alcohol, benzene, chloroform and petroleum ether. The optical rotation indicates that the *Citrus* oil is Dextrorotatory while *Mentha* oil is levorotatory against the plane polarized light. Apart presence of phenolics content was also detected in the both the oil (Table-2).

Table 2
Various physico-chemical properties of Mentha arvensis and Citrus aurantifolia

Parameters	*Mentha arvensis* oil	*Citrus aurantifolia* oil
Colour	Pale yellow	Whitish yellow
Odour	Pungent	Aromatic pleasant
Specific gravity	0.9564 at 25^{0}C	0.8778 at 25^{0}C
Optical rotation	- 23^0 0´ at 20^{0}C	+ 46^0 28´ at 20^{0}C
Refractive Index	1.2024 at 20^{0}C	1.4738 at 20^0 C
Solubility		
Acetone	Soluble (1:1)	Soluble (1:1)
Absolute alcohol	Soluble (1:1)	Soluble (1:1)
90% alcohol	Soluble (1:1)	Soluble (1:1)
Benzene	Soluble (1:1)	Soluble (1:1)
Chloroform	Soluble (1:1)	Soluble (1:1)
Petroleum ether	Soluble (1:1)	Soluble (1:1).
Acid number	2	18.63
Saponification value	35.234	153.867
Ester value	33.234	135.237
Phenolic content	Present	Present
pH	4.0 (Acidic Nature)	4.0 (Acidic Nature)

(III) MIC AND NATURE OF TOXICITY OF M. arvensis AND C. aurantifolia AGAINST THE TEST FUNGUS

To find the MIC of both the oil, concentration of 2000, 1500, 1000, 500ppm (mg/l) of *M. arvensis* and *C. aurantifolia* oil were used for the study. *M. arvensis* exhibited absolute fungitoxic activity at 2000 and 1000 ppm, while 40-50% inhibition was observed at 500ppm (Table 3). However, *C. aurantifolia* oil showed absolute fungitoxic at 2000ppm against both the test fungi while 73-50% inhibition was observed at 1500-500ppm (Table 3). Therefore, the MIC of *Mentha* and *Citrus* oils was assigned to be 1000 and 2000 ppm respectively.

Table 3
Minimum inhibitory concentrations of Mentha arvensis and Citrus aurantifolia.

Concentration ppm	*Mentha arvensis*		*Citrus aurantifolia*	
	Percentage mycelia Inhibition Mean±SD		Percentage mycelia Inhibition Mean±SD	
	Aspergillus flavus	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus niger*
2000	100	100	100	100
1500	100	100	73.93 ± 5.99	86.05±3.74
1000	100	100	58.18 ± 6.47	67.87±3.74
500	39.99 ± 2.57	48.87±2.27	75.15± 3.74	84.24 ±1.72

The fungistatic/fungicidal properties of *M. arvensis* and *C. aurantifolia* oil were tested at their MICs to 3000ppm (2000ppm-3000ppm and 1000-3000ppm respectively) against both the test fungi. At their respective MICs both the oils were observed to be fungistatic against both the test fungus (Table-4). However, the oils became fungicidal at concentrations higher than their MICs.

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Table 4
Nature of toxicity of the oils against Aspergillus flavus and Aspergillus niger

Concentration ppm	***Mentha arvensis* Growth of Test Fungi**				***Citrus aurantifolia* Growth of Test Fungi**			
	Aspergillus flavus		*Aspergillus niger*		*Aspergillus flavus*		*Aspergillus niger*	
	Treated	Re-inoculated	Treated	Re-inoculated	Treated	Re-inoculated	Treated	Re-inoculated
1000	–	+*	–	+*	×	×	×	×
1500	–	–**	–	–**	×	×	×	×
2000	–	–**	–	–**	–	+*	–	+*
2500	–	–**	–	–**	–	–**	–	–**
3000	–	–**	–	–**	–	–**	–	–**

× indicates Concentration is not considered; - indicates no growth of fungus; + indicates growth of fungus;
**** indicates fungi static; ** indicates fungicidal.***

(IV) EFFECT OF INCREASED INOCULUMS ON FUNGITOXICITY OF THE OIL

It has been observed that the oils inhibited the fungal growth of the treatment sets containing even 32 times higher disc (approx. 36736×10^3 spores) of the test fungus indicating the potency of the essential oils to withstand high inoculum density. This is the important potential of the oils to be exploited as botanical fumigant. Reticent fungal growths against increased inoculums reveal strong fungitoxic property of both the oils at their respective MIC (Table 5 and 6).

Table 5
Antifungal activity of Mentha arvensis and Citrus aurantifolia on the increased inoculum density of Aspergillus flavus

Number of inoculated discs	**Approx number of Spore**	**Mycelia Growth of *Aspergillus flavus***		
		Control	**Treatment at MIC(s)**	
			Mentha arvensis	***Citrus aurantifolia***
1	1148×10^3	+	–	–
2	2296×10^3	+	–	–
4	4592×10^3	+	–	–
8	9184×10^3	+	–	–
16	18368×10^3	+	–	–
32	36736×10^3	+	–	–

- ***Indicates no growth of fungus; + indicates growth of fungus***

Table 6
Inhibitory effect of Mentha arvensis and Citrus aurantifolia oils on the increased inoculums density of Aspergillus niger

Number of inoculated discs	**Approx. number of spore**	**Mycelia growth of *Aspergillus niger***		
		Control	**Treatment at MIC(s)**	
			Mentha arvensis	***Citrus aurantifolia***
1	836×10^3	+	–	–
2	1672×10^3	+	–	–
4	3344×10^3	+	–	–
8	6688×10^3	+	–	–
16	13376×10^3	+	–	–
32	26752×10^3	+	–	–

- Indicates no growth of fungus; + indicates growth of fungus

(IV) LONG TERM STORAGE AND HIGH TEMPERATURE RETAINS THE FUNGITOXICITY OF THE OIL

The data in Table-7 illustrate long shelf lives of both the oils. The oil of *M. arvensis* remained active and unaltered upto seven months while *C. aurantifolia* remained absolutely effective and could retain their toxicity up to 180 days against both the test fungi at their respective MIC. Beyond that the antifungal properties of oils was found to decline.

Table 7
Effect of long term storage on the fungitoxic activity of the oils at their MIC(s)

Storage of the oils (in days)	*Mentha arvensis*		*Citrus aurantifolia*	
	Percentage mycelia Inhibition ± SD		Percentage mycelia Inhibition ± SD	
	Aspergillus flavus	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus niger*
30	100	100	100	100
60	100	100	100	100
90	100	100	100	100
120	100	100	100	100
150	100	100	100	100
180	100	100	100	100
210	100	100	83.89±4.16	87.78±4.16
240	85.56±4.16	89.44±0.79	83.33±4.08	85±4.08

Effect of high temperature (40-80^{0}C) on the retention of the fungitoxic property of the oil was also tested. Both the oils were found to withstand the high temperature without any alteration in the activity when tested against the test fungus at their respected MICs (Table-8). The data predicts that the oils remained effective even up to 80^{0}C the maximum temperature taken into consideration showing the thermo stable nature of the oil.

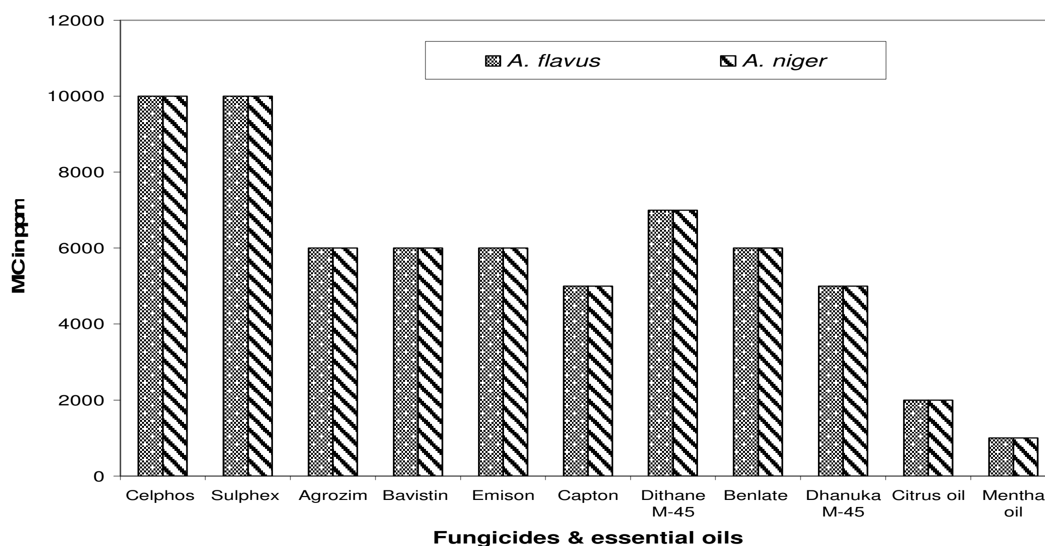
Table 8
Effect of high temperature on the antifungal activity of the oils at their MIC(s)

Oil incubated at temp.^{0}C	*Mentha arvensis*		*Citrus aurantifolia*	
	Percentage mycelia Inhibition ±SD		Percentage mycelia Inhibition ±SD	
	Aspergillus flavus	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus niger*
40	100	100	100	100
60	100	100	100	100
80	100	100	100	100

(V) M. arvensis **and** *C. aurantifolia SHOWED BETTER RESPONSE AT LOWER MIC AS COMPARED WITH SYNTHETIC FUMIGATES*

The antifungal properties of both the oils were compared with some synthetic fungicides (as mentioned in the materials and methods). The synthetic fumigates showed absolute fungitoxicity at higher MIC than the essential oils tested in present study (Table 4). Thus the oils have been found to be more efficacious than the synthetic fungicides.

Figure 3
Comparative efficacy of M. arvensis and C. aurantifolia with some commercially available synthetic fumigate



DISSCUSSION

Essential oils as natural occurring phytochemical are used since centuries with wide application as natural flavoring agent, pesticides, medicinal, health purposes and are thought to play an important role in the plant defence mechanism against phyto-pathogenic microorganism [22]. Various plant materials are believed to have antimicrobial activity and many essential oils have been reported to exhibit antifungal activities with no side effects on humans and animals [23, 24, 10, 25, 26]. Recent finding on the success of essential oil as biodegradable and eco friendly fungi toxicants have shown the possibilities for their exploitation as natural fungicides [27, 28]. The present study demonstrates a report on the anti-fungal activity of seven extracted oils screened against *A. flavus* and *A. niger*. However, *M. arvensis* and *C. aurantifolia* were found to exhibit absolute toxicity against the growth of test fungus with good percentage recovery of oil as compared to all the other oil and was further selected for detail observation. The quality of essential oils depends on a number of physico-chemical parameters as specific gravity, optical rotation, refractive index, solubility in different organic solvents, acid number, saponification value, ester value and phenolic contents. Both the oils were further standardized for their physico-chemical properties where the acidic nature and phenolic content was detected in both the oils. It might be that the fungitoxic property of oil could be due to the presence of phenolic active constituents in the leave of *M. arvensis* and *C. aurantifolia* which supports the initial finding where essential oils with predominant alcoholic compounds have been shown to be slightly less active than compounds containing phenolic structures [29, 30]. The methods used in this study for the estimation of fungitoxic property of essential oil are a well-tried method. Initially, we examined the MICs of both the oil and further the study for antifungal experiments were performed at their respective MICs. The study marks that the oils articulate fungistatic nature at their respective MICs but however, at higher concentration they expressed fungicidal activity. The reason and mechanism behind this activity still need to be clarified and explored. Interestingly, it was also noted that these oil act as a fungitoxic agent against the increased inoculum density of test fungi. This is considered as an important characteristic of the oils to be exploited as botanical fumigant. The most distinctive point of the observation was long shelf lives of both the oils at room temperature ranging upto 180-210 days. Apart it was also noted thast

the fungi toxic properties of the oils donot alters even if the oil is exposed to high temperature i.e. upto 80^0C. Application of essential oils as antimicrobial agents can be a remarkable field of investigation due to their non toxic effect to mammals. Although extensive research has been done within this field further research is still required to known the exact mechanism of action of these essential oil on fungi so, that it could be explored and applicable as a strong antifungal compound against fungal infection either in plants or animals.

CONCLUSION

Essential oils have the potential to be used as antifungal agent both for medical and commercial applications and could be a good source as complementary and alternative medicines against fungal infection.

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Conflict of Interest

Conflict of interest declared none.

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