

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST FOUR FOOD-BORNE FUNGAL STRAINS

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Uleiurile esențiale (EOs) obținute din plante au fost utilizate de secole ca agenți antimicrobieni. Creșterea rezistenței bacteriilor la antibiotice precum și eforturile de a obține conservanți naturali în fabricarea produselor alimentare au crescut interesul pentru posibile aplicații ale Eos.

*Scopul acestui studiu este de a determina activitatea antimicrobiană a patru uleiuri esențiale împotriva unor tulpini de microorganisme ca *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus* și *Penicillium hirsutum* care alterează frecvent produsele alimentare.*

Essential oils (EOs) obtained from plant material have been used for centuries as antimicrobial agents. The increase of bacterial resistance to antibiotics and the efforts to develop natural preservatives in food manufacturing has increased interest in possible applications of Eos.

*The aim of this investigation was to determine antimicrobial activity of four Essential oils against *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus* and *Penicillium hirsutum* molds.*

Keywords: Essential oils, antimicrobial activity, *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus*, *Penicillium hirsutum*

1. Introduction

Essential oils (EOs) obtained from plant material have been used for centuries as antimicrobial agents. The increase of bacterial resistance to antibiotics and the efforts to develop natural preservatives in food manufacturing have increased interest in possible applications of EOs.

Most studies use EOs as antimicrobial agents incorporated into the food [1-3].

However the use of these agents as active antimicrobial compounds in packaging materials has been less developed and only some works study this application [4].

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile, ethereal

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oils or simply as the "oil of" the plant from which they were extracted, such as oil of clove.

Essential oils are generally extracted by distillation. Other processes include expression, or solvent extraction. They are used in perfumes, cosmetics, soap and other products, for flavoring food and drink, and for scenting incense and household cleaning products [5, 6].

Various Essential oils have been used medicinally at different periods in history. Medical application proposed by those who sell medicinal oils range from skin treatments to remedies for cancer, and are often based on historical use of these oils for these purposes. Such claims are now subject to regulation in most countries, and have grown vaguer to stay within these regulations [7].

Interest in Essential oils has revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by Essential oils have curative effects. Oils are volatilized or diluted in carrier oil and used in massage, diffused in the air by a nebulizer or by heating over a candle flame, or burned as incense, for example [8].

The techniques and methods first used to produce essential oil were first mentioned by Ibn al-Baitar (1188-1248), an Andalusian physician, pharmacist and chemist.

Antimicrobial activity of the oils depends on the major constituents and their concentration. The inhibitory effects of Essential oils are mainly due to the major component. The small amounts of minor components might also contribute the antimicrobial activity of the oils.

Essential oils are produced by plants as secondary metabolites in particular cells or formed as glandular hairs [9].

Antimicrobial properties of essential oils depend on genus, species, and geographical area (climatic factors) of spices.

Dorman and Deans [10] examined the antimicrobial activity of the volatile oils of black pepper, clove, geranium, nutmeg, oregano and thyme against 25 different genera of bacteria including animal and plant pathogens, food poisoning and spoilage bacteria. All the bacterial strains tested showed some degree of sensitivity to volatile oils.

Phenolic compounds possess the highest antimicrobial properties followed by alcohols, aldehydes, ketones, ethers and hydrocarbons. The essential oils of spices are composed of a complex composition of compounds. Bioactive ingredients in spices such as sulfides, thiols, terpenes and their derivatives, phenols, glycosides, alcohols, aldehydes, and esters have been investigated for their properties. The majority of the antimicrobial components of spices are phenol compounds with a hydroxyl group (-OH). Presence of -OH group was found to be responsible for antimicrobial properties of thymol. Basilico and Basilico [11] reported that at

1000 ppm essential oils of oregano and mint inhibited the growth of *Aspergillus ochraceus* and ochratoxin.

The aim of this investigation was to determine antimicrobial activity of four Essential oils against *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus* and *Penicillium hirsutum* molds.

2. Experimental set-up

Strains

Four food-borne fungal strains were tested for the antimicrobial activity of Essential oils from lemon (*Citrus lemon*), mint (*Mentha piperita*), juniper (*Juniperus communis*) and rosemary (*Rosmarinus officinalis*): *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus* and *Penicillium hirsutum*. Essential oils used are from Fares Orastie company.

Culture media

These strains were cultivated onto potato-dextrose agar (PDA), at 30 °C, for 7-9 days to complete sporulation. The growth rate was assigned by measurement of diameters of colonies in Petri dishes, on PDA at 30 °C.

Antimicrobial vapor assay

The base of the Petri dish (1) containing culture medium was inoculated with molds (a) 2-20 µL of the essential oil (b) was placed on the cover of the Petri dish (2).

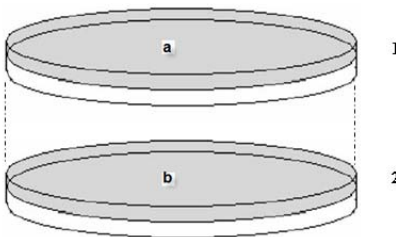


Fig. 1 Schematic of the antimicrobial vapor assay

Radial growth rate and inhibition ratio

In order to estimate the radial growth rate of strains the maximum diameter of colonies was measured after 216-528 hours and the ratio diameter/time was calculated.

The inhibition ratio was estimated using the formula (1) [12]:

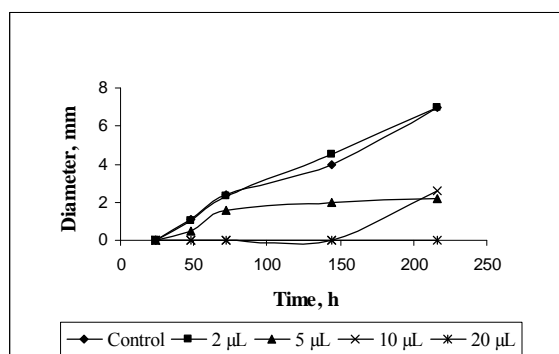
$$\text{Inhibition ratio (\%)} = \frac{C - E}{C} \cdot 100 \quad (1)$$

Where C is the diameter of mold colony from control plate and E is the diameter of the mold colony growth in experiment plate which contains the essential oil.

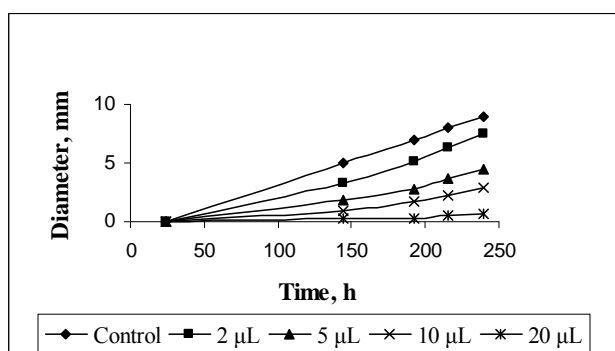
3. Results and discussions

The aim of this investigation was to determine antimicrobial activity of four Essential oils against *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus* and *Penicillium hirsutum* as food contaminant molds.

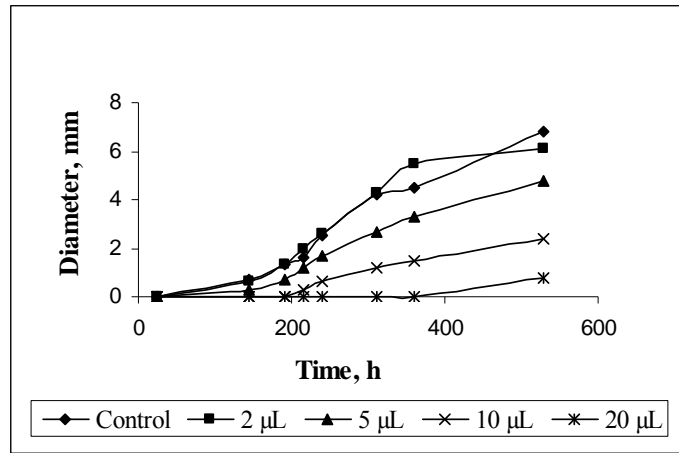
The diameter of fungal colonies was measured to draw the growth curve for each mold in control plates and in the presence of Essential oils.



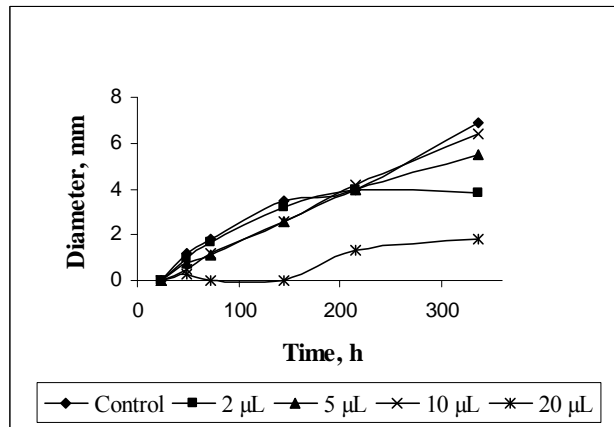
a)



b)



c)



d)

Fig. 2 Growth curve in presence of mint oil of: a) of *A. niger*; b) *F. oxysporum*; c) *M. purpureus*;

d) *P. hirsutum*

As it can be seen in the above figures maximum antifungal activity was exhibited by the oils of mint. For this oil, in all cases is observed a lag phase of few days and the colonies have the smallest diameters.

The inhibition ratio shows a strong action of mint oil against the four tested fungal strains, with values higher than 70 % in all plates with 20 microliters of added oil.

In all cases it has been shown that the mint essential oil presents the most powerful action against fungal colonies, so it has the highest inhibition ratio. The

juniper and rosemary oils have almost the same inhibition action and the lemon oil is the least efficient. For all the fungal strains the inhibition growth depends on the amount of oil applied to Petri lid, but the relationship is not linear.

Radial growth rate

The diameter of cultures was measured in control dishes and in the experimental plates containing the essential oil and there were calculated the average of growth rates (Tables 1-4).

Table 1

The average of growth rates for *Aspergillus niger*

<i>Aspergillus niger</i>	Concentration, μL				
	0	2	5	10	20
	Growth rate, mm/h				
lemon oil	0.032	0.031	0.030	0.025	0.022
mint oil	0.032	0.032	0.012	0.009	0
juniper oil	0.032	0.030	0.028	0.022	0.010
rosemary oil	0.032	0.032	0.032	0.018	0.016

Table 2

The average of growth rates for *Fusarium oxysporum*

<i>Fusarium oxysporum</i>	Concentration, μL				
	0	2	5	10	20
	Growth rate, mm/h				
lemon oil	0.037	0.034	0.038	0.032	0.031
mint oil	0.037	0.025	0.02	0.016	0.0045
juniper oil	0.037	0.033	0.035	0.027	0.026
rosemary oil	0.037	0.04	0.026	0.034	0.034

Table 3

The average of growth rates for *Monascus purpureus*

<i>Monascus purpureus</i>	Concentration, μL				
	0	2	5	10	20
	Growth rate, mm/h				
lemon oil	0.011	0.012	0.011	0.012	0.011
mint oil	0.011	0.013	0.009	0.004	0.001

juniper oil	0.011	0.011	0.012	0.012	0.012
rosemary oil	0.011	0.011	0.980	0.011	0.008

Table 4

The average of growth rates for *Penicillium hirsutum*

<i>Penicillium hirsutum</i>	Concentration, μL				
	0	2	5	10	20
	Growth rate, mm/h				
lemon oil	0.02	0.024	0.015	0.022	0.022
mint oil	0.02	0.011	0.016	0.019	0.005
juniper oil	0.02	0.009	0.018	0.011	0.01
rosemary oil	0.02	0.014	0.02	0.02	0.015

The mint, lemon, juniper and rosemary oils show different antifungal activities; the most effective against all tested strains was the mint oil. The diameters of treated colonies were smaller than the control, depending on the volume of oil added on the cover of Petri dish.

Morphological changes of fungal strains compared to control plate

Volatile oils show a significant effect on both growth rate and morphological structure of fungi. Several studies [13-15] have demonstrated the action of essential oils on plasma membrane whose structure and function are altered and the transport of nutrients is modified.

All Essential oils were found to inhibit the growth of fungal strains of *Aspergillus*, *Fusarium*, *Monascus* and *Penicillium* tested species in a dose-dependent manner.

The mint oil induces the most important changes in the macroscopic appearance of fungal colonies and the addition of 20 microliters dramatically inhibit the growth of mold and the colonies appear white and adherent to the medium surface.

For example, on microscopic slides of *Aspergillus niger* – control culture it can be seen a large number of black conidiospores in chains (fig. 3). The microscopic preparation of treated culture with volatile oil shows the reproductive hyphae with naked vesicles, the phialides and a reduced number of conidiospores (fig. 4).

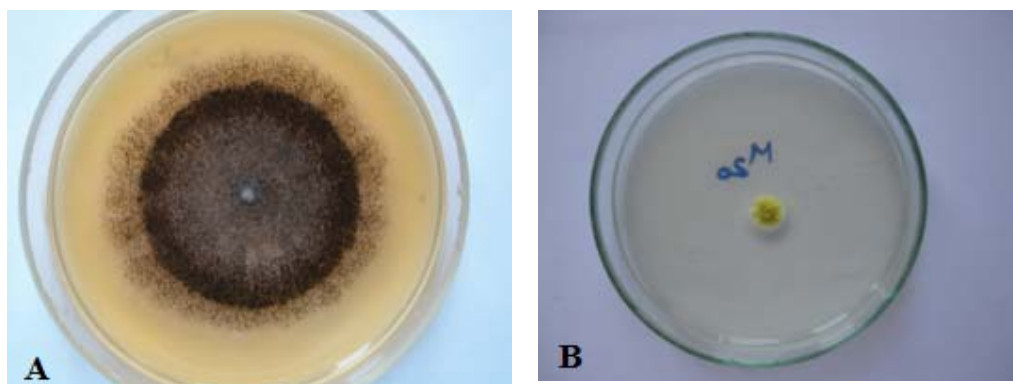


Fig. 3 The morphological aspect of *Aspergillus niger* strain grown on PDA medium for a) control and b) in Petri dish with 20 μ L mint oil

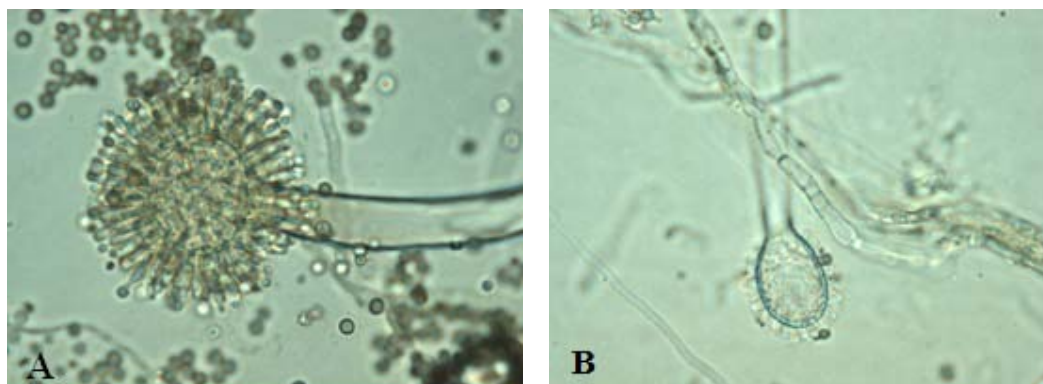


Fig. 4 The microscopic aspect of reproductive formations for *Aspergillus niger* a) control and b) in presence of 20 μ L mint oil (x 200)

Spongial production was also inhibited by the essential oils tested. Light microscopic observation on fungal hyphae exposed of volatile phase of oil revealed considerable morphological alterations in hyphae. Microscopic observation of fungal hyphae exposed to the essential mint oil vapour showed degenerative changes in the hyphal morphology compared to control. After exposure to contact or volatile phases, hyphae appeared degraded and large vesicles are also visible.

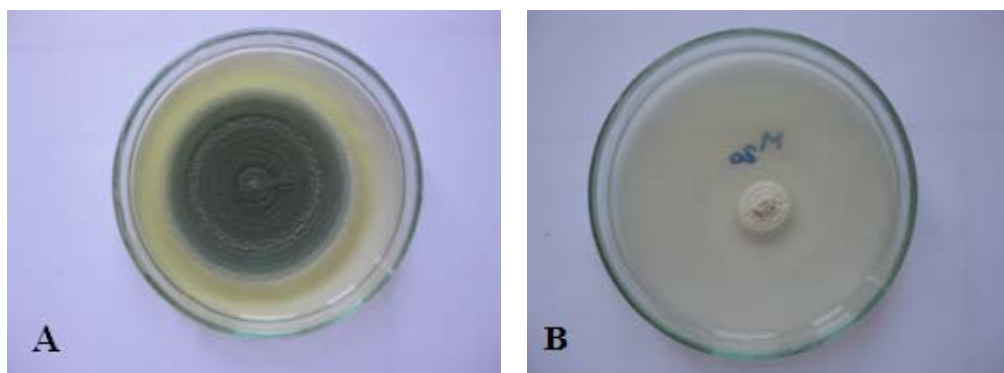


Fig. 5 The morphological aspect of *Penicillium hirsutum* strain grown on PDA medium for a) control and b) in Petri dish with 20 μ L mint oil

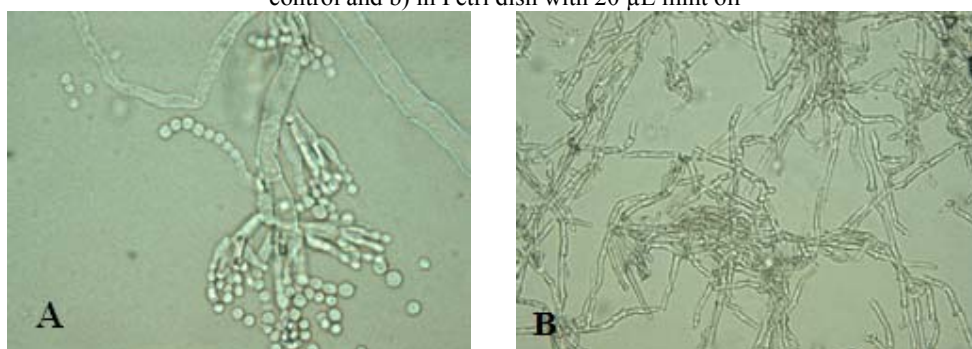


Fig. 6 The microscopic aspect of reproductive formations for *Penicillium hirsutum* a) control and b) in presence of 20 μ L mint oil (x 200)

To study the oil-induced changes, microscopic preparation were made from the fungal colonies and the mycelium appearance was observed. In most cases, the addition of volatile oil led to smaller colonies and partial loss of spore formation (see as colorless colonies).

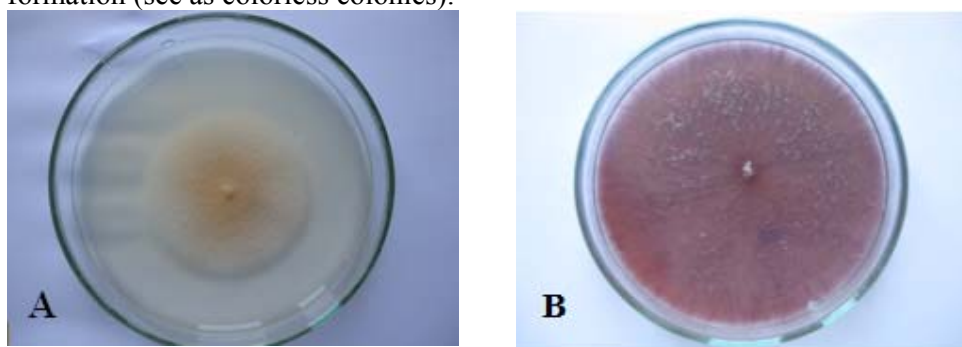


Fig. 7 The morphological aspect of *Fusarium oxysporum* strain grown on PDA medium for a) control and b) in Petri dish with 20 μ L mint oil

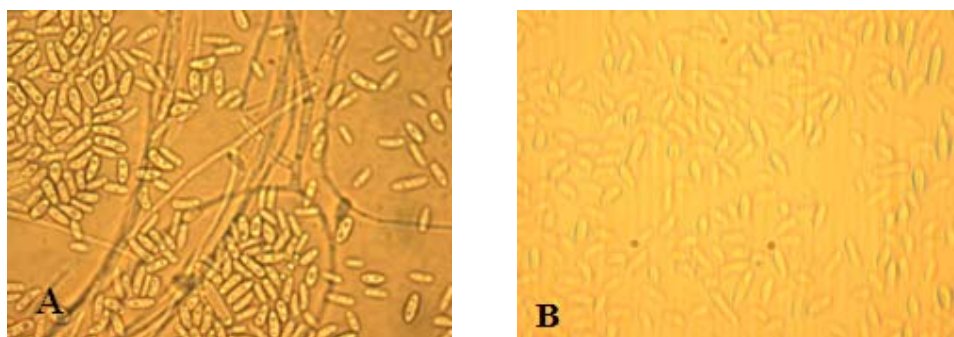


Fig. 8 The microscopic aspect of reproductive formations for *Fusarium oxysporum* a) control and b) in presence of 20 μ L mint oil (x 200)

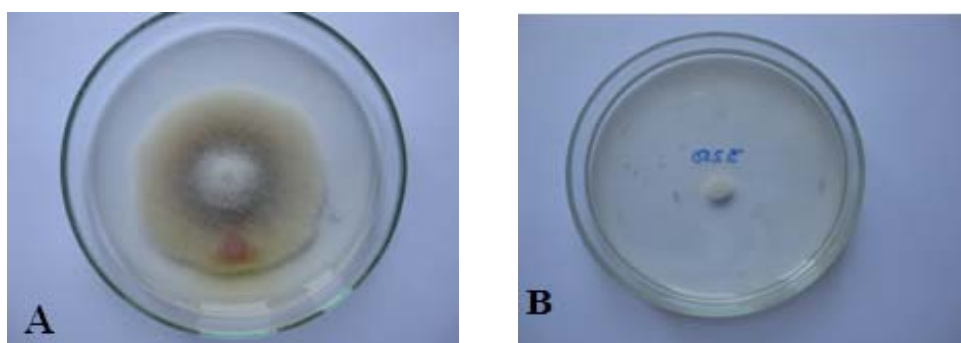


Fig. 9 The morphological aspect of *Monascus purpureus* strain grown on PDA medium for a) control and b) in Petri dish with 20 μ L mint oil

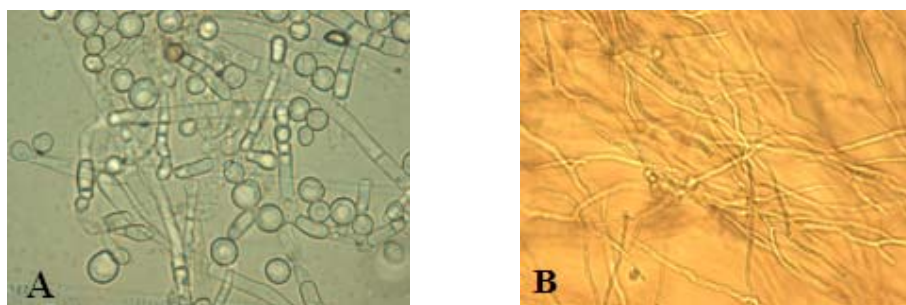


Fig. 10 The microscopic aspect of reproductive formations for *Monascus purpureus* a) control and b) in presence of 20 μ L mint oil (x 200)

The same changes were observed on microscopic preparations of *Penicillium hirsutum* (fig. 5-6) and *Monascus purpureus* (fig. 9-10). For the control culture it can be seen many spores in chains, that are more rare in the

cultures with mint oil. A reduced number of conidiospores was observed for *Fusarium oxysporum* culture in mint oil vapours (fig.7-8).

4. Conclusions

Volatile compounds from plants, especially Essential oils, have antimicrobial activity against a variety of food borne fungi. In this study, we tested the effect of essential oils on mycelial growth of *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium hirsutum* in vitro. We also investigated the effect of essential oils on hyphal morphology under light microscopy.

The results of this study confirm that Essential oils from aromatic plants such as lemon, mint, juniper and rosemary, possess antimicrobial activity.

The mint, lemon, juniper and rosemary oils show different antifungal activities; the most effective against all tested strains was the mint oil. The diameters of treated colonies were smaller than the control, depending on the volume of oil added on the cover of Petri dish.

Light microscopy of untreated food-borne fungi revealed normal mycelia; however hyphae of the strains grown on media with Essential oils revealed alterations in the morphology of the hyphae and in the sporulation process. The lipophilic properties of oil components might have also aided in the ability of the oil to penetrate the plasma membrane and alterate the morphology of this structure.

The volatile oils exhibited considerable inhibitory effects against all the microorganisms under test while their major components demonstrated various degrees of growth inhibition.

These inhibitory effects are interesting in connection with the prevention of mycotoxin contamination in many foods and they could be used instead of synthetic antifungal products.

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