

## ANTIFUNGAL ACTIVITY OF SOME ESSENTIAL OILS ON COTTON FABRICS

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Pathogenic microorganisms, bacteria and fungi such as *Trichophyton interdigitale* may easily develop inside footwear (cotton linings), causing “foot fungus” or “athlete’s foot”, a fungal infection that affects the skin, especially in the interdigital space and nails. Previous studies have shown that some essential oils have antibacterial and antifungal effect. The aim of the present study was to test antifungal activity of five essential oils (origanum, clove, orange, lavender and cinnamon) applied onto the cotton fabric used for shoe lining, with the purpose of preventing mycoses caused by *Trichophyton interdigitale*. The antifungal activities of the tested essential oils against *Trichophyton interdigitale* revealed that origanum and cinnamon oils, as well as the combination of origanum-clove-orange and clove-lavender-cinnamon completely inhibited the growth of *Trichophyton interdigitale* on cotton fabrics, with potential application in footwear production and/or exploitation.

Keywords: essential oil, cotton fabrics, *Trichophyton interdigitale*

### INTRODUCTION

The warm and humid environment inside the footwear facilitates the development of various harmful microorganisms that may degrade the material; therefore it is necessary to develop new products with low toxicity and environmentally friendly treatment of cotton used as shoe linings.

Pathogenic fungi that grow on human skin are *Trichophyton interdigitale*, *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum gypseum*. These fungi cause *tinea pedis*, condition also known as “foot fungus” or “athlete’s foot”. These fungi are located between the toes, on the soles or nails, causing onychomycosis. Foot fungus is highly contagious and can be spread easily by previously worn shoes of a person affected because the spores remain on the material it is lined with. Through the same mechanism “foot fungus” can relapse after being removed by treatment. A shoe lined with a material with both antifungal properties could prevent the spread of fungal infections, and reinfection of the wearer.

To date, treatments for disinfection of footwear based on ultraviolet (Ghannoum *et al.*, 2012) and ozone (Gupta *et al.*, 2013) have been successfully tested, but to prevent colonization bacteria and fungi lining, the application of substances is required which at the same time have a low toxicity for both the wearer and the environment. Other studies developed for incorporating antimicrobial technologies into leather compounds matrix to control and eradicate microorganisms associated with the surface of leather (Deselnicu *et al.*, 2005). Cotton fabrics exposed to oxidizing plasma and subsequently treated with silver nanoparticles and titanium dioxide showed antifungal effect (Surdu *et al.*, 2014; Chirila *et al.*, 2014).

Synthetic fungicides presently used are potentially harmful to both human health and for the environment and therefore, production and marketing is regulated and monitored continuously by European directives and regulations of the European Parliament and Council (Deselnicu, D.C. *et al.*, 2014; Deselnicu, V. *et al.*, 2014). There is no EU legislation in regulating specifically the content of chemicals in footwear and other leather products, but the use of chemicals is restricted by REACH (EC1907/2006).

## Antifungal Activity of some Essential Oils on Cotton Fabrics

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Essential oils extracted from plants may be an alternative to conventional fungicides. Antimicrobial activities of essential oils extracted from plants have been reported by: Nzeako *et al.*, 2006, showing antimicrobial activities of clove and thyme extracts; Chee and Lee (2007) showed antifungal activity of clove essential oil and its volatile vapour against Dermatophytic Fungi; Radwan *et al.* (2014) showed in a study regarding antifungal activity of thyme, clove and cinnamon essential oils on *Candida albicans* and moulds isolated from different sources that thyme oil completely inhibited the growth of different fungal isolates at concentrations of 0.25, 0.5 and 1%; Surdu *et al.* (2014) studied improvement of the resistance to *Candida albicans* and *Trichophyton interdigitale* of some woven fabrics based on cotton.

The aim of this work is to test antifungal activity against *Trichophyton interdigitale* of five essential oils - cinnamon, lavender, organum, orange and clove - on cotton fabrics used as lining in footwear production.

### MATERIALS AND METHODS

#### Materials

*Cotton fabrics* used as footwear lining; samples of 1.5/1.5 cm were used.

*Essential oil* isolated from cinnamon (*Cinnamomum verum*).

*Essential oil* isolated from lavender (*Lavandula angustifolia*).

*Essential oil* isolated from organum (*Origanum vulgare*).

*Essential oil* isolated from orange (*Citrus sinensis*).

*Essential oil* isolated from clove (*Eugenia caryophyllata*).

The essential oils were obtained by hydro distillation in a continuous extractor type Clevenger (Berechet, 2015).

*Biologic Material: Trichophyton interdigitale*

#### Methods

*Chemical composition of essential oils* was determined by gas chromatography-mass spectrometry (GC-MS) with Agilent 6890 N.

*Antifungal activity* against *Trichophyton interdigitale* was performed following Standard SR EN ISO 20645:2004 - Textile fabrics – Determination of antibacterial activity - Agar diffusion plate test.

Two series of experiments were made: a) with each essential oil, and b) with a combination of 3 essential oils (with a rate of 1:1:1).

Applying essential oils on cotton fabric samples was made by dropping 0.2 ml oil on the surface of 1.5 x 1.5 cm<sup>2</sup>.

In each Petri dish Dextrose Agar Sabouraud culture medium was poured. Both cotton samples treated with essential oils alone, with a combination of essential oils and untreated were placed in each Petri dish in the center of the surface of the culture medium, then the culture medium was seeded in 3 points around the sample, as an equilateral triangle. There have been two parallel samples. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analyzed after 3, 7, 14, 21 and 28 days.

*Optical microscopy images* were captured using a Leica stereomicroscope S8AP0 model with optic fiber cold light source, L2, with three levels of intensity, and magnification 40X.

## RESULTS AND DISCUSSION

### Composition of Essential Oils

*Essential oil of clove (Eugenia caryophyllata)*. The essential oil has 13 components and the major one is eugenol (96.999%).

*Essential oil of cinnamon (Cinnamomum verum)*. The essential oil has 30 components and the major one is cinnamaldehyde (84.125%).

*Essential oil of lavender (Lavandula angustifolia)*. Analysis allowed the identification of 32 components and the majors ones are geraniol (26.349%), camphor (23.193%) and eucalyptol (20.821%).

*Essential oil of origanum (Origanum vulgare)*. Analysis allowed the identification of 30 components and the majors ones are thymol (64.413%) and carvacrol (27.624%).

*Essential oil of orange (Citrus sinensis)*. The essential oil has 13 components and the major one is limonene (97.825%).

### Biological Activity

#### *Tests of Each Essential Oil*

Table 1 presents the images of antifungal activity of essential oils against *Trichophyton interdigitale* after 3, 7, 14, 21 and 28 days.

After 3 days a zone of inhibition of 2.5 cm appeared around samples treated with essential oil of clove. After 7 days colonies of *Trichophyton interdigitale* have appeared, keeping the inhibition zone of 2.5 cm. No inhibition zone diameter has changed by the end of the experiment.

The essential oil of lavender has not inhibited the growth, while orange essential oil has favored the growth of *Trichophyton* colonies.

Essential oils of origanum and cinnamon completely inhibited the growth of *Trichophyton* by the end of the experiment.

On the control sample, *Trichophyton interdigital* grew all over the culture medium after 3 days, after which no changes have occurred until the end of the experiment.

#### *Tests of Combinations of Three Essential Oils*

Four combinations of tree essential oils in the rate of 1:1:1 were tested, as follows:

- b1) origanum-clove-cinnamon;
- b2) origanum-clove-orange;
- b3) clove-lavender-cinnamon;
- b4) clove-lavender-orange.

Table 2 presents the images of antifungal activity of the fourth combination (b1-b4) of the essential oils against *Trichophyton interdigitale* after 3, 7, 14, 21 and 28 days.

The combination of essential oils b1) origanum-clove-cinnamon partially inhibited the growth of dermatophytes, with an inhibition zone of 1.5 cm.

The combination of essential oils b2) origanum-clove-orange and b3) clove-lavender-cinnamon completely inhibited the growth of dermatophytes

The combination of essential oils b4) clove-lavender-orange has a strong inhibiting effect on the growth of *Trichophyton* with an inhibition zone of 1 cm.

## Antifungal Activity of some Essential Oils on Cotton Fabrics

Combinations of essential oils b2 and b3 were the most effective in controlling *Trichophyton interdigitale*, and can be used to treat cotton lining or other footwear components to prevent transmission of mycoses.

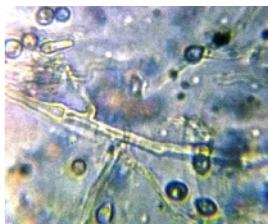


Figure 1. *Trichophyton interdigitale* (image 40x with Leica stereomicroscope S8AP0)

Table 1. Antifungal activity of essential oils against *Trichophyton interdigitale* on cotton fabrics

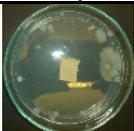
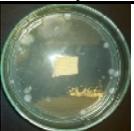
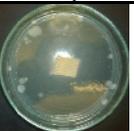
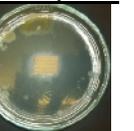
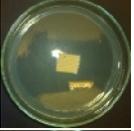
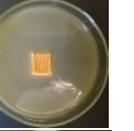
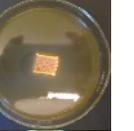
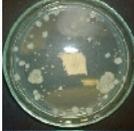
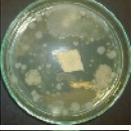
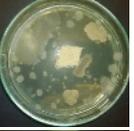
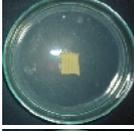
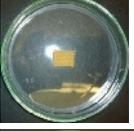
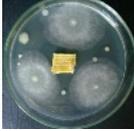
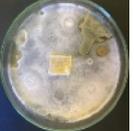
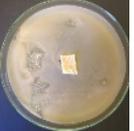
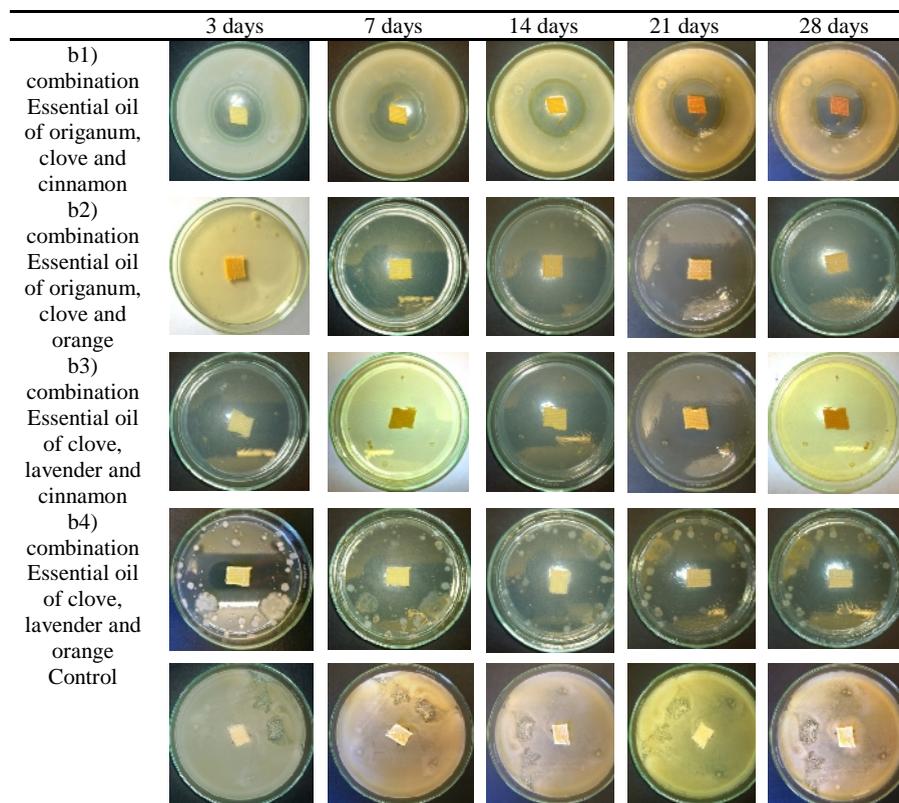
	3 days	7 days	14 days	21 days	28 days
Essential oil of clove					
Essential oil of cinnamon					
Essential oil of lavender					
Essential oil of origanum					
Essential oil of orange					
Control					

Table 2. Antifungal activity of some combination of essential oils against *Trichophyton interdigitale* on cotton fabrics



## CONCLUSIONS

According to this study, among the tested essential oils, origanum and cinnamon have the highest long-term antifungal activity as they inhibited the growth of *Trichophyton interdigitale* completely. Antifungal activity of essential oils is due to active components: thymol and carvacrol and cinnamaldehyde.

Certain combinations of the oils led to a synergistic effect, which is interesting in view of potentiating their inhibition of *Trichophyton interdigitale* colonization and infectiousness.

The results of this study may have potential for use in the development of applications in footwear component sanitation.

### Acknowledgements

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