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Comparative sporicidal effects of volatile oils

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Abstract

In this paper, ten concentrated essential oils (juniper, common thyme, pine, peppermint, silver fir, eucalyptus, fennel, tarragon, caraway and wild thyme) were investigated for their antifungal activity against two mould types (*Aureobasidium sp.* and *Alternaria sp*.) isolated from masonry. The action of essential oils was investigated using the antibiogram method. Results indicated that the antifungal activity of the essential oils is different. The action of wild thyme, common thyme and fennel essential oil appeared the most interesting, with strong fungicidal effect on both moulds tested, followed by tarragon, caraway and eucalyptus. Pine has different action, depending on the mould type. Peppermint, juniper and silver fir seem to have very small or no inhibitory action on moulds isolated from stone. Some volatile oils induce modifications in moulds pigmentation; in the presence of tarragon and caraway (for one mould type), or thyme and peppermint (for the other mould type), no coloration of the colonies appears.

Keywords: Sporicidal, essential oils, antifungal activity,

1. Introduction

A major concern of the specialists today is the moulds growth on surfaces and structures[1]. Problems associated with fungal growth on structures refer to the aesthetic deterioration and potential degradation of the material[2]. Moulds as *Alternaria, Aspergillus* and *Penicillium*, fungi with large dispersal produce pigments giving the black or grey coloration of outdoors or *Cladosporium* and *Penicillium*, identified as frequent indoors [3]. Various workers [4-6] consider that the microscopic fungi cause serious health problems.

In order to prevent the moulds spore growth, treatment of surfaces with sporicidal products is often realised[7,8]. The known biocides have different compositions[9]. The interaction of biocides with microorganisms is various[10]. For example, Bronopol and the ammonium quaternary salts action at the cell membrane level or with proteins[11] [12].

A modern solution is to use the essential oils as biocides. Recent studies showed the inhibitory action of thyme or geranium oil on *Aspergillus niger*, *Trichoderma viridae* and *Penicillium chrysogenum*[13].

Preliminary studies of the authors [14] showed that the antifungal activity of the essential oils is different, depending on the mould type and on the essential oil used. On *Penicilliium sp*. and *Alternaria sp*. isolated from stone, the action of wild thyme, common thyme and fennel essential oil appeared the most interesting, with strong fungicidal effect on both moulds tested, followed by tarragon, caraway and eucalyptus [15].

In this paper, the antifungal effect of ten volatile oils extracted from plants on two fungi is investigated. The oils used are obtained from juniper, common thyme, pine, peppermint, silver fir, eucalyptus, fennel, tarragon, caraway and wild thyme. The volatile oils are tested on superior moulds isolated from masonry. The

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objective of this study is to evaluate the sporicidal effect of the volatile oils on the moulds analysed, in order to incorporate them in some industrial biocides.

2. Materials and Method

Volatile oils were obtained from dried leaves, flowers and aerial part of ten plants by steam distillation for five hours. The plants used were: Juniper (*Juniperus communis*), common thyme (*Thymus vulgaris*), pine (*Pinus sylvestris*), peppermint (*Mentha piperita*), silver fir (*Abies alba*), eucalyptus (*Eucalyptus spp*.), fennel (*Foeniculul vulgare*), tarragon (*Artemisia dracunculus*), caraway (*Carum carvi*) and wild thyme (*Thimus serpyllum*).

The antifungal activity of the essential oils was evaluated on two moulds isolated from masonry in Sibiu, Romania. After isolation on Czapek-Dox substrate, the moulds were identified by colonial analysis and optical microscopy as *Aureobasidium sp*. and *Alternaria sp*. More investigations are necessary to identify the two species isolated. In this paper, the two moulds are abbreviated as SE3 (*Aureobasidium sp*.) and SN2 (*Alternaria sp*.).

The action of essential oils was tested on mould spores. For the obtaining of spores, pure cultures from each mould type were cultivated on malt broth for 10 days. Spores were harvested from the aerial central part of mycelia and poured in sterile water. 1 ml of the aqueous suspension obtained was distributed in Petri dishes and Czapek-Dox cultivation medium was poured over the spores. For the investigation of the action of essential oils on spores, Petri dishes immediately after the addition of spores suspension were used.

The essential oils antifungal activity was measured using the Kirby-Bauer method [16] usually used to determine the antibacterial response of different antibiotics. Small round paper discs were impregnated with the non-diluted volatile oils and distributed directly on the Petri dishes cultivated with spores. The inhibition zone of each essential oil was appreciated.

3. Results and Discussion

Some images of the action of essential oils on spores grown in Petri dishes are presented in Figures 1 and 2. The inhibition areas are clear observed on colonies formed by spores.

As observed in Figures 1 and 2, pigmentation disappear ad the use of some volatile oils. It is the case of tarragon and peppermint for SE2 and thyme, peppermint, fennel, caraway and wild thyme for SN2. Similar results were obtained with other two fungi tested [15].

This action could be very useful for the use of volatile oils in biocidal compounds.

The antifungal efficiency of essential oils tested is expressed as the inhibition zone around each paper disc. The measured zones are presented in Table 1.

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Table 1. Screening of the biocidal action of volatile oils on two mould isolated from stone

Volatile oil	**Biocidal action of the volatile oil on SE3**	**Biocidal action of the volatile oil on SN2**
juniper	+	0
common thyme	++++	++
pine	+	++
peppermint	++	++
silver fir	0	+
eucalyptus	++	+
fennel	+++	+
tarragon	++	+
caraway	++	+
wild thyme	+++	++

0 : no action; + : low biocidal action; ++ : middle biocidal action; +++ : high biocidal action; ++++ : very high biocidal action.

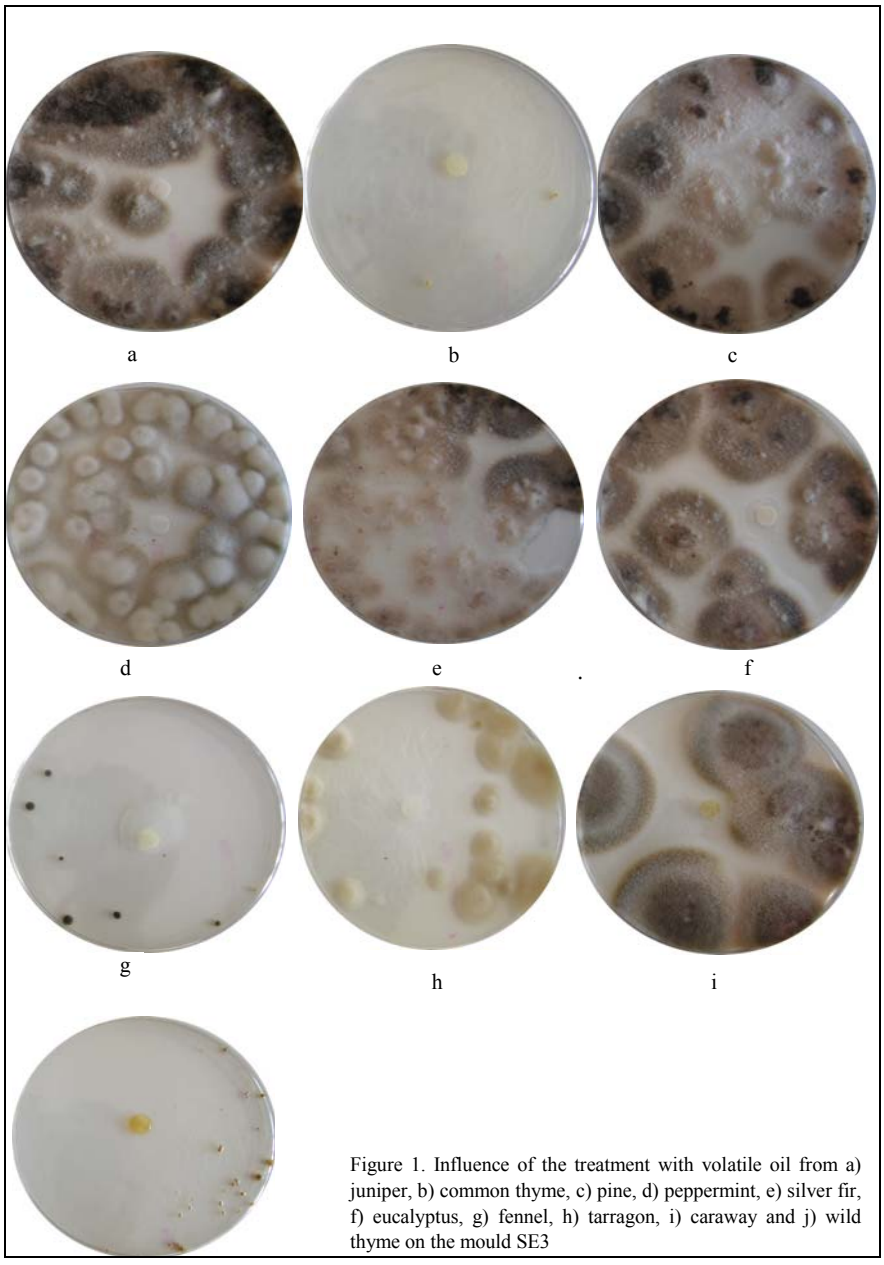

Figure 1. Influence of the treatment with volatile oil from a) juniper, b) common thyme, c) pine, d) peppermint, e) silver fir, f) eucalyptus, g) fennel, h) tarragon, i) caraway and j) wild thyme on the mould SE3

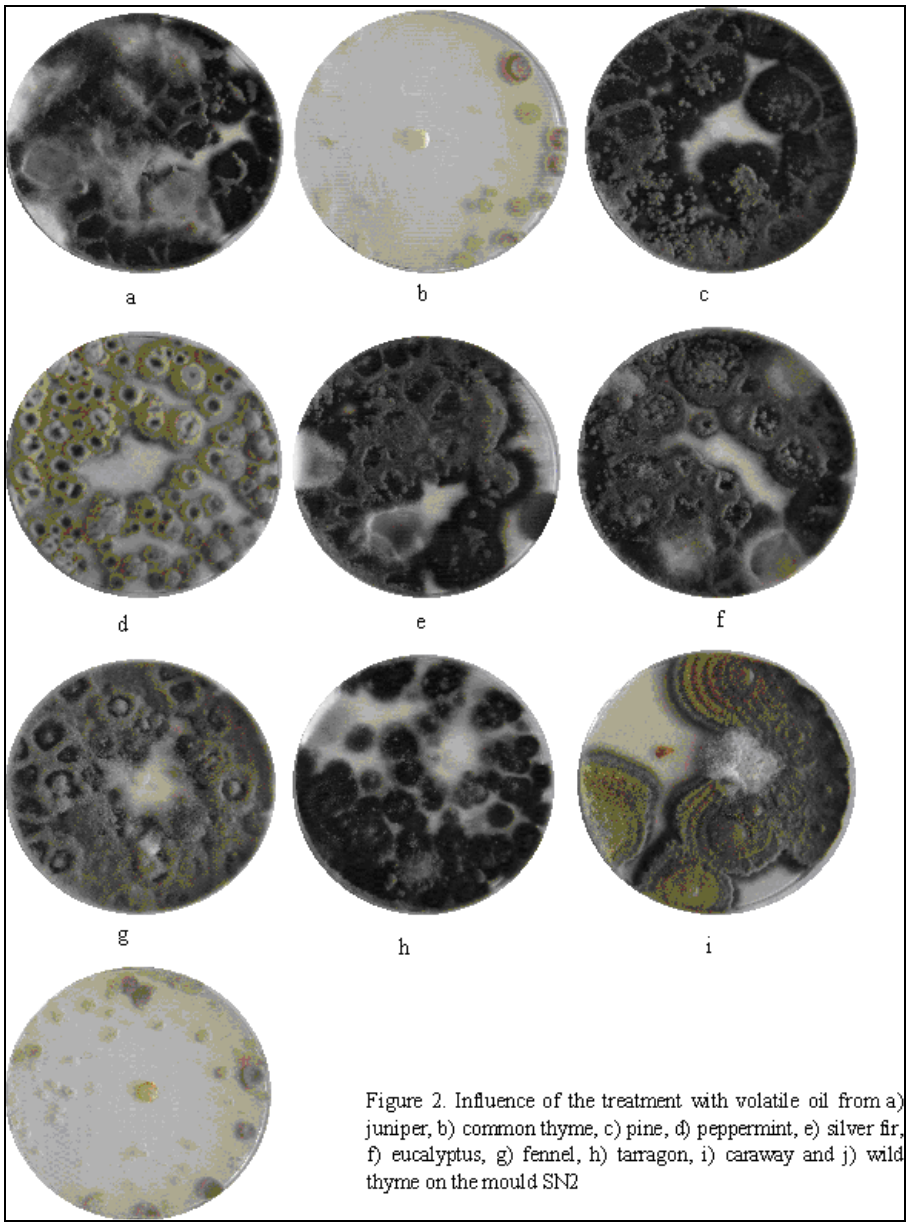

Figure 2. Influence of the treatment with volatile oil from a) juniper, b) common thyme, c) pine, d) peppermint, e) silver fir, f) eucalyptus, g) fennel, h) tarragon, i) caraway and j) wild thyme on the mould SN2

4. Conclusion

The For this research, ten essential oils – juniper, common thyme, pine, peppermint, silver fir, eucalyptus, fennel, tarragon, caraway and wild thyme – were extracted from plants.

Two moulds were isolated from masonry and were used to test the sporicidal activity of the essential oils. The results indicated that the antifungal activity of the volatile oils is different, depending on the mould type and on the oil used.

The actions of common and wild thyme oil, together with fennel volatile oil are the most powerful, with good fungicidal effect on both mould tested, followed by peppermint, pine, eucalypt, tarragon and caraway. Silver fir and juniper seem to have no action or very low influence on mould spores.

These results are in high accordance with previous researches on fungi isolated from stone [15]. As a final conclusion of this research, the uses of thyme and/or wild thyme oil are recommended to be used as sporicidal agents in biocidal formulations.

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