ACTIVITY OF SOME ESSENTIAL OILS AGAINST COMMON SPOILAGE FUNGI OF BUILDINGS

— research paper —

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Abstract: In this paper, six concentrated essential oils (common thyme, wild thyme, juniper, pine, silver fir and fennel) were extracted and characterised using GC-MS. Then, they were investigated for their antifungal action against four mould types isolated from surfaces belongings to the genera *Penicillium*, *Alternaria* and *Aureobasidium*. The antifungal activity of essential oils was investigated using the antibiogram method. Results indicated that the action of the essential oils is different due to their composition and is depending on the mould type. The action of wild thyme, common thyme and fennel essential oil appeared the most interesting, with strong fungicidal effect on all mould spores tested, most probably due to their composition in alcohols like borneol, cineol, tymol, cimol, carvacrol, α-terpineol in thyme, tymol and carvacrol in wild thyme and anetol and estragol in fennel. Pine has lesser antifungal activity, depending on the mould type; *Penicillium* sp. and *Aureobasidium sp.* are relatively resistant to this volatile oil, whereas *Alternaria* species are more susceptible. Juniper and silver fir seem to have very small or no inhibitory action on all moulds tested.

Keywords: volatile oil, thyme, juniper, pine, silver fir, fennel, antifungal

INTRODUCTION

Specialists concerns on buildings are oriented on the moulds growth on surfaces and structures (Gutarowska and Zakowska, 2002). The principal problem is that microscopic fungi cause serious health problems, as asthma

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(Jovanovic et al., 2004) or infections (McCormick et al., 2010). Also, moulds as *Aspergillus* and *Penicillium* with large dispersal produce mycotoxins (Pitt, 2000). They are responsible for the so-called ”sick building syndrome” (Straus, 2009).

Different solutions for the moulds development were found, as the treatment of surfaces with biocidal products (Lindner, 2004). The known biocides have disinfectant action, the result being dependent on their interaction with microorganisms; for example, Bronopol and the ammonium quaternary salts action at the cell membrane level or with proteins (Thomas et al., 2003) (Denyer and Stewart, 1998).

A modern orientation is the use the essential oils as biocides because the plant extracts are generally assumed to be more acceptable and less hazardous than the synthetic compounds (Magan and Aldred, 2007). A relatively large number of volatile oils extracted from plants show antifungal activity (Kalemba and Kunicka, 2003). For example, thyme or geranium oil showed inhibitory action of on *Aspergillus niger*, *Trichoderma viridae* and *Penicillium chrysogenum* (Yang and Clausen, 2007).

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 buildings. The 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.

This research has the goals to extract and characterise five volatile oils from the spontaneous Romanian flora and to realise a biological screening of the action of the volatile oils on four mould types isolated from two types of building materials: stone and bricks (masonry).

MATERIALS AND METHODS

Extraction and characterisation of the volatile oils

Volatile oils were obtained from dried leaves, flowers and aerial part of six plants. The plants used were: Juniper (*Juniperus communis*), pine (*Pinus sylvestris*), silver fir (*Abies alba*), fennel (*Foeniculum vulgare*), common thyme (*Thymus vulgaris*) and wild thyme *(Thimus serpyllum)*.

For obtaining and dosing of the volatile oil, the volatile oils were extracted through steaming for five hours using a modified neo-Clevenger equipment. The content was compared to the moisture less vegetal material.

The volatile oils were analyzed through by GC-MS. The analysis has been performed with a Hewlett Packard 5890 III gas chromatograph equipped with a mass detector MS 5972.
The chromatographic column used was a HP5-MS capillary column made of quartz, with a non-polar stationary phase consisting of 95% methyl and 5% phenyl polysilloxan. The constructive characteristics of the column are: length 30 m; inner diameter 0.25 mm; thickness of the stationary phase 0.25 μm. Helium was used as carrier gas (1:1 mL/min). The injection temperature was 60°C, incrementing with 3°C/min to 240°C.
The Chem. Information, Library Wiley 275 program was used to identify the components. They have been identified through the comparison of the mass spectrum with the one existing in the computer database.

Fungi
The antifungal activity of the essential oils was evaluated against four types of moulds isolated from two old buildings on Malt-agar. The moulds were identified using identification keys (Dan et al., 1999) as belonging to the genera *Penicillium*, *Alternaria* and *Aureobasidium*. Four of these moulds were used in this experiment: two species of *Alternaria (Alt1* and *Alt2*), *Penicillium sp.(Pen1)* and *Aureobasidium sp (Aur1)*.

Antifungal activity of volatile oil
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 spores addition suspension were used.
Antifungal activity of the essential oils was evaluated by the agar diffusion method (Boyle et al., 1973). The undissolved oil samples were not applied to sterile paper discs (8 μL of solution per Whatmann No. 1 disc of 5 mm diameter). The discs were placed on the surface at the centre of agar plates inoculated with sporal suspension (one mould type per Petri dish). The Petri dishes were then incubated at 28°C for 48 h, followed by the measurement of the diameters of zones of inhibition during two weeks. Inhibition of fungal growth was measured as zone diameters (mm) at 4-equidistant points taken from the centre of the inhibition zone, and the average value taken. The antifungal action was calculated with the formula:

*Antifungal action =diameter of inhibition zone/diameter of Petri dish * 100*

All experiments were carried out in triplicate and the reported data represents average values. Samples without volatile oils were used as a control.

RESULTS AND DISCUSSIONS

After GC-MS analysis, the compounds identified belong to four different classes:

- terpenic hydrocarbons (p-cimene, α-pinene, β-pinene, camfene, γ-terpinene, myrcene, limonene, carene, triciclene, fenchene, p-mentane, mentene, sabinene, terpinolene, β-felandrene);
- sesquiterpene (α-selinene, β-selinene);
- esters (bornyl acetate, linalyl acetate);
- alcohols (tymol, carvacrol, borneol, cineol, terpineol, anetol, estragol) (Table 1).

The antifungal efficiency of essential oils tested is presented in Table 1.

Table 1. Composition and screening of the biocidal action of six volatile oils on four moulds isolated from buildings

Botanical name	**Major compounds**	**Screening of the antifungal action**			
		Aur1 (brick)	*Alt1* (brick)	*Alt2* (stone)	*Pen1* (stone)
Thymus serpyllum	borneol, cineol, tymol, cimol, carvacrol, α-terpineol, β-cariofilene, myrcene, caffeic and rozmarinic acid, etc.	93	50	100	35
Thymus vulgaris	tymol, carvacrol, p-cimene, α-pinene, β-pinene, camfene, γ-terpinene, mircene, limonene, carene, triciclene, fenchene, p-mentane, mentene, sabinene, terpinolene, β-felandrene, bornyl acetate, linalyl acetat, nerol, citronellol, etc.	100	80	100	60
Juniperus communis	α-pinene, sabinene, p-cimene, γ-terpinene, α-terpinene, α-cubebene, β-carofilene, β-felandrene, citronellol, camfene, cadinene, junipene, Δ^3-carene, etc.	15	2	9	2
Pinus	α-pinene, β-pinene, α, β-	10	10	2	0

silvestris	felandrene, limonene, Δ^3-carene, bornyl acetate, etc.				
Abies alba	α-pinene, limonene, bornyl acetate, lauric aldehide, triciclene, α-tuyone, camfene, sabinene, β-mircene, β-felandrene, α-terpinene, p-cimene, terpinolene, camphor, citronellil acetate, humulene, etc.	4	3	0	0
Foeniculum vulgare	trans and cis-anetol, estragol	95	8	100	20

The composition of the six volatile oils obtained is very different. Both *Thymus* species and fennel oils contain especially alcohols, whereas the volatile compounds from juniper and fir are terpenic hydrocarbons.
The action on spores of the essential oil extracted from both thyme types and from fennel is the highest, most probably because they have alcohols as major compounds.
Pine oil shows low inhibitory effect on spores, whereas juniper oil and fir oil inhibit in lower measure the spores germination. As their composition indicates, they have preponderantly terpenic hydrocarbons, which are all slightly antiseptic with a lower antifungal action.

CONCLUSIONS

For this research, six essential oils – juniper, common thyme, pine, silver fir, fennel and wild thyme – were extracted from plants.
Two moulds were isolated from buildings 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 pine. Silver fir and juniper seem to have no action or very low influence on mould spores.
As a final conclusion of this research, the uses of thyme and/or wild thyme oil are recommended to be used as antifungal agents in biocidal formulations used on buildings surfaces.

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