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IN VITRO RESEARCH ON THE INHIBITORY EFFECTS OF FENNEL, SAGE AND SEABUCKTHORN ESSENTIAL OILS ON SOME FOOD SPOILAGE FUNGI

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Abstract

Essential oils from natural plants are antimicrobial agents that can be used to control food spoilage and pathogenic food; they have long been used as flavoring agents in beverages and food. The antimicrobial activity of essential oils is attributed to a number of small terpenoids and phenolic compounds that provide antifungal or antibacterial activity. The experimental research carried out in this work focus on the in vitro study of the antimicrobial effect of fennel and sage essential oils on two food spoilage molds with the evaluation of the minimal lethal concentration (MLC). The fungi used in experimental work were Aspergillus niger and Penicillum expansum from Faculty of Biotechnologies collection. Minimum lethal concentrations were determined using a modified disc diffusion method in agar after puncture the fungus in the center of the Petri dishes. Sage and fennel essential oils proved to be the strongest antifungic agents, the minimum volumes that inhibited the growth and development of two fungi ranging between 14 to 19 µL. Seabuckthorn essential oil, even in higher doses of 250-300 µL, did not show antifungal activity.

Key words*: sage oil, fennel oil, seabuckthorn oil, in vitro, modified microdiffusion method, in vitro antimicrobial activity.*

INTRODUCTION

Different chemical and synthetic chemicals have been used in the food industry as antimicrobials to prevent the development of food microorganisms, but the current trend in using preservatives has led to the use of essential oils. Essential oils (EO) have the potential to prevent the development of micro-organisms, which contribute to the degradation of food. Due to the composition of essential plant oils and their high antimicrobial extracts and spectrum, associated with their low toxicity, they are potential natural food preservatives (Conner, 1993).

The essential oil of *S. officinalis* comprises α- and β-t-butone monomers, camphor, 1,8-cineol and borneol, and sometimes higher amounts of sesquiterpene, α-humulin and β-cariopilin. Di- and triterpenes have been found in leaves (Máthé et al., 2007), i.e. manol. There is a high chemical variability among *S. officinalis* essential oils, however, it can be generally argued that the predominant α- and β-thujone constituents (Newall et al., 1996). *S. officinale* seed oil has antimicrobial properties, mainly attributed to the presence of tijons (Bradley, 2006; Newall et al., 1996).

Fennel (*Foeniculum vulgare* Mill*., Apiaceae*) is a well known aromatic plant species. Mature fruits and essential oil of fennel are used as flavoring agents in food products suchas liqueurs, bread, pickle, pastries and cheeses.

The essential oil of fennel are mainly concentrated in the fruits and provide the unique aroma and taste, they are composed of aroma of several monoterpenes and phenylpropanoids. Trans-anethole, often is the most prevalent constituent, counts for the anise taste,fenechone provides the bitterness and estragole (methyl-chavicol) the sweetness. According to some studies esssential oil of fennel and its seed extracts have been reported to have antimicrobial and anticonidic activity (Abed, 2007).

Seabuckthorn is used as a functional food ingredient - the beans are particularly rich in vitamin C and flavonoids. Both soft parts (pulp

and bark) and seeds contain oil and high levels of tocopherols and vegetal sterols (Kallio et al., 2002). Sea buckthorn oil is characterized by a unique fatty acid content compared to other vegetable oils.
This oil contains rare palmitooleic acid (omega-7) which is a component of skin lipids and stimulates regenerative processes in the epidermis and healing of wounds. Seabuckthorn oil contains saturated fatty acids in the form of palmitic acid (30-33%), stearic acid and has a wide range of essential unsaturated fatty acids (UFA), especially so-called PUFAs (polyunsaturated fatty acids); these include alpha-linolenic acid (omega-3), gamma-linolenic acid (omega-6), linoleic acid (omega-6), oleic acid (omega-9) and eicosanoic acid (omega-9).
The research have focused on the *in vitro* study of the antimicrobial effect of fennel, sage and seabuckthorn essential oils on two food spoilage molds (*Aspergillus niger, Penicillium expansum*) with the evaluation of the minimal lethal concentration (MLC).

MATERIALS AND METHODS

Antimicrobial agents and strains

Three types of essential oils from *Foeniculum vulgare*, *Salvia officinalis* and *Hippophae rhamnoides*, purchased from the company Hofigal (Bucharest, Romania), were used to carry out the experiments. Fungi *Penicillum expansum and Aspergillusniger* were provided from collection of Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania. The fungus cultures were prepared on PDA plate and incubated at 25°C for 7 days.

Antifungal assay

The antifungal activity of essential oils was determined by modified disc diffusion method on PDA and the medium was prepared according to the instructions on the package.
Subsequently, filter paper disks (6 mm Ø; Whatman) were placed on the surface of Petri dishes and impregnated with different quantities of essential oils from 3 µl to 20 µl (Figure 1).
In case of seabuckthorn essential oil was used higher doses of 250-300 µl.

Fig. 1. The distribution of Whatman filter paper disks (Φ = 6 mm) impregnated with EO in the Petri plates

Finally, 2 µl of spore suspension of each fungus culture (the initial concentration is 10^6 ufc/ml) was inoculated on culture medium in the center of the plate. All determinations were performed in three replicates and the results were statistical analysed. The negative control were performed with paper disks with ethanol, used for EO extraction. Also, the positive control were tested. The dishes were sealed with parafilm to prevent the evaporation of essential oils and incubated for 7 days at 25°C. The efficacy of the treatment was evaluated after 7 days of incubation by measuring the diameter of the inhibition zone.

Minimum lethal concentration (MLC) determination

Medium was inoculated with 2 µl spore suspension of strains *Aspergillus niger* or *Penicillum expansum* activated in PDA, to achieve a concentration of 10^6 spores/ml. Filter paper disks with different quantities of essential oils were placed on the surface of the medium. The control sample consisted of a plate with culture media without essential oils. All experiments were conducted in 3 replicates and results were statistical analysed. The plates were incubated for 7 days at 25°C. The colony diameters of fungal species used in treatment and control samples were measured.
The minimum lethal concentration (MLC) was defined as lowest concentration inhibiting visible growth of the tested fungi as the samples were measured.

RESULTS AND DISCUSSIONS

Antifungal activity of fennel, sage and seabuckthorn essential oils was determined against two antifungal strains *Aspergillus niger* and *Penicillium expansum*.

The obtained results showed that the diameter of the colony growth is significantly depended by the dose of EO and the fungal species tested (Figures 2 and 3).

The rate of fungal inhibition is directly proportional to the concentration of tested EO. Result showed that sage and fennel oils have antifungal activity at 19 µl (MLC), after 7 days at a temperature of 25°C, against *Apergillus niger*. In the same conditions, against the development of *Penicillum expansum* the minimum lethal concentration (MLC) was 14 µL for both fennel and sage EO. Following the experimental results, seabuckthorn oil has no antifungal activity, at the same condition of incubation, microorganisms growing normally even at rates of 250-300 µl.

For *Aspergillus niger* at lower concentration (3 µl) fennel EO is stronger than sage EO, as the colony diameter (cm) decreased from 7,3 cm for control group to 5,4 cm, while for sage EO decreased to 6,4 cm.

Also, we noticed that the 50% decrease of colony diameter compared to the control group was generated by 9 µl fennel EO for *P. expansum* and 6-7 µl for *A. niger*.

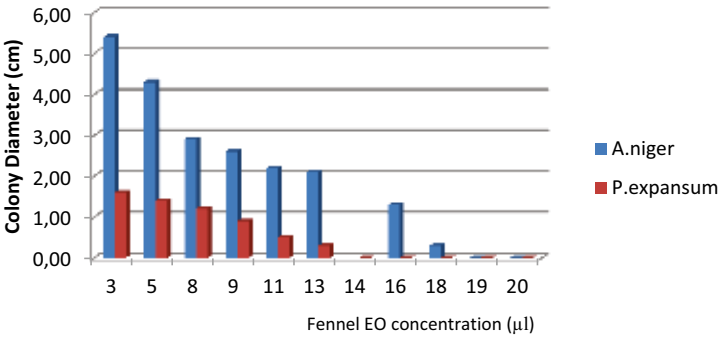

Figure 2. *Aspergillus niger* and *Penicillum expansum* average colony diameter (cm) after 7 days of incubation in PDA medium exposed fennel essential oils (EO)

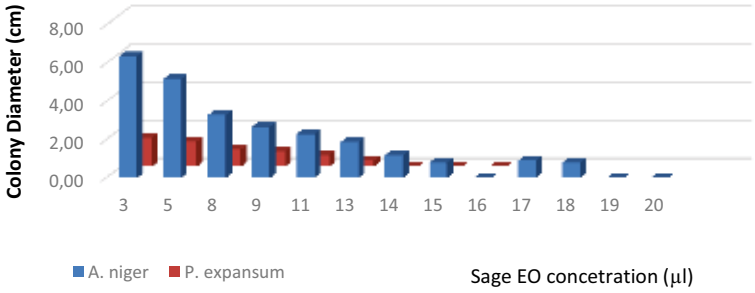

Figure 3. *Aspergillus niger* and *Penicillum expansum* average colony diameter (cm) after 7 days of incubation in PDA medium exposed sage essential oils (EO)

The investigation of the antimicrobial activity of the tested natural essential oils showed that only sage and fennel EO have antifungal effects while seabuckthorn EO even in higher doses do not has antifungal activity (Table 1).

Table 1. *Aspergillus niger* and *Penicillum expansum* colony diameter (cm) after 7 days of incubation in PDA medium exposed seabuckthorn essential oil

Essential oil	Fungal strains	Control group Colony diameter cm/day	Concentration essential oils (µl)	Colony Diameter (cm)/ day 7	Standard deviation (SD)
Seabuckthorn	*Aspergillus niger*	7.5	250	7.2	± 0.51
			300	6.7	± 0.15
	Penicillum expansum	3.5	250	3.4	± 0.12
			300	3.1	± 0.06

CONCLUSIONS

The research showed that two types of essential oils (fennel and sage EO) inhibited the growth of the tested fungi, while in the presence of seabuckthorn oil fungi developed normally and even in high doses 250-300 µl seabuckthorn EO has not antifungal effects. Also, the results of this study showed that the tested oils, sage and respectively fennel EO, have a different pattern of action in their antifungal activity. Both, sage and fennel essential oils, can be used as natural antifungal agents.

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