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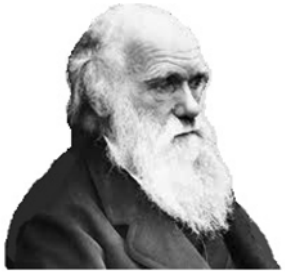

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Antifungal activity of PLA foils covered with ethylocelulose containing essential oils

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ABSTRACT

The purpose of the research work was to examine the antifungal properties of cumin, rosemary and fennel essential oils. The goal of the study was also to determine antifungal activity of coatings containing the essential oils. The results of the study demonstrated that cumin, rosemary and fennel essential oils exhibited antifungal properties. The most sensitive strains were *Botrytis cinerea* and *Mucor circinelloides.* The cumin oil was found to be the most active. The growth of *Botrytis cinerea* and *Mucor circinelloides* was not observed even on medium containing 0.1% of cumin oil. The growth of *Aspergillus niger, Aspergillus clavatus* and *Rhizopus oryzae* was not noticed on mediums containig 0.5% of the oil. The similar results were obtained for fennel essential oil. 0.5% concentration of the oil in medium inhibited the growth of *Botrytis cinerea, Mucor circinelloides, Aspergillus clavatus, Rhizopus oryzae*. The rosemary oil did not inhibited the growth of fungi strains in concentration of 0,1%.

***Keywords*:** antifungal properties, essential oils, active coatings

1. INTRODUCTION

Plants are abundant source of natural products, down the ages they have been used for obtaining a large number of acitve compounds with medicinal properties. Many plants contain extensive variety of phytochemical compounds that shown antibacterial, antiviral, antifungal, antioxidant activity and are used in green-biosynthesis of active chemicals [1-5]. One of the

group of active compounds from plants are essential oils. An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils have been used medicinally throughout history. Many plants are rich source of essential oils [2-5].

Modification of packaging materials by coating with antimicrobial agents is one of the way to create „active packaging" [6]. The development of natural antimicrobial coatings, leading to active packaging, may be uncompromising achievment, significantly affecting the quality of the packaged goods [7]. The active compunds should inhibit the growth of undesireable microflora responsible for spoilage of material inside the packaging. Bacteria nad fungi are considered as a main spoilage causing factors. Currently, many natural compounds are used to modify the packaging materials such as products of bacterial and fungal metabolism, plants and spices extracts, organic acids, essential oils and enzymes [6-9].

2. EXPERIMENTAL

2. 1. Material

The test microorganisms used in this study were obtained from a Czech Collection of Microorganisms (CCM). The strains used in this study were: *Aspergillus clavatus* CCM F-660, *Aspergillus niger* CCM 8189, *Botrytis cinerea* CCM F-16, *Mucor circinelloides* CCM 8328 and *Rhizopus oryzae* CCM 8076.

There were used: Sabouraud broth and Sabouraud agar (Merck KGaA), Tween 20 (Sigma), ethylcellulose (Sigma), ethanol (Sigma), cumin essential oil (Vera-Nord), rosemary essential oil (Sabana) and fennel essential oil (Vera-Nord). The media were prepared according to Merck protocol.

2. 2. Methods

An examination of the Minimal Inhibition Concentration (MIC) of the essential oils was carried out using Sabouraud agar and Sabouraud broth media. In first step of the experiments, 0,1%; 0,2%; 0,3%; 0,4%; 0,5% and 1% essential oils (each oil separately) and 20 drops of Tween 20 were added into each medium. As next step, fungal cells of *A. niger*, *A. clavatus*, *B. cinerea*, *S. chartatum*, *M. circinelloides* and *R. oryzae* were pre-grown in Sabouraud broth for 48 h at 25 °C. After incubation, the biomass was homogenized and plated on Petri dishes of Sabouraud agar containing essential oils or devoid of active substances. The Petri dishes were wrapped with parafilm and incubated at 25 °C for 48 h. After incubation, the growth of fungal colonies diameter was observed.

10 g of ethylocelulose was introduced into 100 mL of 96% ethanol. The mixture was mixed 1 h using magnetic stirrer (Ika) with 1500 rpm and used to cover the PLA films to obtain coatings devoid of active substance. 5 g of essential oil (each oil separately) was added to 100 ml of 10% ethylocelulose in ethanol and mixed 1 h using stirrer (Ika) with 1500 rpm. The mixtures were used to obtain the coatings containing essential oils as active agents.

PLA films were covered using Unicoater 409 (Erichsen) with a roller with diameter of 40 μm. The coatings were dried 10 min in temperature of 50 °C. The PLA films that were not covered served as control samples. The PLA films with ethylocelulose coatings were control samples as well. The film samples were cut into square shape (3×3 cm).

A determination of the antifungal properties of the coated films was carried out using Sabouraud agar and Sabouraud broth mediums. The method used to test antimicrobial

susceptibility was based on diffusion method. In first step of the experiments, fungal cells of *A. niger*, *A. clavatus*, *B. cinerea*, *S. chartatum*, *M. circinelloides* and *R. oryzae* were pre-grown in Sabouraud bulion for 48 h at 25 °C. After incubation, the biomass was homogenized and plated on Petri dishes with Sabouraud agar. The samples were then incubated for 2 h at 4 °C. After the incubation the films samples were put onto medium. The Petri dishes were wrapped with parafilm and incubated at 25 °C for 48 h. After incubation the presence of inhibition zones was checked.

3. RESULTS

The results of the study demonstrated that cumin, rosemary and fennel essential oils exhibited antifungal properties. The most sensitive strains were *Botrytis cinerea* and *Mucor circinelloides*. The cumin oil was found to be the most active. The growth of *Botrytis cinerea* and *Mucor circinelloides* was not observed even on medium containing 0.1% of cumin oil. The growth of *Aspergillus niger* [Fig. 1], *Aspergillus clavatus* and *Rhizopus oryzae* was not noticed on mediums containig 0.5% of the oil (Table 1).

The similar results were obtained for fennel essential oil. 0.5% concentration of the oil in medium inhibited the growth of *Botrytis cinerea*, *Mucor circinelloides*, *Aspergillus clavatus*, *Rhizopus oryzae* (Table 1). The rosemary oil did not inhibited the growth of fungi strains in concentration of 0,1%.

Table 1. The influence of essential oils on growth of fungi strains.

		Strain				
Oil	**Concentration [%]**	*B. cinerea*	*M. circinelloides*	*A. niger*	*A. clavatus*	*R. oryzae*
cumin	**0.1**	+++	+++	-	++	-
	0.2	+++	+++	-	++	-
	0.3	+++	+++	-	++	-
	0.4	+++	+++	-	++	-
	0.5	+++	+++	+++	+++	+++
fennel	**0.1**	++	-	-	-	-
	0.2	++	-	-	-	-
	0.3	++	-	-	-	-

	0.4	++	-	-	-	-
	0.5	+++	+++	+++	+++	+++
rosemary	**0.1**	-	-	-	-	-
	0.2	+	-	-	-	+
	0.3	+	+	-	+	+
	0.4	+	+	+	++	+
	0.5	++	++	++	++	+

The growth: +++: completely inhibited; ++: reduced significantly; +: reduced slightly; - : non-inhibited.

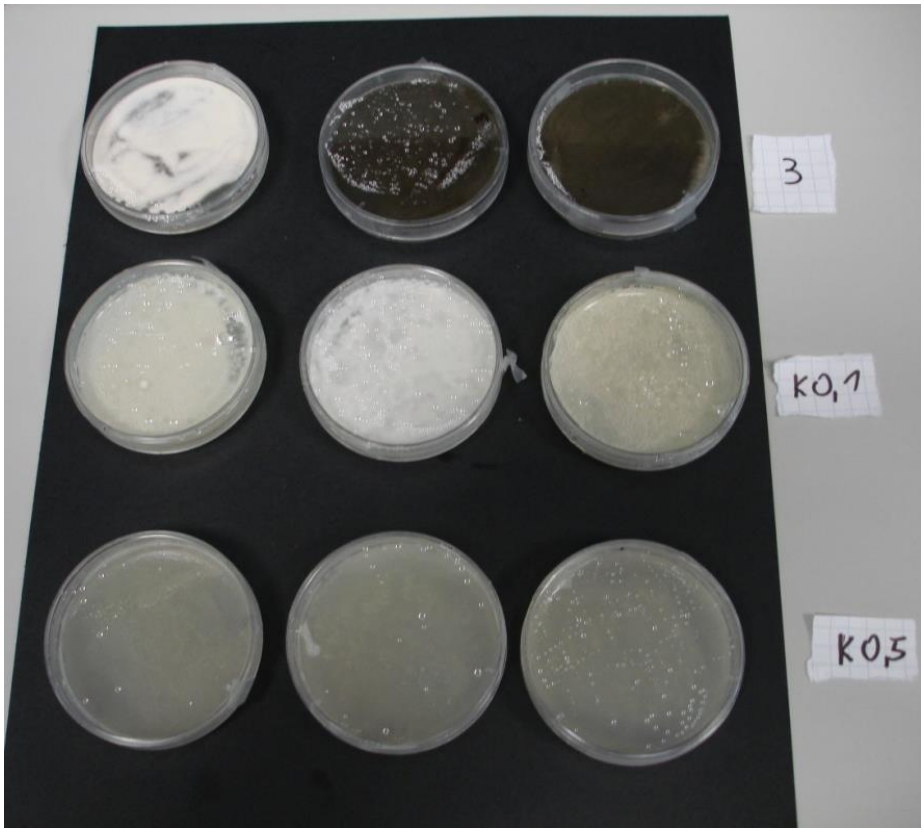

Fig. 1. The influence of 0.1% and 0.5% cumin oil on the growth of *Aspergillus niger*

The results of the study demonstrated that coatings containing cumin, fennel and rosemary oils were not active against *Botrytis cinerea*, *Mucor circinelloides*, *Aspergillus clavatus*, *Rhizopus oryzae* and *Aspergillus niger* strains because inhibition zones were not observed.

4. DISCUSSION AND CONCLUSIONS

The best results of the research work were obtained for cumin oil. Those results are consist with results of Uniyal et al. [10] who proved the antimicrobial properties of the cumin oil against *Aspergillus fumigatus* and *Aspergillus niger*. The authors confirmed also antifungal activity of fennel oil. Fennel oil is known from antifungal activity towards some species [12]. Bansod and Rai [11] confirmed that fennel oil can inhibit the growth of *Aspergillus niger*. The rosemary oil was also found as active against chosen microorganisms. Similat results were obtained by Sousa et al. [13] who confirmed that *Aspergillus niger* was sensitive against rosemary oil. Several authors modified polylactide foil by coatings with antimicrobial agents [6-7]. The ethylocelulose coatings containing essential oils were inactive against fungal strains. This results are contrarty to work of Kwiatkowski et al. [7] who found that polylactide films covered with ethylcellulose and essential oils shown antibacterial activity. It is tempting to suggest that propably some formulations of essential oils and ethylcellulose may be inactive towards some microorganisms and the influence of coating material on ability of incorporated antimicrobial agent activity is important factor. The use of active antimicrobial compounds incorporated in packaging material is now getting more attention. The other essential oils were found to be useful as additive to coatings. It ensures microbial food safety for consumer, and can be useful for the extension of shelf life of the products [14-17].

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