Studies on some essential oils using as fungicides during pickled pelts production

by

Eser EKE BAYRAMOĞLU , Gürbüz GÜLÜMSER

Ege University, Faculty of Engineering, Department of Leather Engineering,

35100 Bornova-İZMİR – TÜRKİYE

eser.eke@ege.edu.tr

and

İsmail KARABOZ

Ege University, Faculty of Sciences, Department of Biology, Basic and Industrial Microbiology Section, 35100 Bornova-İZMİR – TÜRKİYE

In this study the applicability of *Origanum minutiflorum* (oregano), *Laurus nobilis* (laurel leaf), *Foeniculum vulgare* (fennel), *Schinus molle* essential oils as fungicide versus fungus which grows on leather during pickling process was studied. In order to control the study, 0.04% 2-Thiocyano-methylthiobenzotiazole (TCMTB) and 0.02% N-octyl-isothiazolinone (N-OITZ) containing commercial fungicides were also used on pelt weight of leather.

During the microbiologic tests the growth of mold species that cause problems in leather industry like *Aspergillus niger, Alternaria alternata, Penicillium rubrum and Trichoderma viride* were investigated over against these essential oils and fungicides.

As a result of this study it was observed that the antifungal activity of oregano essential oil is the strongest and the effect got stronger with increasing concentration rates. When all experiments done were compared, it was found that the leathers treated with oregano essential oil were even more resistant against test molds than commercial fungicide containing leather samples.

Keywords: Pickled pelts, Essential oils, Fungi, Fungicides, Oregano

INTRODUCTION

Pickling has already been mentioned as a method of preservation and, where a full pickle has been given, the skin may be stored or kept in this state for some time before the next process.

The difference between the preservative and other pickling is the usage of salt in high amounts like 11-12 ºBe' and lowering pH values of pelts to pH 1,5-2. Some researchers noted that fungus growth is inhibited when highly acidic pickle baths are used, and therefore the application of fungicides is not necessary . However, in spite of this inhibition, acid hydrolyze occurs in leather under these conditions, and low acid use in pickled pelts is not recommended. pH level measuring between 1.4 and 1.6 in leathers inhibits fungus growth, yet hydrolyzes in leather proteins may cause pH increase, which provides a more ideal environment for the fungi. For this reason they recommended using fungicide if pelts will be stored more than three weeks.[1]

Currently, TCMTB (2-Thiocyanatomethylbenzthiazole), N-OITZ (N-octil-isothiazolinone), OPP (orto fenil fenol), PCMC (p-chloro-m-cresol), Carbendazim, Merkaptobenzothiazole, TCP (tri-chlorophenol), p-Nitrophenol etc. are active material of fungicides that are commonly used in leather industry. But these chemicals are generally harmfull to human health and nature.[2,3,4,5] A number of widely used fungicides may not be employed for leather in the future due to regulations or other restrictions. Some microorganisms can develop resistance to microbicides as a result of restrictions and the use of insufficient amount of biocide than necessary. [6,7] Thus, leather market necessitates new biocides harmless to human health and natural life.

Some plants possess essential oils which have anti-microbial activity. [8] The antimicrobial activity of essential oils and their derivatives was recognized long time ago. Ibn-i Sina was the first man, who invented the essential oils by steam distillation techniques and used (980-1037).[9] As essential oils and their components are natural products, they are becoming increasingly popular, and such antimicrobial agents are being used in many different sectors such as pharmacy, food industry, bakery, etc.

Currently many studies have been carried out to display the antimicrobial activity of essential oils. [10,11,12,13,14,15,16,17,18,19,20,21,22] In this study, completely natural products were used as fungicides in different percentages and it was compared with commercial fungicides.

The objective of this study was to determine whether the essential oils of *Origanum minutiflorum, Laurus nobilis* , *Foeniculum vulgare* and *Schinus molle* can be

used as alternative to fungicides in the pickling process, and to compare it with widely used commercial fungicides.

Materials and Methods

Materials

Raw skin

In this study, dry salted Turkish domestic sheep skins were used in order to obtain pickled pelts.

Fungicide

In the experiments, two different fungicides were used: one of them was N-octyl-isothiazolinone (N-OITZ), while the other one was 2-Thiocyano-methylthiobenzotiazole (TCMTB) based. The studies at lower rates have been conducted with fungicides. The reason for this was to compare the results with the commercially available materials, which are reported to have the same effect at the same dosage. The amounts indicated in the catalogues were used after the necessary contacts with the experts of the companies were made, and two different materials were selected for the study. A special attention has been paid in order to purchase the recently produced materials containing active material.

Molds

Species of mold used in this study are *Aspergillus niger, Alternaria alternata* (NRRL 10593), *Penicillium rubrum and Trichoderma viride*(NRRL 1608) .

Essential oils

Essential oils of *Origanum minutiflorum, Laurus nobilis* (laurel leaf), *Foeniculum vulgare* (fennel) and *Schinus molle* (fruit) were used. The chemical composition of the essential oils which were used in the experiments are given in tables.

Table 1- *Origanum minutiflorum*[23]

Constituents	***O.minutiflorum*** **Essential oil** **%**
carvacrol	78.8
γ -terpinen	3.7
p-cimen	3.5
β-cariophillen	1.9
α-pinene	1.3
myrsen/ α-phellandren	1.1
α-terpineol/ borneol	1.1
thymol	1.1

Table 2-Composition of the Essential oil of *Schinus molle* [24]

Constituents	***Schinus molle*** **Essential oil (fruit)** **%**
α- phellandrene	22.1-38.1
β- phellandrene	10.4-11.8
limonene	9.6-11.6
α- conidol	5.6-7.2

Table 3-Composition of the Essential oil of *Foeniculum vulgare*[25]

Constituents	**Essential oil %**
(E)- anethole	73.7
limonene	9.5
carvon	6.5
Methyl cavikol	3.2
anisaldehyde	1.7

Table 4-Composition of the Essential oil of *Laurus nobilis* [26]

Constituents	***Laurus nobilis* (leaf)** **Essential oil %**
1,8- cineole	56.7
α-terpineol / terpenil acetate	7.0
sabinene	6.8
α-pinene	4.9
β-pinene	3.6
terpinen-4-ol	2.5
p-cymene	1.6
trans-pinocarveol	1.4
γ-terpinene	1.0

Media

Malt Extract Agar (M.E.A)(Merk) was used in keeping stocked cultures and obtaining fresh cultures in the experiments.

Methods

Obtaining the Pickled Pelt Samples Used in the Experiment

Raw skins were processed so as to obtain pickled pelts. For all pickled pelts production, one standard garment leather processing method was applied.

The group of pickling pelts was obtained during preserved pickling process. The features of the pelts are as follows:

1- Preserved pickled pelt without fungicide (pH:1.5)

2-Preserved pickled pelt obtained by using N-octyl-isothiazolinone (N-OITZ)-based fungicide (0.02%, the amount advised by the chemical company)

3- Preserved pickled pelt obtained by using 2-Thiocyano-methylthiobenzotiazole (TCMTB) based fungicide (0.04% , the amount advised by the chemical company)

4- Preserved pickled pelt obtained by using *Origanum minutiflorum* essential oils (3 different dosages: 0.5%, 1% and 2%).

5- Preserved pickled pelt obtained by using *Laurus nobilis* essential oils (3 different dosages: 0.5%, 1% and 2%).

6- Preserved pickled pelt obtained by using *Foeniculum vulgare* essential oils (3 different dosages : 0.5%, 1% and 2%).

7- Preserved pickled pelt obtained by using *Schinus molle* essential oils (3 different dosages: 0.5%, 1% and 2%).

Activating Mold Species:

Mold species used in the experiments were horizontally prepared and separately incubated in the tubes with M.E.A ., and fresh cultures were obtained through incubation at 27 ^{o}C for a week.

Microbioiogical Test Standards:

ASTM D 4576-86 (Reapproved 1991) microbiological test method is used for mold growth resistance of wet blue and other leather samples. In this study, this test standard was appllied.[27] Specimens of the pelt were placed in petri-dishes on inoculated nutrient agar-medium and incubated for four weeks. Following this period, the specimens were detected and evaluated respectively in terms of the mold growth.

After 7, 10, 14, 17, 21, 24 and 28 days of incubation periods, the formation of an inhibition zone around the specimens were visually assessed using the following rating scale:

Growth	Assessment	Rating
Specimen with growth	Innadequate	1
Growth on edge of specimen	Inadequate [with note: growth on specimen in (mm)]	2
Growth on cut edges No inhibition zone, no growth on the sample	Still good (limit of efficacy)	2-3
Specimen free of growth; Visible inhibition zone	Good [with note: size of inhibition zone in (mm)]	3

Rating numbers are displayed in figures 1, 2, 3, 4.

Figure 1:***Trichoderma virid* e** –

Specimen covered with growth (1)

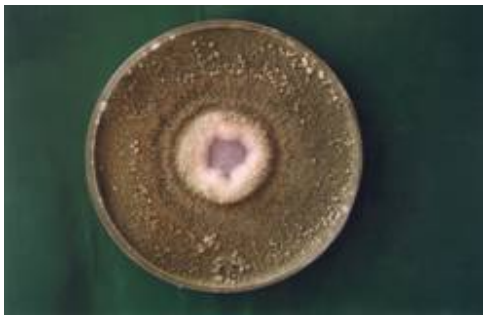

Figure 2:***Trichoderma viride*** –

Growth on edge of specimen (2)

Figure 3: ***Aspergillus niger*** – Growth on cut edges (2-3)

Figure 4: ***Aspergillus niger*** - Specimen free of growth; visible inhibition zone - (3)

Inhibition zone: Growth free zone around the specimen

RESULTS AND DISCUSSION

The results of experiments are reported in tables. During studies it was observed that *Origanum minutiflorum* essential oil had a stronger antifungal effect than the other essential oils and this effect improves with increasing concentrations . As clearly seen in the tables, 2 % of essential oil of *Origanum minutiflorum* was found to be the best antifungal effect even better than the effect of commercial fungicides, which are commonly used in quantities on the market. Even though these amounts were reported to be commercially appropriate, the results of the experiments revealed the contradictory especially for the use of *A. niger* and *T. viride*.

Even in the experiments carried out with *Penicillium rubrum* and *Alternaria alternata* test fungi, 1% of essential oil of *Origanum minutiflorum* had enough antifungal effect. Guynot et al. reported that essential oils of some oregano species have antifungal effect on *Penicillium* species, and this information supported the results of our study. [19]

Besides the fungus inoculated trials, it was determined that %2 of *Origanum minutiflorum* essential oil also had an effect on fungus which contaminated especially pickled pelts during the tests. The fungus which contaminates most during the tests is found to be *Aspergillus flavus* and it was observed that this fungus was effected, and no growth was seen the pelts which treated with %2 *Origanum minutiflorum* essential oil. This situation is shown in Table 9.

During the course of the experiments it was determined that the amounts of essential oils except *Origanum minutiflorum* essential oil, did not have adequate protective effect

against test fungus. However, when compared with the non-preservative containing pickled leathers it was observed that especially laurel leaf had an effect against *Alternaria Alternata.* On the other hand this effect was not adequate to protect the pelts during the test period .