ANTIFUNGAL ACTIVITY OF SELECTED ESSENTIAL OILS AGAINST THE FUNGAL SPECIES OF THE GENUS EUROTIUM BY CONTACT VAPOUR

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ABSTRACT

The aim of this study was evaluation of the antifungal activity of 5 essential oils (EOs), we used concretely thyme, clove, basil, jasmine and rosemary, by vapour contact against the fungal species Eurotium rubrum, E. chevalieri and Eurotium sp. Each fungus was inoculated in the centre on Czapek Yeast Autolysate Agar (CYA) plates. Plates were tightly sealed with parafilm and incubated for 7, 14, and 21 days at 25 ± 1 °C (three replicates were used for each treatment). Volatile phase effect of concentration 50 µl of the essential oils was found to inhibit on growth of E. rubrum, E. chevalieri and Eurotium sp. Complete growth inhibition of the isolates by EOs of thyme and clove was observed. The essential oil (EO) of basil had antifungal activity on growth of E. chevalieri only after 7th and 14th days of the incubation, but in case of Eurotium sp on all days of cultivation. Only E. rubrum was sensitive to jasmine EO and E. chevalieri to basil EO after all days of the incubation. Data was evaluated statistically by 95.0 % Tukey HSD test. The conclusions indicate that volatile phase of combinations of thyme oil and clove oil showed good potential in the inhibition of growth of Eurotium spp. EOs should find a practical application in the inhibition of the fungal mycelial growth in some kind of the food.

Keywords: Eurotium chevalieri, Eurotium rubrum, Eurotium sp., essential oils, vapour, antifungal activity

INTRODUCTION

Fungi are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by retard their nutritive value and sometimes by producing mycotoxins (Bajaj et al., 1975). In bakery processing, the most common type of microbial spoilage is mould growth and in many cases it is the major factor governing shelf-life (Earle and Putt, 1984). Xerophilic moulds are food spoilage organisms of low moisture food such as bakery products (Abellana et al., 1999, Hocking and Pitt, 1980; Samson, 1989; Vega et al., 1998), dried fruit (Pitt and Christian, 1966) and grain flours (Beuchat and Hwang, 1996). The number of resistant fungal pathogenic and toxigenic species is rising (Deising et al., 2008) and the need for a new antifungal substances and alternative treatments are becoming increasingly obvious. The food industry at present is facing a tremendous pressure from consumers for using chemical preservatives to prevent the growth of food borne and spoiling microbes. The increasing demand for safe and natural food, without chemical preservatives, led many researchers to investigate the antimicrobial effects of natural compounds (Rasooli et al., 2008). Between these compounds belong essential oils (EOs). EOs of plants are of growing interest both in the industry and scientific research because of their antibacterial, antifungal, and antioxidant properties and make them useful as natural additives in foods (Pattnaik et al., 1997). The antimicrobial activity of spices and EOs is well recognized. Several studies have reported results on their preservative action (Mishra and Dubey, 1994; Paster et al., 1995; Montes – Belmont and Carvajal, 1998; Vázquez et al., 2001). Eugenol, thymol and carvacrol, for example, are well known phenolic representatives found in clove, thyme and oregano oils and have been demonstrated to have an inhibitory activity against both bacteria (Hao et al., 1998; Ullée et al., 1998; Dídy et al., 1994) and fungi (Scora and Scora, 1998; Mahmoud, 1994; Bouchnourd and Flegel, 1982). The mechanism of phenolic toxicity towards fungi is based on the inhibition of fungal enzymes, which contain -SH groups in their active sites (Cowen, 1999; Celimele et al., 1999). For this reason, these substances can be used as safe and effective alternatives to synthetic preservatives (Wilson et al., 1997).

The objective of our study was evaluation of the antifungal activity of 5 EOs by vapour contact against the selected fungal species of the genus Eurotium.

MATERIAL AND METHODS

Species of microscopic fungi

A total of three isolates, were used. These isolates, two Eurotium rubrum, Eurotium chevalieri and one isolate Eurotium sp. were obtained from different Slovak wheat grains (Triticum aestivum L.). These isolates belong to the microorganisms’ collection at the Department of Microbiology of the Slovak Agricultural University in Nitra. They were inoculated on Czapek Yeast Autolysate Agar (CYA) plates.

Essential plant oils

The essential oils used in this study were of thyme, clove, basil, jasmine and rosemary. They were all supplied by Calendula company a.s. (Nová Ľubovňa, 238 A, Slovakia). Essential oils were extracted by hydro distillation and its quality and stability were certified by suppliers.

Effect of essentials plants oil

Evaluation by filter paper was making by the method adapted from Guynot et al. (2003). Plates were kept in an inverted position. A sterilized filter paper (square of 1 x1 cm) was placed in the centre of the lid and 50 µl of pure essential oil were added to the paper. Blank were made by adding 50 µl of water to it. Each fungus was inoculated in the centre on Petri plates with needle–inoculated. Plates were tightly sealed with parafilm and incubated for 7th, 14th, and 21st days at 25 ± 1 °C (three replicates were used for each treatment). Diameters of the growing colonies were measured weekly with the aid of ruler. Data was evaluated statistically by 95.0 % Tukey HSD test.

RESULTS AND DISCUSSION

The antimicrobial compounds in plant materials are commonly found in the EOs fractions obtained by steam or supercritical distillation, pressing, or extraction by liquid or volatile solvents. The traditionally most well – known antimicrobial species and herbs are clove, cinnamon, chilli, garlic, thyme, oregano and rosemary. But also bay, basil, sage, anise, coriander, allspice, marjoram, nutmeg, cardamom, mint, parsley, lemongrass, celery, cumin, fennel and many others.
have been reported to have an inhibitory effect toward microorganisms (Deans and Ritchie, 1987; Hill et al., 1997; Hammer et al., 1999; Elgayyar et al., 2001). In this study was evaluated the ability of 5 essential oils to inhibit bakery spoilage fungi – species of genus Eurotium. Although there have been many reports of the antibacterial and antifungal effects of EOs per se (Al-Burtamani et al., 2005; Holley and Patel, 2005) there are fewer on essential oils vapours. Vapours are generally more effective than the oils against fungi (Lopez et al., 2005; Tullio et al., 2007). The aim of our study was to find the activity of the volatile components of essential oils clove, basil, jasmine, thyme and rosemary against fungal growth of Eurotium chevalieri, Eurotium rubrum and Eurotium sp. The combination of EOs had significant impact on the growth of all tested isolates (Table 1).

### Table 1 Antifungal activity of tested essential oils to Eurotium spp.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Days</th>
<th>Essential oils (mean colony diameter in mm ± SD)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clove</td>
<td>Basil</td>
</tr>
<tr>
<td>Eurotium rubrum</td>
<td>7th</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>0 ± 0</td>
<td>10.67 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>21st</td>
<td>0 ± 0</td>
<td>35 ± 0</td>
</tr>
<tr>
<td>Eurotium chevalieri</td>
<td>7th</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>21st</td>
<td>0 ± 0</td>
<td>3 ± 1,73</td>
</tr>
<tr>
<td>Eurotium sp.</td>
<td>7th</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>21st</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Data in the column followed by different letters are significantly different in 95.0 % Tukey HSD test, P < 0.05

Among the EOs, the ones that totally inhibited growth of all isolates were clove and thyme regardless of time of days of cultivation. Basil, jasmine and rosemary were also able to inhibit the growth of mould, but depending of the isolates and time of incubation. The most sensitive isolate was Eurotium chevalieri. The growth of Eurotium chevalieri was inhibited by 4 from 5 tested EOs. Clove, thyme and rosemary had antifungal activity against Eurotium chevalieri at all days (7th, 14th and 21st) of the incubation. The essential oil (EO) of basil was effective only after 7th and 14th days of the incubation. On the other hand jasmine, in comparison with control, had positive or stimulating effect on the growth of this isolate after 14th and 21st days of cultivation (Figure 1). Isolate Eurotium rubrum was sensitive to clove, jasmine and thyme at all days of incubation, but basil and rosemary had antifungal activity only after 7th days of incubation (Figure 2). Eurotium sp. was sensitive to clove and thyme at all days of the incubation, as well as E. chevalieri and E. rubrum, but only Eurotium sp. had shown sensibility to basil at all days of incubation. Result showed that EOs jasmine had no the notable effect on the growth Eurotium sp., but rosemary, in comparison with the control, had inhibiting effect on its growth (Figure 3).

Nielsen and Rios (2000) had proven that the volatile compounds from mustard, cinnamon, garlic and clove EOs were efficient in the control of common bread spoilage fungi. Omidbeigi et al. (2007) studied antifungal activity of EOs of thyme, summer savory and clove in culture medium and tomato paste against Aspergillus flavus. A. flavus were inoculated in SDB (Sabouraud Dextrose Broth) and tomato paste and then 0, 50, 200, 350 and 500 ppm of EOs were added to each sample and then kept at 25 ± 0.5°C for 2 months. Result showed that all EOs cloud inhibits the growth of A. flavus and the thyme oil and summer savory, showed the strongest inhibition at 350 ppm and 500 ppm, respectively. Rasooli and Abyaneh (2004) tested inhibition of Aspergillus parasiticus growth and its aflatoxin production exposed to the EOs extracted from two varieties of thyme. They concluded that substitution of currently used antifungal and aflatoxin inhibiting chemicals by natural compounds such as thyme is recommended. Also Blooma and Etcheverry (2008) in their study analysed five essential oils (anise, boldus, mountain thyme, clove and griseb) and their inhibitory effect on growth and accumulation of mycotoxins by Aspergillus section flavi. All of these essential oils showed significant impact on aflatoxins accumulation, but the most effective were boldus, poleo and mountain thyme EOs.

![Eurotium chevalieri](image)

Figure 1 Effect of tested EOs on the growth of Eurotium chevalieri. Three replications were done for each treatment. P < 0.05, with the respect to the control.
Figure 2 Effect of tested EOs on the growth of Eurotium rubrum. Three replications were done for each treatment. P < 0.05, with the respect to the control.

Figure 3 Effect of tested EOs on the growth of Eurotium sp. Three replications were done for each treatment. P < 0.05, with the respect to the control.

Guynot et al. (2003) studied volatile fraction of 16 EOs for activity against the more common fungi causing spoilage of bakery products (Eurotium amstelodami, E. repens, E. rubrum, Aspergillus flavus, A. niger and Penicillium cyclopium). They found that 5 EOs (cinnamon leaf, clove, bay, lemongrass and thyme) had potential antifungal activity against all species tested.

Our results showed that the most effective EOs are thyme and clove and the weakest EO was jasmine. Basil and rosemary appeared to be less effective, but many of authors had different results. Basílico and Basílico (1999) tested inhibitory effect of EOs of oregano, mint, basil sage and coriander, on the mycelial growth and ochratoxin A production by Aspergillus ochraceus NRRL 3174. Cultures were incubated on yeast extract-sucrose (YES) broth, at concentration 0, 500, 750 and 1000 ppm of essential oils during 7th, 14th and 21st days at 25 °C. They found oregano and mint completely inhibited A. ochraceus and its production of ochratoxin A up to 21 days, while basil was only effective up to 7 days. But Atanda et al. (2007) proved that EOs of sweet basil optimal protective dosage of 5 % (v/v) was fungistatic on Aspergillus parasiticus CFR 223 and aflatoxins produced in vitro and on fungal development on sorghum grains with a residual effect that lasted for 32 days. Dambolena et al. (2010) tested inhibitory effect of basil EO of Ocimum basilicum and O. gratissimum L. against Fusarium verticillioides and fumunosin production. Their result showed that the oils from different location had antifungal activity and antimycotoxinogenic property. Soylu et al. (2010) studied antifungal activity of EOs, such as origanum, lavender and rosemary against Botrytis cinerea. Volatile phase effects were consistently found to be more effective on fungal growth than contact phase effect. But rosemary and lavender essential oils were inhibitory at relatively higher concentrations (25.6 μg/ml).

However, comparison of the data obtained by different studies is difficult, because of differences in plants extract compositions, in methodologies followed to assess antimicrobial activity and in microorganisms chosen to be tested (Hammer et al., 1999).

CONCLUSION

The conclusions indicate that volatile phase of combinations of thyme oil and clove oil showed good potential to inhibit growth of Eurotium spp. Even though that EOs such as basil, rosemary and jasmine had not antifungal activity like thyme and clove EOs, they should find a practical application in the inhibition of the fungal mycelial growth. In our study we tested the antifungal activity of EOs against common fungi causing spoilage of bakery products for 7th, 14th and 21st days of cultivation. Results showed that all of the tested EOs had antifungal activity after 7th days of cultivation, except jasmine, which appeared to be effective only to isolate E. rubrum after all days of culture. According to all, bakery products haven’t longer shelf life, therefore EOs can be use as preservative materials on bakery products, but further safety studies are necessary.

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