INHIBITORY ACTIVITY OF THE ONION ESSENTIAL OIL ON GROWTH Cladosporium cladosporioides, Emericella nidulans, AND Eurotium spp.

INHIBITORSKA AKTIVNOST ETARSKOG ULJA CRNOG LUKA NA RAST Cladosporium cladosporioides, Emericella nidulans, I Eurotium spp.

Sunčica KOCIC-TANACKOV*, Gordana DIMIC*, Ljiljana MOJOVIC**, Jelena PEJIN*, Jelica GYOZDANOVIĆ-VARGA***

* University of Novi Sad, Faculty of Technology, 21 000 Novi Sad, Bulevar cara Lazara 1, Serbia
** University of Belgrade Faculty, of Technology and Metallurgy, 11 000 Beograd, Karmegjeva 4, Serbia
***Institute of Field and Vegetable Crops, 21 000 Novi Sad, Maksima Gorkog 30, Serbia
e-mail:sunicicat@uns.ac.rs

ABSTRACT

This study was aimed at investigating the antifungal potential of onion (Allium cepa L. cultivar Kupusinski jabučar) essential oil against Cladosporium cladosporioides, Emericella nidulans, and Eurotium spp. isolated from food. The antifungal determination of onion essential oil was performed using the agar plate method. Applied concentrations of the onion essential oil (3.5, 7.0, 14.0, and 28.0 µL/100 mL) caused the delay or absence of fungal growth with different inhibitory effects on deceleration in the growth rate. Onion essential oil at a concentration of 14.0 µL/100 mL demonstrated a fungicidal effect (MFC) on the growth of C. cladosporioides and Eurotium spp. and inhibitory effect (MIC) for E. nidulans, while at a concentration of 28.0 µL/100 mL showed MFC on the growth of E. nidulans. Microscopic investigations showed that the application of onion essential oil caused changes in micro-morphology of the investigated fungi (hypophae and reproductive organs deformation). This study proved that the tested onion essential oil could potentially be used as a protective agent against food-borne fungi.

Key words: onion essential oil, antifungal activity.

REZIME

Ova studija imala je za cilj da ispitati antifungalni potencijal etarskog ulja crnog luka (Allium cepa L. Kupusinski jabučar) prema Cladosporium cladosporioides, Emericella nidulans, Eurotium amstelodami, E. herbariorum, E. chevalieri i E. rubrum izolovanih iz hrane. Antifungalna ispitivanja izvedena su metodom agar ploča. Primjenjene koncentracije etarskog ulja crnog luka (3.5, 7.0, 14.0 i 28.0 µL/100 mL) uzrokovala su izostanak ili odlaganje rasta gljiva, sa različitim inhibitornim delovanjem na brzinu rasta. Koncentracija etarskog ulja od 14.0 µL/100 mL bila je fungicidna (MFC) za C. cladosporioides, E. amstelodami, E. herbariorum, E. chevalieri, E. rubrum i inhibitorna (MIC) za E. nidulans. Koncentracija od 28.0 µL/100 mL fungicidno (MFC) je delovala na E. nidulans. Mikroskopska posmatranja su pokazala da je pod uticajem etarskog ulja crnog luka došlo do mikromorfoloških deformacija gljiva (deformacije hija i reproduktivnih organa). Ova istraživanja su pokazala da se ispitivano etarsko ulje crnog luka može koristiti kao potencijalni zaštitni agens protiv fungalne kontaminacije hrane.

Ključne reči: etarsko ulje crnog luka, antifungalna aktivnost, gljive, hirne.

INTRODUCTION

Fungi can not only lead to the food spoilage and change in sensory properties, but also produce a variety of toxic metabolites, some of which are considered carcinogenic agents. Most important are the aflatoxins produced by fungi of the genus Aspergillus (A. flavus, A. parasiticus), followed by ochratoxin A, produced by Aspergillus ochraceus, A. carbonarius, A. aliuscuis, Penicillium verrucosum, P. nordicum and toxins of Fusarium species (zearalenone, fumonisins, deoxinivalenol, etc.) (Samson et al., 2004; Pitt and Hocking, 2009; Kocić-Tanackov and Dimić, 2012). Fungi develop well on substrates with reduced humidity, which is their advantage in comparison with the majority of bacteria and yeast (Kocić-Tanackov and Dimić, 2012). They are regularly present in herbal agricultural products. Animal products can contain mycotoxins originating from contaminated feed and other additives. Their complete elimination from food-stuff is highly unlikely, because of their resistance to thermal treatments that are used in the food industry. The concentrations of mycotoxins in food are usually not high, but the concerning fact is that they have a cumulative character. This means that introduction of low doses in the organism during a certain period of time enhances their effect (Duraković and Duraković, 2003; Diz, 2005; Sinovec et al., 2006). It is known that essential oils extracted from certain aromatic plants inhibit the growth of microorganisms (Burt, 2004; Tajkarimi et al., 2010). Because of that, there is significance for the food industry in the application of essential oils and spice plant extracts as bio-preservatives, which should reduce the use of synthetic preservatives and additives in controlling fungi that contaminate food. As complex mixtures, essential oils of spices consist of a large number of different compounds (aldehydes, monoterpenes, phenols, alcohols, esters, ketones, etc.) and their antimicrobial activity is linked to their major components (Burt, 2004; Tajkarimi et al., 2010). Onion and garlic are used since ancient times as indispensable ingredients in the preparation of various foods and in medicine due to their positive effects on human health. The main biologically active components of onion and garlic are sulfur compounds (di-allyl-trisulphide, diallyl-disulphide, diallyl-sulphide, dimethyl-trisulphide, methyl-propyl-trisulphide, dimethyl-tetrasulphide, etc.), proven to exhibit antimicrobial properties (Lanzotti, 2006; Benkeblia and Lanzotti, 2007; Corzo-Martinez et al., 2007; Kocić-Tanackov et al., 2012). Several studies indicate the inhibitory potential of onion essential oils on fungi (Yin and Tsao, 1999; Hisieh et al., 2001; Irkin and Korukluğlu, 2007; Dimić et al., 2008; Kocić-Tanackov et al., 2012). The aim of this paper was to test the effect of onion essential oil on fungi isolated from food.

MATERIAL AND METHOD

Onion essential oil

Essential oil of onion (Allium cepa L. cultivar Kupusinski jabučar) (dry material content - 15.41%; mineral material content - 0.6 %; total sugars content - 3.99%; vitamin C content - 22.77 mg/100g; earliness - 130 days; storability- one month after harvest) were obtained by steam distillation. Ripe bulbs of fresh onions (500 g) were cleaned and milled in a blender and...
then distilled for 3 h in an apparatus for distillation by Clevenger, with 500 mL of distilled water. The oil was collected in petroleum ether, which was eventually evaporated (in glass tube) at room temperature. The resulting oil, yield of 0.042 %, were stored at +4°C.

**Fungal strains**

The following fungal strains were used as test microorganisms: Cladosporium cladosporioides (Fres.) de Vries, Emericella nidulans (Edam) Vuill, Eurotium amstelodami L. Mangin, E. herbariorum Link, E. chevalieri L. Mangin, and E. rubrum Jos Kööng et al. isolated from cakes and fresh salads from different varieties of ready-for-use vegetables. Isolation of fungi from food was determined according to the method described by Samson et al. (2004) and Pitt and Hocking (2009). Obtained pure cultures of Cladosporium, Eurotium, and Emericella genera were identified according to the keys for determination (colony diameter, color and texture; microscopic characteristics – hyphae and conidiophore appearance, size and shape of vesicles, metulae, phialides, and conidia) described by Klich (2002), Samson et al. (2004), and Pitt and Hocking (2009). Isolated and identified fungal cultures were kept on PDA (Potato Dextrose Agar) (C. cladosporioides) and Czapek agar (Emericella and Eurotium species) at 4°C as part of the collection of the Laboratory for Food Microbiology at the Faculty of Technology, University of Novi Sad, Serbia.

**Determination of the onion essential oil effect on the fungi growth**

The agar plate method was applied to test the antifungal activity of the onion essential oil. The basic medium for the anti-fungal tests was PDA. The medium was divided into equal volumes (150 mL), poured into Erlenmeyer flasks (volume 250 mL) and autoclaved at 121 °C for 15 min, and cooled to 45 °C. The onion essential oil, was poured into Erlenmeyer flasks (volume 250 mL), poured into Erlenmeyer flasks (volume 250 mL) and poured into Erlenmeyer flasks (volume 250 mL). The oil was collected in petroleum ether, which was eventually evaporated (in glass tube) at room temperature. The resulting oil, yield of 0.042 %, were stored at +4°C.

Monitoring of macroscopic and microscopic features of fungi

Macroscopic and microscopic features of fungi as well as their changes which occurred in the presence of onion essential oil were also observed. The macroscopic features were observed using a stereoscopic microscope (Technival 2, Carl Zeiss, Jena, Germany) and the microscopic features using a light microscope (Aristoplan, Leitz, Wetlzar, Germany).

**RESULTS AND DISCUSSION**

Applied concentrations of the onion essential oil caused the delay or absence of fungi growth with different inhibitory effects on deceleration in the growth rate (Figures 1a-6a).

![Fig. 1. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of C. cladosporioides](image)

At the lowest concentration (3.5 µL/100 mL) growth onset of E. nidulans, E. herbariorum, and E. chevalieri was delayed for two days, E. rubrum for three days, E. amstelodami for five days, and C. cladosporioides for six days. At a concentration of 7.0 µL/100 mL the growth of E. nidulans and E. herbariorum was observed on the 5th day, E. amstelodami on the 9th day, C. cladosporioides on the 10th day, while the latest recorded growth was in E. rubrum (13th day). Onion oil at a concentration of 14.0 µL/100 mL showed fungicidal activity (MFC) towards Eurotium species and C. cladosporioides. At this concentration growth of E. nidulans was delayed until the 23rd day, which is the MIC for this fungi, while the highest concentration used (28.0 µL/100 mL) was fungicidal for this species of fungi. Decrease of fungi growth rate with the increase of onion essential oil content in a solid medium was more pronounced in C. cladosporioides and E. rubrum, than with other species of fungi, indicating their higher sensitivity (Figures 1a-6a).
Fig. 2. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. nidulans.

Fig. 3. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. herbariorum.

Fig. 4. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. rubrum.

Fig. 5. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. chevalieri.
Growth inhibitions of the studied fungi under the effect of different concentrations of onion essential oil during a fourteen day period are shown in Figures 1b-6b and in Table 1. Lowest concentration of onion essential oil (3.5 μL/100 mL) had an inhibitory effect on the growth of all fungi. Weak inhibition was observed in *E. herbariorum* and *E. amstelodami* (16.7 and 17.6% respectively), and slightly more significant (from 22.7 to 26.9%) in *E. nidulans*, *E. chevalieri* and *E. rubrum*. *C. cladosporioides* showed the greatest sensitivity at this concentration with a growth inhibition of 43.5% (Figures 1b-6b, Table 1). With increasing concentrations (7.0 μL/100 mL) of onion essential oil in the medium there was a significant growth inhibition of *E. rubrum* and *C. cladosporioides* with inhibition values of 76.9 and 65.4%, respectively. Growth of other species was inhibited from 33.5 to 45.5%. Complete growth inhibition of tested fungi was achieved by applying essential oil at concentrations of 14.0 and 28.0 μL/100 mL (Figures 1b-6b, Table 1).

**Table 1. Inhibition (%) of fungal growth after 14 days of incubation on 25°C**

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Concentration of onion essential oil (μL/100 mL)</th>
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<tbody>
<tr>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td><em>E. nidulans</em></td>
<td>22.7</td>
</tr>
<tr>
<td><em>E. herbariorum</em></td>
<td>16.7</td>
</tr>
<tr>
<td><em>E. rubrum</em></td>
<td>26.9</td>
</tr>
<tr>
<td><em>E. chevalieri</em></td>
<td>24.3</td>
</tr>
<tr>
<td><em>E. amstelodami</em></td>
<td>17.6</td>
</tr>
<tr>
<td><em>C. cladosporioides</em></td>
<td>43.5</td>
</tr>
</tbody>
</table>

Other researchers also report on the inhibitory effects of al- liums’ (onion, garlic, scallions, leeks, shallots and Chinese onion) essential oil and extracts on the growth of fungi and biosynthesis of their toxic secondary metabolites - mycotoxins.

*Yin and Tsao* (1999) investigated antifungal effect of seven herbs from *Allium* family. According to their results, garlic showed the highest antifungal activity against three tested *Aspergillus* species (*A. niger*, *A. flavus* and *A. fumigatus*). *Benkebla* (2004) confirmed inhibitory effect of onion and garlic essential oils against *A. niger*, *Penicillium cyclopium* and *Fusarium oxysporum*. *Dimić et al.* (2008) point out the antifungal activity of onion essential oil against *Penicillium commune*, *P. aurantiogriseum*, *P. griseofulvum*, *P. corylophilum* and *Aspergillus ochraceus*. *Kocić-Tanackov et al.* (2009) examined the effect of scallions on *Allium ampeloprasum* and onion (*Allium cepa* cultivar Junski srebrnjak and *A. cepa* cultivar Kupusinski jabučar) on the growth of three fungi (*Aspergillus tamarii*, *P. griseofulvum* and *E. amstelodami*) isolated from spices. The strongest inhibitory activity against *A. tamarii* was demonstrated by the oil of *A. cepa* cultivar Kupusinski jabučar, and against *P. griseofulvum* it was the oil of *A. ampeloprasum*. Growth of *E. amstelodami* was completely inhibited under the effect of onions (cultivars Kupusinski jabučar and Junski srebrnjak), which is consistent with the results obtained in this study.


Components isolated from the onion bulbs also showed antifungal activity against fungi. So tiopropanal-S-oxide (lachrymatory factor), isolated from onion, has inhibited the sporulation of *Aspergillus parasiticus* (*Sharma et al.*, 1981). Fistulosin, antifungal compound isolated from Velsh onion bulbs, had expressive antifungal activity against few fungi, especially *Penicillium roqueforti* and *Aspergillus oryzae* (*Phay et al.*, 1999). Allicepin, a peptide extracted from onion bulbs, showed inhibitory activity against *Botrytis cinerea, Fusarium oxysporum, Mucorphaeella arachidicola* and *Physalospora pirciola* (*Wang and Ng*, 2004).

Tested onion essential oils, besides limiting the growth of the colonies, have also caused changes in the macro-and micromorphology of fungi. The presence of essential oils in a medium at concentrations of 7.0 μL/100 mL resulted in decrease of conidia- tion in all tested fungi. There has also been formation of irregular folds on the colonies and uplift of the central part in *E. nidulans*, *E. chevalieri*, and *E. rubrum*. Micromorphological observations have revealed the frequent fragmentation of the hyphae and deformation of the cell wall, deformation and reduction of reproductive organs in all the tested fungi. These changes indicate possible deformations at the cellular level, as well as inhibiting the synthesis of lipids, proteins and nucleic acids, changing the lipid profile of the cell membrane and inhibiting the synthesis of the fungal cell wall (*Gupta and Porter*, 2001; *Rassoli et al.*, 2006; *Sharma and Tripathi*, 2006; *Corzo-Martinez et al.*, 2007).

**CONCLUSION**

The results of this study demonstrate the significant antifungal activity of the onion essential oil on the mycelial growth of *Cladosporium cladosporioides*, *Emericella nidulans*, and *Eurotium* spp., which is in agreement with our previous research (*Dimić et al.* 2008; *Kocić-Tanackov et al.* 2009; *Kocić-Tanackov et al.* 2012).

![Graph](image-url)
et al. 2012) as well as with the results obtained by other researchers (Hasan and Mahmoud 1993; Yin and Tsao 1999; Benkeblia, 2004). On the basis of these results, the onion essential oil could be used for food protection against the fungal growth, especially in food with compatible flavour (e.g. sausages, salads). Further research should be directed to optimization of antifungal oil concentration with product sensory properties.

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REFERENCES


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