Biblid: 1821-4487 (2013) 17; 1; p 12-16
UDK: 547.913:582.573.16

Original Scientific Paper
Originalni naučni rad

INHIBOTORY 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.

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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 micromorphology of the investigated fungi (hyphae 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 ispita antifungalni potencijal etarskog ulja crnog luka (Allium cepa L. kultivar 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. Primenjene koncentracije etarskog ulja crnog luka (3,5; 7,0; 14,0 i 28,0 μL/100 mL) uzrokovale 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 hifa 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, hrana.*

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. alliacus, 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 foodstuff 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; Diaz, 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 (diallyl-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; Hsieh et al., 2001; Irkin and Korukluiglu, 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 Köing 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 antifungal 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 were added to the PDA to achieve the following concentrations: 0, 3.5, 7.0, 14.0 and 28.0 μL/100 mL. The PDA containing different concentrations of the onion essential oil was poured into sterile Petri dishes (∅ 9 cm), 12 mL per dish. The seven-day fungal cultures grown on PDA were used to prepare the fungal spore suspension tests (initial spore count of 10^6 spores/mL). For each essential oil dose and fungal species, including the controls (onion essential oil was not added in the PDA medium), the dishes were centrally inoculated by spreading 1 μL of a spore suspension (10^3 spores/mL). After the inoculation, the Petri dishes were closed with parafilm. The effect of the onion essential oil on fungal growth was evaluated by a daily measurement of the diameter of the radial colony growth during 14 days of incubation at 25±2 ºC. The parafilm was removed from the Petri dishes in which no colony growth was observed after 14 days, and the dishes were further incubated for 16 days (30 days in total) at 25±2 °C. In the Petri dishes in which fungal growth was observed from 15th to 30th day, the concentration of onion essential oil used was considered to be the minimal inhibitory concentration (MIC). If there was no visible fungal growth after 30 days, the fungal spores were transferred using a wet cotton baton to the PDA in which no onion essential oil was added, and were incubated for 5 days at 25±2ºC for the determination of fungicide effect (MFC). The inhibitory effect of the onion essential oil on fungal growth after 14 days was calculated from the following formula (*Pandey et al., 1982*):

$$I\ (\%) = (C-T)/C \cdot 100$$

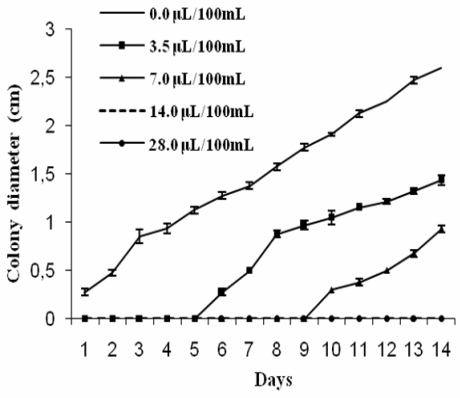
where I is the inhibition (%), C is the colony diameter in the control dish (cm) and T is the colony diameter in the test dish (cm) (10). Each antifungal test was carried out in 3 series and 2 replications. Values are presented as means±SD of six measurements.

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).



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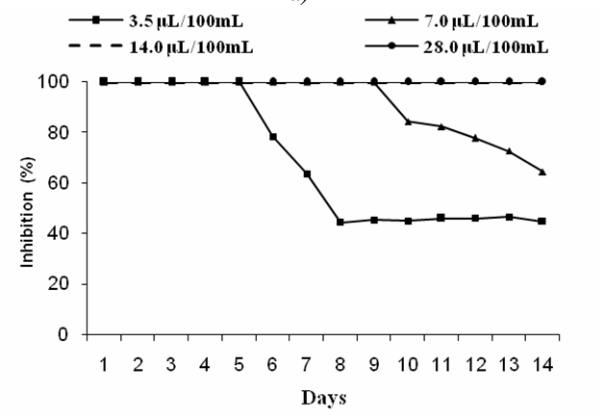

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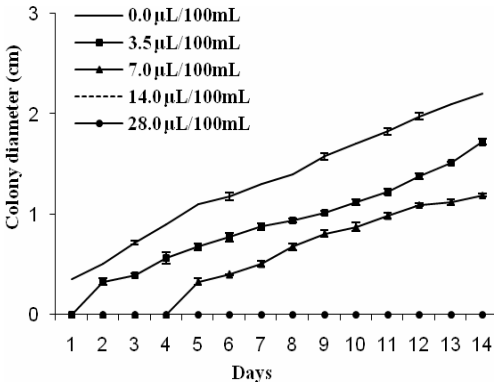
Fig. 1. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of C. cladosporioides

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. chevalieri* on the 7th 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).



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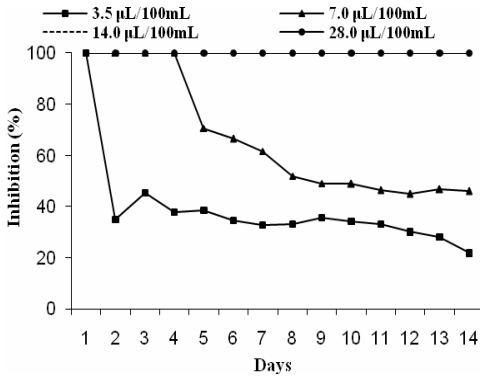

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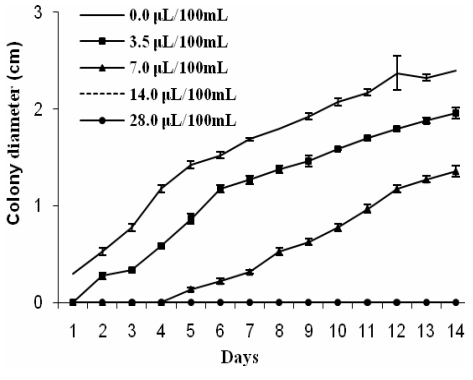
Fig. 2. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. nidulans



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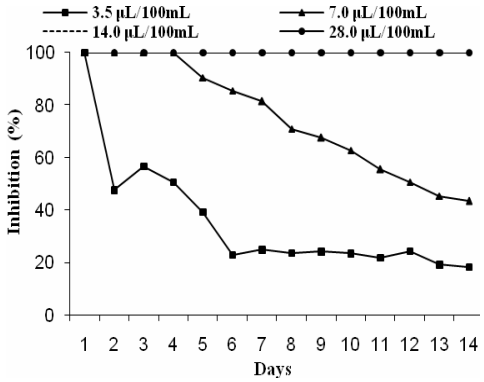

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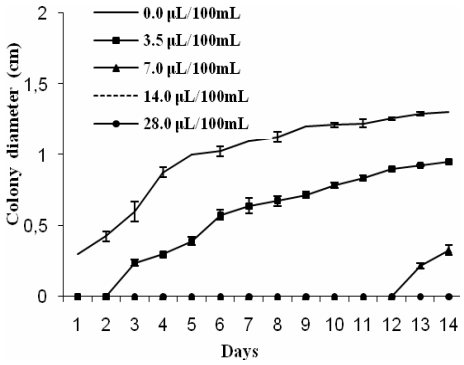
Fig. 3. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. herbariorum



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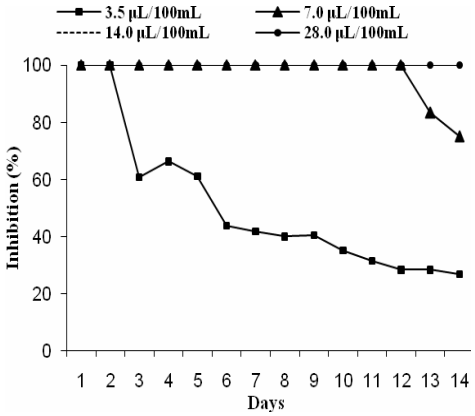

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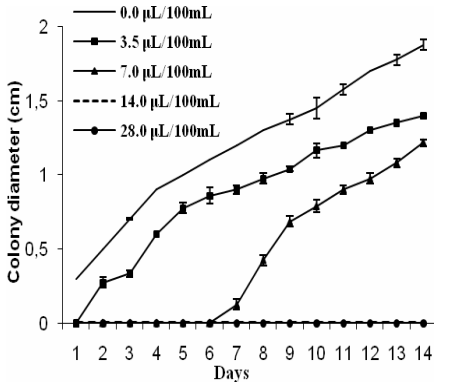
Fig. 4. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. rubrum



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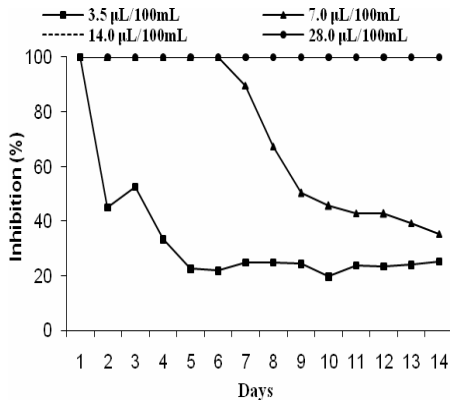

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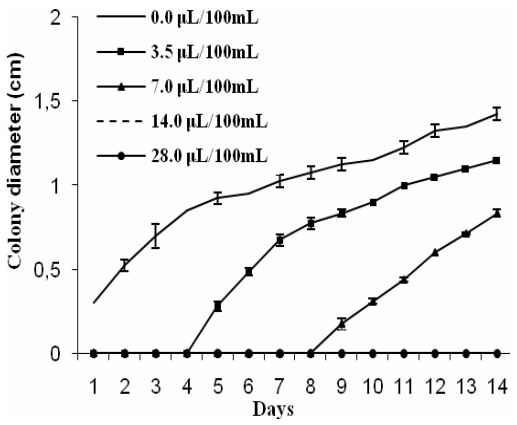
Fig. 5. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. chevalieri



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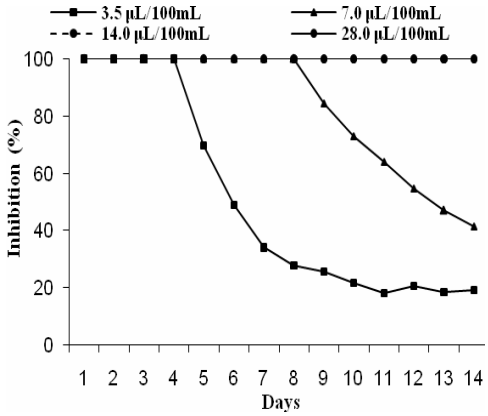

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Fig. 6. Effect of the onion essential oil on the growth rate (a) and growth inhibition (b) of E. amstelodami

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

Fungi	Concentration of onion essential oil (µL/100 mL)			
	3.5	7.0	14.0	28.0
E. nidulans	22.7	45.5	100.0	100.0
E. herbariorum	16.7	41.7	100.0	100.0
E. rubrum	26.9	76.9	100.0	100.0
E. chevalieri	24.3	33.5	100.0	100.0
E. amstelodami	17.6	39.3	100.0	100.0
C. cladosporioides	43.5	65.4	100.0	100.0

Other researchers also report on the inhibitory effects of alliums' (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*). *Benkeblia (2004)* confirmed inhibitory effect of onion and garlic essential oils against *A. niger*, *Penicillium cycloprium* 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 *Aspergillis 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.

Hsieh et al. (2001) noticed high sensitivity of *A. niger* towards combined extract of cornelberry, cinnamon nad oriental onion (1:6:6, vol/vol/vol). Results of *Dimić et al. (2007)* showed that there was a complete growth inhibition of *Eurotium* spp. and *Aspergillus sydowii*, at a concentration of 2% garlic extract in the medium. *Hitokoto et al. (1980)*, *Hasan and Mahmoud (1993)*, and *Kocić-Tanackov et al. (2012)* showed that onion and garlic essential oils can infulence the synthesis of sterigmatocystin and aflatoxins, toxic metabolits of *Aspergillus* species.

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 *Aspergilus oryzae* (*Phay et al., 1999*). Allicepin, a peptide extracted from onion bulbs, showed inhibitory activity against *Botrytis cinerea, Fusarium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora piricola* (*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 conidiation 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 hyphe 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) 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.

ACKNOWLEDGMENT: This paper is a result of the research within the project TR 31017, 2011-2014, supported by the Ministry of Education, Science and Technology, Republic of Serbia.

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Received: 11.03.2013. Accepted: 01.04.2013.