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INFLUENCE OF *ALLIUM AMPELOPRASUM* L. AND *ALLIUM CEPA* L. ESSENTIAL OILS ON THE GROWTH OF SOME YEASTS AND MOULDS

ABSTRACT: Essential oils extracted from spices, as natural antimicrobial agents, attract particular attention due to their possible role in food protection from microorganisms, and their nontoxicity, in contrast to the synthetic preservatives. In this work, inhibitory effect of *Allium ampeloprasum* and two onions (*Allium cepa*), Junski srebrnjak and Kupusinski jabučar, essential oils in different concentrations (1, 4, 7 and 10%) on three yeasts (*Saccharomyces cerevisiae, Candida tropicalis* and *Rhodotorula* sp.) and three moulds (*Aspergillus tamarii, Penicillium griseofulvum* and *Eurotium amstelodami*) was investigated. All three essential oils showed the strongest inhibitory effect against *S. cerevisiae* in concentration of only 1%. Among onions, Kupusinski jabučar essential oil had stronger influence to *C. tropicalis*, while *Allium ampeloprasum* essential oil did not show any influence on this yeast. *Rhodotorula* sp. was influenced only by *Allium ampeloprasum* essential oil. The strongest inhibitory effect on *A. tamarii* showed Kupusinski jabučar (57% of inhibition, in concentration of 10%), while on *P. griseofulvum*, the strongest influence showed *Allium ampeloprasum* essential oil (78.3% of inhibition, in concentration of 10%). Junski srebrnjak and Kupusinski jabučar essential oils, in concentrations of 7 and 10% respectively, completely inhibited the growth of *E. amstelodami*.

KEY WORDS: *Allium* essential oils, antimicrobial activity, moulds, yeasts

INTRODUCTION

Fungi, as a large group of microorganisms, are common food contaminents and the cause of food deterioration. Besides, they are responsible for unpleasant odour and production of very toxic secondary metabolities — mycotoxins and alergen compounds. The most frequently isolated species of filamentous fungi from food belong to the genus *Aspergillus, Penicillium, Cladosporium, Mucor, Rhizopus, Fusarium, Alternaria, Eurotium, Emericella* etc. (Jay et al., 2005; Montville and Matthews, 2005; Škrinjar et al., 2005; Stanković et al., 2007) and yeasts to the genus *Saccharomyces, Rhodotorula, Candida, Debaromyces, Hansenula, Kloeckera, Lodderomyces,* *Torulopsis* etc. (Davis, 2003; Jay et al., 2005; Montville and Matthews, 2005). An attempt to decrease the growth and evolution of fungi, as well as the synthesis of toxic metabolites, is achieved by the addition of synthetic preservatives. In recent years, there has been an increasing interest for the application of essential oils, obtained from spices and other herbs, as alternative antimicrobial agents in food for human nutrition. Also, the advantage of spice extracts is that they do not contain microorganisms which contaminate natural spices (Karan et al., 2005).

Garlic (*Allium sativum*) and onion (*Allium cepa*) are widely used as culinary ingredients and in food industry for taste improvement. Garlic has also been known for centuries as a spice with a very wide range of application, but also as a herb with important healing features (Harris et al., 2001). According to morphology and flavour, *Allium ampeloprasum* is very similar to garlic. It is mostly called as great-headed garlic, elephant garlic or pearl onion. The main active antifungal agents from garlic and onion essential oils are the degradation products of allicin, including diallyl trisulphide (DATS), diallyl disulphide (DADS), diallyl sulphide (DAS), which have greater antifungal effect than allicin (Tansey and Appleton, 1975; Corzo-Martinez et al., 2007), fistulosin (octadecyl 3-hydroxyindole), that has been isolated from welsh onion (Phay et al., 1999), antifungal proteins and peptides (allicepin, novel, isolated from onion bulb) (Lam et al., 2000; Wang and Ng, 2001; Wang and Ng, 2004), steroid saponins (eruboside-B, isolated from garlic bulb) (Matsuura et al., 1988). Garlic and onion germs are especially rich in essential oils (Stanković and Nikolić, 2002; Corzo-Martinez et al., 2007).

Content and composition of these active compounds, as well as their antimicrobial activity, are influenced by variety, area, climatic and storage conditions (Russo et al. 1998; Ceylan and Fung, 2004). For these reasons, the aim of this work was to investigate the antimicrobial activity of *Allium* plants esential oils from the region of Vojvodina against some food contaminating fungi.

MATERIAL AND METHODS

Allium plants. *Allium ampeloprasum* and two types of onion (*Allium cepa*), Junski srebrnjak and Kupusinski jabučar, grown at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, were used for obtaining essential oil.

Test microorganisms. Three yeasts: *Rhodotorula* sp. (isolated from air), *Candida tropicalis* (clinical isolate), *Saccharomyces cerevisiae* 112 Hefebank Weinhenstephan, and three moulds: *Aspergillus tamarii, Penicillium griseofulvum* and *Eurotium amstelodami* (isolated from spices) were used for antimicrobial investigations. Microorganisms were maintained on Sabouraud maltose agar slants (SMA), at temperature of 4°C. For this experiment, 48 hours and 7 days old cultures of yeasts and moulds were used, respectively.

Essential oil extraction. Essential oils were extracted by steam distillation. They were chopped in small pieces and mashed with domestic blender (Braun Minipimer MR 400). Sample was transferred into a 2 l flask and mixed with distilled water (1:1 ratio), and the Clevenger apparatus was installed. The system was heated during 3 h and the essential oil was collected in petroleum ether layer, in the oil separator tube. When the extraction time ran out, the petroleum ether layer was collected in a centrifuge tube and left at room temperature to evaporate the solvent. The tube, containing essential oil, was sealed with rubber stopper and stored in refrigerator.

Assessment of yeast growth inhibition. The assay was carried out by disc-diffusion test (L e b o f f e and P i e r c e, 2005). The tested concentrations of essential oils were 1, 4, 7 and 10%. One mililiter of inoculum (10^5 cfu/ml) was added to 10 ml SMA, which was consequently placed in 0 9 cm Petri dishes. Sterile 5 mm paper discs, after gelling, were placed in the centre of agar medium and soaked with 10ml prepared concentrations of ethanol-water solution of essential oils. Dishes were incubated at 25°C for 48—72 h, and the zones of inhibition showing the presence of antimicrobial activity were measured. All tests, including the control test (ethanol-water solution), were done in triplicates.

Assessment of mould growth inhibition. For each isolate, a conidial spore suspension (10^6/ml) was prepared in medium which contained 0.5% Tween 80 and 0.5% agar in distilled water (N i e l s e n and R i o s, 2000). Inoculation was performed with 1 ml of spore suspension (10^3 spores/ml) in the standard Petri dishes containing centred SMA medium. Steril disc (5 mm) was placed in the centre of every dish cover, and 10 ml of essential oil in concentrations of 1, 4, 7 and 10% were added. Then, the plates were closed with parafilm and left to incubate at 25°C for up to 7 days. The colony diameters were measured every day.

RESULTS

Antimicrobial activity of essential oils against yeasts

From Tab. 1, it can be seen that essential oil from *Allium ampeloprasum*, and the lowest concentration, had inhibitory influence only on *S. cerevisiae*. The zone of inhibition was 10 mm.

The increased concentration of essential oil did not show any influence on *C. tropicalis*, whereas the growth of *Rhodotorula* sp. and *S. cerevisiae* was completely inhibited.

Tab. 1 — Influence of *Allium ampeloprasum* essential oil on yeast growth

concentrations / yeasts	1%	4%	7%	10%
Rhodotorula sp.	—	ng	ng	ng
C. tropicalis	—	—	—	—
S. cerevisiae	10	ng	ng	ng

zone of inhibition is expressed in mm; — no activity; ng — no growth

Essential oil of onion (*Allium cepa*) Junski srebrnjak did not show inhibitory effect on *Rhodotorula* sp. (Tab. 2). Concentrations of 1 and 4% inhibited the growth of other two yeasts, *C. tropicalis* and *S. cerevisiae*, with inhibition zones of 13 and 14 mm, and 14 and 16 mm, respectively, and complete inhibition at concentration of 7%.

Tab. 2 — Influence of onion (*Allium cepa*) Junski srebrnjak essential oil on yeast growth

concentrations / yeasts	1%	4%	7%	10%
Rhodotorula sp.	—	—	—	—
C. tropicalis	13	14	ng	ng
S. cerevisiae	14	16	ng	ng

zone of inhibition is expressed in mm; — no activity; ng — no growth

Data displayed in Tab. 3 show strong influence of 1% of onion (*Allium cepa*) Kupusinski jabučar essential oil on the growth of *S. cerevisiae* (21 mm inhibitory zone), weaker influence on *C. tropicalis* (10 mm inhibitory zone), and capability to completely stop their growth at concentration of 4%. *Rhodotorula* sp. in this case did not show any sensitivity.

Tab. 3 — Influence of onion (*Allium cepa*) Kupusinski jabučar essential oil on yeast growth

concentrations / yeasts	1%	4%	7%	10%
Rhodotorula sp.	—	—	—	—
C. tropicalis	10	ng	ng	ng
S. cerevisiae	21	ng	ng	ng

zone of inhibition is expressed in mm; — no activity; ng — no growth

Antimicrobial activity of essential oils against moulds

Allium ampeloprasum essential oil in concentration of 1% showed low inhibition of the growth of tested moulds (Tab. 4). The growth of *A. tamarii* (8.9%) was weakly inhibited in concentration of 4%, while *E. amstelodami* and *P. griseofulvum* showed higher sensitivity (23.2 and 18.7%). To achieve a similar effect on *A. tamarii*, 10% of essential oil was needed. At this level, the growth of *P. griseofulvum* was significantly reduced by 78.3%, and of *E. amstelodami* by 61.6%.

Tab. 4 — Inhibition of mould growth (%) influenced by *Allium ampeloprasum* essential oil

concentrations / yeasts	1%	4%	7%	10%
A. tamarii	43	8.9	14.8	23.2
P. griseofulvum	8.7	18.7	34.8	78.3
E. amstelodami	8.1	23.2	54.0	61.6

Results obtained for (*Allium cepa*) Junski srebrnjak (Tab. 5) indicate that *E. amslelodami* was very sensitive mould, with higher growth reduction in comparison to other two moulds, which could be seen with already 1% of oil, and complete inhibition was at the level of 7% of essential oil. The growth of *A. tamarii* was equivalent to the control sample under influence of 1% of oil, with 0.9% of inhibition. Generally, essential oil of Junski srebrnjak was the least active against *A tamarii*.

Tab. 5 — Inhibition of mould growth (%) influenced by onion (*Allium cepa*) Junski srebrnjak essential oil

concentrations / yeasts	1%	4%	7%	10%
A. tamarii	0.9	6.5	9.3	20.0
P. griseofulvum	2.2	6.5	14.5	19.6
E. amstelodami	29.7	40.5	100	100

1% essential oil of onion (*Allium cepa*) Kupusinski jabučar had less than 10% inhibitory effect on *A. tamarii* and *P. griseofulvum*. High concentrations (7 and 10%) lowered the growth of these moulds by 18.5 and 57% (*A. tamarii*) and 21.7% (*P. griseofulvum*). *E. amstelodami* was completely inhibited with concentration of 10% (Tab. 6).

Tab. 6 — Inhibition of mould growth (%) influenced by onion (*Allium cepa*) Kupusinski jabučar essential oil

concentrations / yeasts	1%	4%	7%	10%
A. tamarii	8.3	15.7	18.5	57.0
P. griseofulvum	5.7	12.2	21.7	21.7
E. amstelodami	27.0	46.0	67.6	100

Under impact of *Allium ampeloprasum* essential oil (Figure 1), of three investigated moulds, only the growth of *E. herbariorum* at lowest concentration of essential oil (1%) was delayed for two days, in comparison to the control. The growth of *A. tamarii* at concentration of 4% was delayed for two and three days, when used at higher concentrations. The absence of *P. griseofulvum* for three and six days, and the absence of *E. herbariorum* for five and six days in the presence of 7 and 10% of oil, indicates their higher sensitivity. With concentrations over 4%, stronger inhibitory effect on the growth rate of all investigated moulds can be noticed. An increase in the quantity of oil lengthens the time needed for mould colonies to reach the size of control sample colonies (more than 7 days).

Among onions, (*Allium cepa*) Kupusinski jabučar showed stronger inhibitory effect on the germination and growth rate of *A. tamarii* and *E. herbariorum* than of *P. griseofulvum* (Figure 2). *P. griseofulvum* was already noticed in the first two days, at all applied concentrations, while *A. tamarii* was

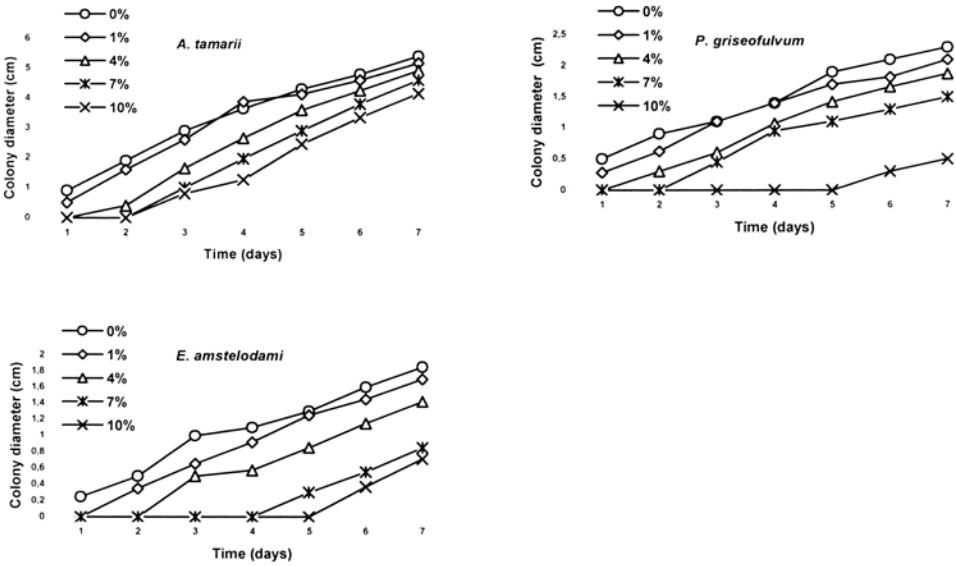

Fig. 1. Effect of essential oil of *Allium ampeloprasum* on the growth of moulds

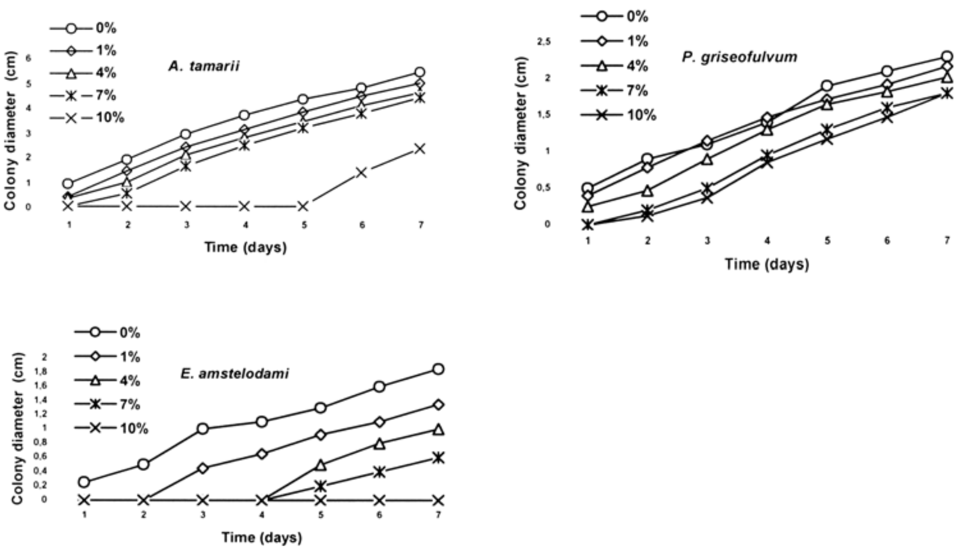

Fig. 2. Effect of essential oil of onion (*Allium cepa*) Kupusinski jabučar on the growth of moulds

noticed at concentration of up to 7%, and the growth was suppressed until the sixth day at the highest concentration. The most inhibited mould, with the lowest rate of development was *E. amstelodami*. Although *A. tamarii* and *P. griseofulvum* both showed at the concentration of up to 7%, stronger influence of essential oil was noticed on *A. tamarii*.

DISCUSSION

These investigations showed that *Allium* essential oils have the potential to inhibit the growth of yeasts and moulds. Essential oil of *Allium ampeloprasum* showed inhibitory effect on *Rhodotorula* sp. and *S. cerevisiae*, while *C. tropicalis* did not show any sensitivity. Essential oils of onion (*Allium cepa*) Junski srebrnjak, and particularly Kupusinski jabučar were more effective against *C. tropicalis* and *S. cerevisiae*. However, the latest two oils did not show any effect on *Rhodotorula* sp. All three oil samples had the strongest influence against *S. cerevisiae*. Essental oil of Kupusinski jabučar showed the strongest inhibitory activity against the investigated yeasts.

Conner and Beuchat (1984) pointed out the higher sensitivity of yeasts (*Candida lypolitica, Debaromyces hansenii, Hansenula anomala, Kloeckera apiculata, Lodderomyces elonginosporus, Rhodotorula rubra, S. cerevisiae* and *Torulopsis glabrata*) towards the influence of garlic, in comparison to onion. The onion essential oil inhibited only the growth of *S. cerevisiae*. The results of Dimić et al. (2008) showed good antimicrobial activity of onion essential oil against yeasts.

Among the tested moulds in this study, *Allium ampeloprasum* essential oil showed the strongest inhibitory effect on *P. griseofulvum. A. tamarii* turned out to be the most resistant mould towards the influence of essential oils. The highest inhibitory effect on *A. tamarii* was showen by the essential oil of Kupusinski jabučar. The most sensitive was *E. amstelodami*. The growth of this mould was completely stopped at concentration of 7% (Junski srebrnjak) and 10% (Kupusinski jabučar).

Yin and Tsao (1999) investigated the antifungal effect of seven herbs from *Allium* family. According to their results, garlic showed the highest antifungal activity against three *Aspergillus* species investigated. Fistulosin, antifungal compound isolated from Velsh onion, had expressed antifungal activity against few mould species, especially *P. roqueforti* and *A. oryzae* (Phay et al., 1999). Hsieh et al. (2001) noticed high sensitivity of *A. niger* towards combined extract of cornelberry, cinnamon and oriental onion (1:6:6, vol/vol/vol). Benkebala (2004) confirmed the inhibitory effect of onion and garlic on *A. niger, P. cycloprium* and *F. oxisporum*. Hitikoto et al. (1980) and Hasan and Mahmoud (1993) showed that essential oils of caraway, clove, onion and garlic can influence the synthesis of sterigmatocystine and aflatoxins, toxic metabolits of *Aspergillus* species. Dimić et al. (2008) point out the antifungal activity of onion essential oil against *P. commune, P. aurantiogriseum, P. griseofulvum, P. corylophilum* and *A. ochraceus*. Investigations about the influence of different spice extracts on the growth of fungi showed that besides limiting the growth, they also cause changes in the morphology of colonies (Dimić et al., 2007a, 2007b), as well as changes on cellular level (destruction of fungal cells, decreasing the oxygen uptake, reducing the cellular growth, 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) (Tansey and Appleton, 1975; Adetumbi et al., 1986; G h a n n o u m, 1988; G u p t a and P o r t e r, 2001; R a s s o l i et al., 2006, C o r z o - M a r t i n e z et al., 2007).

CONCLUSION

Results obtained in this investigation point out that essential oils of investigated *Allium* plants could be useful in controlling the development of yeasts and moulds in different foods, acting directly against microorganisms of food deterioration, or with surroundings, in case of packaged foods.

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ИНХИБИТОРНИ УТИЦАЈ ЕТАРСКИХ УЉА *ALLIUM AMPELOPRASUM* L. И *ALLIUM CEPA* L. НА РАСТ НЕКИХ КВАСАЦА И ПЛЕСНИ

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Резиме

Етарска уља екстрахована из зачина као природни антимикробни агенси привлаче посебну пажњу због улоге коју би могла имати у заштити хране од микроорганизама и за разлику од синтетичких конзерванаса нису токсична. У раду је испитиван инхибиторни утицај различитих концентрација (1, 4, 7 и 10%) етарских уља *Allium ampeloprasum* и две сорте црног лука (*Allium cepa*) Јунски сребрњак и Купусински јабучар на три квасца (*Saccharomyces cerevisiae, Candida tropicalis* и *Rhodotorula* sp.) и три плесни (*Aspergillus tamarii, Penicillium griseofulvum* и *Eurotium amstelodami*). Сва три етарска уља имала су најјачи инхибиторни ефекат према *S. cerevisiae* већ при концентрацији од 1%. Од црних лукова, етарско уље (*Allium cepa*) Купусински јабучар је јаче деловало на *C. tropicalis*, док етарско уље *Allium ampeloprasum* није показало ефекат према овом квасцу. На *Rhodotorula* sp. једино је деловало етарско уље *Allium ampeloprasum*. Најјачи инхибиторни ефекат према *A. tamarii* је показало етарско уље (*Allium sera*) Купусински јабучар (57% инхибиције при концентрацији од 10%), а према *P. griseofulvum* етарско уље *Allium ampeloprasum* (78.3% при концентрацији од 10%). Раст *E. amstelodami* је потпуно инхибиран при 7 и 10% етарских уља (*Allium cepa*) Јунски сребрњак и Купусински јабучар, респективно.