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ANTIFUNGAL ACTIVITY OF THE BASIL (*Ocimmum basilicum* L.) EXTRACT ON *Penicillium aurantiogriseum, P. glabrum*, *P. chrysogenum*, AND *P. brevicompactum*

Sunčica D. Kocić-Tanackov[a], Gordana R. Dimić[a], Dušanka J. Pejin, Ljiljana V. Mojović[b], Jelena D. Pejin[a] and Ilija J. Tanackov[c]*

[a] University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[b] University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000, Belgrade, Serbia
[c] University of Novi Sad, Faculty of Technical Sciences, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia

This study was aimed at investigating the antifungal potential of basil (Ocimmum basilicum L.) extract against toxin-producing Penicillium spp. (P. aurantiogriseum, P. glabrum, P. chrysogenum, and P. brevicompactum) isolated from food.

The basil extract composition was determined by the GC-MS method. The major component identified in the extract was estragole (86.72%).

The determination of the antifungal activity of basil extract on Penicillium spp. *was performed using the agar plate method. Basil extract reduced the growth of Penicillium spp. at all applied concentration levels (0.16, 0.35, 0.70, and 1.50 mL/100mL) with the colony growth inhibition from 3.6 (for P. glabrum) to 100% (for P. chrysogenum).*

The highest sensitivity showed P. chrysogenum, where the growth was completely inhibited at the basil extract concentration of 1.50 mL/100mL. The growth of other Penicillium spp. was partially inhibited with the colony growth inhibition of 63.4 % (P. brevicompactum), 67.5% (P. aurantiogriseum), and 71.7% (P. glabrum).

Higher concentrations (0.70 and 1.50 mL/100mL) reduced the growth of the aerial mycelium of all tested Penicillium species. In addition, at the same extract concentrations, the examination of microscopic preparation showed the deformation of hyphae with the frequent occurrence of fragmentations and thickenings, occurrence of irregular vesicle, frequently without metulae and phialides, enlarged metulae.

The results obtained in this investigation point to the possibility of using basil extract for the antifungal food protection.

KEY WORDS: Basil extract, antifungal activity, *Penicillium* spp., food

* Corresponding author: Sunčica D. Kocić-Tanackov, University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia, e-mail: suncicat@uns.ac.rs

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INTRODUCTION

Penicillium species are videly spread in the nature and are frequent contaminants of food. Food products with the medium (0.75 - 0.9 a_w) and low (0.75 a_w) moisture content, as well as the acid medium favour their development. These species are frequent contaminants of storage products such as fruits, vegetables, spices, sausages, cheese, grain and grain products (flour, bread, cakes), etc. (1-3).

The metabolic activity of this species results in spoilage of food products and high economic damage. On the other hand, the toxin-producing species (*P. aurantiogriseum, P. brevicimpactum, P. chrysogenum, P. glabrum, P. expansum, P. nordicum, P. rugulosum, P. solitum, P. verrucosum,* etc.) biosynthesize toxic metabolites – mycotoxins (ochratoxin A, roquefortine C, patulin, citrinin, nephrotoxic glycopeptides, verrucosidin, citromycetin, botryodiploidin, mycophenolic acid, etc.). The consumption of food products contaminated with these mycotoxins, both by human and animal, leads to the occurrence of mycotoxicoses, which, further, have cytotoxic and cancerogenic effects on human cells (liver cells in the first place) (1-5).

Extracts and essential oils extracted from spices and other herbs, as well as their biologically active components, have attracted of attention many authors to investigate their antimicrobic activity (6-9). In food production and processing, the extracts and essential oils obtained from spices and other herbs are important in the food prevention from microorganisms, especially of short shelf-life products, which are the most sensitive to microbiologic spoilage (bread, bakery products, cakes, salads, fresh fruit and vegetable). Their use in the food industry decreases the use of synthetic preservers and additives, and, at the same time, improves the freshness and sensory of the product quality.

The paper presents a study of the antifungal effect of basil extract on the growth of toxin-producing *Penicillium* species isolated from food.

EXPERIMENTAL

Basil extract

For the testing of the antifungal activity, a commercially available, food grade basil extract was provided from ETOL Tovarna arom in eteričnih olj d.d., Celje, Slovenia.

Determination of basil extract composition

The composition of the extracts was determined by Gas Chromatography – Mass Spectrometry (GC-MS) analysis carried out on a Varian T2100 GC-MS instrument equipped with data processor. A fused silica capillary column VF-5MS (30 m x 0.25 mm i.d., 0.25 μm film thickness, Varian) was used for the separation of the sample components. The carrier gas, ultra pure helium, was passed through moisture and oxygen traps at a constant flow rate of 0.62 cm^3/min. The following temperature program was used: injector temperature 230°C, initial temperature 40°C (held 5 min), temperature increase 5°C/min to 200 °C and held at this temperature for 25 min. The mass spectrometer was

operated in the electron ionization mode. The data acquisition was carried out in the scan mode (range 50-550 m/z). The injection volume was 1 μl. The compounds were identified by matching the mass spectra with NIST Mass Spectra Library stored in the GC-MS database.

Fungal strains

The following fungal strains from the genus *Penicillium* were used as test microorganisms: *P. aurantiogriseum* Dierckx, *P. glabrum* (Wehmer) Westling, *P. chrysogenum* Thom, and *P. brevicompactum* Dierckx. The fungal cultures were isolated from cakes and fresh salads from different varieties of ready-for-use vegetables and maintained on Potato Dextrose Agar (PDA) (Merck, Darmstadt) at 4 °C as a part of the collection of the Laboratory for Food Microbiology at the Faculty of Technology, University of Novi Sad, Serbia.

Determination of the basil extract effect on the *Penicillium* spp. growth

The agar plate method was applied to test the antifungal activity of basil extracts. 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 extracts were added to the PDA to achieve the following concentrations: 0, 0.16, 0.35, 0.70, and 1.50 mL/100mL. The PDA containning different concentrations of basil extract was poured into sterile Petri dishes (ϕ 9 cm), 12 mL per dish.

The seven-day fungal cultures grown on PDA were used to prepare the fungal spore suspension tests. Suspensions of the fungi were prepared in a medium containing 0.5% Tween 80 and 0.2% agar dissolved in distilled water and were adjusted to provide initial spore count of 10^6 spores/mL by using a haemocytometer. For each extract dose and fungal species, including the controls, the dishes were centrally inoculated by spreading 1 μL of a spore suspension (10^3 spores/mL), using an inoculation needle. After the inoculation, the Petri dishes were closed with parafilm.

The effect of the basil extract on fungal growth was evaluated by a daily measurement of the diameter of the radial colony growth during 14 days of incubation at 25±2ºC. 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 15^{th} to 30^{th} day, the concentration of basil extract 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 basil extract was added, and were incubated for 5 days at 25±2 ºC for the determination of fungicide effect (MFC).

The inhibitory effect of the basil extract on fungal growth after 14 days was calculated from the following formula:

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

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).

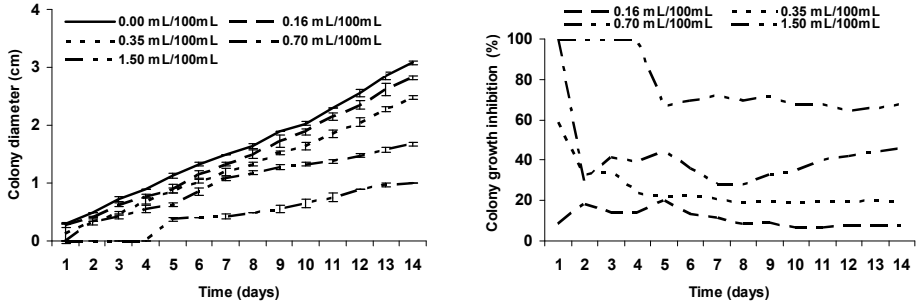
Each antifungal test was carried out in 3 series (2 replications in each series). Values are presented as means±SD of six measurements.

The changes in the macroscopic and microscopic features of the fungi were also observed and compared to the controls. The macroscopic features of spores were observed using a binocular, magnifying glass Technival 2, Carl Zeiss and the microscopic features using a microscope Aristoplan, Leitz.

RESULTS AND DISCUSSION

The basil extract at the investigated concentrations exhibited the capacity to reduce or inhibit the growth of the *Penicillium* species. The effect of the basil extract on the *Penicillium* spp. growth is presented in Figures 1-4. Table 1 shows the inhibitory effect (%) of the basil extract on the colony growth of *Penicillium* species on the 14th day of incubation.

The concentrations of basil extract, 0.7 and 1.5 mL/100mL resulted in the delay or no growth of investigated moulds with different inhibitory effect on growth rate decline. The extract concentration of 0.7 mL/100 mL delayed the growth of *P. aurantiogriseum*, *P. glabrum* and *P. brevicompactum* for 1 day. The germination of *P. chrysogenum* at this concentration was noticed on the first day. The highest applied concentration (1.5 mL/ 100 mL) was minimal fungicidal concentration (MFC) for *P. chrysogenum,* while the growth of other molds was delayed by 4 (*P. aurantiogriseum*), 7 (*P. brevicompactum*), and 8 (*P. glabrum*) days (Figures 1-4). The growth rate decline with the increase of the basil extract content in the PDA medium was more expressed in *P. aurantiogriseum* and *P. chrysogenum*, compared to the other two molds, pointing to their higher sensitivity (Figures 1-4).



(a) (b)

Figure 1. The influence of basil extract on the growth rate (a) and colony growth inhibition (b) of *P. aurantiogriseum*

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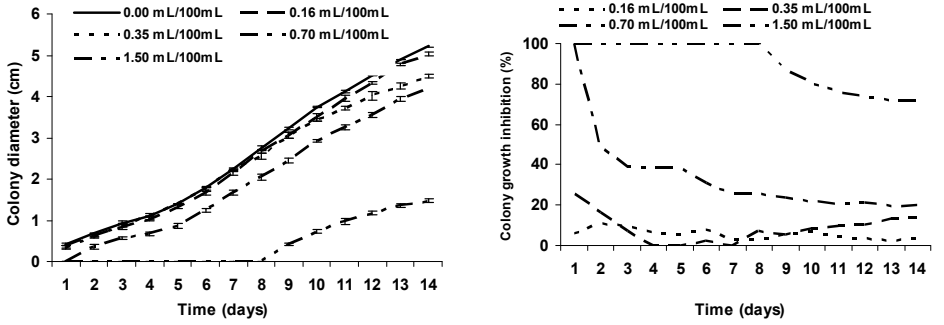
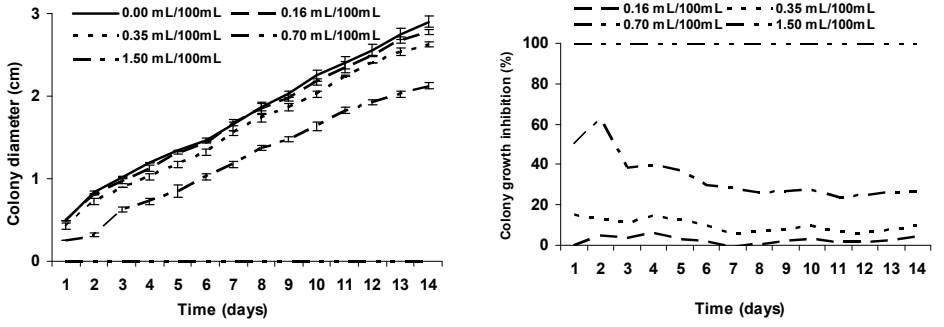


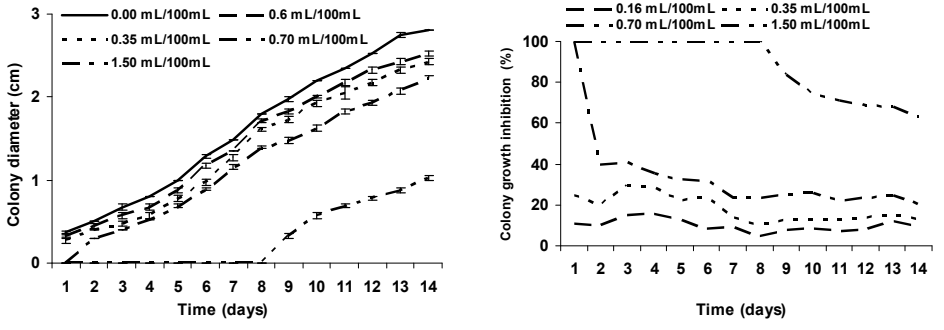
(a) (b)

Figure 2. The influence of basil extract on the growth rate (a) and colony growth inhibition (b) of *P. glabrum*



(a) (b)

Figure 3. The influence of basil extract on the growth rate (a) and colony growth inhibition (b) of *P. chrysogenum*



(a) (b)

Figure 4. The influence of basil extract on the growth rate (a) and colony growth inhibition (b) of *P. brevicompactum*

The lowest applied basil extract concentration (0.16 mL/100mL) inhibited the growth of all investigated fungi at a low inhibition level, from 3.6 to 9,8%. The weakest antifungal effect of the extract at this concentration was observed against *P. glabrum*, with the inhibition rate of 3.6%, while the growth of other fungi was decreased by 4.3% (*P. chrysogenum*), 7.3% (*P. aurantiogriseum*), and 9.8% (*P. brevicompactum*). The increased extract concentration (0.35 mL/100mL) in agar medium affected the increase of the growth inhibition of the investigated fungi from 9.5% (*P. chrysogenum*) to 18.7% (*P. aurantiogriseum*). The growth of *P. aurantiogriseum* was inhibited significantly (44.7%) at the concentration of 0.7 mL/100mL, and the reaction of the other investigated fungi was similar, with the inhibition degree from 19.9% (*P. glabrum*) and 26.7% (*P. chrysogenum*) (Table 1).

The strongest antifungal effect at the highest applied extract concentration (1.5 mL/100mL) was observed for *P. chrysogenum*. The growth of this fungi was completely stopped at this concentration, while in the other fungi, the growth was partially inhibited with high inhibition degree of 63.4% (*P. brevicompactum*) and 71.7% (*P. aurantiogriseum*) (Table 1).

Table 1. Inhibitory effect (%) of basil extract on the colony growth of *Penicillium* species after 14 days of incubation

Fungi	Basil extract concentration (mL/100mL)			
	0.16	0.35	0.70	1.50
	Colony growth inhibition (%)			
P. aurantiogriseum	7.3	18.7	44.7	67.5
P. glabrum	3.6	13.8	19.9	71.7
P. chrysogenum	4.3	9.5	26.7	100.0
P. brevicompactum	9.8	13.4	20.5	63.4

These results are in accordance with our previous investigations, showing a strong antifungal activity of basil extract towards *Fusarium* spp. (*F. oxysporum*, *F. proliferatum*, *F. subglutinans,* and *F. verticillioides*) isolated from cakes. Their growth was completely inhibited at the extract concentration of 1.5 mL/100mL (11). The antifungal investigations performed by Adigüzel et al. (12) by disc-difusion method are in contrast to our findings. They reported the inefficiency of the ethanol, methanol and hexane basil extracts on the growth of *Alternaria aternata*, *Aspergillus flavus, F. oxysporum* and *Penicillium* spp. at the concentrations of 300 μg/disc.

However, a number of studies report on strong antifungal action of basil essential oil. Doube et al. (13) using the agar plate method, showed that basil oil, in a concentration of 1.5 mL/L inhibited completely the growth of 22 species of molds, including the aflatoxigenic strains *Aspergillus parasiticus* and *A. flavus*.

Zollo et al. (14) reported that basil oil inhibited completely the growth of *Candida albicans* and *A. flavus* at a concentration of 5000 ppm, during 7 days of incubation, using microdilution method. Soliman and Badeaa (15) found that basil oil acts as a fungistatic agent against *F. verticillioides* in a concentration of 2000 ppm, and as a fungicid agent in concentration of 3000 ppm. The results presented by Fandohan et al. (16) showed a complete growth inhibition of *F. verticillioides* at concentrations higher than 2.7 μL/mL.

The antifungal activity of tested basil extract depends on the content of major and minor components. The content of estragol (methyl chavicol) was the highest in the investigated basil extract (86.72%). Besides estragol the basil extract contained: *trans*-*alfa*-bergamotene (2.91%), eucalyptol (2.67%), *trans*-ocimene (1.04%), cadinol (0.74%), linalol – syn. linalool (0.72%), methyl-eugenol (0.71%), δ-cadinene (0.49%), camphor (0.42%), *β*-elemene (0.37%), δ-guaiene (0.29%), menthol (0.27%), *β*-pinene (0.23%), limonene (0.22%), bornyl acetate (0.21%), *α*-pinene (0.16%), *β*-caryophyllene (0.15%), myrcene (0.15%), sabinene (0.13%), fenchone (0.13%), γ-muurolene (0.12%), borneol (0.10%), menthone (0.10%), *β*-sesquiphelladrene (0.10%), cubenol (0.11%), carvone (0.07%), *β*-selinene (0.07%), *cis*-*α*-bergamotene (0.06%), camphene (0.05%), *cis*-ocimene (0.05%), *p*-cymene (0.05%), *α*-humulene (0.04%), aromadendrene (0.03%), terpinolene (0.02%), γ-terpinene (0.02%), *α*-guaiene (0.01%). The majority of authors address linalol, estragol, eugenol and methyl cinnamate as the major antimicrobial components of basil extracts and essential oil (17-23). The study performed by Lis-Balchin et al. (18) point to a strong antifungal effect of oil which contained estragol as the main component on the growth of *Aspergillus niger*, *A. ochraceus*, and *Fusarium culmorum* (inhibition growth of 71.0 to 94.76%). These results are in accordance with the results and investigations of Baratta et al. (19), who found that estragol type oil inhibited the growth of *A. niger* by 93.1%. Reuveni et al. (17) investigated the effect of eteric basil oil components on the growth of *Rhizopus nigricans* and *F. oxysporum*. They found linalol and estragol to be more efficient against *R. nigricans* (100% of inhibition), compared to eugenol (38.1% of inhibition). Eugenol exhibited stronger inhibition towards *F. oxysporum* (100% of inhibition), in contrast to linalol and estragol, where the inhibition values were 26.4 and 30.3%, respectively.

Besides the effect of growth reduction, the investigated extract at higher concentrations caused the macro- and micromorphologicl changes of the fungi. The extract concentrations of 0.70 and 1.5 mL/100mL reduced the growth of the aerial mycelium in all tested species. In addition, at the same extract concentrations, the examination of the microscopic preparation showed deformation of hyphae, with frequent occurrence of fragmentations and thickenings, occurrence of irregular vesicle, frequently without metulae and phialides, enlarged metulae. These macro- and micromorphologic changes point to the possible changes at cellular level (reduction in the cellular growth, decrease in the oxygen uptake, inhibition of the synthesis of lipids, proteins and nucleic acids, changes in the lipid profile of the cell membrane and inhibition of the synthesis of the fungal cell wall) due to the action of extract components with functional groups of cellular enzymes (23-25).

CONCLUSIONS

This study proved that the tested basil extract can be used as a protective agent against *Penicillium* spp, frequent contaminants of food.

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АНТИФУНГАЛНА АКТИВНОСТ ЕКСТРАКТА БОСИЉКА (*Ocimmum basilicum* L.) НА РАСТ *Penicillium aurantiogriseum, P. glabrum*, *P. chrysogenum* И *P. brevicompactum*

Сунчица Д. Коцић-Танацков[а]*, *Гордана Р. Димић*[а], *Душанка Ј. Пејин*[а], *Љиљана В. Мојовић*[б], *Јелена Д. Пејин*[а] *и Илија Ј. Танацков*[в]

[а] Универзитет у Новом Саду, Технолошки факултет, Булевар цара Лазара 1, 21000 Нови Сад, Србија
[б] Универзитет у Београду, Технолошко-металуршки факултет, Карнегијева 4, 11000 Београд, Србија
[в] Универзитет у Новом Саду, Факултет техничких наука, Трг Доситеја Обрадовића 6, 21000 Нови Сад, Србија

Овај рад приказује антифунгални потенцијал екстракта босиљка на раст токсинпродукујућих *Penicillium* врста (*P. aurantiogriseum, P. glabrum*, *P. chrysogenum* и *P. brevicompactum*) изолованих из хране.

Састав екстракта босиљка одређен је GC-MS методом и у највећем проценту садржавао је естрагол (86,72%). Утицај екстракта на раст плесни испитан је методом агар плоча. При свим примењеним концентрација екстракта (0,16; 0,35; 0,70 и 1,50

mL/100mL) редукован је раст *Penicillium* spp. уз инхибицију раста колонија од 3,6 (*P. glabrum*) до 100% (*P. chrysogenum*).

Највећу осетљивост показала је плесан *P. chrysogenum*. Њен раст је био потпуно спречен при концентрацији од 1,5 mL/100mL. Раст осталих *Penicillium* spp. је делимично инхибиран са високим процентом инхибиције раста колоније од 63,4 (*P. brevicompactum*), 67,5 (*P. aurantiogriseum*) и 71,7% (*P. glabrum*). Више концентрације екстракта (0,70 и 1,5 mL/100mL) су редуковале мицеларни раст свих тестираних врста. Такође, при овим концентрацијама су у микроскопском препарату уочене деформације хифа са честом фрагментацијом и задебљањима, везикуле неправилног облика, појава проширених метула, али често без метула и фијалида.

Добијени резултати указују на могућност коришћења екстракта босиљка у антифунгалној заштити хране.

Кључне речи: Екстракт босиљка, антифунгална активност, *Penicillium* spp., храна

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