Зборник Матице српске за природне науке / Proc. Nat. Sci., Matica Srpska Novi Sad, № 113, 173—177, 2007

UDC 633.822:665.52

Milica Ljaljević Grbić, Jelena Vukojević, Marina Soković, Dragoljub Grubišić*, Mihajlo Ristić***

Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Takovska 42, 11000 Belgrade, Serbia
* Institute for Biological Research "Siniša Stanković", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia
** Institute of Medicinal Plant Research "dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia

THE EFFECT OF *NEPETA RTANJENSIS* ESSENTIAL OIL ON TEST MICROMYCETES MYCELIA GROWTH

ABSTRACT: The antifungal activity of *Nepeta rtanjensis* Diklić et Milojević essential oil on mycelia growth has been performed by macrodilution method. The most efficient impact of *N. rtanjensis* essential oil on mycelia growth *in vitro* was found in *Alternaria* species with the same value of minimal inhibitory quantity (MIQ) of 0.6 µl/ml. *Bipolaris spicifera* and *Cladosporium cladosporoioides* had MIQ values of 1.0 µl/ml whereas *Trichoderma viride* with MIQ value of 1.6 µl/ml showed the most efficient defense against the essential oil examined. The values of minimal fungicidal quantity (MFQ) in *Alternaria* sp. 2, *B. spicifera* and *C. cladosporioides* match the MIQ values, whereas MFQ values in *Alternaria* sp.1 is 0.8 µl/ml, and in *T. viride* 1.8 µl/ml.

KEY WORDS: Antifungal activity, essential oil, micromycetes, *Nepeta rtanjensis*

INTRODUCTION

Nepeta rtanjensis (*Lamiaceae*) is an endemic and critically endangered (CR B_{2c}) aromatic plant which grows only on few localities on the Rtanj mountain in southeastern of Serbia (Diklić, 1999).

Nepeta species are widely used in folk medicine because of their medical properties. The essential oil of *N. rtanjensis* possesses strong antibacterial effect against different strains of *Staphylococcus aureus*, even more stronger than most synthetic antibiotics (Stojanović et al., 2005). The main component of essential oil of *N. rtanjensis* is 4aα, 7α, 7aβ nepetalactone. In wild population of *N. rtanjensis* oil amount of 4aα, 7α, 7aβ nepetalactone is 86.4%, while in oil of cultivated plants this component is presented with 77.9% (Chalchar et al., 2000; Stojanović et al., 2005). It is well known

that fungal infection can be a great threat to plant, animal and human health. Medicinal plants are good source of natural products with strong antimicrobial activities without any harmful effects. The use of natural antimicrobial compounds is important in the control of human, animal and plant diseases of microbial origin.

The aim of our investigation was to evaluate the antifungal activity of *N. rtanjensis* essential oil against mycelia growth of the selected fungi.

MATERIALS AND METHODS

N. rtanjensis was collected on the experimental fields of the Institute for Biological Research "Siniša Stanković", Belgrade. The plants were rapid micropropagated *in vitro*, transferred to the Greenhouse for acclimatization, and subsequently planted in an experimental field (M i š i ć et al. 2005). Herbal material was deposited at the Herbarium of Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, Belgrade (16064 BEOU).

The essential oil was isolated from air-dried aerial part of *N. rtanjensis* by hydrodistillation in a Clevenger type apparatus within two hours. Analyses of this oil were performed by GC (-FID) and GC/MS on fused silica capillary column PONA (crosslinked methyl silicone gum, 50 m x 0.2 mm, 0.5 μm film thickness). For these purposes Hewlett-Packard, model 5890, series II gas chromatograph, equipped with split-splitless injector, was used. Sample solution in ethanol (0.2%) was injected in split mode (1:100) at 250°C. Detector temperature was 300°C (FID), while column temperature was linearly programmed from 40°—280°C, at a rate of 2°C/min. In the case of GC/MS analysis, Hewlett-Packard, model 5971A MSD was used. The transfer line was kept at 280°C. The identification of each individual compound was made by comparison of their retention times with those of pure components, matching mass spectral data with those from Wiley library of 138 000 MS spectra. For library search PBM based software package was used.

Among the tested organisms were two groups of micromycetes: the autochtonous species (*Alternaria* sp. 1 from leaves and *Alternaria* sp. 2 from seeds of *N. rtanjensis*) and selected fungal species (*Cladosporium cladosporioides*, *Trichoderma viride* and *Bipolaris spicifera*) from Mycotheca at the Department of Algology, Mycology and Lichenology, Faculty of Biology, University of Belgrade.

The fungi were maintained on malt agar (MA). The cultures were stored at +4°C and subcultured once a month. In order to investigate the antifungal activity of essential oil, the mycelial growth test with malt agar was used (I s h i i, 1995). The minimum inhibitory quantity (MIQ) of oil necessary for the inhibition of mycelial growth of the fungal strain was determined. Different concentrations of essential oil (0.6—1.4 μg/mL) were diluted in Petri dishes with malt agar (MA). All fungal species were tested in triplicate. Essential oils were added into molten malt agar (MA) and poured into Petri dishes. The tested fungi were inoculated at the centre of the plates. Plates were incubated for three weeks at room temperature, and after this period MIQ and

MFQ were determined. Petri plates with commercial fungicide, Quadris (0.6—6.0 μg/mL), were used as a control.

RESULTS AND DISCUSSION

The analysis of chemical composition of essential oil showed the prevalence of 4aα, 7α, 7aβ nepetalactone (79.89%). It was shown that the essential oil isolated from *N. rtanjensis* has strong antifungal activity against all examined micromycetes (MIQ 0.6—1.6 μg/mL), (Table 1).

Tab. 1 — Minimal inhibitory quantity (MIQ) and minimal fungicidal quantity (MFQ) of the tested micromycetes

Micromycetes	MIQ (μl/ml)	MFQ (μl/ml)
Alternaria sp. 1	0.6	0.8
Alternaria sp. 2	0.6	0.6
Bipolaris spicifera	1	1
Cladosporium cladosporioides	1	1
Trichoderma viride	1.6	1.8

The oil quantity in amount of 0.6—0.8 μg/mL inhibited the growth of *Alternaria* species mycelia. The minimal inhibitory quantity of oil for *C. cladosporioides* and *B. spicifera* was 1.0 μg/mL. The highest MIQ (1.4 μg/mL) of oil was against *T. viride*. The commercial fungicide, Quadris, showed lower antifungal activity than *Nepeta* oil, with MIQ of 3.0—4.0 μg/mL. Quadris inhibited mycelial growth of *C. cladosporioides, B. spicifera* and *Alternaria* species at 3.0—4.0 μg/mL. *T. viride* was also the most resistant fungi on Quadris, with MIQ higher than 6.0 μg/mL.

In previous investigations of antifungal activity of different oils it can be seen that *A. alternata* was more sensitive than *T. viride* (Sokvić et al., 2002). The strong resistance of *T. viride* was also observed in previous investigations of essential oil antifungal activity. Analyses of antifungal activity of some essential oils, *Achillea atrata* and *Lauraceae* plants, showed that *T. viride* is the most resistant fungi (Ristić et al., 2004; Simić et al., 2004).

Our research proved that essential oil from *N. rtanjensis* has strong antifungal activity, and that it can inhibit the growth of mycelia of some fungi. The antifungal activity of essential oils isolated from other *Nepeta* species are also reported. Iridodial b-monoenol acetate isolated from the essential oil of *Nepeta leucophyla*, and actidine isolated from *Nepeta clarkei*, showed strong antifungal activity. Iridodial b-monoenol acetate was the most effective against *Sclerotium rolfsii*, while actidine was highly active against *Macrophomina phaseolina*. Both fungi are soybean pathogens. The essential oil from *Nepeta hindostana* has inhibitory effect on *Pythium aphanillermatum*, *P. debaryanum* and *Rhyzoctonia solani* (Saxena et al., 1996).

Because of low mammalian toxicity and biodegradable abilities as well as strong antimicrobial activity of essential oils, they can be used as bioagents (Oxenham et al., 2005).

REFERENCES

Diklić, N. (1999): *Nepeta rtanjensis Diklić & Milojević*. In: *The Red Data Book of Flora of Serbia 1*, Extinct and Criticaly Endangered Taxa, Ed. V. Stevanović, 153—155.

Stojanović, G., Radulović, N., Lazarević, J., Miladinović, D. & Đoković, D. (2005): *Antimicrobial Activity of Nepeta rtanjensis Essential Oil*, J. Essent. Oil Res. 17, 587—589.

Chalchar, J. C., Gorunović, M. S., Petrović, S. D. & Maksimović, Z. A. (2000): *Composition of the essential oil Of Nepeta rtanjensis Diklić & Milojević, Lamiaceae from Serbia*, J. Essent. Oil Res., 12, 238—240.

Soković, M., Tzakou, Pitarokili, O. D. & Couladis, M. (2002): *Antifungal activities of selected aromatic plants growing wild in Greece*, Nahrurung/ Food, 5, 317—320.

Ristić, M., Soković, M., Grubišić, D. & Kovačević, N. (2004): *Chemical Analysis and Antifungal Activity of the Essential Oil of Acillea atrata L.*, J. Essent. Oil Res., 16, 75—78.

Simić, A., Soković, M., Ristić, M., Grujić-Jovanović, S., Vukojević, J. & Marin, P. D. (2004): T*he chemical composition of some Lauraceae essential oils and their antifungal activities*, Phytoterapy Res., 18 (9), 713—717.

Saxena, J. & Mathela, C. S. (1996): *Antifungal Activity of New Compounds from Nepeta leucophylla and Nepeta clarkei*, Applied and Environmental Microbiology, 702—704.

Oxenham, K. S., Svoboda, P. K. & Walters, R. D. (2005): *Antifungal Activity of the Essential Oil of Basil (Ocimum basilicum)*, Journal of Phytopathology, 153, 174.

УТИЦАЈ ЕТАРСКОГ УЉА *NEPETA RTANJENSIS* НА РАСТ МИЦЕЛИЈЕ ТЕСТ МИКРОМИЦЕТА

Милица Љаљевић Грбић, Јелена Вукојевић, Марина Соковић*,
Драгољуб Грубишић*, Михајло Ристић**
Институт за ботанику и Ботаничка башта „Јевремовац", Биолошки факултет,
Универзитет у Београду, Таковска 43, 11000, Београд, Србија
* Институт за биолошка истраживања „Синиша Станковић",
Булевар Деспота Стефана 142, 11000 Београд, Србија
** Институт за проучавање лековитог биља „др. Јосиф Панчић",
Тадеуша Кошћушка 1, 11000 Београд, Србија

Резиме

Макродилуционом методом тестиран је утицај етарског уља ендемичне биљке *Nepeta rtanjensis (Lamiaceae)* на мицелијални раст микромицета: *Alternaria* sp. 1, *Alternaria* sp. 2, *Bipolaris spicifera, Cladosporium cladosporioides* и *Trichoderma viride*. Хемијска анализа етарског уља *N. rtanjensis* показала је апсолутну доминацију 4aα, 7α, 7aβ непеталактона (79.89%). Најефикаснији утицај на раст мицелије *in vitro* забележен је код врста рода *Alternaria* са истом вредношћу минималне инхибиторне количине (МИК) од 0.6 μl/ml. *Bipolaris spicifera* и *Cladosporium cladosporioides* су имали МИК 1.0 μl/ml, док је *Trichoderma viride*, са вредношћу

МИК од 1.6 μl/ml, показала највећу отпорност на дејство испитиваног уља. Вредности минималне фунгицидне количине (МФК) се код *Alternaria* sp. 2 (0.6 μl/ml), *B. spicifera* (1.0 μl/ml) и *C. cladosporioides* (1.0 μl/ml) поклапају са вредностима МИК, док је код *Alternaria* sp. 1 МФК 0.8 μl/ml а код *T. viride* 1.8 μl/ml. Етарско уље *N. rtanjensis* показало је јако антифунгално дејство на раст мицелије тестираних микромицета.