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Original scientific paper

Elicitation effects of a synthetic 1,2,4,5-tetraoxane and a 2,5-diphenylthiophene in shoot cultures of two *Nepeta* species

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Abstract. The presented study aimed to investigate the elicitation possibility for the production of main secondary metabolites in *Nepeta cataria* L. and *N. pannonica* L. plants, by exposing them to synthetic compounds belonging to tetraoxanes and thiophenes group. The effect of DO63 (1,2,4,5-tetraoxane) and DOVF15 (2,5-diphenylthiophene) on the production of *cis–trans*-nepetalactone (NL) and rosmarinic acid (RA) in two *Nepeta* species, was investigated in shoots grown on the culture medium with the addition of synthetic compounds in the concentrations ranging from 0.1 to 2 mg L^{-1}. The content of targeted metabolites in tested *in vitro* shoots depended on the type and concentration of applied synthetic compounds. Application of DO63 in the concentration range 0.1–1 mg L^{-1} affected only NL production in both *Nepeta* species resulting in its increased content, while production of RA was not influenced in the treated shoots. Addition of DOVF15 caused decreased RA content in *N. pannonica* shoots and an increase in *N. cataria* shoots, whereas NL production was not affected. The presented results reveal the possibility of DO63 and DOVF15 application for the elicitation of the main secondary metabolites production in species from the genus *Nepeta.*

Keywords: 1,2,4,5-tetraoxanes; 2,5-diphenylthiophenes; *cis–trans*-nepetalactone; rosmarinic acid; *Nepeta cataria*; *Nepeta pannonica*.

INTRODUCTION

Plant species belonging to the genus *Nepeta* (the largest genus of family *Lamiaceae* comprised of approximately 300 species), produce various secondary metabolites (SM) such as terpenoids (monoterpenoids, diterpenoids, triterpenoids and sesquiterpenoids) and phenolic compounds.[1] In folk medicine *Nepeta* species

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are used in the treatment of gastrointestinal disorders, cough and asthma due to their spasmolytic and myorelaxant activities.[2] Their anti-inflammatory,[3] cytostatic activity,[4] as well as phytotoxic effect on plants,[5–8] behavioral effect on cats,[9,10] and repellent activity against some insects[11] are well known. The plethora of published literature data explain antibacterial, antifungal and antiviral activity of many different species from genus *Nepeta*.[12–19] Majority of the pharmacological and biological activities of *Nepeta* species are generally attributed to nepetalactone, an iridoid monoterpene lactone, which is dominantly present in the essential oils (EOs) of these plants.[17,20–23] Nepetalactones ($C_{10}H_{14}O_2$) exist in the form of 8 stereoisomers, *i.e.*, 4 diastereoisomers and their corresponding enantiomers,[24] but only 7*S* diastereoisomers are present in the natural environment. Stereochemistry of nepetalactones determines their biological activity.[11,25] *cis*–*trans*-Nepetalactone (NL) is the only stereoisomer that exists in *Nepeta pannonica*.[7] This stereoisomer is the dominant nepetalactone stereoisomer in *N. cataria* plants, although another stereoisomer (*trans*–*cis*-nepetalactone) is also present, as minor constituent.[26–31] Besides the nepetalactones, the most important bioactive substances in *Nepeta* species are phenolic acids.[1,23] In the *Lamiaceae* family the most abundant phenolic acids are rosmarinic and caffeic acids,[32] but the presence of rosmarinic acid (RA) is limited to *Nepetoideae* subfamily.[33] The methanol extracts of certain *Nepeta* species possess considerable antioxidant activity, which was attributed to phenolic acids, primarily to rosmarinic acid.[6] RA exhibits a variety of pharmacological properties such as antibacterial, antioxidant,[34] anticancerogenic[35] and antiviral,[36] including anti-HIV activity[37]. Due to its biological activity RA is often used in pharmaceutical, cosmetic and food industries.[38–41]

In vitro plant culture technologies have become of major industrial prominence regarding large scale plant multiplication, plant propagation and improvement, elimination of plant diseases, production of secondary metabolites, and their utilization as a research tool.[42] The application of elicitors is one of the most effective strategies to increase *in vitro* productivity of the plant culture,[43–46] in a time-saving manner.[47] Plant elicitors are compounds that can initiate morphological and physiological responses, including plant defense compound stimulation.[48] Commonly *in vitro* tested chemical elicitors are phytohormones, primarily salicylic and jasmonic acid.[49,50] Recently, the application of different synthetic compounds indicated their potential in elicitation of the secondary metabolism in some plants.[51–54]

In this work, synthetic compounds tetraoxane DO63 (1,2,4,5-tetraoxane) and tiophene DOVF15 (2,5-diphenylthiophene) were tested for their elicitation potential of *in vitro* grown *N. cataria* and *N. pannonica* shoots. Tetraoxane DO63 was initially developed as antimalarial agent[55] that was capable to generate oxygen radicals as principal active intermediates responsible for biological activity.[56–58] On the other hand, thiophene derivatives are very well known for their remark-

able pharmacological activities as anti-inflammatory agents or as serotonin antagonists used in treatment of Alzheimer's disease.[59] Derivative DOVF15 was previously shown to moderate binding affinities to Ab plaques, which are produced during development of Alzheimer's disease.[60] The assumption of the presented study was that application of DO63 could cause oxidative stress in treated plants, while DOVF15 might interfere with the protein component of enzymes. The cumulative effect could stimulate production of certain secondary metabolites as a part of activated plant defense mechanisms.

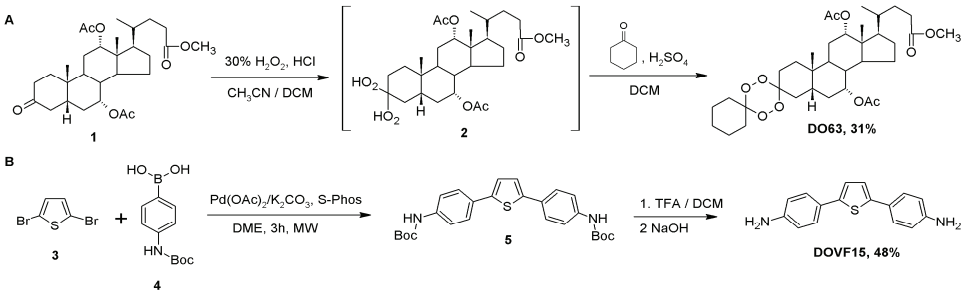
EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded on Varian Gemini-200 spectrometer (at 200 and 50 MHz, respectively) and on Bruker Ultrashield Advance III spectrometer (at 500 and 125 MHz, respectively) employing indicated solvents (*vide infra*) using tetramethylsilane (TMS) as the internal standard. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. ESI mass spectra of ligands were recorded on 6210 time-of-flight LC–MS instrument (G1969A, Agilent Technologies) in positive ion mode with CH_3CN/H_2O. Reactions carried out employing MW conditions were performed using a Biotage Initiator Eight Robot with an automatic sampler (USA and Sweden).

Chemical compounds used as elicitors

Methyl 7α,12α-diacetoxy-3,3-[cyclohexilidenebis(dioxy)]-5β-cholan-24-oate (DO63, Scheme 1A). Tetraoxane DO63 was obtained according to the procedure described by Šolaja *et al*.[55] Briefly, methyl 7,12-diacetoxy-5β-cholan-24-oate (**1**) was transformed into *gem*-dihydroperoxide **2** using 30 % H_2O_2, under acid catalysed peroxyacetalysation. To the solution of dihydroperoxide **2** (500 mg, 0.90 mmol) in CH_2Cl_2 (14 mL) cyclohexanone (1.80 mmol) was added at r.t., and the reaction mixture was cooled down while stirring in an ice-bath. After 30 min, 599 μL of ice-bath cooled H_2SO_4:CH_3CN mixture (1:10, *V*/*V*) was added dropwise. The reaction mixture was stirred at 0 °C for 15 min, and after usual work-up crude product was purified by column chromatography (dry-flash, SiO_2, eluent heptane/EtOAc (8:2)) to assure 31 % yield of tetraoxane. All spectral and analytical data were identical to literature reported.

2,5-Bis(4-aminophenyl)thiophene (DOVF15, Scheme 1B). Derivative DOVF15 was synthesized applying modified Pd-catalyzed procedure (Lopez *et al.*,[61] Gonzales *et al.*[62] and Chandra *et al.*[60]). Mixture of $Pd(OAc)_2$ (28 mg, 0.13 mmol) and SPhos (84 mg, 0.20 mmol) in 1,2-dimethoxyethane (DME, 16 mL) was stirred at room temperature in an inert atmosphere (Ar) for 10 min. 2,5-Dibromothiophene (**3**, 0.15 mL, 1.24 mmol) was added, and subsequently 2 M water solution of K_2CO_3 (3 mL, 6 mmol). After 5 min stirring at room temperature, *N*-Boc-4-aminophenylboronic acid 4 (588 mg, 2.48 mmol) was added and the reaction mixture was heated in microwave (MW) oven for 3 h at 100 °C. Solvents were removed under reduced pressure; residue was dissolved in dichloromethane (20 mL), washed twice with 25 mL of saturated Na_2CO_3 solution and once with brine, and subsequently dried using anhydrous Na_2SO_4. After solution was concentrated to a quarter of the initial volume, 5 ml of trifluoroacetic acid (TFA) was added, and mixture was stirred at room temperature for 5 h. Following solvent removal under reduced pressure, residue was dissolved in 250 mL dichloromethane, washed three times with 50 mL of saturated Na_2CO_3 solution and once with brine, and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure. Yield (195 mg, 59 %). All spectral and analytical data were identical to literature reported.



Scheme 1. A – synthesis of 1,2,4,5-tetraoxane, DO63;[55] B – synthesis of 2,5-diphenyl-tiophenes, DOVF15.[60-62]

Plant material

Seeds of *Nepeta pannonica* (syn. *nuda*) L. were collected in 2007 at Rimski Šančevi (Vojvodina, Serbia), while seeds of *N. cataria* L. were purchased in 2008 from Grugapark Essen (Germany). Seeds were kept at 10 °C until use.

Nepeta seeds were surface sterilized in 20 % solution of commercial bleach (0.8 % active chlorine) for 10 min and rinsed 5 times in sterile distilled water. The germination of seeds was induced by 24 h treatment with 1 mM solution of GA_3 containing 500 mg L^{-1} nystatin. Seeds were washed 5 times with sterile distilled water and subsequently transferred into 350 mL glass jars closed with polycarbonate caps, each containing 70 mL of culture medium (CM): a modified MS medium[63] with half-strength macro- and micro-elements, and supplemented with 20 g L^{-1} sucrose and 7 g L^{-1} agar. Prior to sterilization in an autoclave at 114 °C for 25 min, pH of CM was adjusted to 5.8. Glass jars were kept in growth chamber at 25±2 °C temperature and 16/8 h light/dark regime (irradiance of 70 µmol m^{-2} s^{-1}).

To obtain genetically uniform material, plants were micropropagated using one-node stem segments as explants. Sub-cultivation was performed every 4 weeks. Four month-old *N. pannonica* and *N. cataria* cultures were used in the experiments.

Experimental design

One-node stem segments of *N. pannonica* or *N. cataria* were placed in 350 mL glass jars containing 75 mL CM. After 2 weeks explants were transferred onto CM supplemented with DO63 or DOVF15 in concentrations: 0, 0.1, 0.25, 0.5, 0.75, 1 and 2 mg L^{-1}). DO63 was previously dissolved in ethanol (1 mg L^{-1}), filter sterilized (pore size 0.2 µm), and added into autoclaved CM; DOVF15 was dissolved in deionized water pH 3 (1 mg L^{-1} final concentration) and was added into CM before sterilization by autoclaving at 114 °C for 25 min. Fresh and dry weights (FW and DW, respectively) of explants were measured two weeks later. The experiments were repeated three times, using 21 explants each.

Phytochemical analysis

Preparation of the plant extracts. The plant material, *in vitro* grown shoots of *Nepeta cataria* and *N. pannonica*, was air-dried and stored at room temperature until use. Each sample (250 mg) was converted into fine powder by liquid nitrogen (LN) and soaked in 1 mL of methanol (Zorka, Šabac, Serbia). After 2 h, at 4 °C, samples were further extracted in an ultrasonic bath (Bandelin Sonorex, Germany) for 15 min, and subsequently centrifuged (Heraeus Biofuge Stratos Centrifuge, Thermo Electron Corporation, Germany) at 12000*g* for 15 min at 4 °C. The supernatants were filtered using Econo filter, pore size 0.2 µm (Agilent

Technologies, Santa Clara, CA, USA) and kept at 4 °C until analyses. The extractions were performed in triplicates.

Ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC––MS/MS) identification of nepetalactone and rosmarinic acid. Separation and identification of components in methanolic extracts of the two Nepeta species were performed using Dionex Ultimate 3000 UHPLC system equipped with diode array detector (DAD), and connected to TSQ Quantum Access Max triple-quadrupole mass spectrometer (Thermo Fisher Scientific, Basel, Switzerland). Elution was performed at 40 °C on Syncronis C18 column (100 mm×2.1 mm, 1.7 μm particle size) from Thermo Fisher Scientific. The mobile phase consisted of water (A) + 0.1 % formic acid and acetonitrile (B), which were applied in the gradient elution previously described.[23] The flow rate was set to 0.4 mL min^{-1}, and the wavelengths to 225, 260 and 320 nm, and the injection volume was 5 μL.

TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI) source, was used with vaporizer temperature kept at 300 °C, and the ion source settings as follows: spray voltage 4500 V, sheet gas (N_2) pressure 40 AU, ion sweep gas pressure 1 AU and auxiliary gas (N_2) pressure at 10 AU, capillary temperature at 275 °C, skimmer offset 35 V. Collision-induced fragmentation experiments, product ion scanning (PIS) and selected reaction monitoring (SRM) experiments, were performed using argon as the collision gas, and the collision energy was set to 30 eV. Xcalibur software (version 2.2) was used for instrument control, data acquisition and analysis.

Rosmarinic acid (RA) was identified by direct comparison to the commercial standard, based on its UV and MS spectra, characteristic MS/MS fragmentation patterns and retention time. A 1 mg L^{-1} of stock solution was prepared in methanol, and further diluted with methanol to yield 10 mg L^{-1} working solution. Essential oil of *N. rtanjensis*, containing 72 % of *trans–cis*-nepetalactone and 16 % of *cis–trans*-nepetalactone was used as standard for nepetalactone identification and quantification, as previously described.[23]

Quantification was performed based on the calibration curves for each standard. Total amounts for each compound in the samples were expressed as mg per g of fresh weight, and are shown as percentage of the values measured in the control (Fig. 1). Calibration curves revealed good linearity, with r^2 values for rosmarinic acid and *cis–trans*-nepetalactone of 0.9992 and 0.9996, respectively. Limit of detection *(LOD*) and limit of quantification (*LOQ*), as revealed by UHPLC–MS/MS analyses in SRM experiment, were 0.02 and 0.06 μg mL^{-1} for RA, and 0.03 and 0.09 μg mL^{-1} for *cis–trans*-nepetalactone, respectively.

Statistical analyses

Statistical analyses were performed using Statgraphics software, version 4.2 (STSC Inc. and Statistical Graphics Corporation, 1985-1989, USA). The data were subjected to one-way analysis of variance (ANOVA). Differences between means were evaluated by Fisher's LSD test calculated at the confidence level of $P \leq 0.05$.

RESULTS AND DISCUSSION

It is considered that elicitors activate an array of defense reactions; including the induced or enhanced synthesis of plant defensive secondary metabolites (SM) in order to ensure survival, persistence and competitiveness of plants.[64] The exact mechanism of elicitation in plants is vaguely elucidated and various assumptions have been proposed like Ca^{2+} as a messenger, factors affecting cell membrane integrity, inhibition/activation of intracellular pathways and changes in osmotic

stress *etc*.[64] Different elicitors do not follow the same string of events. The route varies depending on the origin, specificity, concentration, phytochemical environment, stage of plant growth or level of plant nutritional uptake, *etc*. Abiotic elicitors are characterized by the non-biological origin, and usually include inorganic salts, Cu and Cd ions, Ca^{2+} or high pH.[64] Synthetic compounds have been proven to possess respectable potential for the elicitation of the SM production in plants and the application of these substances is therefore increasing.

Previous studies revealed rosmarinic acid is the major phenolic compound in the *Nepeta* species, [1,23,32,65–68] including *N. cataria* and *N. pannonica*. *Cis–trans*--nepetalactone is reported to be the major terpenoid constituent in *N. cataria*,[21,23] while *N. pannonica* is generally characterized by the low amount of volatiles and possesses only trace amounts of this iridoid monoterpenoid.

The UHPLC/DAD analysis and UHPLC/(−)HESI–MS/MS data confirmed the presence of RA, a caffeic acid derivative, in the samples analysed. The peak corresponding to RA was identified at *Rt* = 4.83 min, revealing characteristic deprotonated molecule *m*/*z* $[M-H]^-$ of 359, and UV absorbtion maxima at λ_{max} of 240 and 320 nm (Fig. 1A). MS/MS spectrum of RA showed characteristic fragments at *m*/*z* of $[M-H-C_9O_4H_8]^-$ 179 and *m*/*z* $[M-H-C_9O_3H_6]^-$ 197 (Fig. 1B), assignable to caffeic acid and quinic acid moieties, respectively. According to the UHPLC/DAD and UHPLC/(+)HESI–MS/MS data, peak corresponding to *cis*–*trans*-nepetalactone was chromatographically separated at *Rt* = 6.56 min, with

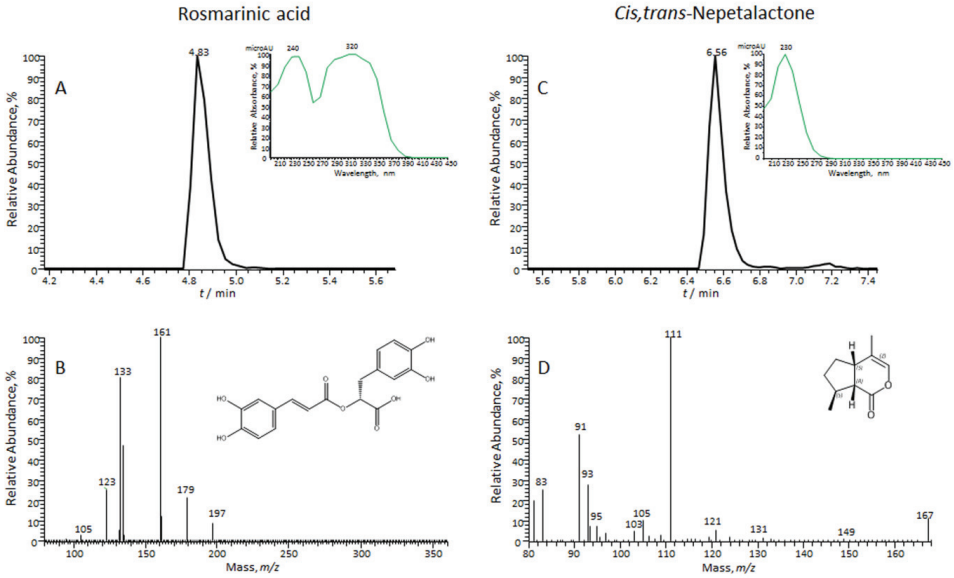


Fig. 1. Product ion scanning (PIS) chromatograms and DAD spectra of rosmarinic acid (A) and *cis–trans*-nepetalactone (C), and corresponding MS/MS spectra (B and D).

protonated molecule *m*/*z* $[M+H]^+$ of 167, and λ_{max} of 230 nm (Fig. 1C). MS/MS spectrum obtained for *cis,trans*-nepetalactone showed fragments at *m*/*z* of $[M+H-C_4H_8]^+$ 111, *m*/*z* $[M+H-C_5H_{16}]^+$ 91, and *m*/*z* $[M+H-C_6H_{12}]^+$ 83 (Fig. 1D).

Two characteristic MS^2 fragments of each compound were selected and utilized in the SRM experiment for the accurate quantification of targeted chemical species, as previously described:[23] *m*/*z* of 135 and 197 for RA, and *m*/*z* of 77 and 111 for *cis–trans*-nepetalactone (Fig. 2).

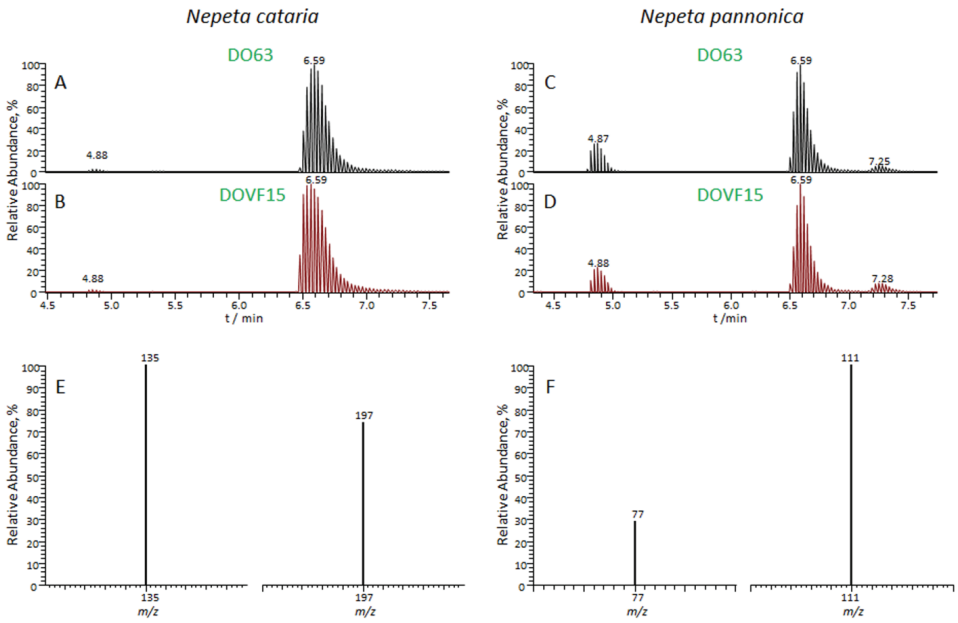


Fig. 2. Selected reaction monitoring (SRM) chromatograms of *N. cataria* and *N. pannonica* shoots grown on CM treated with DO63 (A and C) or DOVF15 (B and D) elicitors, and corresponding MS/MS spectra of rosmarinic acid (E) and *cis–trans*-nepetalactone (F) identified in samples.

Both analyzed plant species have approximately the same content of rosmarinic acid (about 1 mg g^{-1} DW), whereas *N. cataria* is characterized by a high content (about 40 mg g^{-1} DW), and *N. pannonica* by a low content (about 1.5 mg g^{-1} DW) of *cis–trans*-nepetalactone. The content of rosmarinic acid and *cis–trans*-nepetalactone influenced by the synthetic elicitors DO63 and DOVF15, was further analyzed. The application of synthetic compounds was performed two weeks after cutting the explants in order to minimize the effects of wounding on the secondary metabolite production.[69–72] The wounding activates plant defense responses, including accumulation of terpenoids and phenolic compounds, which is often accompanied by *de novo* synthesis or increased activity of enzymes involved in their biosynthetic pathways.[64]

Nepetalactone accumulation in *N. pannonica* and *N. cataria* explants was enhanced after two weeks of DO63 treatments. The maximum increase of NL content in *N. pannonica* shoots was observed on DO63 doped medium in the range from 0.1 to 1 mg mL^{-1} (Fig. 3A). The NL measure was approximately 4.5 times higher in the plants treated with 0.75 mg mL^{-1} DO63 in comparison to the control. Similarly, the treatment with DO63 in the same range of concentrations increased the NL content in *N. cataria* explants roughly 1.4 times (Fig. 3C). The application of DO63 did not have effects on the content of rosmarinic acid in *N. pannonica* and *N. cataria* shoots (Fig. 3B and D). According to the presented results, DO63 showed moderate stimulatory effects on the production and accumulation of NL, especially in *N. pannonica* plants which generally contain much less NL than *N. cataria*. On the other hand, addition of DOVF15 affected the production and accumulation of rosmarinic acid in the treated *Nepeta* shoots, which leads to a higher content of RA in explants of *N. cataria* (0.75 and 1 mg L^{-1}) or to decrease of RA in *N. pannonica* shoots (0.5 mg L^{-1}; Fig. 3B and D). RA production was increased more than 3.5 times in *N. cataria* shoots after appli-

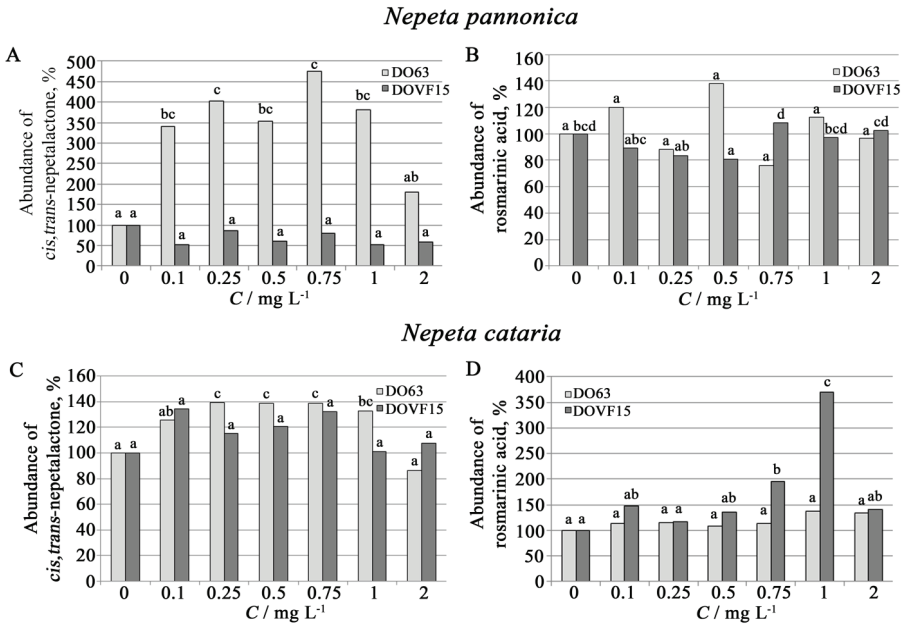


Fig. 3. Contents of secondary metabolites in: *Nepeta pannonica* seedlings, *cis–trans*-nepetalactone (A) and rosmarinic acid (B) and *N. cataria* seedlings, *cis–trans*-nepetalactone (C) and rosmarinic acid (D). One-node stem segments of *N. pannonica* and *N. cataria* plants were grown for 2 weeks on CM and subsequently on CM supplemented with DO63 or DOVF15 (0, 0.1, 0.25, 0.5, 0.75, 1 and 2 mg L^{-1}) for 2 weeks. The values with the same letter above bars belong to statistically homogenous groups ($P \leq 0.05$), according to Fischer's LSD test.

cation of 1 mg L^{-1} DOVF15 (Fig. 3D) or decreased by 20 % in comparison to the control plants of *N. pannonica* (Fig. 3B). Synthetic thiophene showed no effects on NL production and accumulation. In general, different effects of the rosmarinic acid and nepetalactone production were observed in *N. pannonica* and *N. cataria* explants treated with DOVF15.

There are scarce literature data describing stimulation of nepetalactone production in plants. Mišić *et al.*[73] demonstrated that nepetalactone accumulation in *Nepeta rtanjensis* shoots was significantly affected by the type and levels of carbohydrates applied in culture media. The most efficient carbon source was glucose as described by Mišić *et al.*[73] Exogenous auxins and polyamines were previously reported to enhance rosmarinic acid production in hairy root culture of *N. cataria*.[74] Salicylic acid stimulates the biosynthesis of rosmarinic acid in *Coleus forskohlii* hairy root,[75] while sodium salicylate did not not affect RA production in sage.[50] Elicitation with vanadyl sulfate led to 2.8 times increased RA production compared to the control in *Lavandula vera* MM cell suspension cultures.[76] Similarly, benzothiadiazole, a synthetic activator of plant systemic acquired resistance, significantly enhanced the elicitation of RA production in suspension cultures of *Agastache rugosa*.[77]

Growth of *N. pannonica* and *N. cataria* explants was not affected by the treatments with DO63 and DOVF15 regardless of applied concentrations (0.1–2 mg L^{-1}); specifically, fresh and dry weights of treated explants did not significantly differ from the control group (Fig. 4).

Furthermore, morphological alterations were not observed regardless of the type or concentration of the applied compounds. Considering that production of either NL or RA increased, although the growth of explants was not changed, DO63 and DOVF15 were recognized as potential agents for enhancing SM biosynthesis and accumulation. Since the application of DO63 only affected NL production, whereas DOVF15 only influenced the production of RA, it could be concluded that these two compounds actually have an impact on biosynthetic pathways of different SM groups: terpenoids (monoterpenoids) and phenolics (phenolic acids). General biochemical responses, induced by the application of elicitors involve elicitor binding to an elicitor-binding sites or receptors in plant plasma membranes, rapid changes in protein phosphorylation patterns and protein kinase activation, mitogen-activated protein kinase (MAPK) activation, NADPH oxidase activation, G-protein activation, cytoplasm acidification, *etc.*[64,78] Defense response genes expression during elicitation process is well documented as well as production of reactive oxygen species (ROS) and involvement of oxidative burst.[64,78] Due to its complexity and intricacy, elicitation process is still under investigation and is not yet revealed. Presented results with DO63 and DOVF15 implicate that these compounds have a good potential in the elicitation of two groups of secondary metabolites. However, the mechanism of elicitation

induced by DO63 and DOVF15, as well as their effect on the accumulation of wider range of compounds and/or SM classes is still to be further studied.

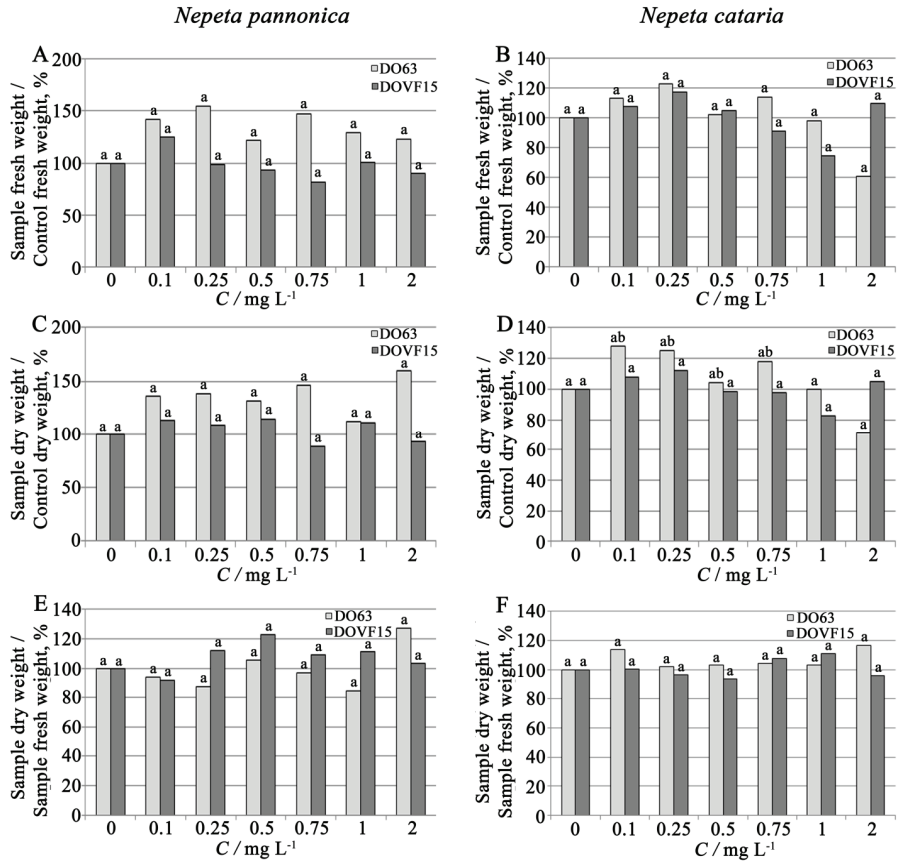


Fig. 4. Fresh and dry weight (FW and DW) and content of DW in FW of *Nepeta pannonica* and *N. cataria* seedlings. One-node stem segments of *N. pannonica* and *N. cataria* plants were grown for 2 weeks on CM and subsequently on CM supplemented with DO63 or DOVF15 (0, 0.1, 0.25, 0.5, 0.75, 1 and 2 mg L^{-1}) for 2 weeks. The values with the same letter above bars belong to statistically homogenous groups ($P \leq 0.05$), according to Fischer's LSD test.

CONCLUSION

Synthetic compounds DO63 and DOVF15 could be recommended as elicitors in stimulation of the secondary metabolites production belonging to the group of phenolic acids and monoterpenoids, respectively. Further efforts should be focusing on achieving, directly or indirectly, practical application of these elicitors in the production of NL and RA, not only in *Nepeta* species, but also in numerous other species containing these compounds. This could be applicable in pharmaceutical, food, and cosmetics industry. However, widespread utilization of

tested compounds requires detailed selection of the optimal plant development stage, estimation of the effective dose and timing of the treatment,[64] but also environmental risk assessments. The real challenge would be to avoid negative effects of synthetic compounds on normal growth and development of plants, but at the same time boost the production of secondary metabolites of interest. Future work is conducted towards investigation of the DO63 and DOVF15 elicitation effects on a variety of plant species and on other groups of secondary metabolites, but also toward testing of different synthetic compounds.

SUPPLEMENTARY MATERIAL

Characterization data for the synthesized compounds are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ЕЛИЦИТАЦИЈА СЕКУНДАРНИХ МЕТАБОЛИТА У ИЗДАНЦИМА ДВЕ ВРСТЕ РОДА *Nepeta* ГАЈЕНИМ *in vitro* ПРИМЕНОМ СИНТЕТИЧКИХ ЈЕДИЊЕЊА ТИПА 1,2,4,5-ТЕТРАОКСАНА И 2,5-ДИФЕНИЛТИОФЕНА

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Истраживање је било усмерено ка испитивању продукције главних секундарних метаболита у изданцима две врсте рода *Nepeta* изложеним дејству потенцијалних елицитора, синтетичких једињења из групе тетраоксана и тиофена. Ефекат DO63 (из групе 1,2,4,5-тетраоксана) и DOVF15 (из групе 2,5-дифенилтиофена) на продукцију *cis–trans*-непеталактона (NL) и рузмаринске киселине (RA) код две врсте рода *Nepeta*, *N. pannonica* L. и *N. cataria* L., испитиван је у изданцима гајеним на ½ MS хранљивим подлогама којима су додата синтетичка једињења у опсегу концентрација од 0,1 до 2 mg/L. Садржај циљаних метаболита у испитиваним изданцима гајеним у *in vitro* условима зависио је од типа и концентрације примењених синтетичких једињења. Третман са DO63, посебно у опсегу концентрација од 0,1 до 1 mg/L, утицао је на повећану продукцију NL код обе испитиване врсте *Nepeta*, док на продукцију RA није имао ефекта. Третман са DOVF15 је условио смањење садржаја RA у изданцима *N. pannonica* и повећање садржаја RA у изданцима *N. cataria,* док на продукцију NL није било утицаја. Резултати указују на могућност и значај примене DO63 и DOVF15 у елицитацији продукције главних секундарних метаболита код биљака рода *Nepeta*.

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