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APPLICATION OF PTR-MS IN DETECTION OF VOLATILE COMPOUNDS: *IN VITRO* CULTURE OF THREE *NEPETA* SPECIES

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Here we demonstrate the possibilities of PTR-MS (Proton Transfer Reaction Mass Spectrometer, Ionicon Analytik, Innsbruck, Austria) application in the detection of volatile organic compounds (VOCs) under *in vitro* conditions. After their accumulation in the glandular trichomes of *in vitro* grown plants, VOCs are released from the leaf surfaces and spread over the culture vessels, in which the plants are cultivated. PTR-MS offers the possibility of sensitive VOCs detection without sample preparation or chromatography [1].

Iridoid monoterpenoid, nepetalactone, the dominant compound in essential oils of majority medicinal and aromatic *Nepeta* species (fam. *Lamiaceae*), is the compound of interest in our investigations. The various biological activities of *Nepeta* species are usually ascribed to this volatile compound. PTR-MS was used for the measurements of nepetalactone content in the atmosphere of glass jars.

Shoot cultures of *Nepeta rtanjensis* Diklić & Milojević, *N. sibirica* L. and *N. nervosa* Royle & Bentham were grown on ½ MS culture medium, under long day conditions (16/8 h light/dark cycle), at a temperature of 25±2°C, and a relative humidity of 60-70%. Qualitative and quantitative content of nepetalactone in shoots of three *Nepeta* species were determined by HPLC-DAD analyses [2]. The presence of these compounds in the atmosphere of culture vessels was confirmed by the PTR-MS [3,4]. High concentration of this monoterpenoid in the atmosphere of glass jars [ppbV] on its protonated mass m/e=167, was detected in *N.sibirica* and especially in *N.rtanjensis* shoot cultures. For *N. nervosa*, nepetalactone was detected only in traces. We further investigated the effect of different carbohydrate sources (sucrose, fructose and glucose) in culture medium on the accumulation of nepetalactone in shoot cultures of *N.rtanjensis*, *N.sibirica* and *N. nervosa* and its subsequent release. Qualitative and quantitative content of nepetalactones varied among different species, and was influenced by the sugar source [4]. Furthermore, HPLC-DAD analysis of nepetalactone content in shoot cultures of three different genotypes of *N.rtanjensis*, combined with PTR-MS detection of this volatile compound in the atmosphere of glass jars, was suggested to be efficient in selection of nepetalactone-rich genotypes of this species [5]. In words of nepetalactone accumulation and its relief from the leaf surface, significant differences between *N. rtanjensis* genotypes were observed.

PTR-MS also offered the possibility to investigate the allelopathic potential of nepetalactone [6]. Seeds of garden cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and love-lies-bleeding (*Amaranthus caudatus* L.) were cocultivated with shoots of *N. rtanjensis*, *N. sibirica* and *N. nervosa*. The increase in nepetalactone

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content in the atmosphere of glass jars was strongly correlated with the decrease of *L. sativum* and *L sativa* seed germination and seedling growth. This effect was more pronounced in cultures with *N sibirica* and especially *N. rtanjensis.* The inhibition of seed germination was not observed for *N.* nervosa, which was previously reported to contain nepetalactone only in traces. Allelopathic potential of nepetalactone was not observed for *Lotus corniculatus* and *Amaranthus caudatus.*

In conclusion, PTR-MS offers a great possibility for our further research aimed at determining various biological activities of nepetalactone, but also of other VOCs under *in vitro* conditions**.**

Reference

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