Original Paper

Effect of growing season upon microbial status of peppermint (*Mentha* × *piperita* L.) rhizosphere

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In this study we assessed the number of microorganisms in rhizosphere of peppermint (*Mentha* × *piperita* L.) as one of the important aromatic species in Serbia. The trial was set up on chernozem soil at experimental field of Bački Petrovac, Institute of Field and Vegetable Crops, Novi Sad (northern Serbia) in 2012, 2013 and 2014. Rhizosphere soil samples were collected from both conventional and organic grown peppermint. In each year of investigation two samplings (June and July) were performed for microbiological analysis. Soil samples were analyzed by the standard methods in four replications and average number of microorganisms was calculated at 1.0 g absolutely dry soil. The results were analyzed in accordance with three-way model of analysis of variance. The obtained results showed that a significantly higher number of ammonifiers, azotobacters and free nitrogen-fixing bacteria was recorded in 2012 compared to 2013 and 2014. Also, a significantly higher number of azotobacters and free nitrogen-fixing bacteria was obtained in organic than in conventional growing. In addition, significant differences in number of fungi and actinomycetes were recorded between the sampling terms, i.e. significantly higher numbers of these groups of microorganisms were found in the second term.

Keywords: *Mentha* × *piperita*, peppermint, microorganisms, conventional and organic growing, rhizosphere

1. Introduction

Peppermint (*Mentha* × *piperita* L.) is a perennial herb belonging to the *Lamiaceae* family. This plant species is native to Europe where it is both cultivated and naturalised in many European countries and in North America. The genus *Mentha* L. comprises many species, but only four are reported to be cultivated commercially: *Mentha arvensis* L., *Mentha* × *piperita* L., *Mentha citrata* Ehrh. and *Mentha spicata* L. (Chand et al., 2004). Herb of these plants is used in medicine, cosmetics and food industry, therefore, these species are widely grown around the world. Species of the genus *Mentha* are among the plants which have often been used for their therapeutic properties (Andro et al., 2013). Essential oil of *Mentha* L. has many interesting facilities, for example antibacterial and antioxidant properties (Gulluce et al., 2007).

The rhizosphere is the soil region that is influenced by plant roots and is characterized by a high microbial activity. The microbial community composition in the rhizosphere is important for the performance of the plant (Sylvia and Chellemi, 2001). Many species of microorganisms, most of which are found in the rhizosphere have beneficial effects on plant growth and on crop yield and quality (Van Loon, 2007). The diversity and structure of microorganisms communities is plant–specific and varies over time (Barriuso et al., 2005). Diversity of microorganisms is affected by the plant age, the season and the soil conditions (Hrynkiewicz et al., 2010). Microbes that are closely associated with roots in the rhizosphere are likely to be primarily influenced by patterns of root exudation that are known to vary both quantitatively and qualitatively between plant species (Gransee and Wittenmayer, 2000). The release of root exudates, influenced by plant species, plant age and root zone, creates unique and attractive environment for microbial growth and activity (Merbach et al., 1999). Many rhizosphere interactions between different factors are known, demanding further investigations to determine this problem (Huang et al., 2014).

In this study we assessed the number of microorganisms in rhizosphere of peppermint (*Mentha* × *piperita* L.) as one of the important aromatic species in Serbia.

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2. Material and methods

The trial was set up on chernozem soil at experimental field of Bački Petrovac, Institute of Field and Vegetable Crops, Novi Sad (northern Serbia) in 2012, 2013 and 2014.
Rhizosphere soil samples were collected from both conventional (Con) and organic (Org) grown peppermint. Two sampling terms (June and July) were performed in each year of investigation to collect samples for microbiological analysis.

Soil samples were analyzed by the serial–dilution method followed by plating on different selective mediums. The total number of microorganisms was determined on agarized soil extract (10^7) and number of ammonifiers on MPA medium (10^6) (Pochon and Tardeux, 1962). The method of fertile drops on Fjodorov medium (10^2) was used to determine Azotobacter number (Anderson, 1958). The number of fungi was determined on Czapek-Dox medium, and actinomycetes on a synthetic medium (10^4). The number of cellulolytic microorganisms was done on Waksman-Carey medium (10^5). Fjodorov medium was used for determination of N-fixing microorganisms (10^6). The microbiological analyses were done in four replications and the average number of microorganisms was calculated at 1.0 g absolutely dry soil (Jarak and Đurić, 2004).

The results were analyzed in accordance with three-way model of analysis of variance (ANOVA) using Statistica software (StatSoft Inc., 2013). Means between the levels of the factors were separated by Tukey' HSD using a 5% level of significance.

3. Results and discussion

Data presented in Table 1 show that the average soil temperature at 0.1 m depth in June and July of 2012 (as well as air temperature) was higher than in 2013 and 2014, possibly indicating a positive impact upon an increase in numbers of microorganisms of individual groups. Plant adaptations to drought stress may cause changes in below ground C input through higher root production and turnover. This may in turn influence the functional structure and activity of the microbial community in the rhizosphere (Grayston et al., 1998). The extreme moisture deficiency that we observed with the current prolonged drought can have direct consequences on soil microorganisms and their activities.

The obtained results (Table 2) showed that a significantly higher number of ammonifiers, azotobacters and free nitrogen-fixing bacteria was recorded in 2012 compared to 2013 and 2014. Also, a significantly higher number of azotobacters and free nitrogen-fixing bacteria was obtained in organic than in conventional growing. In addition, significant differences in number of fungi and actinomycetes between the sampling terms were recorded, i.e. significantly higher numbers of these groups of microorganisms were found in the second term (July). Wang et al. (2012) ascertained higher abundance and diversity of total cultivable bacteria and fungi in organic soils. Gaur and Kaushik (2012) reported significant differences in the number of species of mycorrhizal fungi between different years, locations and also between studied medicinal plants. In previous study (Adamović et al., 2015) differences between four medicinal and aromatic plants in different

Table 1 Mean monthly temperatures in °C and sum of precipitations in mm in 2012, 2013 and 2014 at Bački Petrovac (Serbia) locality

Month/Parameter	**Year**		
	2012	**2013**	**2014**
May			
Soil temperature at 0.1 m depth	18.8	19.2	18.0
Air temperature	17.9	17.7	16.9
Precipitation	40.4	95.5	178.5
June			
Soil temperature at 0.1 m depth	25.2	22.3	23.9
Air temperature	23.4	20.2	21.1
Precipitation	62.3	124.5	23.2
July			
Soil temperature at 0.1 m depth	27.8	26.1	24.6
Air temperature	25.2	22.7	22.4
Precipitation	44.3	35.4	146.0

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Table 2 Number of microorganisms in rhizosphere of peppermint in three years, two sampling terms and conventional and organic growing systems

Group of microorganisms	Year			Sampling period		Growing system	
	2012	2013	2014	I	II	Con	Org
Total microbial number	246[a]	238[a]	241[a]	237[a]	246[a]	237[a]	246[a]
Ammonifiers	192[a]	104[b]	89[b]	102[a]	155[a]	129[a]	128[a]
Azotobacters	156[a]	70[b]	85[b]	107[a]	100[a]	75[b]	133[a]
N-fixing bacteria	302[a]	99[b]	174[b]	202[a]	182[a]	143[b]	241[a]
Fungi	14[a]	15[a]	9[a]	9[b]	17[a]	11[a]	15[a]
Actinomycetes	27[a]	14[a]	25[a]	14[b]	30[a]	21[a]	23[a]
Cellulolytic bacteria	13[a]	10[a]	10[a]	10[a]	12[a]	11[a]	11[a]
Cellulolytic actinomycetes	13[a]	13[a]	8[a]	10[a]	13[a]	12[a]	11[a]
Cellulolytic fungi	7[a]	9[a]	9[a]	7[a]	9[a]	6[a]	11[a]

The different letter above the number indicates a significant difference at $P < 0.05$

growing systems and sampling terms were recorded. In peppermint, the highest microbial number for several groups of microorganisms was detected. Solaiman and Anawar (2015) stated that most of the microorganisms in the rizosphere are related to plant species. The rhizosphere effect is mainly based on the reproduction and distribution of microorganisms influenced by root growth and the environment (Bürgmann et al., 2005), and the well-studied rhizosphere effect describes the phenomenon that, in comparison with bulk soil, the biomass and activity of microorganisms is enhanced as a result of exudation of compounds by the root (Raaijmakers et al., 2009). Karthikeyan et al. (2008) found that, in four medicinal plants, the microbial population is greater in the rhizosphere when compared to bulk soil. However, Berg and Smalla (2009) observed that some other medicinal plants, for example chamomile, thyme and eucalyptus, contained unique antimicrobial metabolites in their exudates which influenced the structure and function of microbial communities. Many microbes present in rhizosphere have neutral effect on plants, while others positively or negatively affect host development and health via complex interactions. Particular and specific interactions between plants and microbial groups need to be compatible at a physiologic level (Compant et al., 2005).

4. Conclusions

Peppermint plays an important role in the microbial status of rhizosphere. It can be concluded that 2012 growing season (for three microorganisms groups), organic growing system (for two microorganisms groups) and the second sampling term – July (for two microorganisms groups) exhibited significantly higher effect upon increase in microbial number of peppermint rhizosphere.

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