

Microbial Abundance in Rhizosphere of Medicinal and Aromatic Plant Species in Conventional and Organic Growing Systems

Dušan Adamović · Ivica Đalović · Nastasija Mrkovački

Summary: This study was aimed at comparing the abundance of microorganisms in the rhizosphere of four different medicinal and aromatic plant species (basil, mint, dill and marigold) grown under both conventional and organic management on the chernozem soil at the experimental field of Bački Petrovac (Institute of Field and Vegetable Crops, Novi Sad, Serbia). Two sampling terms (June 1 and July 18, 2012) were performed to collect samples for microbiological analyses. The microbial abundance was higher in organic than in conventional system while at the same time significant differences were obtained only with dill rhizosphere. The differences in number of microorganisms belonging to different groups relied upon both plant species and sampling term. Thus, in mint, the recorded number of azotobacters and fungi was significantly higher whereas the number of ammonifiers was significantly lower. The present results indicate that organic growing system affected the abundance of microorganisms in rhizosphere of species investigated, especially in the second term of sampling.

Keywords: basil, dill, marigold, microbial abundance, microorganisms, mint, organic agriculture, rhizosphere

Introduction

Microorganisms play an important role in soil fertility because they oxidise organic matter and promote the biogeochemical cycles of carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) (Balloni & Favilli 1987). The microbial activity in soil is controlled by several environmental factors such as availability of C, mineral nutrients and growth factors, water availability, temperature and pH, composition of soil microflora and ecological interactions between microorganisms (Nannipieri et al. 2003, Barbosa et al. 2013).

On the other hand, World Health Organization (WHO) has defined medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs (WHO 2008). So, medicinal herbs are well known as sources of phytochemicals, or active compounds that are widely sought-after for their natural properties (Rasouli–Sadaghiani et al. 2010). The production of natural substances by plants is affected by both genotype and environmental conditions (Sharafzadeh & Ordoorkhani 2011). Growth, quality and health of medicinal plants are highly influenced and controlled by their

microbiota via microbial metabolism and host interactions. Medicinal plants harbour a distinctive microbiome due to their unique and structurally divergent bioactive secondary metabolites that are most likely responsible for the high specificity of the associated microorganisms (Qi et al. 2012). Fundamentally important in plant nutrition, growth promotion and disease interactions are microorganisms (bacteria) associated with plant root. Therefore, a considerable interest has been aroused in characterizing the structure and function of rhizosphere communities. Plant–microbe interactions can positively influence plant growth through a variety of mechanisms, including fixation of atmospheric nitrogen by different classes of proteobacteria (Moulin et al. 2001), increased biotic and abiotic stress tolerance imparted by the presence of endophytic microbes (Scharndl et al. 2004) and direct and indirect advantages imparted by plant growth–promoting rhizobacteria. Plant species effects are important variables in determining the bacterial species composition of the rhizosphere. The results are different community structures for various species grown in the same soil (Grayston et al. 1998, Miethling et al. 2000, Patkowska & Konopiński 2014). Effects of plant species are probably due to

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differences in the composition of root cell components and root exudates (Merbach et al. 1999). Soils also exert strong effects on rhizosphere communities (different pH, aeration and physical and chemical characteristics) (Gelsomino et al. 1999, Carelli et al. 2000). The number of bacteria in the rhizosphere relies upon mineral nutrition factors such as nitrogen and iron (Yang & Crowley 2000) and long-term fertilization (Stępień et al. 2014) which change the composition of the rhizosphere community. In some cases, the effect of soil type was greater than that of the plant species (Buyer et al. 1999) whereas in studies of Grayston et al. (1998) and Miethling et al. (2000), plant species had a stronger effect on the community composition than soil type. Here we selected four different plant species grown in the same soil to establish plant effects on microbiological community composition of rhizosphere.

The present investigation was carried out to compare the abundance of microorganisms in rhizosphere of four medicinal and aromatic plant species (basil, mint, dill, and marigold) in two growing systems (conventional and organic).

Materials 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). The preceding crop in both growing systems (conventional and organic) was wheat. Soil samples were collected at depth of 0-30 cm and analysed prior to crop planting (Tab. 1).

Rhizosphere soil samples were collected from both conventional and organic grown four medicinal and aromatic plant species, namely basil (*Ocimum basilicum* L.), mint (*Mentha × piperita* L.), dill (*Anethum graveolens* L.), and marigold (*Calendula officinalis* L.). Samples for microbiological analyses were collected in two sampling terms (June 1 and July 18, 2012). Soil samples were analysed by the serial-dilution method followed by plating on different selective mediums.

Total number of microorganisms was determined on soil extract agar (10^6) and number of ammonifiers on MPA medium (10^6) (Pochon & Tardeux 1962). The method of fertile drops on Fjodorov medium (10^2) (Anderson 1965) was used to determine *Azotobacter* number. The number of fungi was determined on Czapek–Dox medium, and actinomycetes on a synthetic medium (10^4). The number of cellulolytic microorganisms was determined on Waksman-Carey medium (10^5). Fjodorov medium was used for determination of N-fixing microorganisms (10^6). The microbiological analyses were done in three replications and the average number of microorganisms was calculated at 1.0 g absolutely dry soil (Jarak & Đurić 2004). The variables were analysed in accordance with three-way model of analysis of variance (ANOVA) (Tab. 2) using Statistica software (StatSoft Inc. 2013).

All sources of variation in the model were considered as fixed. In order to ensure the validity of the model assumptions, data were transformed by square root transformation. Means between the levels of the factors were separated by Tukey' HSD and letter groupings was generated using 5% level of significance.

Table 1. Analysis of soil samples from conventional and organic plots

Growing system	pH in KCl	pH in H ₂ O	CaCO ₃ %	Humus %	Total N %	Al-P ₂ O ₅ mg/100 g	Al-K ₂ O mg/100 g	Organic C %
Conventional	7.50	8.32	2.51	2.37	0.176	34.7	32.3	1.623
Organic	7.42	8.19	2.80	2.48	0.188	21.2	24.8	1.734

Table 2. Analysis of variance of microbial abundance of three factor experiment ANOVA ($-\log_{10}$ (P) values)

Source of variation	Basil	Mint	Dill	Marigold
Replication	0.11	0.24	0.73	1.27
Growing system (A)	0.12	1.09	1.39	1.18
Group of microorganisms (B)	>4.0	>4.0	>4.0	>4.0
Date of sampling (C)	>4.0	0.07	1.02	0.46
A × B	0.95	1.49	0.02	0.39
A × C	1.13	0.35	3.10	0.92
B × C	>4.0	0.29	>4.0	>4.0
A × B × C	1.28	0.70	0.85	0.12

Results and Discussion

The comparison between rhizosphere of four conventionally and organically grown medicinal and aromatic plants showed that a higher number of microorganisms was found in organic system, but no significant differences were obtained except for dill (Tab. 3).

With respect to the tested microbial groups, there were also no significant differences in total microbial number and number of azotobacters and actinomycetes between four plant species (Tab. 3).

A significantly lower number of ammonifiers was evidenced in mint rhizosphere than in rhizosphere of other plants. The number of free living N fixers in mint and marigold rhizosphere was significantly higher than in rhizosphere of basil and dill, being similar to the number of both fungi and cellulolytic bacteria. Significant differences between sampling terms were recorded only in basil, namely, the number of microorganisms was higher in second term, with exception for mint (Tab. 3). These data point to a significant effect of plant species on rhizosphere microbial properties. The results show that organic variant highly influenced the abundance of microorganisms in all the species investigated, in the second sampling term in particular.

When the number of microorganisms in rhizosphere was analysed, the most effective plant species was mint, followed by marigold, dill and basil. When attention is directed for microbial abundance, mint promoted the increase in the total number of microorganisms, number of azotobacters, free nitrogen-fixing and cellulolytic microorganisms and reduced the number of ammonifiers. On the other hand, marigold affected the increase of the total number of microorganisms, ammonifiers, azotobacters and fungi. Finally, basil increased the number of ammonifiers and reduced the total number of microorganisms and fungi. Significant differences in the number of ammonifiers

were obtained between the rhizosphere of mint and rhizosphere of other studied species, then in the number of free nitrogen fixers between the rhizosphere of marigold and mint as well as between the rhizosphere of basil and dill. Significant differences in the number of fungi between the rhizosphere of marigold and mint and between rhizosphere of basil and dill were also recorded (Tab. 3). The same regularity was observed in the number of cellulolytic microorganisms, i.e. between the rhizosphere of mint and marigold where a significantly higher number of microorganisms was recorded than in rhizosphere of basil and dill.

The diversity and composition of a microbial community in the rhizosphere can be affected by several factors including plant species, soil type (Hoitink & Boehm 1999), soil management practices (Delcá & Stere 2013), microbial interactions (Hedges & Messens 1990) and other environmental variables. The rhizosphere of most crops may have some effect on the soil microbial communities within the first 0.1 m of soil. Root exudates from different plants can stimulate the growth of unique bacterial and fungal populations in the vicinity of roots. Thus, the determination of size and composition of soil microbial communities associated with different rhizospheres under organic farming practice is essential in order to evaluate above- and below-ground agroecosystem health and functioning. Our results are in agreement with those of Ramesh et al. (2012) who analysed the differences in microbial abundance and diversity between rhizosphere of four medicinal plants species. Significant differences in the number of species of mycorrhizal fungi between different years, locations and also between three studied medicinal plants, including the basil, were reported by Gaur & Kaushik (2012). In addition, our results confirmed previous studies indicating that both root exudates and plant species strongly determine the microbial composition of the rhizosphere, producing plant genotype-specific community structures in the same soil

Table 3. Microbial abundance in rhizosphere of medicinal and aromatic plant species in conventional and organic growing systems

Plant species	Basil	Mint	Dill	Marigold
Growing system				
Conventional	86.1±83.6 ^a	119.3±97.9 ^a	95.4±81.8 ^b	96.7±93.7 ^a
Organic	95.6±105.3 ^a	158.3±153.2 ^a	112.9±100.9 ^a	131.9±138.1 ^a
Group of microorganisms				
Total number of microorganisms	180.7±140.7 ^a	289.7±80.9 ^a	188.3±48.9 ^a	230.7±101.1 ^a
Number of ammonifiers	166.9±79.3 ^a	148.1±66.3 ^b	198.5±86.0 ^a	223.7±77.6 ^a
Total number of <i>Azotobacter</i>	78.9±35.4 ^b	155.8±39.6 ^b	115.1±39.1 ^b	97.2±63.5 ^b
Number of N-fixing microorganisms	130.9±112.4 ^a	302.3±124.3 ^a	157.1±101.9 ^{ab}	192.8±153.7 ^a
Number of fungi	9.8±8.6 ^d	14.6±5.9 ^c	8.9±2.3 ^d	10.2±5.4 ^c
Number of <i>Actinomycetes</i>	33.5±4.9 ^c	27.1±11.0 ^c	34.3±10.0 ^c	22.7±3.4 ^c
Number of cellulolytic microorganisms	22.9±14.5 ^{cd}	33.9±17.8 ^c	26.6±14.4 ^{cd}	35.2±20.8 ^c
Date of sampling				
June 1	71.2±78.6 ^b	142.2±135.9 ^a	98.2±92.1 ^a	101.4±98.3 ^a
July 18	110.5±105.5 ^a	135.4±124.0 ^a	110.0±92.1 ^a	127.2±135.9 ^a

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

The same is true for a long-term field trial in which organic and conventional agricultural systems were compared, where microbial biomass was higher in soils from organic plots (Tu et al. 2005, Liu et al. 2007). Fraser et al. (1994) reported 10-26% increase in microbial biomass under organic management. Other researchers have shown that incorporation of organic amendments increased soil microbial activity (Elliott et al. 1994), microbial diversity (Girvan et al. 2004) and densities of bacteria (van Bruggen & Semenov 2000). Results of Das & Dkhar (2011) showed that application of organic fertilizers enhanced the microbial population and increased rhizosphere soil physical and chemical properties compared to inorganic fertilizers and control. Okur et al. (2009) reported that soil organic C and soil microbial biomass as well as protease, urease, alkaline phosphatase, and dehydrogenase activity were significantly higher in the organic system than in the conventional system. Araujo et al. (2008) showed that the highest organic carbon and soil microbial biomass C levels occurred in organic system plots compared to the conventional system, and that the soil respiration was significantly enhanced by the organic system plots. On the contrary, some studies found that there were no differences in bacterial biodiversity (Lawlor et al. 2000) or in fungal communities (Franke-Snyder et al. 2001) between organically or conventionally managed soils. In contrast, Donnison et al. (2000) found that a change in management had no effect on soil nutrient status, soil microbial biomass, and soil microbial activity. Shannon & Johnson (2002) compared the microbiology of organically and conventionally managed soils and showed that percentage of metabolically-active bacteria present was not determined by management practice, while total and active fungi were found to be more abundant in organically-managed soils. The total bacterial and fungal communities revealed similar colonization patterns in *Matricaria chamomilla* and *Calendula officinalis* compared with *Solanum distichum* (Koberl et al. 2013). This effect may be intensified due to a close relationship between plants of *Asteraceae* family that produce similar bioactive metabolites. Furthermore, the first two are annual herbs while *Solanum distichum* is a perennial plant that provides longer time-frame to specifically select a stable associated microbiome. Tamilarasi et al. (2008) enumerated the total heterotrophic bacteria, actinomycetes and fungi from the rhizosphere and non-rhizosphere soil of 50 selected locally available medicinal plants. In all the plants, population of microorganisms was higher in the rhizosphere soil than in the non-rhizosphere soil. Higher abundance and diversity of total culturable bacteria and fungi were ascertained in organic soils by various methods (Wang et al. 2012). The nitrogen fixing and ammonia-oxidizing bacteria were found to

play an important role in N cycling, even though some studies mainly focused on total bacteria and fungi. Orr et al. (2011) stated that management regime affecting both the total bacterial community and the free-living diazotroph community could be secondary to other factors such as time of sampling and previous crop.

Conclusions

Our results show that the organic growing system had a great influence on abundance of microorganisms in all the species studied, especially in the second term of sampling.

Generally, obtained results indicated that mint promoted the increase in the total number of microorganisms, number of azotobacters, free nitrogen-fixing and cellulolytic microorganisms and reduced the number of ammonifiers. Marigold affected the increase of the total number of microorganisms, ammonifiers and fungi. Basil increased the number of ammonifiers and reduced the total number of microorganisms and fungi. Dill increased number of ammonifiers and reduced number of nitrogen-fixing microorganisms and fungi. A better understanding of nutrients recycling in conventional and organically grown plants therefore demands a comprehensive knowledge and monitoring of microbes involved in N and C cycle under different farming systems.

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Brojnost mikroorganizama u rizosferi lekovitih i aromatičnih biljnih vrsta u konvencionalnom i organskom sistemu proizvodnje

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Sažetak: Cilj istraživanja bio je da se uporedno ispita brojnost mikroorganizama u rizosferi četiri lekovite i aromatične biljne vrste (bosiljak, pitoma nana, mirođija i neven) koje su gajene na konvencionalan i organski način na zemljištu černozeu na eksperimentalnom polju u Bačkom Petrovcu (Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija). Uzorci za mikrobiološku analizu uzeti su u dva termina (1. jun i 18. jul 2012. godine). Brojnost mikroorganizama bila je veća u organskom sistemu u odnosu na konvencionalni, ali je značajna razlika zabeležena samo za rizosferu mirođije. Razlike u broju mikroorganizama koji pripadaju različitim grupama zavisile su kako od biljne vrste tako i od termina uzorkovanja. Tako je na primer kod pitome nane zabeležen visoko značajno veći broj azotobaktera i gljiva, dok je broj amonifikatora bio značajno manji. Dobijeni rezultati ukazuju da organski način gajenja više utiče na brojnost mikroorganizama u rizosferi ispitivanih vrsta, posebno u drugom terminu uzorkovanja.

Cljučne reči: bosiljak, brojnost mikroorganizama, mikroorganizmi, mirođija, neven, organska poljoprivreda, pitoma nana, rizosfera