European Journal of Sustainable Development (2017), **6**, 3, 423-429 ISSN: 2239-5938
Doi: 10.14207/ejsd.2017.v6n3p423

Beneficial Role of Biofertilization on Yield Related Characteristics of Two Apple Cultivars and Soil Microorganisms under Orchard Conditions

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Abstract
Intensive development of agriculture and increasing utilization of synthetic nitrogen fertilizers significantly contributes to a series of undesirable effects and results in excessive environmental pollution. Approximately half of all nitrogen applied to boost agricultural production escapes its intended use and is lost to the environment. Over the past years, fruit growing practice has been focused on a new concept, which relies primarily on the application of microbial inoculants i.e. biofertilizers. In line with the concept, we conducted comparative study of the effect of bio- and chemical fertilizer applications on yield-related characteristics of 'Granny Smith' and 'Čadel' apple cultivars and microbiological properties of the soil. The yield-related characteristics of the tested plants were evaluated for generative potential parameters i.e flowering intensity, fruit set intensity, final fruit set intensity and yield (kg tree^{-1}; t ha^{-1}).The microbiological properties of soil were monitored by determining the total microbial count, numbers of soil fungi, actinomycetes, oligonitrophilic bacteria and *Azotobacter*. The analysis of the results points to the fact that the efficient apple nutrition management should ensure both enhanced and sustainable production. This approach seems to contain a certain potential as an appropriate technique in commercial apple production, which may improve yield-attributing characteristics and soil fertility.

Keywords: bacterial fertilizer, (Malus domestica Borkh), soil biological properties, generative potential

1. Introduction

Fertilization is one of the basic agro-technical practice in plant production, which contributes most to the efficiency and intensity. The active biological roles of N element on metabolism activities in fruit trees, with special emphasis on yield production, fruit quality and improving performance of roots, make N-fertilizers some of the most widely used fertilizers in the world (Mengel and Kirkby, 1987; Westwood, 1993; Raese and Drake, 1997). However, improper nitrogen fertilization practice (excessive and indiscriminate use) may cause disturbances in the functioning of the entire agro-system, with a negative impact on the development of soil microorganisms and fertility of farming land (Styła and Sawicka, 2009). According to Barabasz and Vořišek (2002), incorrect agro-technical treatments and irrational application of fertilization may contribute to the development of different noxious compounds in soil environments (nitrozoamines, mycotoxins) acting unfavourably upon soil microorganisms, as well as upon cultivated plants and fertility of arable soils.
In addition to that, treatment with chemical fertilisers in delicate environment (water protection area, region of rivers and other watercourses, landscape and national parks) is

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ecologically controversial and causes partial leaching of nutrients, especially nitrogen and phosphorus. According to Bockman et al. (1990), more than 50% of the applied chemical fertilizers are not adopted by plants, but lead to their loss in different ways instead.
Despite negative effects on the environment, the increase in chemical fertilizer consumption followed by the increase of the world population and the need for greater food production will have been increased several times by the year of 2050.
To overcome those and to achieve and maintain the optimum balance between growth and productivity, modern fruit production is focused on monitoring various parameters of growth in the plantations, from the early spring until the fruit-bearing season, while sustaining regular checks of the soil's productive capacity (Miller, 2002), as well as on using fertilizers that are less harmful to the environment and less expensive for consumers. Current trends are focused on the use of the microbial inoculants, so called biofertilizers. Mosa et al. (2015) report that the biofertlization of fruits is considered a healthy alternative and/or supplement to chemical fertilizers. Pešaković et al. (2015; 2017) quote that the application of biofertilizers in apple production leads to increasing the number and microbial diversity in apple rhizosphere, which results in improving the production capacity.
The aim of this paper is to examine the impact made by the type of fertilizer and apple genotype on the most significant parameters of generative potential and microbiological activity of the soil.

2. Material and Method

The study was conducted at the apple production plantation of the Fruit Research Institute in Čačak, Republic of Serbia (43^{o} 53' N latitude, 20^{o} 20' E longitude, 225 m altitude) and included two apple cultivars i. e. 'Granny Smith' and 'Čadel'. The plantation was set up using standard one-year-old plantings grafted on the M9 rootstock, planted at the 4 x 1 m planting distance (2.500 trees per ha^{-1}).
In the course of the study, two types of fertilizer were used – the chemical fertilizer (Multi-Comp Base 14-13-20 + 2MgO + ME) and two bio-fertilizers (PGPR 1 and PGPR 2).
The Multi-Comp Base 14-13-20 + 2MgO + ME is a highly soluble chemical fertilizer from the 'Haifa Chemicals Ltd' production programme. PGPR 1 represents a pure culture of *Gram*-negative nitrogen-fixing bacteria *Klebsiella planticola* TSHA-91. The bacterial titer in the inoculum was in the range 20-40 x 10^{6} cm^{-3}. PGPR 2 is a microbiological fertilizer, which contains bacteria of the *Azotobacter*, *Pseudomonas* and *Bacillus* genera, as well as natural vitamins, enzymes and growth stimulators.
The fertilizers were applied using the drip irrigation system (in a concentration of 0.3% with 30 mm water (30 l /m^{2}), applying the Venturi pipe, in three periods during the vegetation period (between the end of June until beginning of August). The irrigation periods were determined taking into consideration the condition of soil moisture, which was recorded using a tensiometer, as well as precipitation data.
Determining the intensity of flowering, as well as of the initial and final fruit set was accomplished using a 1–5 point scale. Yield per tree and unit area was determined during the phase of physiological maturity by measuring the fruit mass and was expressed in kg tree^{-1}, i.e. t ha^{-1}.

Soil samples for microbiological analysis were taken at the end of the vegetation season. Total numbers of microorganisms, number of fungi, actynomicetes, *Azotobacter* and oligonitrophils were determined as colony forming units (CFUs) on agar plates by Serial Dilution Plate method. The medium used for enumeration of total numbers of microorganisms, fungi, actynomicetes and oligonitrophils was soil agar, Czapek's medium, Krasilnikov medium, Fyodorov medium, respectively. *Azotobacter* number was done on Fyodorov medium by fertile drops method (Pochon and Tardideux, 1962). The incubation for total number of microorganisms and actinomycetes took 7 days, and 5, 4–5 and 2 days for fungi, oligonitrophils and *Azotobacters*, respectively, at 28^{o}C temperature. The numbers of microorganisms was calculated as per 1.0 *g* of absolutely dry soil.
The data was subjected to analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The Least Significance Difference (LSD) was used to compare treatment means and treatments declared different at $p = 0.05$ level of significance.

3. Results and Discussion

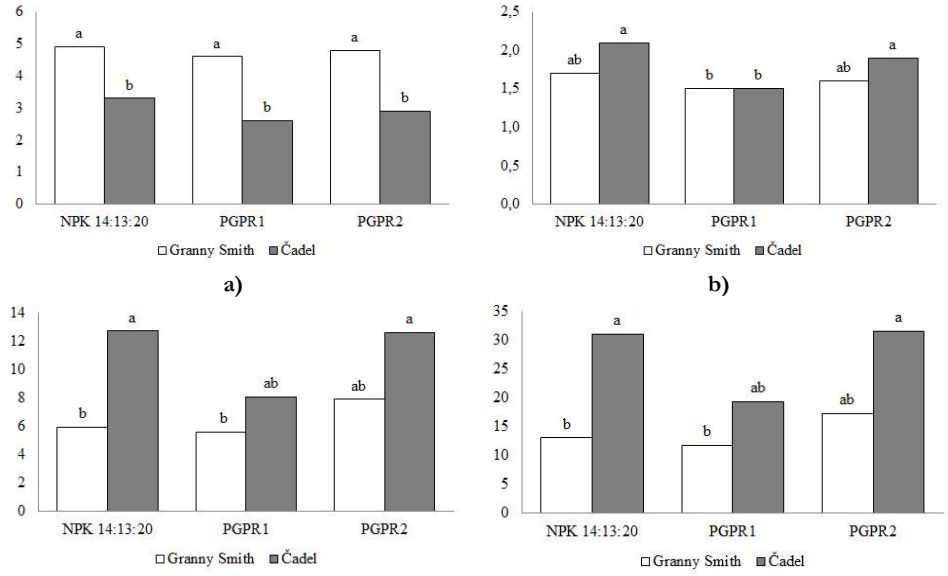
The variance analysis (Table 1) revealed that the parameters of generative potential (except final fruit set intensity) was significantly influenced by both variability factors, as well as their interaction (except fruit set intensity).
Flowering intensity, fruit set intensity, final fruit set intensity, yield per tree and yield per unit area ranged from 2.93 to 4.77, 1.57 to 2.03, 1.50 to 1.90, 6.48 to 11.14 kg tree^{-1} and 14.00 to 27.29 t ha^{-1}, respectively. The flowering intensity, fruit set intensity, and final fruit set intensity were the highest in treatment with chemical fertilizer (4.10, 1.95, 1.90, respectively), whereas the yield per tree and per unit area were the highest in the treatment with PGPR 2 (10.25 kg tree^{-1} and 24.37 t ha^{-1}). All the inoculated PGPR strains contributed to the increase in fruit yield of apple when compared to control but it was strongly depended on rootstocks, cultivars and treatments. Similar reports were obtained by Aslantaş et al. (2007) in their study of the effects of rootstocks (M9 and MM106), cultivars ('Granny Smith' and 'Stark Spur Golden') and growth promoting rhizobacteria (OSU-142, OSU-7, BA-8 i M-3) on the tree growth and yield at apple. Authors stated that bacterial applications including *Pseudomonas* and *Bacillus* strains can stimulate growth and increase yield in apple. Favourable effects of plant growth promoting rhizobacteria (*Agrobacterium rubi* A-18, *Bacillus subtilis* OSU- 142, *Burkholderia gladioli* OSU-7 and *Pseudomonas putida* BA-8) on growth and leaf nutrient content of 'Starking Delicious', 'Granny Smith', 'Starkrimson Delicious', 'Starkspur Golden Delicious' and 'Golden Delicious' apple cultivars grafted on semi-dwarf rootstock MM-106 have been confirmed by the results obtained by Karakurt and Aslantas (2010).The applications of bacterial strains increased the leaf number and area as well as number of annual shoots and their diameter, although OSU-7 application suppressed annual shoot length. In general, the parameters of generative potential were found to be worse in 'Granny Smith' fruits, except for flowering intensity.

Table 1. Impact made by the fertilizer and genotype on the generative potential parameters in apple

Factor		Flowering intensity (1–5)	Fruit set intensity (1–5)	Final fruit set intensity (1–5)	Yield (kg tree^{-1})	Yield (t ha^{-1})
Fertilizer (A)	NPK 14:13:20	4.10±0.55 a	1.95±0.46 a	1.90±0.38 a	9.34±2.62 a	22.02±6.63 b
	PGPR 2	3.60±0.68 b	1.60±0.32 b	1.50±0.16 b	6.84±1.54 b	15.54±3.69 c
	PGPR 1	3.85±0.63 b	1.85±0.41 a	1.75±0.37 a	10.25±2.99 a	24.37±7.69 a
Cultivar (B)	'Granny Smith'	4.77±0.06 a	1.57±0.19 b	1.60±0.09 a	6.48±0.69 b	14.00±1.67 b
	'Čadel'	2.93±0.53 b	2.03±0.40 a	1.83±0.36 a	11.14±2.62 a	27.29±6.63 a
ANOVA						
A		*	*	*	*	*
B		*	*	ns	*	*
A × B		*	ns	*	*	*

Values within each column followed by the same small letter are not significantly different at $p \leq 0.05$ by LSD test.

The analysis of the interaction effect of all treatments (Figure 1) revealed that fruit set intensity (2.30), final fruit set intensity (2.10) and yield per tree (12.73 kg tree^{-1}) were highest in 'Čadel' fruits in treatment with chemical fertilizer. On the other hand, flowering intensity (4.90) was highest in 'Granny Smith' fruits in the same fertilizer, and yield per unit area (31.50 t ha^{-1}) was highest in 'Čadel' fruits in treatment with PGPR 2. Parameters of generative potential, except flowering intensity, showed an identical trend in terms of lowest value.



***** The same small letters represent non-significant differences at $P \leq 0.05$ by LSD test

Figure 1. Impact made by the interaction effect fertilizer/genotype on the flowering intensity (a), final fruit set intensity (b), yield per tree (c) and yield per unit area (d)

The results obtained in the study of the impact made by the applied fertilizer and genotype on the total numbers of microorganisms, fungi, actinomyces, *Azotobacter* and oligonitrophilics in the soil of the experimental apple orchard are presented in Table 2.

The results of the variance analysis revealed a significant impact of fertilizer on all analysed groups of microorganisms, whereas the genotype had an important impact on the number of fungi, *Azotobacter* and oligonitrophils.
The values for the total number of microorganisms were in the range between 21.83 and 36.50. The largest total number of microorganisms was determined in the PGPR 2 treatment (36.50), which was considerably higher compared the chemical fertilizer treatment (26.50) and treatment with PGPR 1 (21.83).
A significantly higher number of actinomycetes in the apple rhizosphere was recorded in the PGPR 2 treatment (41.50), whereas cultivar produced no significant effect.

Table 2. Impact made by the fertilizer and genotype on the total number of microorganisms, number of fungi, actinomyces, *Azotobacter* and oligonitrophilics in the soil of the experimental apple plantation

Factor		Total number of microorganisms	Number of			
			Fungi	Actynomicetes	*Azotobacter*	Oligonitrophils
		(CFU·10^6 g d.m. $soil^{-1}$)	(CFU·10^5 g d.m. $soil^{-1}$)	(CFU·10^5 g d.m. $soil^{-1}$)	(CFU·10^2 g d.m. $soil^{-1}$)	(CFU·10^5 g d.m. $soil^{-1}$)
Fertilizer (A)	NPK 14:13:20	26.50±6.51 b	27.00±8.15 a	30.33±6.26 b	8.50±1.54 b	14.17±3.82 b
	PGPR 1	21.83±5.80 c	21.00±5.74 b	16.67±2.39 c	10.67±2.42 a	22.83±4.02 a
	PGPR 2	36.50±7.46 a	20.00±3.31 b	41.50±7.47 a	10.67±1.71 a	22.33±6.15 a
Cultivar (B)	'Granny Smith'	27.78±7.01 a	27.44±5.47 a	29.11±5.52 a	13.33±1.21 a	22.67±4.37 a
	'Čadel'	28.78±4.49 a	17.89±3.56 b	29.89±6.11a	6.56±2.75 b	16.89±3.37 b
A		*	*	*	*	*
B		ns	*	ns	*	*
A × B		ns	ns	ns	ns	ns

Values within each column followed by the same small letter are not significantly different at $p \leq 0.05$ by LSD test.

The most favourable impact on the number of *Azotobacter* and oligonitrophils in the apple rhizosphere was made by the PGPR 1 fertilizer (10.67 and 22.83, respectively) and PGPR 2 (10.67 and 22.33, respectively), whereas the lowest number of aforementioned groups microorganisms was recorded in the treatment with chemical fertilizer (8.50 and 14.17, respectively).
The number of *Azotobacter* and oligonitrophils in 'Granny Smith' (13.33 and 22.67, respectively) was significantly higher, compared to 'Čadel' (6.56 and 16.89, respectively).
Similar results were observed in our previous investigations into the effect of biofertilizer applications on microbial activity in the rhizosphere of the cvs 'Gloster 69', 'Morens Jonagored' and 'Hapke Delicious' (Pešaković et al., 2013; Pešaković et al., 2017). Stimulating effect of the applied biofertilizers are not only an indicator of a pronounced nitrogen-fixing capacity of introduced bacterial strains, but is also the indicative of a number of phenomena, i.e., intensification of photosynthesis process, inhibition of phytopathogens or synthesis of phytohormones (Sukhovitskaja et al., 2004), and other plant growth stimulators, targeted detoxification of heavy metals and high salt concentrations, and exocellular polysaccharide synthesis (Park et al., 2005; Biari et al.,

2008).
On the other hand, the highest number of fungi in the rhizosphere of the examined apple cultivars was obtained under chemical fertilizer treatment (27.00) whereas the lowest count determined in treatment with PGPR2 fertilizer (27.00). This phenomenon is most likely a consequence of changes in physical and chemical properties of soil, as well as changes in the structure of soil microbial cenosis (Stark et al., 2007). The authors point out that in such circumstances, an increase in population of toxinogenic and pathogenic fungi may cause a decline in numbers, especially *Gram*-negative bacteria and other poorly competitive species of soil microorganisms. Wołoszyk and Nowak (1993), Myśków et al. (1996) found that repeated chemical fertilization, particularly with high doses of nitrogen, can cause strong acidification of soils and thus increase the development of fungi which is in accordance with our results. Myśków et al. (1996) have found that the development of microfloral communities in soil depends also on the type of the applied nitrogen fertilizer. These authors confirmed in their studies the presence of the greatest number of fungi in the soil fertilized with ammonium sulphate.
Significant differences in the number of fungi in soil existed between the 'Granny Smith' and 'Čadel', which could be explained by differences in the exudate production.

4. Conclusion

The analysis of the results points to the fact that introduction of biofertilization in apple production can contribute to advancement of the existing growing technology, i.e., to establishing an organisation of this production in a manner that satisfies basic postulates of sustainable agriculture.

5. Acknowledgements

This study is the part of the project No. 31093 financed by Ministry of Education, Science and Technological Development of the Republic of Serbia.

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