



## EFFECT OF INDIGENOUS *PSEUDOMONAS* SP. AND *BACILLUS* SP. STRAINS ON YIELD AND MAIN CHEMICAL GROWTH PARAMETERS OF RADICCHIO

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**Summary:** *Pseudomonas* sp. and *Bacillus* sp. belong to plant growth promoting rhizobacteria which are able to colonize the plants roots and stimulate growth. In this study, the effect of two indigenous plant growth promoting rhizobacterial strains *Pseudomonas* sp. Q4 and *Bacillus* sp. Q10 and their mixture (mix Q4+Q10) on content of the main chemical growth parameters (nitrogen, phosphorus, potassium, calcium and magnesium) and the yield of dry biomass of radicchio (*Cichorium* spp. var. *rossa di treviso*) aerial parts and root, was investigated. The study was carried out with stagnosol type of soil in pot experiments under semi-controlled conditions in the Institute of Soil Science (Belgrade), in the period from July to October in 2013. Phosphorus was determined by spectrophotometer, potassium - by flame emission photometry and total nitrogen and carbon - using elemental CNS analyzer, while calcium and magnesium were determined by AAS. The data on yield of both aerial parts and root dry biomass of radicchio showed that its treatment with Q4 and Q10 strains, as well as with their mixture, caused noticeably increase in this parameter in relation to the control, whereby the strain Q4 was more effective for aerial parts, while mix Q4+Q10 - for roots. The obtained data on the studied chemical parameters of radicchio root and aerial parts were in total accordance with their yield. Concluding, studied strains have a potential in promoting the biomass yield and main chemical growth parameters of both aerial parts and root of radicchio.

**Key words:** PGPR strains, radicchio, dry biomass yield, chemical growth parameters, Stagnosol

### INTRODUCTION

Radicchio "Rossa di Treviso" is the classic red tall Italian radicchio, belonging to the genus *Cichorium* and family Asteraceae. All the red types of radicchio now being cultivated derive from red-leaved individuals belonging to the botanical variety *foliosum* (Barcaccia et al. 2003). The plant is characterized by upright red and white striped leaves, with large pure white stems. The leaves are very crunchy and long. It grows well in most soils (the best are soils with a pH of 5.5-6.8), but loose, fertile ones that have plenty of nutrients and good drainage are ideal. It is a very early variety that is suitable for harvesting from the end of summer and throughout the winter, meaning it has high resistance to cold. Radicchio also grows well in containers, pots, or raised beds (<http://www.seedaholic.com/chicory-radicchio-rossa-di-treviso-early.html>).

Radicchio is usually consumed as fresh vegetable. As stated in previous study (Rangarajan and Ingall, 2001), in the US interest in the cultivation of this vegetable in particular has grown because of the popularity that has in the market, as a part of the pre-mixed salad, prepared for consumption. The increasing commercial interest expressed in radicchio during the last years is justified by both leaf characteristics and their nutritional features (Filippini et al. 2011). The chemical composition of vegetables in general is an important source of minerals involved in various metabolic processes in the human body (Ćustić et al. 2003). Red chicory features considerable antioxidant properties, and it contains carotenoids, vitamins A, B<sub>6</sub>, K, as well as macro- and micro- elements, such as phosphorus, potassium, zinc, copper, iron, etc. (Ćustić et al. 2000, Mulabagal et al. 2009). Due to the presence of large quantities

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of sesquiterpene lactones, such as lactucin, 8-desoxylactucin and lactucopicrin, the plant has a certain kind of bitterness (Peters and Van, 1998).

Many vegetable crops, including radicchio, are typically consumed without further processing after harvest and it is important that chemicals are not present in or on any part of the plant (Banchio et al. 2008). Therefore it is very important to identify the growth measurements of radicchio grown avoiding the chemical fertilizers and pesticides and achieving optimal growth and yields. Nowadays, the use of biofertilizers in production plays an important role as a supplement to improve the growth and yield of several agricultural, horticultural and medicinal plants (Lugtenberg and Kamilova, 2009). This biological approach is nevertheless considered to be more environment friendly in the long term.

One of the emerging research area for improving the plant growth and yield and for the control of different phytopathogenic agents is the use of plant growth promoting rhizobacteria (PGPR). They are able to improve plant growth by increasing the rate of seed germination and seedling emergence, minimizing the adverse effects of external stress factors, and protecting plants from soil-borne pests and diseases. In this respect, different isolates of fluorescent *Pseudomonas* and *Bacillus* species take prominent place (Cattelan et al. 1999). These bacteria belong to PGPR which are able to colonize the plants roots and stimulate the growth. These bacteria use various mechanisms for their action: production of antibiotics, HCN, plant hormones, the ability to solubilize mineral phosphates and other nutrients and antagonism towards phytopathogenic microorganisms.

Regarding the aforementioned comments, the aim of this study was to examine the effect of two indigenous plant growth promoting rhizobacterial strains *Pseudomonas* sp. Q4 and *Bacillus* sp. Q10 and their mixture (mix Q4+Q10) on the main chemical growth parameters (content of nitrogen, phosphorus, potassium, calcium and magnesium) and yield of dry biomass of radicchio (*Cichorium* spp. var. *rossa di treviso*) aerial parts and root.

## MATERIAL AND METHODS

The bacterial strains *Pseudomonas* sp. Q4 and *Bacillus* sp. Q10 were isolated from different host plants in Serbia. Taxonomic characterization of the isolates was based on tests for Gram staining, cytochrome oxidase, catalase, fermentation/utilization of glucose, lactose and sucrose, utilization of citrate, ability to degrade urea and 16S rDNA analyses (Jošić et al. 2012, Jošić et al. 2012a, Jošić et al. 2015). The bacteria were grown in liquid King's B medium (KB) for 24 h on orbital shaker at 120 rpm. Concentrations were determined spectrophotometrically (OD<sub>600</sub>) and all experiments were conducted using 10<sup>6</sup> CFUml<sup>-1</sup>.

**Plant material and pot experiments.** The effect of PGP rhizobacteria was studied on radicchio (*Cichorium* spp.) variety *rossa di treviso*, in pot experiments under semi-controlled conditions in the glasshouse of the Institute of Soil Science (Belgrade). The experiment was conducted during the first week of July until the second week of October in 2013, in plastic pots with 3 kg of homogenized soil - Stagnosol (WRB, 2014). Four experimental variants were set up in three replications, as follows: Control (V1); *Pseudomonas* sp. strain Q4 (V2); *Bacillus* sp. strain Q10 (V3); *Pseudomonas* sp. strain Q4 and *Bacillus* sp. strain Q10 mixture (mix Q4+Q10 - V4). In every plastic pot ten radicchio seeds were sown on July 4<sup>th</sup>. After the emergence on July 20<sup>th</sup> five plants per pot were left. The inoculation of the soil and radicchio plants in each pot was carried out with the solution containing 150 ml of distilled water and 50 ml of pure liquid inoculums of *Pseudomonas* sp. Q4 and *Bacillus* sp. Q10 strains, as well 100 ml of distilled water and 100 ml of pure liquid inoculum mixture (50 ml of Q4 and 50 ml of Q10). This treatment was performed one day after reducing the plants number in the phenophase of rooting. Control (V1) was treated with solely 200 ml of distilled water.

**Soil preparation and analysis.** The soil samples were air-dried, crushed and passed through a sieve ( $\leq 2$  mm). Before setting up the experiment soil physical and chemical properties were studied. Soil granulometric composition was analyzed by determination of particle size distribution in mineral soil material, using the standardized method by sieving and sedimentation (ISO 11277:2009(E) 2009). Chemical soil properties were determined using the following chemical analyses: soil acidity (pH in 1M KCl, v/v - soil:1M KCl=1:5) was analyzed potentiometrically, using glass electrode (SRPS ISO 10390:2007, 2007); humus content was determined using Kotzman method (Jakovljević et al. 1985); available phosphorus (P<sub>2</sub>O<sub>5</sub>) and potassium (K<sub>2</sub>O) were analyzed by Al-method according to Egner-Riehm (Riehm, 1958), where K<sub>2</sub>O was determined by flame emission photometry and P<sub>2</sub>O<sub>5</sub> by spectrophotometer after color development with ammonium molybdate and stannous chloride. Calcium (Ca) and magnesium (Mg) were extracted by ammonium acetate followed by determination on atomic adsorption analyzer SensAA Dual - GBC Scientific Equipment Pty Ltd, Victoria, Australia (Wright and Stuczynski, 1996).

**Preparation and analysis of the plant material.** Radicchio plants were grown according to the standard growing methods until October 14<sup>th</sup>, when all studied relevant parameters of the plant growth were measured/analyzed. Samples of the plant material were air-dried and the yield was measured (g per pot). The radicchio plants were dried at 105°C for a period of 2 hours and weighed and for all treatments and replicates the following main chemical growth parameters of the plant aerial parts and root were analyzed: nitrogen (N) was analyzed on elemental CNS

analyzer Vario EL III (Nelson and Sommers, 1996); phosphorus (P) and potassium (K) contents were determined by method named as "wet" combustion, i.e. they were heated to boiling with the mixture of concentrated sulfuric and perchloric acids. In the obtained solution, P was determined by spectrophotometer with molybdate, and K - by flame emission photometry (Jakovljević et al. 1985); calcium (Ca) and magnesium (Mg) were determined by Atomic Absorption Spectrometry (AAS) (Wright and Stuczynski, 1996), where the plant material was converted to a solution by method named as "dry" combustion, i.e., first by heating at 550°C (for several hours) and then by treating the obtained ash with hydrochloric acid (Miller, 1998).

**Data analysis.** The obtained data on soil properties represent the arithmetic means of three replicates and standard deviation values. The effects of V1, V2, V3 and V4 treatments on the studied chemical parameters and yield of the plants were evaluated using the analysis of variance (SPSS 20.0, Chicago, USA), followed by Duncan's Multiple Range Test (DMRT). Significant differences between means were tested by the LSD test at  $P = 0.05$ .

## RESULTS

**Soil properties.** Table 1 displays the results of soil physical and chemical properties. The studied Stagnosol is a clay loam according to the soil textural class determined on the basis of particle size distribution, having very acid reaction and medium humus content, with a very low content of available phosphorus and medium provided with available potassium. It is medium provided with calcium and with high content of magnesium.

Table 1. Physical and chemical properties of Stagnosol (means  $\pm$  standard deviation value)

Granulometric composition (fraction, %)		Chemical properties	
Bulky sand (2-0.2 mm)	5.0 $\pm$ 0.59	pH in 1M KCl	4.45 $\pm$ 0.01
Miniature sand (0.2-0.02 mm)	25.9 $\pm$ 1.33	Humus (%)	2.26 $\pm$ 0.47
Dust (0.02-0.002 mm)	45.1 $\pm$ 1.22	Available P <sub>2</sub> O <sub>5</sub> (mg 100g <sup>-1</sup> )	3.73 $\pm$ 0.28
Clay (< 0.002 mm)	24.0 $\pm$ 0.21	Available K <sub>2</sub> O (mg 100g <sup>-1</sup> )	19.80 $\pm$ 1.54
Total sand (> 0.02 mm)	30.9 $\pm$ 1.05	Available Ca (mg 100g <sup>-1</sup> )	240.00 $\pm$ 19
Dust + clay (< 0.02 mm)	69.1 $\pm$ 1.05	Available Mg (mg 100g <sup>-1</sup> )	35.00 $\pm$ 3.89

**Chemical growth parameters and dry biomass yield of radicchio aerial parts.** Table 2 displays the data on chemical growth parameters (available macroelements) in radicchio aerial parts influenced by the treatment applied. The obtained results of N, P, K, Ca and Mg contents showed that the treatment of radicchio with both *Pseudomonas* sp. and *Bacillus* sp. strains, as well as with their mixture, had positive effect on all these parameters in aerial plant parts in relation to the control, whereby the strain Q4 was slightly more effective than Q10 and mix Q4+Q10. However, this influence was statistically significant at  $P < 0.05$  only regarding the content of K and Mg, while for the contents of other macroelements there were no statistical differences between the treatments. In addition, improved mineral nutrition promoted the plant growth (Image 1), positively affecting the dry biomass yield of radicchio aerial parts. In general, certain effects of both strains, as well as their mixture, on radicchio growth in relation to the control were noticed after a week of the treatment. The obtained data on dry biomass yield of the aerial parts were in accordance with chemical growth parameters, meaning that the yield was the highest in radicchio treated with Q4 strain, and this influence was statistically significant at  $P < 0.05$  (Table 2).

**Chemical growth parameters and dry biomass yield of radicchio root.** Table 2 displays the data on chemical growth parameters (available macroelements) in radicchio roots affected by the treatment applied. The obtained results of N, P, K, Ca and Mg contents showed that the treatment of radicchio with both *Pseudomonas* sp. and *Bacillus* sp. strains, as well as with their mixture, had positive effect on all these parameters in plant roots in relation to the control, whereby the *Pseudomonas* sp. Q4 and *Bacillus* sp. Q10 strains pronounced lower effects than their mixture. However, this influence was statistically significant at  $P < 0.05$  only regarding the content of Mg, while for the contents of other macroelements there were no statistical differences between the treatments. Accordingly, improved mineral nutrition promoted the root growth (Image 1), reflecting on the dry biomass yield of radicchio roots. The obtained data on dry biomass yield of the root were in accordance with chemical growth parameters, meaning that the highest yield was registered in pot treatment with the mixture of Q4 and Q10 strains, and this influence was statistically significant at  $P < 0.05$  (Table 2).

Table 2. Available macroelements in radicchio aerial parts and root and the yield of dry biomass depending on the treatment

Variants	Yield (g/pot)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Aerial parts						
V1-control	2.86±0.76 <sup>b</sup>	1.06±0.52 <sup>a</sup>	0.59±0.06 <sup>a</sup>	1.71±0.13 <sup>b</sup>	1.33±0.02 <sup>a</sup>	0.32±0.00 <sup>c</sup>
V2- <i>Pseudomonas</i> sp. strain Q4	4.34±0.75 <sup>ab</sup>	1.91±0.09 <sup>a</sup>	0.66±0.05 <sup>a</sup>	2.61±0.45 <sup>a</sup>	1.84±0.23 <sup>a</sup>	0.50±0.03 <sup>a</sup>
V3- <i>Bacillus</i> sp. strain Q10	3.96±0.04 <sup>ab</sup>	1.23±0.40 <sup>a</sup>	0.63±0.01 <sup>a</sup>	1.78±0.08 <sup>b</sup>	1.66±0.31 <sup>a</sup>	0.35±0.02 <sup>bc</sup>
V4-mix Q4+Q10	4.29±0.29 <sup>a</sup>	1.57±0.60 <sup>a</sup>	0.64±0.04 <sup>a</sup>	2.43±0.46 <sup>ab</sup>	1.61±0.19 <sup>a</sup>	0.37±0.01 <sup>b</sup>
P value	*	NSD	NSD	*	NSD	***
LSD (0.05)	1.02	0.838	0.086	0.623	0.399	0.034
Root						
V1-control	1.16±0.23 <sup>b</sup>	1.02±0.59 <sup>a</sup>	0.62±0.17 <sup>a</sup>	1.84±0.13 <sup>a</sup>	1.32±0.05 <sup>a</sup>	0.28±0.03 <sup>c</sup>
V2- <i>Pseudomonas</i> sp. strain Q4	1.46±0.03 <sup>b</sup>	1.10±0.64 <sup>a</sup>	0.62±0.14 <sup>a</sup>	2.41±0.13 <sup>a</sup>	1.40±0.06 <sup>a</sup>	0.33±0.01 <sup>b</sup>
V3- <i>Bacillus</i> sp. strain Q10	1.85±0.08 <sup>a</sup>	1.22±0.60 <sup>a</sup>	0.63±0.02 <sup>a</sup>	2.47±0.28 <sup>a</sup>	1.71±0.41 <sup>a</sup>	0.34±0.02 <sup>b</sup>
V4-mix Q4+Q10	1.93±0.26 <sup>a</sup>	1.89±0.01 <sup>a</sup>	0.68±0.00 <sup>a</sup>	2.74±0.63 <sup>a</sup>	2.02±0.85 <sup>a</sup>	0.41±0.02 <sup>a</sup>
P value	**	NSD	NSD	NSD	NSD	***
LSD (0.05)	0.34	0.995	0.211	0.671	0.885	0.039

LSD - least significant differences at P=0.05; \*\*\* - statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; NSD - statistically not significant; DMRT was used to compare different variants at P≤0.05, where values followed by the same letter in a column are not significantly different.

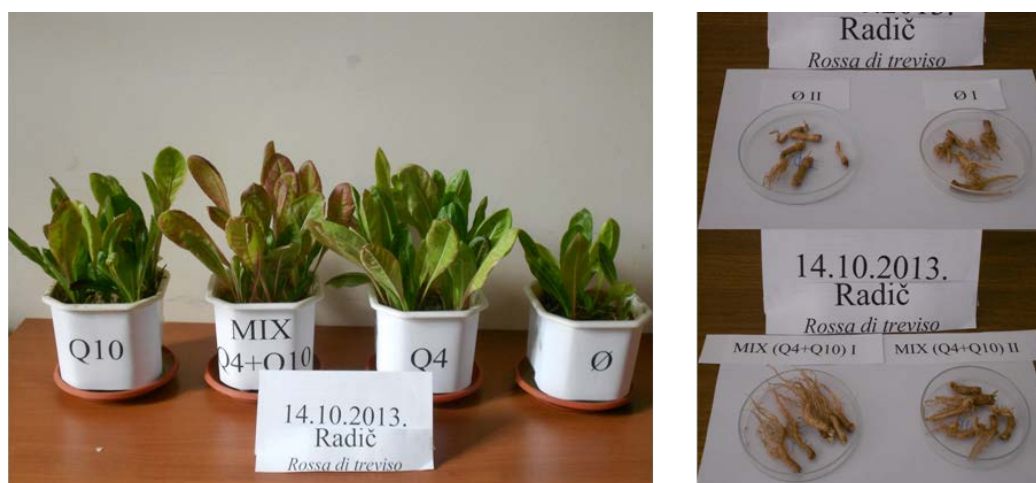


Image 1. Growth and development of the radicchio aerial parts and roots in different treatments

## DISCUSSION

The use of PGPR-based products has certain advantages over conventional chemicals: they are considered safer than many of the chemicals now in use; they do not accumulate in the food chain; properly-developed biocontrol agents are not considered harmful to ecological processes or the environment, etc. (Lucas García et al. 2004).

According to Podile and Kishore (2006), PGPR and their interactions with plants are exploited commercially and hold great promise for sustainable agriculture. Applications of these associations have been investigated in maize, wheat, oat, barley, peas, canola, soy, potatoes, tomatoes, lentils, radicchio and cucumber (Gray and Smith, 2005, Jošić et al. 2012, Zdravković et al. 2015).

Our objective was to compare some plant growth promoting rhizobacterial properties of indigenous *Pseudomonas* sp. strain Q4 and *Bacillus* sp. strain Q10 through their effect on the main chemical growth parameters (content of N, P, K, Ca and Mg) and yield of dry biomass of radicchio aerial parts and root. *Pseudomonas* isolate was selected on the basis of previous studies that confirmed its intrinsic antibiotic resistance and plant growth promoting traits: phosphate solubilization, siderophore and indoleacetic acid production, enzymatic activity (Jošić et al. 2012,

Jošić et al. 2012a, Zdravković et al. 2015). In addition, *Bacillus* sp. has the ability to inhibit the growth of other strains. Many strains of *Bacillus* have been reported to produce substances that act as growth inhibitors for other microorganisms (Lunares et al. 1994).

The present data on N, P, K, Ca and Mg contents showed that the treatment of radicchio with both *Pseudomonas* sp. and *Bacillus* sp. strains, as well as with their mixture, had positive effect on all these parameters in aerial plant parts in relation to the control, whereby the strain Q4 was slightly more effective than Q10 and mix Q4+Q10. However, this influence was statistically significant only regarding the content of K and Mg. On the other hand, the data on the content of these elements in radicchio root indicated better effect of the Q4 and Q10 mixture, with statistically significant influence only regarding the content of Mg. Similar results were obtained in previous studies that refer to other tested crops (Vikram et al. 2007, Ordookhani et al. 2011). Sivasakthi et al. (2014) reported that phosphate solubilizing *Bacillus* sp. stimulates plant growth through enhanced P nutrition increasing the uptake of N, P, K and iron (Fe). The increase in N, P and K contents in crops inoculated with PGPR *Pseudomonas* and *Azotobacter* strains was also reported by Vikram et al. (2007) and Ordookhani et al. (2011). This is also consistent with the observation that plants inoculated with PGPR take up N, P, K and microelements more efficiently from the soil (Cakmakci et al. 2006).

The bacteria assayed not only affected the chemical growth parameters, but also the dry biomass yield of radicchio. Accordingly, improved mineral nutrition promoted the growth of the plant aerial parts and root, positively affecting the dry biomass yield of both, and they were in accordance with chemical growth parameters. Similar data were obtained in the glasshouse trials of Adesemoye et al. (2008), indicating an increase in dry biomass yield from 29 to 83% for plants treated with *Bacillus subtilis* and *Pseudomonas aeruginosa* at 60 days after planting in relation to non-treated control. The potential of *Bacillus* sp. to increase the yield, growth and nutrition of raspberry plants under organic growing conditions was reported in trials of Orhan et al. (2006). In addition, specific strains of *Pseudomonas fluorescens* have been used as seed inoculants on crop plants to promote growth and increase yields. These strains in field trials rapidly colonize roots of potato, sugar beet and radish and cause a yield increase up to 44% (Sivasakthi et al. 2014). Similarly, improved lettuce biomass yields up to 30%, when the strains of *Pseudomonas chlororaphis* were applied on plants, were determined by Cipriano et al. (2016).

## CONCLUSION

The data on studied chemical growth parameters and dry biomass yield of radicchio showed that its treatment with *Pseudomonas* sp. Q4 and *Bacillus* sp. Q10 strains, as well as with their mixture, caused markedly increase in this parameter in relation to the control. More precisely, the strain Q4 was more effective for aerial parts, while mix Q4+Q10 - for roots. These effects were statistically significant at  $P < 0.05$  only regarding the content of K and Mg in aerial parts, and content of Mg in roots, respectively. The obtained data on radicchio dry biomass yield were in accordance with chemical growth parameters. Accordingly, the yield of aerial plant parts was the highest in radicchio treated with Q4 strain, with no statistical significance between the treatments, while the highest yield of plant roots was registered in pot treatment with the mixture of Q4 and Q10 strains, with statistically significant effect at  $P < 0.05$ . Concluding, studied strains have a potential in promoting the biomass yield and main chemical growth parameters of both aerial parts and root of radicchio.

## ACKNOWLEDGEMENT

This research is a part of Project III46007, supported by Ministry of Education, Science and Technological Development, Republic of Serbia

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**UTICAJ AUTOHTONIH SOJEVA *PSEUDOMONAS* SP. I *BACILLUS* SP. NA PRINOS I OSNOVNE HEMIJSKE PARAMETRE RASTA RADIČA**

**Izvod:** Ispitan je uticaj autohtonih sojeva koji pripadaju rizosfernim bakterijama - stimulatorima rasta biljaka (PGPR), *Pseudomonas* sp. Q4 i *Bacillus* sp. Q10, kao i njihove kombinacije (Q4+Q10), na sadržaj osnovnih hemijskih parametara rasta (azot, fosfor, kalijum, kalcijum i magnezijum) i prinos suve mase nadzemnog dela i korena radiča (*Cichorium* spp. var. *rossa di treviso*). Ogled je izveden u vegetacionim sudovima sa zemljištem tipa stagnosol, u periodu od jula do oktobra 2013. godine. Fosfor je određen spektrofotometrijski, kalijum - plamenom emisionom fotometrijom, azot na elementarnom CNS analajzeru Vario EL III, dok su kalcijum i magnezijum određeni atomskom apsorpcionom spektrometrijom. Dobijeni podaci o prinosu suve mase nadzemnog dela i korena radiča pokazuju da je tretman biljaka radiča sojevima Q4 i Q10, kao i njihovom kombinacijom, izazivao vrlo primetno povećanje ovog parametra u odnosu na kontrolu, pri čemu je soj Q4 efikasnije delovao na nadzemni deo biljaka, a kombinacija Q4+Q10 - na koren. Rezultati dobijeni ispitivanjem hemijskih parametara rasta nadzemnog dela i korena radiča su bili u potpunosti u skladu sa dobijenim prinosom. Može se zaključiti da testirani sojevi *Pseudomonas* sp. Q4 i *Bacillus* sp. Q10, delujući sami ili kombinovano, imaju veliki potencijal u povećanju prinosa biomase i osnovnih hemijskih parametara rasta biljke radiča.

**Ključne reči:** PGPR sojevi, radič, prinos suve biomase, hemijski parametri rasta, Stagnosol

**Received / Primljen:** 20.06.2017.

**Accepted / Prihvaćen:** 24.12.2017.