In vitro and *in vivo* effects of *Pseudomonas* spp. and *Bacillus* sp. on *Fusarium acuminatum, Botrytis cinerea* and *Aspergillus niger* infecting cucumber

Jasmina Zdravković[1*], Milan Ugrinović[1], Milan Zdravković[1], Slaviša Đorđević[2], Snežana Pavlović and Dragana Jošić[3]

[1] *Institute of Vegetables, Karadjordjeva 71, 11420 Smederevska Palanka, Serbia*

[2] *University of Belgrade, Faculty of Agriculture, Nemanjina 8, 11080 Zemun, Serbia*

[3] *Institute of Soil Research, Teodora Drajzera 7, Belgrade, Serbia*

(*jzdravkovic@institut-palanka.co.rs)

SUMMARY

Cucumber (*Cucumis sativus* L) is an important member of the *Cucurbitaceae* family. Production of healthy nursery is necessary for high-quality production of this crop in greenhouses and in fields. With the idea of minimizing the use of pesticides and mineral fertilizers to preserve soil quality, we investigated the effects of plant growth promoting bacteria (PGPB) on growth promotion and protection of cucumber plants from phytopathogenic fungi. The effects of *Pseudomonas* spp. strains with different antifungal activities and *Bacillus* sp. Q10 strain with PGP activity were tested on cucumber plants. Antagonistic activity of *Pseudomonas* spp. against the growth of several phytopathogenic fungi isolated from cucumber: *F. acuminatum, B. cinerea* and *A. niger*, was observed. The influences of overnight cultures, supernatants and heat-stable antifungal factors were tested on the phytopathogenic fungi *in vitro. Pseudomonas* sp. K35 and K24 strains were more effective than *P. chlororaphis* Q16 and *Pseudomonas* sp. K27, showing 70-80% of fungal growth inhibition regardless of culture or fraction applied. The good antagonists that belong to pseudomonads and *Bacillus* sp. Q10 strain were used as mixtures for estimation of plant growth and health promoting effects on cucumber plants. Growth dynamics differed depending on the applied strain of *Pseudomonas* sp. The M3 treatment (a mixture of *Bacillus* sp. Q10 and *P. chlororaphis* Q16) stimulated the initial phase of growth, while M4 (a mixture of *Bacillus* sp. Q10 and *Pseudomonas* sp. K24) resulted in the maximal height at the final measurement. Significant differences in leaf and plant weight (M4), and leaf weight (M5, containing K35 strain) were found after the treatments. No significant differences in chlorophyll and NBI level were observed in any of the tested combinations. The obtained results suggested that M3 was suitable for stimulation of the early phase of cucumber growth, while the mixtures M4 and M5 improved plant protection and stimulated the later phases of cucumber growth.

Keywords: Rhizobacteria; *Pseudomonas* spp.; *Bacillus* sp.; Plant growth; Plant diseases; Cucumbers

INTRODUCTION

PGPR (Plant Growth Promoting Rhizobacteria) include bacterial species that live in close association with plants and have beneficial effects on their host. PGPR affect plants in various ways: they synthesize the compounds necessary for plant nutrition, facilitate the absorption of certain nutritional substances from soil, and reduce or prevent the sensitivity of plants to soil pathogens, inducing their systemic resistance. The influence of PGPR can be direct (promoting plant growth through plant hormone production and absorption of nutritional substances) and indirect (prevention of harmful effects of pathogenic microorganisms) (Hayat et al., 2010). Biological products for vegetable nutrition based on PGP microorganisms are considered to be an alternative to chemical fertilizers. Microbiological treatment can stimulate plant growth and considerably decrease the amount of fertilizers necessary in growing substrates (Sripontan et al., 2014), both in organic and conventional systems of vegetable growth. Beneficial microorganisms, such as biological control agents (BCAs) and PGPRs, can play a key role in this major challenge (Raaijmakers et al., 2009). Strains of to the genera *Bacillus* and *Pseudomonas* have been used in experimental tests on a wide range of economically important crops (Kokalis-Burelle et al., 2006; Meyer et al., 2010). The PGPR included indigenous strains belonging to *Pseudomonas* and *Bacillus* genera selected from different soils in Serbia. Those strains have exhibited numerous PGP traits (Djuric et al., 2011; Josic et al., 2012), stimulated plant growth (Jarak et al., 2012), and some of them demonstrated an ability to inhibit several phytopathogenic fungi (Jošić, et al., 2012 a,b,c; Protolipac et al., 2012; Starovic et al., 2013).

Cucumber (*Cucumis sativus* L) is one of the most popular members of the family Cucurbitaceae and the second most important vegetable crop after tomato in Western Europe (Eifediyi & Remison, 2010). Historical records about cucumber cultivation date back 5000 years (Wehner & Guner, 2004). Many pathogens of this plant affect its growth and yield. *Fusarium* spp. are among the phytopathogenic fungi that cause deleterious effects on cucumber plants and yields. These fungi survive and overwinter in greenhouse and field soils for a long time and it is very difficult to eliminate them from soil by chemical fungicides. Cultural practices and biological control measures have been reported as successful in minimizing such deleterious effects of these fungal pathogens (Ling et al., 2010; Eifediyi & Remison, 2010; Cao et al., 2011). *Fusarium acuminatum* Ellis &

Everhart causes diseases of many plants, including those in the *Cucurbitaceae* family. This is a toxic fungus than can be infectious both for people and warm-blooded animals. *F. acuminatum* produces the T-2 toxin, which is the most toxic representative among trichothecene mycotoxins (Wing et al., 1994). Grey mould disease caused by the necrotrophic fungus *Botrytis cinerea* Pers is a devastating agent of more than 200 plants, including vegetables. Grey mould is economically very significant since it causes great losses, especially in vegetable and fruit production. *B. cinerea* is controlled mainly by applying fungicides, but biological control has recently become a sound alternative to chemicals (Couderchet, 2003). The ochratoxin A- producing *Aspergillus niger* has also been isolated from cucumber fruits. *A. niger* was found as a saprophyte in the seed of many field crops, vegetables and herbs (Stević et al., 2012). It causes grey mould in fruits and vegetables during their shelf life and transport. *A. niger* produces several micotoxins, including ochratoxin A as the most important one (Vuković et al., 2009).

The aim of this study was to investigate the effects of several bacterial strains on growth inhibition of phytopathogenic fungi isolated from cucumber: *F. acuminatum*, *B. cinerea* and *A. niger*, as well as their promoting effects on the cucumber plants. Bacterial antagonists that belong to *Pseudomonas* and *Bacillus* genera were used as a mixture for plant growth promotion and protection. We tested their effects on growth dynamics of transplanted plants, on the nitrogen balance index (NBI) and the content of chlorophyll in transplanted cucumber plants. The ultimate objective was to find optimal combinations of bacteria for formulating a microbiological fertilizer for cucumber seedling production.

MATERIAL AND METHODS

Bacterial strains and their antagonistic activity to phytopathogenic fungi isolated from cucumber

The bacterial strains *Bacillus* sp. Q10, *Pseudomonas chlororaphis* Q16, and *Pseudomonas* sp. K24, K27, K35 and N3 were isolated from different soils in Serbia. Taxonomic characterization of the isolates was performed 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 (unpublished data). 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_{600}) and all experiments were conducted using 10^6 CFUml^{-1}.

The phytopathogenic fungi *F. acuminatum*, *B. cinerea* and *A. niger* were isolated from cucumber plants and fruits (private collection of S. Pavlović). The antagonistic activity was tested on Waksman agar (WA) (Wolf et al, 2002) using overnight cultures of bacteria optimized to 10^6 CFUml^{-1}. The influence of: a) the whole culture (PK) b) extracellular metabolites (supernatant) (SN) and c) heat stable antifungal factors (HSAFs) were tested on the phytopathogenic fungi. SN was obtained by centrifugation of overnight bacterial cultures at 10000 rpm for 8 min. HSAFs, as the termostable extracellular metabolites, were obtained after heat treatment of bacterial supernatant at 70°C for 30 min. Bacterial agents (10μl) were applied near the edges of Petri dishes, while 6 mm plugs of fungal mycelia were placed at the centre. Negative control variants contained only mycelia on WA plates. After seven days of incubation at 26°C, fungal growth was measured and the percentage of growth inhibition was calculated using the formula: % Inhibition = [(R-r)/R] x 100, where r is the length of the fungal colony opposite the bacterial colony, and R is the maximum radius of fungal colony in the control (Zarrin et al., 2009).

Plant material and pot experiments

The effects of PGP rhizobacteria were tested on cucumber (*Cucumis sativus* cv Renesansa F1), in a glasshouse of the Institute for Vegetable Crops, Smederevska Palanka, Serbia. Four experimental variants were set up in ten replications: 1. K - Control; 2. M3 - mixed *Bacillus* sp. Q10 and *P. chlororaphis* Q16; 3. M4-mixed *Bacillus* sp. Q10 and *Pseudomonas* sp. K24; and 4. M5 - mixed *Bacillus* sp. Q10 and *Pseudomonas* sp. K35. The mixture of *Bacillus* sp. Q10 and *Pseudomonas* sp. N3 (M1), as well as the mixture of *Bacillus* sp. Q10 and *Pseudomonas* sp. K27 (M2) were excluded from further experiments because of their low antagonistic activities against the tested fungi *in vitro*. The seedlings were transplanted on April 9th to pots sized 10x10 cm, containing the commercial substrate Gramoflor GmbH&Co.KG, Germany, (250 l). Treatment with 3 bacterial mixtures (M3, M4 and M5) was performed 2 days and another treatment one month after sowing. Negative control was treated with the same volume of distilled water. Plant height as a parameter of cucumber growth dynamics was measured on 5 dates following the second treatment (on May 8th, May 12th, May 18th,

May 25th and June 1st). Seedlings were grown according to standard growing methods until June 1st when all other relevant parameters were measured as well, namely:

- Plant height, root length, root mass, number of formed leaves, plant weight, leaf weight;
- Nitrogen balance index (NBI) and chlorophyll level by polyphenol and chlorophyll meter Dualex scientific 4+, Force A, France.

Data analysis

Plant growth dynamics (plant height) after the second treatment was followed on a trend line. The line represents the average state of the observed phenomena trough time. The following model of linear trend (Njegić et al., 1991) was applied:

$$\hat{y} = a + bx$$
$$\text{where: } b = \sum x_t y_t - n \cdot \bar{x} \cdot \bar{y} / \sum x_t^2 - n \cdot \bar{x}^2$$
$$a = \bar{y} - \bar{x} \cdot b$$

Differences among the treatments (K, M3, M4 and M5) regarding the several morphological traits were shown by mono-factorial ANOVA, while the least significant difference (LSD) test was applied to determine the least significant difference between the treatments and control. LSD was calculated at $p \leq 0.05$ according to Gomez and Gomez (1984).

RESULTS

Antagonistic activity of *Pseudomonas* spp. to phytopathogenic fungi isolated from cucumber

The inhibitory activity of *Bacillus* sp. Q10 and five *Pseudomonas* spp. against fungal pathogens responsible for detrimental effects on cucumber plants and fruits were assessed using the dual culture method. Three phytopathogenic fungi isolated from cucumber plants and fruits, namely: *Fusarium acuminatum*, *Botrytis cinerea* and *Aspergillus niger*, were used. Only one strain - *Bacillus* sp. Q10, allowed the same mycelial growth as in control variants (Table 1) and showed no inhibitory effect to any of the tested fungi (Table 2). Mycelial growth and growth inhibition of *F. acuminatum* and *B. cinerea* caused by *Pseudomonas* spp. application are shown in Figure 1 (a and b, respectively). The strain *P. chlororaphis* Q16 had earlier been tested for antifungal activity against fungal pathogens of several medicinal plants and showed good biocontrol potential

(Jošić et al., 2012a,b,c, 2015). In this study, the strain was used for comparison with the more recently isolated strains *Pseudomonas* sp. N3, K24, K27 and K35. *P. chlororaphis* Q16 inhibited *in vitro* mycelial growth of *F. acuminatum* by 45-50%, *B. cinerea* by 55-60% and *A. niger* by 32-37%, depending on bacterial culture or fraction applied (Table 2). Similar values were observed for the *Pseudomonas* sp. K27 strain. The *Pseudomonas* sp. N3 strain inhibited only *B. cinerea* by about 40%. The strain *Pseudomonas* sp. K35 was almost as effective as the strain K24, with inhibition values from 68-80%, compared to 70-80% for K24. These two strains inhibited the mycelial growth of all fungi regardless of culture or fraction applied.

Table 1. Effects of *Pseudomonas* spp. and *Bacillus* sp. Q10 strains on mycelial growth (mm) of different fungal pathogens of cucumber

| Bacterial strain | Bacterial culture /fraction | Radial growth (mm) (diameter ± standard error) | | |
		Fusarium acuminatum	*Botrytis cinerea*	*Aspergillus niger*
/	control	51.50 ± 0.76	52.00 ± 0.00	52.00 ± 0.00
Q10	culture	51.88 ± 0.354	52.00 ± 0.00	52.00 ± 0.00
N3	culture	51.75 ± 0.463	31.63 ± 2.45	52.00 ± 0.00
Q16	culture	26.13 ± 1.81	20.38 ± 1.92	32.25 ± 4.13
	SN	28.25 ± 1.75	21.75 ± 1.28	34.88 ± 1.64
	HSAF	33.38 ± 3.02	23.25 ± 1.39	33.38 ± 3.02
K24	culture	12.50 ± 3.42	9.38 ± 1.77	9.63 ± 1.06
	SN	15.00 ± 2.83	11.75 ± 1.75	12.63 ± 1.19
	HSAF	11.88 ± 0.84	13.13 ± 1.81	11.88 ± 0.84
K27	culture	28.38 ± 2.39	18.63 ± 0.92	25.25 ± 1.67
	SN	32.50 ± 1.60	21.88 ± 0.64	27.38 ± 1.30
	HSAF	26.63 ± 1.51	22.75 ± 1.58	26.63 ± 1.51
K35	culture	14.00 ± 1.07	10.38 ± 3.16	13.13 ± 1.36
	SN	15.50 ± 1.41	11.88 ± 2.64	15.50 ± 0.54
	HSAF	15.38 ± 0.92	12.25 ± 2.25	15.38 ± 0.92

Table 2. Fungal growth inhibition (%) caused by *Pseudomonas* spp. and *Bacillus* sp. Q10 strains

| Bacterial strain | Bacterial culture /fraction | Inhibition of mycelial growth (%) | | |
		Fusarium acuminatum	*Botrytis cinerea*	*Aspergillus niger*
Q10	culture	0	0	0
N3	culture	0	39.18 ± 4.70	0
Q16	culture	49.28 ± 3.30	60.82 ± 3.70	37.98 ± 7.95
	SN	45.16 ± 2.97	58.17 ± 2.46	32.93 ± 3.16
	HSAF	46.02 ± 2.75	55.29 ± 2.67	35.82 ± 5.81
K24	culture	75.76 ± 6.51	81.97 ± 3.40	81.49 ± 2.04
	SN	70.90 ± 5.33	77.40 ± 3.37	76.10 ± 2.28
	HSAF	70.84 ± 5.11	74.76 ± 3.30	77.16 ± 1.60
K27	culture	44.92 ± 4.38	57.25 ± 1.76	51.44 ± 3.21
	SN	36.89 ± 3.04	57.93 ± 1.23	47.36 ± 2.50
	HSAF	34.93 ± 2.63	56.25 ± 3.04	48.80 ± 2.90
K35	culture	72.80 ± 2.29	80.05 ± 6.08	74.7 6± 2.61
	SN	69.90 ± 2.80	77.16 ± 5.08	70.19 ± 1.03
	HSAF	68.91 ± 1.61	76.44 ± 4.33	70.43 ± 1.76

Various effects of different bacterial mixtures on cucumber growth were noticed immediately after the second treatment. Statistically significant differences were interpreted considering average values against the untreated control. The influence on plant height was determined considering the average values for all mixtures. The highest plants were obtained by applying M4 (41.91cm) (Figure 2 A). The average value of the M3 treatment was close to the control and did not

influence cucumber growth significantly (Figure 2 B). The mixtures M4 and M5 had stronger impact on plant growth after the second treatment: M4 had highly significant effect, while M5 was statistically significant (P value $p \le 0.05$) (Table 3). The values of dependent variable regression of plant height were almost identical (Figure 2 C and D). Initial difference in plant height among the treatments was influenced by the first treatment a few days after sowing.
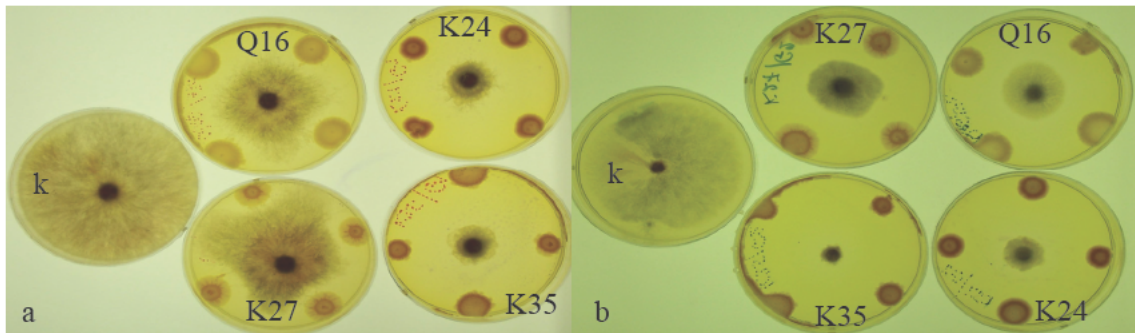

Figure 1. Mycelial growth and growth inhibition of *Fusarium acuminatum* (a) and *Botrytis cinerea* (b) caused by *Pseudomonas* spp. Control - fungus only (k), *P. chlororaphis* (Q16) and *Pseudomonas* spp. strains (K24, K27 and K35).
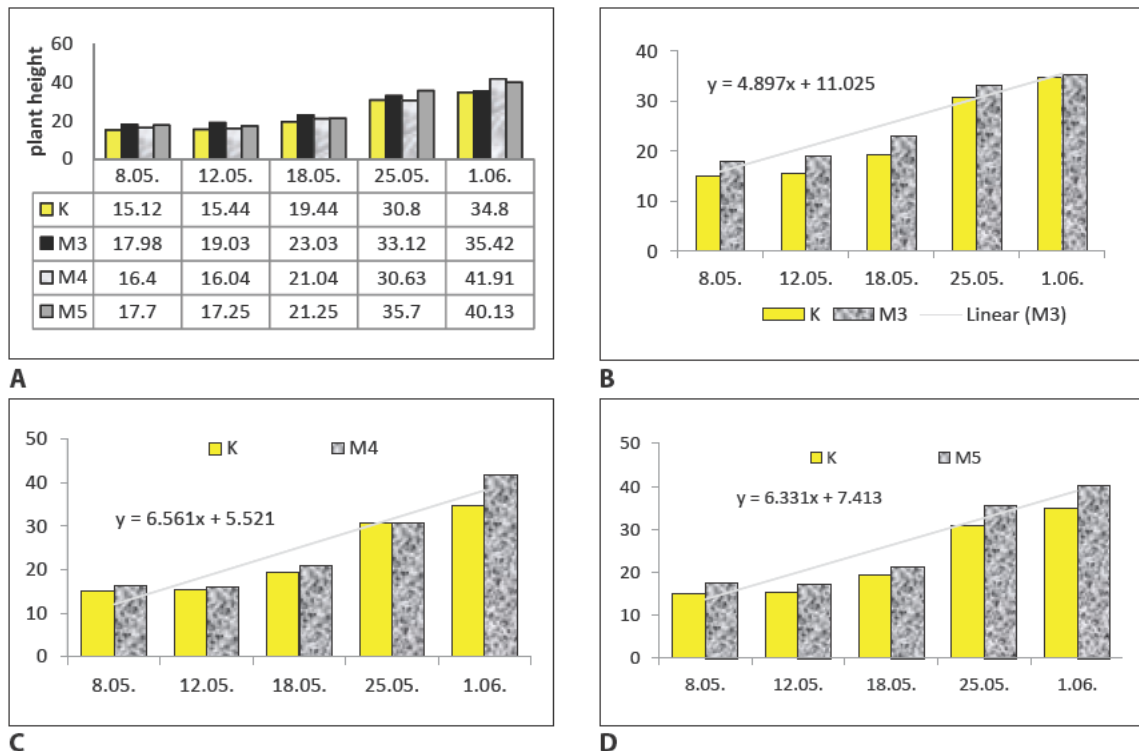

	8.05.	12.05.	18.05.	25.05.	1.06.
K	15.12	15.44	19.44	30.8	34.8
M3	17.98	19.03	23.03	33.12	35.42
M4	16.4	16.04	21.04	30.63	41.91
M5	17.7	17.25	21.25	35.7	40.13

Figure 2. Dynamics of cucumber seedling growth influenced by different PGPR mixtures. (A) Average values of plant height measured every 5 days after the second treatment of substrate for cucumber growth; B) Comparison of plant growth under the influence of M3 mixture and untreated control; C) Comparison of plant growth under the influence of M4 mixture and untreated control; D) Comparison of plant growth under the influence of M5 mixture and untreated control.

Table 3. Morphological traits of cucumber seedlings influenced by different mixtures of *Pseudomonas* spp. and *Bacillus* sp. Q10

Treatment	Plant height (cm)	Number of leaves	Root weight (g)	Plant weight (g)	Leaf weight (g)
K	34.80	7.06	4.64	7.42	4.94
M3 (Q10 + Q16)	35.42	6.76	3.80	7.21	5.30
M4 (Q10 + K24)	41.91**	7.52*	4.22	9.44*	6.09*
M5 (Q10 + K35)	40.13*	7.13	2.56	7.52	6.04*
LSD $_{0.05}$	3.547	0.457	3.014	1.951	0.883
LSD $_{0.01}$	5.373	0.692	4.566	2.955	1.338

The lowest significant differences of leaf weight and plant mass were observed for the mixture M4 at 95% probability level. The lowest significant differences in leaf weight were found for M5.

After two months of growth of cucumber seedlings, several parameters influenced by different combinations of *Pseudomonas* spp. and *Bacillus* sp. Q10, except plant height, were not all statistically different from the control. Chlorophyll and NBI in all tested combinations of *Pseudomonas* spp. and *Bacillus* sp. Q10 were at the level of untreated control (Table 4).

Table 4. Chlorophyll content and nitrogen balance index (NBI) in cucumber leaves

Treatment	Chlorophyll content	NBI
K	18.75	11.08
M3 (Q10 + Q16)	19.35	12.15
M4 (Q10 + K24)	18.05	11.00
M5 (Q10 + K35)	18.25	10.85
LSD $_{0.05}$	7.120	5.498
LSD $_{0.01}$	10.230	7.900

DISCUSSION

Production of quality and healthy nursery is a significant precondition for high-quality production in greenhouses and in open fields. Restricted use of pesticides and mineral fertilizers is a global tendency that creates preconditions for conservation of soils and ecosystems in general (Turan et al., 2014). Therefore, application of plant growth promoting bacterial strains is an environmentally safe control method in nursery and plant production.

In this investigation, the effects of *Pseudomonas* spp. strains with different antifungal activities and *Bacillus* sp. Q10 strain with PGP activity were tested on cucumber plants. The detected phytopatogenic fungi showed the same growth rate in the control as in treatment with the strain *Bacillus* sp. Q10. No inhibitory effect on the mycelial growth of *F. acuminatum* and *Aspergillus*

niger was observed when *Pseudomonas* sp. N3 strain was used in dual culture. The strain *P. chlororaphis* Q16 showed lower antifungal activity against fungal pathogens of cucumber than some medicinal plants (Josic et al., 2012 b). Two *Pseudomonas* strains (K24 and K35) were selected for our pot experiment for their high antifungal activity against all tested fungi that reached 67% and 82%, respectively. All tested fractions – filtrate of supernatant and HSAF, as well as the whole culture of bacteria – were almost equally effective in mycelial growth suppression.

F. acuminatum is a toxic species that infects many plant species. This pathogen has been found on cucumbers in Turkey, on cereal crops in Spain (Marín et al., 2012) and on shallot onions in Georgia (Parkunan & Ji, 2013). Elimination of this pathogen from fields and greenhouses is essential for growing healthy plants. *P. chlororaphis* Q16 inhibited mycelial growth of this fungus 43-52%, which was a lower efficacy than those achieved by *Pseudomonas* spp. K35, which reached 67-75%, and K24 - 82%, the maximum of *F. acuminatum* growth inhibition.

P. chlororaphis Q16 *in vitro* inhibited the mycelial growth of *B. cinerea* 55-60%. We chose the isolates *Pseudomonas* spp. K35 and K24 for further investigation of biological control of *B. cinerea* because of their inhibition rates of 74% and 82%. In future investigation, a planned field trial will include *P. chlororaphis* Q16 because of the production of several antibiotics (Jošić et al, 2012 a,c, 2015) already known as effective at suppressing plant pathogens and related diseases. Microbial agents, such as *Serratia plymuthica* (Azami-Sardooei, 2011) or plant activators such as chitosans (Ben-Shalom et al., 2003), have been reported as important and safe control methods of the grey mould disease caused by *B. cinerea* on cucumber plants.

P. chlororaphis Q16 *in vitro* inhibited mycelial growth of *A. niger* 32-37%, and the applied bacterial culture and fractions achieved similar values. *P. chlororaphis* Q16 had earlier been shown to cause strong inhibitory or even fungicidal effects against the

pathogenic fungi *A. fumigatus*, *A. niger*, *Trichoderma viride* and *Penicillium verrucosum* var. *cyclopium* with the same antifungal potential of its SN and HSAF fractions (Jošić et al., 2015). The inhibition zones in this study ranged from 18-22 mm, which is similar to the values in previous reports, where the inhibition zones of *A. niger* in dual culture with *P. aeruginosa* were 14 mm (Kishore et al., 2005, 2006) and 18 mm (Khanuchiya et al., 2012), while it was 17 mm with *P. fluorescence* (Khanuchiya et al., 2012). Better results in mycelial growth inhibition of *A. niger* were obtained using *Pseudomonas* sp. K35, with inhibition zone values from 37-40 mm, while the best inhibition was observed using *Pseudomonas* sp. K24, which showed 41-45 mm inhibition zones. These results are in concordance with data previosly reported by Lukkania and Surendranatha Reddya (2014) for different fluorescence *Pseudomonas* spp. inhibiting *A. niger* growth and forming inhibition zones of 22.6-55.8 mm.

Our data showed which combination of bacteria had the most favourable effect on cucumber plant growth, and proved a suppressive effect against its phytopathogenic fungi. Different mixtures of strains did not react at the same time or in the same way. Growth dynamics (plant height) differed depending on the applied strain of *Pseudomonas* sp. Treatment M4 - mixed *Bacillus* sp. Q10 and *Pseudomonas* sp. K24, which ultimately achieved the highest plant height (41.91cm), reached in the first height measurement (8.05) a value only slightly higher than the control (16.4 cm). The first treatment of plants with *Bacillus* sp. Q10 mixed with different *Pseudomonas* spp. (two days after sowing) had the greatest impact on initial seedling growth. Treatment M3 - *Bacillus* sp. Q10 mixed with *P. chlororaphis* Q16, stimulated initial growth (17.98 cm) but ultimately had the lowest effect (35.42cm) in the last measurement (1.06). This can be attributed to the effectiveness of *P. chlororaphis* Q16 in stimulating the early phases of development of germinating seeds. In contrast to M3 treatment, the strain *Pseudomonas* sp. K24, which is a constituent of the mixture M4, lent that treatment its strongest impact in the later phases of seedling development, which resulted in maximal seedling height.

Bacterial suspension should be applied during the growth phase when it has the greatest impact, i.e. when enzyme activity is the strongest. Similar results of growth in morphological traits that directly or indirectly impact the yield were found in studies conducted by Dursun et al., (2010), Yolcu et al., (2011), and Moshabaki Isfahani and Besharati, (2012).

We recommend the suspensions M4 and M5 because they had the greatest efficiency regarding the morphological parameters of seedlings, and had the highest level of statistically significant differences against the control, especially for stimulation of the final phase of growth before transplanting. For stimulation of the initial phases of development, the suspension M3 is recommended, which is very important for early production of seedlings for growing in plastic greenhouses, when it is hard to achieve an optimal growing regime, especially temperature.

Comparing the effects of the tested mixtures of bacterial strains against the control we found the chlorophyll content and nitrogen balance index to be at the control level. The result was expected because the seedlings were grown on substrates containing sufficient nutritional matter and the nutritional needs of plants in the control were fully satisfied. A stimulating effect of PGP bacteria was therefore not observed. Our results were not in consistence with data from other studies (Moshabaki Isfahani & Besharati, 2012; Dursun et al., 2010; Yolcu et al., 2011) in which significant differences were detected regarding chlorophyll contents between treated cucumber plants and the control. High levels of chlorophyll influenced by PGPR in vegetable production have been reported for cabbage (increase in plant diameter, height, chlorophyll content of up to 47.5%), (Turan et al., 2014) and some other cultivated plants (Ghazvini et al., 2014). It was found that some soil bacteria have inhibitory action, but strains that increase the level of cytokines were also found, which resulted in better growth, increased level of chlorophyll content, weight of fresh fruit and size of cotyledon leaves of cucumber compared to plants not treated with PGPR (Hussain & Hasnain, 2009). In this study, an absence of negative effects of *Bacillus* sp. Q10, *P. chlororaphis* Q16 and *Pseudomonas* sp. K35 on morphological and physiological parameters of plants was confirmed, as well as a statistically significant increase in plant height when exposed to *Bacillus* sp. Q10 and *Pseudomonas* sp. K24. This combination can even have a protective effect on plants, through the presence of strain K24, which is particularly efficient in suppressing phytopathogenic fungi isolated from cucumber and other plants (unpublished data). Further research on poorer substrates that are used in cucumber seedling production is still to prove the expression of PGP traits. Based on all relevant results, an adequate formulation of bacterial mixtures will be made for promoting the growth of cucumber plants in the best way.

ACKNOWLEDGEMENTS

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In vitro i *in vivo* efekti *Pseudomonas* spp. i *Bacillus* sp. na *Fusarium acuminatum*, *Botrytis cinerea* i *Aspergillus niger* na krastavcu

REZIME

Krastavac (*Cucumis sativus* L) je važan predstavnik familije *Cucurbitaceae*, a proizvodnja zdravog rasada je nephodna za visoku produktivnost u plastenicima i na otvorenom polju. Da bi se umanjila primena pesticida i mineralnih đubriva i pri tome sačuvalo zemljište, ispitan je uticaj bakterija stimulatora biljnog rasta (PGPB) na rast biljaka i zaštitu od patogena. Primenjeni su sojevi *Pseudomonas* spp. sa različitim antimikoznim delovanjem i *Bacillus* sp. soj Q10 sa PGP aktivnošću. Praćena je antagonistička aktivnost *Pseudomonas* spp. na fitopatogene gljive izolovane sa biljaka krastavca: *F. acuminatum, B. cinerea* i *A. niger*. Testiran je uticaj prekonoćne kulture bakterija, supernatanta i termostabilnih antifungalnih faktora na rast ovih fitopatogena. *Pseudomonas* sp. K35 i K24 sojevi, koji pokazuju 70-80% inhibicije rasta gljiva bez obzira na primenjenu kulturu ili frakciju, efektivniji su od *P. chlororaphis* Q16 i *Pseudomonas* sp. K27. Sojevi *Pseudomonas* spp., koji su ispoljili visok stepen antagonizma, kombinovani su sa sojem *Bacillus* sp. Q10 i procenjen je uticaj na rast i zdravstveno stanje biljaka krastavca. U zavisnosti od primenjenog soja *Pseudomonas* sp., razlikovala se dinamika rasta krastavca. Tretman M3 - kombinacija *Bacillus* sp. Q10 i *P. chlororaphis* Q16 uticala je na početnu fazu porasta biljaka, dok je treatman M4 - kombinacija *Bacillus* sp. Q10 i *Pseudomonas* sp. K24, imala najviše uticaja na visinu biljke na kraju merenja. Uočene su značajne razlike za masu lista i masu biljke (M4) i masu lista (M5 sa sojem K35), dok sadržaj hlorofila i nivo NBI nisu imali značajne razlike kod svih ispitivanih kombinacija. Dobijeni rezultati ukazuju da je M3 pogodan za rane faze razvoja biljke, a kombinacije M4 i M5 pogodne su za zaštitu biljaka i u kasnijim fazama porasta biljaka. Potpuna ekspresija PGP svojstava za M4 i M5 može se utvrditi tek posle testiranja na siromašnim supstratima, koji će biti upoređeni sa rezultaima dobijenim na supstratima sa dovoljno hranljivih materija.

Ključne reči: Rizobakterije; *Pseudomonas* spp.; *Bacillus* sp.; Rast biljaka; Bolesti biljaka; Krastavac