

The effect of *Pseudomonas chlororaphis* subsp. *aurantiaca* strain Q16 able to inhibit *Fusarium oxysporum* growth on potato yield

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Summary: This study assesses the potential of antibiotics-producing *Pseudomonas chlororaphis* strains to increase potato yield and to inhibit the mycelial growth of phytopathogenic fungi *Fusarium oxysporum* (Fo) isolated from potato. *P. chlororaphis* subsp. *aurantiaca* strain Q16 (PchlQ16) caused the highest growth inhibition (67.07%) of FoA2 isolate *in vitro*. In field trials the effect of PchlQ16 was measured as the number of stems, number and weight of tubers and a total potato yield of the Rudolph potato variety. Application of *P. chlororaphis* and the number of treatments exhibited a significant effect on the yield. Two treatments of PchlQ16 increased the total yield of tubers from 4.9% to 33.05%, while four treatments from 9.3% to 92.35%, compared to the control. Based on our field results we can recommend a frequent application of PchlQ16 (4 times) during potato growth season. The results of our *in vitro* experiment support these findings as the bacterial strain suppressed growth of *F. oxysporum*. In this investigation PchlQ16 was confirmed as an effective growth promoting agent in potato production and can be highly effective in prevention of *F. oxysporum* infection.

Key words: *Fusarium oxysporum*, inhibitory effects, *Pseudomonas chlororaphis*, rhizobacteria, *Solanum tuberosum* L.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important globally grown crops and has a significant role in human nutrition. The potato is the world's fourth largest food crop after wheat, rice and corn. In Serbia, potatoes are grown on approximately 40,000 ha, with relatively low average yields of around 7 t ha⁻¹ in 2016 (FAOSTAT 2018). Such modest yield is the result of low output of the small farms that prevail in Serbia. At the same time, much higher yields of 30–40 t ha⁻¹ are

obtained by a more intensive production on larger farms (Bročić et al., 2016).

About 40 soil-borne diseases affect potato worldwide and cause severe damage especially on tubers, the economically most important part of the plant (Gudmestad et al., 2007). *Fusarium* dry rot is caused by several *Fusarium* species, among them *F. sambucinum*, *F. culmorum* Fückel, *F. avenaceum* (Fries) Saccardo (Secor & Salas, 2001; Daami-Remadi & Mahjouj, 2006; Peters et al., 2008) including *F. oxysporum* Schlech (Venter et al., 1992; Esfahani, 2005; Ocamp et al., 2007). Potato dry rot usually occurs during storage and can lead to losses in crop quality and yield. It is responsible for severe vascular wilts, and can cause tuber rot. Reduction of the yield caused by dry rot is on average 6%, with possible losses up to 25% (Gashgari & Gherbawy 2013), while almost complete loss of stored commercial potatoes varieties was reported in Turkey (Eken et al., 2000).

Fusarium wilt is mainly managed with chemical soil fumigation and resistant cultivars (Recep et al., 2009). However, synthetic fungicides used to fumigate soil before planting, particularly methyl bromide, lead to

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residue problems and accumulation of toxic pollutants in soil and water (Bunker & Mathur 2001). The issues with controlling *Fusarium* wilt have led to research in biological control, resulting in the development of biopesticides for controlling *Fusarium* wilt without causing environmental pollution (Organisation for Economic Co-operation and Development 2009). Some strains of *Bacillus subtilis* and *Pseudomonas fluorescens* are reported to have reduced *Fusarium* wilt of different plants host (Jamali et al., 2004; Khorsani & Safaie, 2008).

Plant growth promoting (PGP) rhizobacteria have a potential for improving yield and for suppressing soil-borne pathogens (Backer et al., 2018). Many soil microorganisms, including *Pseudomonas* spp., produce different compounds with antifungal and antibacterial properties such as antibiotics, iron-chelating siderophores, cyanide and enzymes (Sindhu & Dadarwal, 2001; Backer et al., 2018). These secondary metabolites have been involved in plant growth stimulation and disease control and their combination is essential for effectiveness of some strains against phytopathogens (Costa et al., 2009; Selin et al., 2010). Susilomati et al. (2011) reported that screening of *Pseudomonas* sp. indigenous to rhizosphere of soybean showed biocontrol activity against soil-borne fungi, mainly *F. oxysporum*. Many *Pseudomonas* strains are known to produce antibiotics such as phenazines (PHZ), pyrrolnitrin (PRN), pyoluteorin (PLT), and 2,4-diacetyl phloroglucinol (DAPG). The role of PHZ in biological control of three *Colletotrichum lindemuthianum* races using *P. chlororaphis* PCL1391 and *P. fluorescens* WCS365 (Bardas et al., 2009) and cypress canker by *P. chlororaphis* subsp. *aureofaciens* strain M71 (Raio et al., 2011) were reported. Different DAPG-producing *P. fluorescens* were involved in growth suppression of the different *F. oxysporum* subspecies: *Fo* f. sp. *pisii* (Landa et al., 2002), *Fo* f. sp. *ciceri* (Saikia et al., 2009), *Fo* f. sp. *lycopersici* (Manikamdan et al., 2010) and *Fo* f. sp. *cubense* (Selvaraj et al., 2014).

In Serbia, phenazines production of *Pseudomonas* spp. was assessed and significant amounts of phenazine-1-carboxylic acid (PCA) and 2-hydroxy-phenazine-1-carboxylic acid (2-OH-PCA) were quantified. A PCR confirmation of the presence of phenazines was revealed. All phenazine-producers, including *P. chlororaphis* strain Q16, were effective against phytopathogenic fungi (Jošić et al., 2012; 2015). Additional high enzymatic activities, a production of siderophores, HCN, IAA and AHLs, as well as good phosphosolubilization capacity placed it among the most promising PGP strains (Jošić et al., 2015).

In this study we tested the inhibitory effects of five PGP *Pseudomonas chlororaphis* strains to *F. oxysporum* isolated from potato (FoA2) and the effect of metabolites of *P. chlororaphis* subsp. *aurantiaca* strain Q16 (PchlQ16), as the best antagonist, on this fungal pathogen. In field trials, we tested treatment frequency of PchlQ16 on the growth promotion and yield of potato (variety Rudolph).

Materials and Methods

Microorganisms and growth conditions

The *Fusarium oxysporum* isolate A2 (FoA2) used in this experiment (GenBank accession number MK621298) was previously isolated from potato tubers in Laboratory of Plant Disease in the Institute for Plant Protection and Environment in Belgrade, Serbia. FoA2 was maintained in Potato Dextrose agar (PDA) and used to assess antifungal activity of *P. chlororaphis* strains.

The complete genome sequences of used bacterial strains *P. chlororaphis* B25 (CP027753), three strains of *P. chlororaphis* subsp. *aurantiaca* Q16 (CP027718), K27 (CP027745) and M12 (CP027715) and *P. chlororaphis* subsp. *aureofaciens* strain C50 (CP027722) were deposited earlier (the accession numbers in DDBJ/503 EMBL/GenBank) (Biessy et al., 2019). Bacterial strains were grown on King B and Waksman media (Jošić et al., 2012).

In vitro antifungal assay

The screening test for antagonism in vitro was performed on Waksman agar medium by dual culture method (Wolf et al., 2002). Overnight cultures (ON) of the bacteria were optimized to 10^7 CFU mL⁻¹ on the basis of spectrophotometric data and placed (10 µL) on the edges of Petri dishes, 3 cm distance from fungal mycelia placed as 1 cm plug in the center. The control variants contained only mycelia of *F. oxysporum* on WA plates. The cultures were incubated at 25 °C, and growth of the fungi was allowed for 7 days after incubation for each of four replicates. The percentage inhibition of the growth of the fungi was calculated using the following formula: $100 \cdot (1 - R_2/R_1)$, where R1 was the radial distance growth of the fungus in a control plate and R2 was the radial distance growth of the fungus in the bacterial treatment.

PchlQ16, showing the highest percentage of growth inhibition of FoA2, was selected for further investigation. The effects of ON culture, extracellular metabolites in cell-free supernatant (CFS), CFS treated with EDTA (ethylenediaminetetraacetic acid disodium salt dehydrate) (CFS-EDTA) and heat-treated cell-free supernatant (HT-CFS) on fungal growth were recorded. To obtain the supernatant fraction, optimized ON culture (10^8 CFU mL⁻¹) was centrifuged twice at 13000 rpm for 5 min., without and with filtration (filter tubes with microporous membrane 0.22 µm) (Merck Millipore Ltd.); one aliquot was treated with 1mM EDTA and one aliquot was heated at 70 °C for 30 min. The control variants contained only mycelia of FoA2 on WA and WA with 1mM EDTA added instead of bacterial culture/fraction. The assay was done as described for the screening test. All fungal inhibition assays were performed in four replicates and repeated three times.

Table 1. Meteorological conditions during the potato growing season (2013 and 2014) in the area of western Serbia

Month	2013		2014	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
April	11.1	44.8	10.4	160.6
May	13.8	149.2	13.1	288.2
Jun	17.4	75.7	17.6	126.3
July	20.5	52.8	19.4	115.8
August	21.6	51.9	19.0	174.5
September	14.7	83.1	15.1	156.0
Average /Total	16.5	457.5	15.8	1021.4

The effect of P. chlororaphis strain Q16 on potato growth and yield

The effect of PchlQ16 and the frequency of treatments on potato yield of Rudolph variety were tested during two years: 2013 and 2014. The experiment was set up on a plot in Jagodnja in Mačva district, western Serbia (44°19'33"N, 19°20'33"E, 759 m a. s. l.) Field experiments were conducted in a split plot method with four replications. The total size of the experimental field was 144 m² divided in 12 equally sized partitions (12 m² each). 40 tubers were planted in each partition. Spacing between rows was 0.7 m and 0.35 m between plants in row. Potato tubers cv. Rudolph were planted manually in the first two weeks of April (5 April 2013 and 11 April 2014).

The soil on the experimental field was a type of acid and brown podzolic soil. The humus content in the surface layer was 3.40%. The total nitrogen content of 0.27% classifies this as rich soil. The soil was acidic as the pH value in H₂O was 4.35, and 3.80 in nKCl. The top soil layer provided readily available phosphorus (19.96 mg 0.1 kg⁻¹ soil) and potassium (K₂O of 36.04 mg 0.1 kg⁻¹ of soil) (personal communication). However, the content of the soluble potassium was insufficient to achieve high yield of potatoes, and this had to be compensated by adding fertilizers. According to the carbonate content, this is a poor calcareous soil. The precipitation level in the growing season was 457.5 in 2013 and 1021.4 mm in 2014, while the average temperature in those growing seasons was 16.5 and 15.8°C, respectively (Table 1). Immediately prior to planting, the tubers were submerged in a 1L culture (10⁶ CFU mL⁻¹) of PchlQ16 (first treatment). During the vegetation period of potato, plants are watered at the stage of intensive vegetative development (second treatment); intensive tuber bulking (third treatment), and after flowering (fourth treatment) using a total of 5L (10⁵ CFU mL⁻¹) culture. During the vegetation period, standard technology methods of growing potatoes were applied and six fungicide treatments were performed in May, June and July using mixture of Metalaksil-m (40 g kg⁻¹) and Mankozeb (640 g kg⁻¹) to protect from Late blight and Early blight. The number of primary stems per plants was measured 65 days after

planting, while after harvest (6 September 2013 and 19 September 2014) the number of tubers per plant and the average weight of tubers per plants were measured. The total yield of tubers was calculated as fresh weight/ha of the harvested tubers.

The obtained results were processed by the analysis of variance (ANOVA, F-test; P ≤ 0.05 and P ≤ 0.01) and the effect of factors. Correlations between the observed parameters were determined by Pearson correlation coefficients (r). Data were processed by the STATISTICA program, version 8 (StatSoftInc, Tulsa, OK, USA).

Results

In vitro antifungal assay

Fungal growth inhibition screening test showed the inhibition ranging from 29 to 72% (Fig. 1). Four bacterial strains of PchlQ16: *P. chlororaphis* B25, *P. chlororaphis* subsp. *aurantiaca* M12 and *P. chlororaphis* subsp. *aureofaciens* C50 inhibited FoA2 isolate more than 50%. The PchlQ16 was selected as the most effective strain for further study due to production of extracellular inhibitory substances.

Antagonistic activity of PchlQ16 against FoA2 was confirmed by additional testing using ON culture, cell-free supernatant, as well as CFS-treated with 1mM EDTA and heat-treated. The maximum inhibition rate

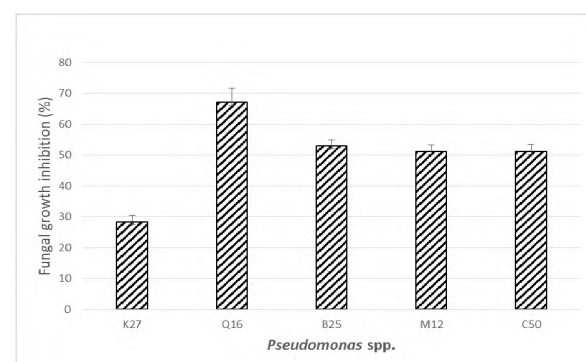


Figure 1. The effect of *Pseudomonas chlororaphis* strains on the growth of *F. oxysporum* A2

was observed in the ON culture and was in concordance with a screening test, even when different concentrations of bacteria were used. FoA2 was inhibited by 67.1% in screening test (10^5 CFU mL⁻¹ inoculum) and 67.5% in the second test, with a 10 × higher concentration (Table 2). All CFS fractions showed a significant decrease of FoA2 growth inhibition comparing to the ON culture. EDTA did not influence fungal growth at all, showing the same values as controls.

The effect of P. chlororaphis strain Q16 on the growth and yield potato

Weather conditions during the vegetation period in 2014 (Table 1) were much more favourable for the growth of potatoes, compared to 2013. Analysis of the results regarding the number of primary stems per plant showed significant differences from the effect of PchlQ16 (factor A) or the number of treatment (factor B) as well as their interaction (A×B) (Table 3). Applying PchlQ16 twice during the potato growing season increased the number of stems per plant by 15.5% (in 2013) to 30.21% (in 2014) compared to the control. Using PchlQ16 four times during the growing seasons was even more effective, increasing the number of stems by 27.5% (in 2013) to 49.65% (in 2014) relative to control.

The lowest number of stems per plant in both years was recorded on a control variant (K) without the use of PchlQ16 (Table 3). Number of stems per plant largely depends on the variety, cultivation technology, the size of seed tubers and physiological age. Number of stems per plant affects the development of above-ground weight, i.e. assimilation surface, the number of set tubers, or total yield (Poštić et al., 2012, Momirović et al., 2016).

Similar results were obtained when the number of tubers per plant was counted. Our results showed a very significant difference when either PchlQ16 (factor A) or the number of treatment (factor B) were taken into account as well as their interaction (A×B) (Table 4). Applying PchlQ16 twice during the potato growing season increased the number of tubers per plant by 16.3% (in 2013) and 38.43% (in 2014) compared to the

Table 2. The effect of *P. chlororaphis* strain Q16 on *F. oxysporum* A2

Treatment	<i>F. oxysporum</i> A2	
	Growth (mm)	Inhibition (%)
Control/ EDTA	80.0 ± 0.82	/
ON culture	26.0 ± 1.63	67.5*
Q16 CFS	33.8 ± 0.96	57.8
CFS- EDTA	35.5 ± 1.29	55.6
HT-CFS	35.0 ± 1.41	56.2

* - significant at 0.05

control. Using PchlQ16 four times during the growing seasons was found to increase the number of tubers per plant from 32.7% (in 2013) to 91.14% (in 2014), relative to control.

The lowest number of tubers per plant in both study years was recorded on the control variant (C) without the application of PchlQ16. Number of tubers per plant is a trait that largely depends on the variety, agro-ecological conditions, cultivation technologies and the size of seed tubers (Tadesse et al., 2001, Poštić et al., 2012, Momirović et al., 2016).

In contrast, the analysis of the average weight of tubers per plant (Table 5) showed no statistically significant difference caused by PchlQ16 (factor A), the number of treatment (factor B), and their interactions. Although the average weight of tubers is a varietal characteristic, it largely depends on the agro-ecological factors, cultivation practices, the number of above ground stems per plant and the number of tubers per plant (Tadesse et al., 2001).

The effect of PchlQ16 on the average weight of tubers per plant in both years was absent (Table 5), due to an increased number of tubers per plant (Table 4). Although the number of tubers per plant had grown, the average tuber weight per plant decreased and vice versa (Poštić et al., 2013).

The analysis of the total yield of tubers showed a highly significant difference with application of

Table 3. The number of primary stems per plant affected by *P. chlororaphis* Q16 and the frequency of treatment in 2013 and 2014

Year	<i>P. chlororaphis</i> Q16 (A)					
	2013			2014		
Treatment (B)	2	4	C	2	4	C
Mean No	3.57	3.94	3.09	3.75	4.31	2.88
Index (%)	115.50	127.50	100.00	130.21	149.65	100.00
	A	B	AB	A	B	AB
F	8.15**	7.89**	4.12**	4.93**	49.48**	4.25**
LSD _{0,05}	0.45	0.36	0.63	0.35	0.28	0.49
LSD _{0,01}	0.62	0.5	0.84	0.47	0.39	0.67

** - significant at 0.01; * - significant at 0.05; ns - not significant; C – control variant

Table 4. The number of tubers per plant affected by *P. chlororaphis* Q16 and frequency of treatment in 2013 and 2014

Year	<i>P. chlororaphis</i> Q16 (A)					
	2013			2014		
Treatment (B)	2	4	C	2	4	C
Mean No	8.21	9.37	7.06	9.69	13.38	7
Index (%)	116.30	132.7	100.00	138.43	191.14	100.00
	A	B	AB	A	B	AB
F	4.50**	10.88**	3.47**	21.66**	129.76**	21.49**
LSD _{0,05}	1.21	0.99	1.71	0.84	0.68	1.18
LSD _{0,01}	1.66	1.36	2.25	1.15	0.94	1.62

** - significant at 0.01; * - significant at 0.05; ns - not significant; C – control variant

Table 5. Average weight (g) of tubers per plant affected by *P. chlororaphis* Q16 and frequency of treatments in 2013 and 2014

Year	<i>P. chlororaphis</i> Q16 (A)					
	2013			2014		
Treatment (B)	2	4	C	2	4	C
Mean weight (g)	75.10	81.70	83.20	53.02	54.94	55.58
Index (%)	90.30	98.20	100.00	95.39	98.85	100.00
	A	B	AB	A	B	AB
F	0.03ns	2.75ns	0.38ns	0.09ns	0.25ns	0.08ns
LSD _{0,05}	10.99	8.98	15.55	6.68	5.45	9.44
LSD _{0,01}	15.08	12.31	21.33	9.15	7.48	12.95

** - significant at 0.01; * - significant at 0.05; ns - not significant; C – control variant

Table 6. Total yield of tubers (t ha⁻¹) affected by *P. chlororaphis* Q16 and the frequency of treatment in 2013 and 2014

Year	<i>P. chlororaphis</i> Q16 (A)					
	2013			2014		
Treatment (B)	2	4	C	2	4	C
Mean yield (t ha ⁻¹)	28.10	29.30	26.80	20.53	29.68	15.43
Index (%)	104.90	109.30	100.00	133.05	192.35	100.00
	A	B	AB	A	B	AB
F	121.38**	76.96**	50.50**	88.67**	396.56**	88.83**
LSD _{0,05}	0.93	0.76	1.31	1.02	0.83	1.44
LSD _{0,01}	1.27	1.04	1.80	1.40	1.14	1.98

** - significant at 0.01; * - significant at 0.05; ns - not significant; C – control variant

Table 7. A correlation between evaluated traits of potato (n=6)

	No. of primary stems per plant	No. of tubers per plant	Average tuber weight	Total yield
No. of primary stems per plant	-	0.918**	-0.156	0,653
No. of tubers per plant		-	-0.427	0.473
Average tuber weight			-	0.569
Total yield				-

Pearson correlation coefficient: *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, respectively

PchlQ16 (factor A) and the number of treatment (factor B), as well as their interaction (A×B) (Table 6). Applying PchlQ16 twice during the potato growing season increased the yield by 4.9% (in 2013) and 33.05% (in 2014) compared to the control.

The lowest total tuber yield in both study years was recorded on the control variant (C) without the use of PchlQ16. The yield of potato tubers depends on the

genetic potential of the varieties, the number of above ground stems and the number of tubers per plant (Knowles et al., 2003, Bus & Wustman 2007, Pošćić et al., 2012, Momirović et al., 2016).

Based on the correlation analysis, the correlation between the number of tubers per plant and the number of above-ground stems is high ($p = 0.01$) (Table 7).

Discussion

Although no natural infection with Fo was observed in the field trial during the two years, PchlQ16 can be applied to prevent infection with *Fusarium* spp. This conclusion is based on the inhibition rate of 67% and 56% for FoA2, when ON culture and different cell-free supernatants were applied respectively. In experiments on the cardoon (*Cynara cardunculus* L.) disease caused by *A. tenuissima* (Jošić et al., 2012), PchlQ16 exhibited a very similar inhibition rate for *in vitro* mycelial growth on WA and for the disease suppression *in vivo* under gnotobiotic conditions (about 43%). The value for heat-stable antifungal factors was lower (34%), similarly to results in this assay, with a decrease from 67 to 56% for FoA2.

Cell-free supernatant significantly reduced the inhibition percent of FoA2 mycelial growth comparing to ON culture. Heat-treated cell-free supernatant (HT-CFS) was used in order to explore the thermo-resistance of the molecule responsible for antagonistic activity of PchlQ16. Similar results for CFS and treated CFS suggest that resistance to EDTA, as non-specific inhibitor of neutral- or metallo-proteases, and the thermo-resistance of extracellular metabolites indicate to non-protein antifungal factors. One possible explanation could be that more than one compound is responsible for the inhibition of Fo. The mycelial growth inhibition of about 40 and 55% caused by all CFS fractions and more than 67% of ON culture for FoA2, identify a potential of PchlQ16 for biological control of Fo in field conditions.

All *P. chlororaphis* strains in this study showed the ability to reduce *F. oxysporum* growth, four of them more than 50%. In our previous study, we reported the highest phenazines production by PchlQ16 among tested strains (Jošić et al., 2012). Most research on *P. chlororaphis* has focused on antibiotic production and its antifungal activities to phytopathogens. Fungal growth inhibition and reduction of *F. oxysporum* f. sp. *radici-lycopersici* pathogenicity on tomato plantlets are reported for *P. chlororaphis* M71 (Puopolo et al., 2011). *P. chlororaphis* strain PA23, which is able to protect canola against sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, produced several antibiotics - pyrrolnitrin, PCA, 2-hydroxy-phenazine, and other exometabolites such as hydrogen cyanide (HCN) and degradative enzymes protease, lipase and chitinase (Poritsanos et al., 2006; Selin et al., 2010).

Besides antibiotic production, other extracellular metabolites are important in effective biocontrol and plant growth stimulation. During assessment of DAPG-producing *P. fluorescens* for the management of *F. oxysporum* on watermelon, Meyer et al. (2016) demonstrated that *P. fluorescens* strains, even *in vitro* inhibiting *F. oxysporum* f. sp. *niveum*, resulted in some inhibition of vine growth in the field and were not effective for enhancing plant vigor or suppressing fungal infection on watermelon. In our earlier study of the capability to control powdery mildew in wheat, seeds treatment with *P. chlororaphis* Q16

improved the plant biomass and N content and decreased powdery mildew disease incidence (Pivić et al., 2015). The antibiotics production, in addition to other PGP traits, can be the favorable traits of PchlQ16 for the biocontrol of Fo in potato production.

Field experiments indicated a positive effect of this strain on the potato growth in two growing seasons. Favourable average temperatures in July and August, accompanied by an optimal amount of precipitation in 2014, contributed to the higher crop productivity then in 2013 as the potatoes were at the stage of intensive tuber bulking. In addition, the higher precipitation value and optimum temperature in 2014 resulted in the increased activity of the microorganisms.

During 2013 PchlQ16 application increased the number of above ground stem per plant by 15.5% and 27.5%; the number of tubers per plant by 16.3% and 32.7%; and the total yield of tubers by 4.9% and 9.3% when applied twice and four times, respectively. In 2014 PchlQ16 application was significantly more effective as the number of above ground stem per plant increased by 30.21% and 49.65%; the number of tubers per plant by 38.43% and 91.14%; and the total yield by 33.05% and 92.35% compared to untreated control, when was applying twice and four times, respectively.

Using PchlQ16 four times during the growing seasons led to an increase in yield ranging from 9.3% (in 2013) to 92.35% (in 2014) compared to the control.

Based on our comprehensive results, we can recommend the use of PchlQ16 as a biological agent during the potato growth season. Further work is recommended to test the inhibition of *Fusarium* spp. in field conditions.

References

- Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian S. & Smith, D. L. (2018). Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Frontiers in plant science*, 9, 1473 (1/17).
- Bardas, G.A., Lagopodi, A.L., Kadoglidou, K. & Tzavella-Klonari, K. (2009). Biological control of three *Colletotrichum lindemuthianum* races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365. *Biological Control*, 49(2), 139–145.
- Biessy, A., Novinscak, A., Blom, J., Léger, G., Thomashow, L. S., Cazorla, F. M., Josic, D. & Fillion, M. (2019). Diversity of phyto-beneficial traits revealed by whole - genome analysis of worldwide - isolated phenazine - producing *Pseudomonas* spp. *Environ Microbiol*, 21, 437-455.
- Bročić, Z., Dolijanović, Z., Poštić, D., Milošević, D. & Savić, J. (2016). Yield, Tuber Quality and Weight Losses During Storage of Ten Potato Cultivars Grown at Three Sites in Serbia. *Potato Research*, 59(1), 21-34.
- Bunker, R.N. & Mathur K. (2001). Integration of biocontrol agents and fungicide for suppression of dry root rot of *Capsicum frutescens*. *Journal of mycology and plant pathology*, 31, 330–334.
- Bus, C.B. & Wustman, R. (2007). The Canon of Potato Science: 28. Seed Tubers. *Potato Research*, 50(3-4), 319-322.
- Costa, R., van Aarle, I.M., Mendes, R. & van Elsas, J.D. (2009). Genomics of pyrrolnitrin biosynthetic loci: evidence for conservation & whole-operon mobility within Gram-negative bacteria. *Environmental Microbiology*, 11(1), 159–175.

- Daami-Remadi, N. & El Mahjoub, M. (2006). Présence en Tunisie d'isolats de *Fusarium sambucinum* résistants aux benzimidazoles. *Biotechnology, Agronomy, Society and Environment*, 10, 7-16.
- Eken, C., Demirci, E. & Sahin, F. (2000). Pathogenicity of the fungi determined on tubers from potato storages in Erzurum, Turkey. *Journal of Turkish Phytopathology*, 29, 61-69.
- Esfahani, M.N. (2005). Susceptibility assessment of potato cultivars to *Fusarium* dry rot species. *Potato Research*, 48, 215-226.
- FAOSTAT (2018) *Online Database* (available at <http://faostat.fao.org/>, accessed May 28, 2018).
- Gashgari, R. & Gherbawy, Y. (2013). Pathogenicity of Some *Fusarium* Species Associated with Superficial Blemishes of Potato Tubers. *Polish Journal of Microbiology*, 62(1), 59-66.
- Gudmestad, N.C., Taylor, R.J. & Pasche, J.S. (2007). Management of soilborne disease on potato. *Australasian Plant Pathology*, 36(2), 109-115.
- Jamali, F., Sharifi-Tehrani A, Okhovvat M, Zakeri Z. & Saberi-Riseh R. (2004). Biological control of chickpea *Fusarium* wilt by antagonistic bacteria under greenhouse condition. *Communications in agricultural and applied biological sciences*, 69(4), 649-51.
- Jošić, D., Protolipac, K., Starović, M., Stojanović, S., Pavlović, S., Miladinović, M. & Radović, S. (2012). Phenazines producing *Pseudomonas* isolates decrease *Alternaria tenuissima* growth, pathogenicity and disease incidence on cardoon. *Archives of Biological Sciences*, 64 (4), 1495-1503.
- Jošić, D., Cirić, A., Soković, M., Stanojković-Sebić, A., Pivić, R., Lepšanović, Z. & Glamočlija, J. (2015). Antifungal Activities of Indigenous Plant Growth Promoting *Pseudomonas* spp. from Alfalfa and Clover Rhizosphere. *Frontiers in Life Science*, 8(2), 131-138.
- Khorsani, G.A. & Safaie, A. N. (2008). Biological control of *Fusarium* wilt of potato using antagonistic strains of bacteria. *Iranian Journal of Plant Pathology*, 44 (173), 1-21.
- Knowles, R., Knowles, L. & Kumar, G.N.M. (2003). Stem number & set relationships for Russet Burbank, Ranger & Umatilla Russet potatoes in the Columbia Basin. *Potato Progress*, 3(13), 1-4.
- Landa, B.B., Mavrodi, O.V., Raaijmakers, J.M., McSpadden Gardener, B.B., Thomashow, L.S. & Weller, D.M. (2002). Differential ability of genotypes of 2,4 - diacetylphloroglucinol - producing *Pseudomonas fluorescens* strains to colonize the roots of pea plants. *Applied and Environmental Microbiology*, 68(7), 3226-3237.
- Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchand, T. & Samiyappan, R. (2010). Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against *Fusarium* wilt of tomato. *Biological Control*, 54(2), 83-89.
- Meyer, S. L. F., Everts, K. L., McSpadden Gardener, B., Masler, E.P., Abdelnabby, H.M.E. & Skantar, A.M. (2016). Assessment of DAPG-producing *Pseudomonas fluorescens* for Management of *Meloidogyne incognita* and *Fusarium oxysporum* on Watermelon. *Journal of Nematology*, 48(1), 43-53.
- Momirović, N., Broić, Z., Stanisavljević, R., Štrbanović, R., Gvozden, G., Stanojković-Sebić A. & Poštić, D. (2016). Variability of Dutch potato varieties under various agroecological conditions in Serbia. *Genetika*, 48(1), 109-124.
- Ocamb, C.M., Hamm, P.B. & Johnson, D.A. (2007). Benzimidazole resistance of *Fusarium* species recovered from potatoes with dry rot from storages located in the Columbia basin of Oregon and Washington. *American Journal of Potato Research*, 84, 169-177.
- Organisation for Economic Co-operation & Development (2009). "Report of Workshop on the Regulation of Biopesticides: Registration & Communication Issues". In: *Series on Pesticides*. <http://www.oecd.org/chemicalsafety/pesticides-biocides/biological-pesticide-registration.htm>, No 44.
- Peters, R.D., MacLeod, C., Seifert, K.A., Martin, R.A., Hale, L.R. Grau, C.R. & MacInnis, S. (2008). Pathogenicity to potato tubers of *Fusarium* spp. isolated from potato cereal and forage crops. *American Journal of Potato Research*, 85, 367-374.
- Pivić, R., Starović, M., Delić, D., Rasulić, N., Kuzmanović, Đ., Poštić, D. & Jošić, D. (2015). Bacterial antagonists *Bacillus* sp. Q3 and *Pseudomonas chlororaphis* Q16 capable to control wheat powdery mildew in wheat. *Romanian Biotechnological Letters*, 20(3), 10448-10460.
- Poritsanos, N., Selin, C., Fern&o,W.G.D., Nakkeeran, S. & de Kievit T.R. (2006). A GacS deficiency does not affect *Pseudomonas chlororaphis* PA23 fitness when growing on canola, in aged batch culture or as a biofilm. *Canadian Journal of Microbiology*, 52, 1177-1188.
- Poštić, D., Momirović, N., Dolijanović, Ž., Broić, Z., Jošić, D., Popović, T. & Starović, M., (2012). Effect of Potato Tubers Origin & Weight on the Yield of Potato Variety Desiree in Western Serbia. *Field and Vegetable Crops Research*, 49(3), 236-242.
- Poštić, D., Starović, M., Popović, T., Bosnić, P., Stanojković-Sebić, A., Pivić, R. & Jošić D. (2013). Selection and RAPD analysis of *Pseudomonas* spp. isolates able to improve biological viability of potato seed tubers. *Genetika*, 45(1), 237-249.
- Puopolo, G., Raio, A., Pierson, L.S. & Zoina, A. (2011). Selection of a new *Pseudomonas chlororaphis* strain for the biological control of *Fusarium oxysporum* f.sp.*radicis lycopersici*. *Phytopathologia Mediterranea*, 50, 228-235.
- Raio, A., Puopolo, G., Cimmino, A., Danti, R., Della, R.G. & Evidente, A. (2011). Biocontrol of cypress canker by the phenazine producer *Pseudomonas chlororaphis* subsp. *aurofaciens* strain M71. *Biological Control*, 58(2), 133-138.
- Recep, K., Fikretin, S., Erkol, D. & Cafer, E. (2009). Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. *Biological Control*, 50(2), 194-198.
- Saikia, R., Varghese, S., Singh, B.P. & Arora, D.K. (2009). Influence of mineral amendment on disease suppressive activity of *Pseudomonas fluorescens* to *Fusarium* wilt of chickpea. *Microbiological Research*, 16(4), 365-373.
- Secor, G.A. & Salas, B. (2001). *Fusarium* dry rot and fusarium wilt. In: Stevenson, W.R., Loria, F., Franc, G.D., Weingartner, D.P., editors. Compendium of potato diseases. St.Paul, mn, USA: APS Press.
- Selin, C., Habibian, R., Poritsanos, N., Athukorala, S.N., Fern&o, D. & de Kievit, T.R. (2010). Phenazines are not essential for *Pseudomonas chlororaphis* PA23 biocontrol of *Sclerotinia sclerotiorum*, but do play a role in biofilm formation. *FEMS Microbiology Ecology*, 71(1), 73-83.
- Selvaraj, S., Ganeshamoorthi, P., And, T., Raguchander, T., Seenivasan, N. & Samiyappan, R. (2014). Evaluation of a liquid formulation of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *cubense* and *Helicotylenchus multicinctus* in banana plantation. *Biological Control*, 59(3), 345-355.
- Sindhu, S.S. & Dadarwal, K.R. (2001). Chitinytic and cellulolytic *Pseudomonas* spp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* spp. cicer in chickpea. *Microbiological Research*, 156(4), 353-358.
- Susilomati, A., Wahyudi, A.T., Lestari, Y., Suwanto, A. & Wiyono S. (2011). Potential *Pseudomonas* isolated from soybean rhizosphere as biocontrol against soil borne phytopathogenic fungi. *Journal of Biosciences*, 18, 51-56.
- Tadesse, M., Lommen, W.J.M. & Struik, P.C. (2001). Development of micropropagated potato plants over three phases of growth as affected by temperature in different phases. *Netherlands Journal of Agricultural Science*, 49(1), 53-66.
- Venter, S.I., Theron, D.J., Steyn, P.J., Ferreira, D.I. & Eicker, A. (1992). A relationship between vegetative compatibility and pathogenicity of isolates of *Fusarium oxysporum* f.sp. *tuberosi* from potato. *Phytopathology*, 82, 858-862.
- Wolf, A., Fritze, A., Hagemann, M. & Berg, G. (2002). *Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal properties. *International Journal of Systematic and Evolutionary Microbiology*, 52(6), 1937-1944.

Uticaj *Pseudomonas chlororaphis* subsp. *aurantiaca* soja Q16 koji inhibira rast *Fusarium oxysporum* na prinos krompira

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Sažetak: U ovom radu je ispitan potencijal sojeva *Pseudomonas chlororaphis* koji proizvode antibiotike da povećaju prinos krompira i da inhibiraju razvoj micelije fitopatogene gljive *Fusarium oxysporum* (Fo) izolovane sa krompira. *P. chlororaphis* subsp. *aurantiaca* soj Q16 (PchlQ16) izazvao je najveću inhibiciju rasta micelije (67,07%) gljive Fo izolata A2 *in vitro*. U poljskim ogledima utvrđivan je uticaj PchlQ16 na broj stabala po biljci, broj krtola i prosečnu masu krtole po biljci i ukupan prinos krompira sorte Rudolph. Primena PchlQ16 i broj tretmana imali su značajan uticaj na prinos krompira. PchlQ16 je povećao ukupan prinos krtola od 4,9% do 33,05% (dva tretmana), odnosno od 9,3% do 92,35% (četiri tretmana) u poredjenju sa kontrolom. Na osnovu ovih rezultata preporučujemo primenu PchlQ16 četiri puta tokom vegetacionog perioda krompira. Rezultati *in vitro* ogleda u kojima je ovaj soj izvršio supresiju razvoja *F. oxysporum* podržavaju ovu preporuku. U ovom istraživanju pokazano je da PchlQ16 deluje kao efektivan stimulator rasta biljaka u proizvodnji krompira i može biti efikasan u prevenciji infekcije gljivom *F. oxysporum*.

Key words: *Fusarium oxysporum*, *Pseudomonas chlororaphis*, rizobakterija, *Solanum tuberosum* L., uticaj inhibicije

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