doi: 10.17221/725/2017-PSE

Use of active microorganisms of the *Pseudomonas* genus during cultivation of maize in field conditions

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ABSTRACT

Holečková Z., Kulhánek M., Hakl J., Balík J. (2018): Use of active microorganisms of the *Pseudomonas* genus during cultivation of maize in field conditions. Plant Soil Environ., 64: 26–31.

The aim of this research is to estimate the influence of a bioeffector (BE) application on dry matter yield and nutrient content (P, K, Ca, Mg, S) in maize (*Zea mays* L.). Between 2014 and 2016, a field experiment with silage maize as a testing plant was realized on sandy loam Cambisol. The application of *Pseudomonas* sp. in combination with phosphorus (rock phosphate (RP) or triple superphosphate (TSP)) and nitrogen fertilizers (ammonium nitrate with urea, ammonium nitrate with limestone, calcium nitrate or ammonium sulfate with a nitrification inhibitor) and with different application strategies was studied. The effects of a bioeffector application on the increase of dry matter yields were not confirmed. An important influence on the BE application and its activity was probably those of soil and site conditions and competition of the researched microorganisms with other present microorganisms. Higher yields of dry matter were shown in treatments where P fertilizers were applied. There was almost no difference between the application of RP and TSP. This could be caused by the fact that the soil had a slightly acidic pH value. In this case, the RP showed similar results to the TSP. The application of bioeffector significantly increased Mg, K and S contents in maize above-ground biomass. An increase of the Ca content was almost significant and a tendency towards a higher average content of phosphorus was also recorded.

Keywords: plant nutrition; bioavailability; biocontrol; bacteria; fungi; organic farming

In past decades, agriculture and crop production were almost completely dependent on mineral fertilizers, and hence on natural sources of nutrients which are scarce and limited. As a result, there is need to develop new alternative ways to improve bioavailability of nutrients from the applied fertilizers. Development of these alternative ways requires understanding of plant-soil relationship, but also good knowledge of agriculture and environment (Whithers et al. 2014). One of many alternative ways is the tested application of products containing live and active microorganisms in plant production. These commercially produced preparations, so-called bioeffectors, contain two main components: live microorganisms (bacteria or fungi) and active natural compounds (plant and herbal extracts, dried seaweeds and soil and compost extracts).

Pseudomonas well suited as biocontrol and growth-promoting agents (Vallabhaneni 2016). These microorganisms can enhance availability of deficient or immobile nutrients in soils after solubilizing their mineral forms. The use of *P. putida* improved the growth and yield of various crops such as rice, tomato or wheat. The application of *P. putida* either alone or in combination with phosphorus improved plant growth, plant uptake (N, P, K) and antioxidative activity (Israr et al. 2016). Yusran et al. (2009) reported that the application

Supported by the Resource Preservation by Application of Bioeffectors in European Crop Production, Project No. 7. RP 312117.

doi: 10.17221/725/2017-PSE

of *Pseudomonas* sp. and *Bacillus amyloliquefaciens* (individually or in combination) into soil in a pot experiment led to improved state of tomato roots. They were healthy and showed significantly higher colonization by arbuscular mycorrhizal fungi. Liu et al. (2015) reported a positive effect of seed inoculation with diazotrophic bacteria on shoot dry weight and yield of maize. Products with this composition can be used in both conventional and organic farming and are developed for a wide range of agricultural and ornamental plants (Hogenhout et al. 2009, Neumann 2012). Most nutrients (mainly phosphorus) in soil are in a form that is barely available for plants. The main effect of bioeffectors should be to improve the bioavailability of nutrients for plants (Lošák et al. 2016). These products also contribute to a better plant health because they are also used against various diseases and pests. By promoting plant growth, they contribute to higher yields and better production quality (Janarthanam 2013, Vallabhaneni 2016, El-Gremi et al. 2017, Holečková et al. 2017).

This work has three main aims: (1) to assess the effect of the *Pseudomonas* sp. application in combination with P fertilizers on silage maize yield, and on the content of selected nutrients (P, K, Ca, Mg, S) in field conditions; (2) to confirm a potential of bioeffector Proradix (PDX) to promote maize growth and acquisition of mineral nutrients in soil with satisfactory available P content in the Czech Republic; (3) to test new, costs-saving, ways of bioeffector (BE) application together with different ways of nitrogen application.

MATERIAL AND METHODS

Small-plot field experiment with different ways of application of Proradix (*Pseudomonas* sp., strain DSMZ 113134, 5 × 10^{10} colony forming units/g, Sourcon Padena, Tübingen, Germany (further only PDX) to silage maize was realized in the years 2014–2016. The small-plot experiment was established at the Humpolec site (49°33'16''N; 15°21'18''E). Characteristics of the experimental field are following: Cambisol, sandy loam, 525 m a.s.l., average yearly temperature and rainfall 8.2°C and 573 mm, pH_{CaCl2}: 5.7. The content of bioavailable nutrients in soil estimated using the Mehlich 3 method is: 71 mg P/kg, 180 mg K/kg, 1200 mg Ca/kg and 120 mg Mg/kg. The plot size was 4.20 × 7.50 m. The distance between rows was 70 cm (6 rows per plot) and the number of seeds was 95 000 per ha. Maize seeds were untreated cv. Colisée in 2014 and 2015 and cv. Kartagos in 2016 (both from KWS, Einbeck, Germany). Twenty randomly selected plants from two centre rows were harvested from each plot.

Experimental design A. Different application strategies and combinations with P fertilizers were tested in the experiment A. For broad application, a dose of 22.7 kg/ha of PDX was used (dose per plot was diluted in 9 L of water) and for local application ten times lower amount (2 L of water solution per plot). Local application was conducted using a spike wheels applicator GFI 3A (Maschinen und Antriebstechnik GmbH, Güstrow, Germany). Broad application was conducted with sowing, where PDX was applied into soil to a depth of 10 cm immediately after spraying at soil surface. Local application was done at the 5th developed leaf. All fertilizers were used before sowing and applied in 10 cm soil profile. Two phosphorus fertilizers were applied with sowing – (i) fine milled rock phosphate – RP (7.9% P) and (ii) triple superphosphate – TSP (21% P). The whole experiment was fertilized with nitrogen (120 kg N/ha in calcium ammonium nitrate 27% N) and potassium (50 kg K/ha in Patentkali 24.9% K, 6% Mg) at sowing. The experimental design is shown in Table 1.

Experimental design B. The main aim of the experiment B (Table 2) was to test the cost-saving application strategies of PDX. PDX was always applied in bands (10 cm depth) into the rows next

Table 1. Experimental design A

Treatment	PDX Application	PDX	N	P	K
		(kg/ha)			
Zero control	0	0	120	0	50
Water control	broad	0	120	0	50
RP	broad	0	120	26	50
TSP	broad	0	120	26	50
PDX + RP	broad	22.7	120	26	50
PDX + TSP	broad	22.7	120	26	50
PDX + RP	local	2.27	120	26	50
PDX + TSP	local	2.27	120	26	50

PDX – bioeffector Proradix; RP – rock phosphate; TSP – triple superphosphate

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Table 2. Shortened experimental design B with terms of bioeffectors and fertilizers application

Treatment	PDX application	PDX (kg/ha)	N (kg/ha)	N application
Zero	–	0	0	–
N1	–	0	80 + 60 + 0	broad
N2	–	0	0 + 80 + 60	broad
Local N	–	0	0 + 140 + 0	local
N1 + PDX li	local	2.27	80 + 60 + 0	broad
N1 + PDX gr	local	2.27	80 + 60 + 0	broad
Local N + PDX li	local	2.27	0 + 140 + 0	local
Local N + PDX gr	local	2.27	0 + 140 + 0	local
N2 PDX liquid	local	2.27	0 + 80 + 60	broad

N1 – 80 kg N/ha 3 days before sowing + 60 kg N/ha at 2–3 developed leaf; N2 – 80 kg N/ha at 2–3 developed leaf + 60 kg N/ha; PDX – bioeffector Proradix (li – liquid; gr – granulated)

to the seeds (Figure 1). Two forms of PDX were used: (i) liquid (PDX li), where the application rate was 9 L of solution per plot or (ii) granulated (PDX gr) form where the granules were made by spraying the PDX stock solution on pumice stone (size 0–3 mm, Palkowitschia s.r.o., Prague, Czech Republic). The final dose of PDX was always 2.27 kg/ha. PDX was always applied 4 days after sowing. The PDX application was combined with three ways of nitrogen fertilizing: (i) 80 kg N/ha 3 days before sowing + 60 kg N/ha at 2–3 developed leaves (N1); (ii) 140 kg N/ha via the CULTAN strategy at 2–3 developed leaves (Figure 1; Local N) and (iii) 80 kg N/ha at 2–3 developed leaves + 60 kg N/ha (N2). For N1 and N2, calcium ammonium nitrate was used.

The aim of the CULTAN method is building of ammonium nitrogen depot in soil using high pressure injectioning. Roots grow around this reserve decreasing the ammonium toxicity through N uptake from the non-toxic reserve surface. As a result, N uptake is continuous and plants have the N supply available during the entire cropping season, which saves the costs for commonly used repeated N application (Sommer 2005).

Experimental design C. Ammonium releases the H^+ proton in microorganisms and plants can therefore improve solubilization of Ca-phosphates as a side effect (Neumann and Römheld 2002).

The aim was hence to test the potential of PDX to release phosphorus from rock phosphate improved with ammonium nitrogen fertilizing. The experimental design is shown in Table 3. The source of nitrogen was calcium nitrate – CN (15% N) or ammonium sulfate + dimethylphenylpiperazinium (DMPP) nitrification inhibitor – AS (21% N). CN was applied in two doses (short before sowing) and AS all at once short before sowing. The nitrate form in CN is very mobile in soil. Because of that, the dose was divided in two parts and the first (bigger) part was applied very shortly (one day) before sowing to be the source of available nitrogen for germinating plants. The second part was applied during vegetation to provide the nitrogen supply during vegetation. RP and TSP were applied also in one dose short before sowing. All fertilizers as well as PDX were applied broad.

Maize from all three experiments was always harvested in dough vegetation stage. For experiment A it was on 3rd September, for experiment

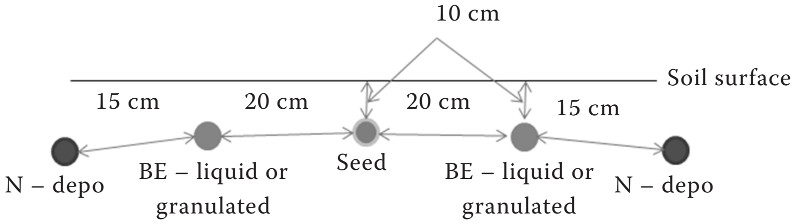

Figure 1. Scheme of the bioeffectors (BE) application together with Local N

doi: 10.17221/725/2017-PSE

Table 3. Experimental design C

Treatment	PDX application	PDX	N	P
		(kg/ha)		
Zero	–	0	0	0
CN	–	0	107 + 54	0
AS	–	0	161	0
CN + RP	–	0	107 + 54	130
AS + RP	–	0	161	130
CN + RP + PDX	broad	22.7	107 + 54	130
AS + RP + PDX	broad	22.7	161	130
CN + TSP	–	0	107 + 54	130
AS + TSP	–	0	161	130

CN – calcium nitrate; AS – ammonium sulfate + dimethylphenylpiperazinium (DMPP) nitrification inhibitor; RP – rock phosphate; TSP – triple superphosphate; PDX – bioeffector Proradix

B on 11th September, and for experiment C on 14th September.

Analyses. Twenty average plants from two centre rows of each plot were always harvested to estimate the silage maize yield. A representative sample of three selected plants from each plot was air dried and finely milled for further analyses. Total content of macronutrients in the above-ground biomass was estimated using dry decomposition (Mader et al. 1998). The extracts were measured by an inductively coupled plasma optical emission spectrometry (ICP-OES, Varian VistaPro, Victoria, Australia). Nutrient uptake was calculated based on the content of elements in the above-ground biomass and the yield of maize from the small-plot.

Data evaluation. Due to different designs of the experiments each year, the data were analysed by ANOVA, where the year, bioeffector application and P fertilization were included. All analyses were carried out by Statistica ver. 12. software (California, USA) at a significance level of 0.05 (Tukey's *HSD* (honest significant difference)).

RESULTS AND DISCUSSION

Three-way analysis of variance (ANOVA) with interactions between external factors (year, bioeffector and P-fertilizer) was used to investigate differences in yield of dry matter and P, K, Ca, Mg and S content from 2014 to 2016 in the above-ground biomass of maize. Differences in dry matter yields over years depending on the application of bioeffector and P-fertilizers are shown in Table 4.

The application of bioeffector or P-fertilizers did not significantly affected dry matter yields. On the other hand, only the effect of year was particularly significant for dry matter yield where the highest content was observed in 2016 and the lowest in 2014.

Differences in the element content over years, application of bioeffector and P-fertilizers are shown in Table 5.

The results show that the application of bioeffector significantly increased the potassium, magnesium and sulfur content in maize above-ground biomass. The increase of Ca content was almost significant and a trend towards a higher average content of P was also observed. Also Lošák et al. (2010) described the effect of year in 2-year field experiments with graded fertilizer doses applied to maize. Application of P-fertilizers did not affect the content of any included element. On the other hand, the effect of year was significant for all elements, as the highest content was observed in 2015 and the lowest in 2016. The highest contents of K, Ca and S were probably caused by higher rainfall in 2015 or by site conditions.

Differences in the element uptake over years, application of bioeffector and P-fertilizers yield of dry matter are shown in Table 6.

The data obtained validated the results of nutrient contents mentioned in Table 5. The application of

Table 4. Effect of Proradix (PDX), phosphorus fertilization and year on the yield of biomass

		Dry matter yield (t/ha)
BE	0	18.6
	PDX	16.1
	P	0.478
P-fertilizer	0	15.1
	RP	19.5
	TSP	20.8
	P	0.035
Year	2014	10.8^{a}
	2015	15.1^{a}
	2016	26.7^{b}
	P	< 0.001

RP – rock phosphate; TSP – triple superphosphate. Different letters are significantly different at the 0.05 level

doi: 10.17221/725/2017-PSE

Table 5. Effect of bioeffector Proradix (PDX), phosphorus fertilization and year on the concentration of elements (mg/kg) in maize above-ground biomass

		P	K	Ca	Mg	S
BE	0	2438	13 728[a]	2688	1453[a]	789[a]
	PDX	2539	15 486[b]	2891	1538[b]	881[b]
	P	0.210	< 0.001	0.095	0.049	0.012
P-fertilizer	0	2329	14 883	2878	1481	857
	RP	2416	14 601	2770	1505	820
	TSP	2521	14 338	2721	1500	829
	P	0.197	0.708	0.629	0.905	0.729
Year	2014	2674[b]	11 072[a]	1757[a]	1545[b]	568[b]
	2015	2851[c]	22 031[b]	5089[b]	1950[c]	1464[c]
	2016	1942[a]	10 718[a]	1523[a]	991[a]	473[a]
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

BE – bioeffector; RP – rock phosphate; TSP – triple superphosphate. Different letters are significantly different at the 0.05 level

bioeffector significantly increased Ca and S uptake in maize above-ground biomass. The application of P-fertilizers led to a slightly increased P uptake by plants. However, a decreasing tendency in Ca and S uptake was observed. Lower calcium and sulfur intake after application of P-fertilizers, compared to the control treatment, could have been caused by chemical sorption of HPO_4^{2-} and calcium. In case of sulfur, there could have been an antagonistic relation between HPO_4^{2-} and SO_4^{2-}. No effects depending on P fertilizing were significant. On the other hand, an effect of year was significant for all elements, as the highest content was observed in 2015 and the lowest in 2014. Also in this case, the highest nutrient uptake for all nutrients was probably caused by higher local rainfall in 2015 or by site conditions. Many authors carried out studies and experiments where they applied *Pseudomonas* and the results showed that the application of bacteria increased resistance of plants to many diseases (Mikicinski et al. 2016, Vallabhaneni 2016, Wu et al. 2017). Therefore, a higher uptake of all nutrients in 2015 could have been caused by the fact that plants began to defend themselves more against pathogens and thus they took up more nutrients.

Israr et al. (2016) reported that this bacteria genus is used as a biological fertilizer which, together with mineral fertilizers, can serve as an effective approach to enhance plant nutrition requirements. And that these microorganisms can increase avail-

Table 6. Effect of bioeffector Proradix (PDX), phosphorus fertilization and year on element uptake (kg/ha) in maize above-ground biomass

		P	K	Ca	Mg	S
BE	0	40.7	219.7	39.2[a]	22.4	11.8[a]
	PDX	40.2	251.1	48.2[b]	24.7	14.5[b]
	P	0.042	0.020	0.035	0.111	0.041
P-fertilizer	0	38.7	256.8	55.1[b]	24.0	15.9[b]
	RP	40.1	214.0	30.8[a]	22.5	9.9[a]
	TSP	45.2	202.0	29.5[a]	23.1	9.9[a]
	P	0.352	0.238	0.522	0.063	0.310
Year	2014	28.6[b]	119.8[a]	18.9[a]	16.7[b]	6.16[a]
	2015	43.6[a]	334.6[c]	79.1[c]	29.4[a]	22.5[c]
	2016	50.6[a]	270.8[b]	38.7[b]	25.6[a]	12.3[b]
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

BE – bioeffector; RP – rock phosphate, TSP – triple superphosphate. Different letters are significantly different at the 0.05 level

doi: 10.17221/725/2017-PSE

ability of deficient or immobile nutrients in soil after dissolution of their mineral forms. This fact has been confirmed for K, Mg and S content, as well as Ca and S uptake. On the other hand, Kifle and Laing (2016) carried out the study, which included experiments with corn where they applied the *Pseudomonas* bacteria. The results showed that the application had a positive effect only on seed germination, not on increased grain yield, dry matter or plant height. This study confirms this fact in the yield of dry matter.

In this research, the influence of bioeffectors on the K, Mg and S content in maize above-ground biomass and also on Ca and S uptake was statistically validated. After P-fertilizers application, Ca and S uptake decreased in contrast to the control treatment without P-fertilizer. A statistically significant effect of experimental year on the content and uptake of all elements and on the yield of dry matter was observed.

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Received on November 11, 2017
Accepted on December 8, 2017
Published online on January 12, 2018