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Improved soil and tomato quality by some biofertilizer products

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Summary

The use of microbial inoculums is a part of sustainable agricultural practices. Among various bioeffectors, the phosphorus-mobilizing bacteria are frequently used.

The objective of this study is to investigate the effect of some industrial biofertilizer inoculums, of containing P-mobilizing bacteria on the quantity and some quality parameters of tomato fruits. Spore-forming industrial *Bacillus amyloliquefaciens* FZB42 (Rhizovital) as single inoculums and combinations with other *Bacillus* strains (Biorex) were applied on *Solanum lycopersicon* Mill. var. Mobil test plant. Soil microbial counts, phosphorus availability, yield and fruit quality, such as total soluble solids (TSS) content and sugars (glucose, fructose) were assessed. The results found that single industrial inoculums of FZB42 product had positive effect on P-availability and fruit quality in the pots. Fruit quality parameters, TSS content, soluble sugars were significantly improved ($p<0.05$). Such better fruit taste was correlated significantly by the most probable number (MPN) microbial counts. Use of such bioeffector products is supported by the positive interrelation among measured soil characteristics and inside healthy quality parameters of tomato fruits.

Keywords: biofertilizers, P-solubilization, *Bacillus* sp., inoculation, tomato quality, Brix value, TSS

Introduction

Sustainable agriculture and healthy food production is one of the most important key aspects nowadays (FAO, 2003). An increasing demand exists for fruits and vegetables of avoiding the use of chemical fertilizers and pesticides (Doran and Zeiss, 2000; Jakab and Kátai, 2013; Németh and Várallyay, 2015). Crucial requirements of reducing artificial chemicals and xenobiotic inputs, especially among horticultural practices (Kassam et al., 2009; Mónok and Füleky, 2017; Madarász et al., 2018).

Various microbial diversity and types of microorganisms are living in the soils, including both bacteria and fungi with known general and/or highly specific nutrient mobilization ability (Schweitzer et al., 2008).

Active microbial soil characteristics were largely ignored, due to the mainly artificial plant nutrient fertilizing practices. Concern needs for the above mentioned reasons for healthy environment. Eco-friendly production methods and chemical-free farming systems are increasing (www.biofector.info).

One of the most accepted and followed alternatives of intensive agricultural practices are the application of bioeffectors (BE), which might include strains of beneficial microbes (Matics and Biró, 2015). Those microbes are known to support plant-growth and development, nutrient and water uptake of plants and they might suppress harmful soil-borne plant pathogens in soil-plant systems. Bioeffectors can successfully integrate into any environment-friendly agricultural practices. The main accepted application of bioeffectors is reducing or diminishing fertilizers and high levels of pesticide inputs.

The direct and indirect beneficial effects and mechanisms of bioeffectors are known. The combination of described mechanisms are possible, and different bacteria or fungal species might have more than one of these functional traits. Schippers et al. (1985) described three different affecting mechanisms: a) increased nutrient supply, nitrogen fixation, phosphorous solubilisation, nutrient exploration or nutrient transport, b) increased defence mechanisms against some soil-borne plant pathogens by inhibiting of the growth; competition for space and for nutrients, antibiosis, parasitism, induced systemic resistance; c) direct enhancement of plant growth, production of hormone-like, plant growth regulating substances.

These protected effects can overlap in many cases and different microorganisms can also have more than one efficient PGPR or plant growth regulating (PGR), hormone-like mechanisms. In several occasions not all these effects might be developed, due to the fact, that they might be modified during the vegetation periods through the various biotic and abiotic environmental (stress) factors (Biró et al., 2000; Carvalhais et al., 2013; Kátai et al., 2015). Specific bioeffective and stress-tolerant microbes therefore are used efficiently at the practice of amelioration, recultivation and/or at remediation practices (Biró et al., 2012; Tállai et al., 2017).

Phosphorus (P) supply is key issue for optimal tomato production. Necessity of P-elements are crucial at early stage of plant growth. Presence of P-mobilizing microorganisms as *Bacillus* spp. bacteria, furthermore the *fluorescens* and *putida* types of *Pseudomonas* spp., which can influence indirectly the uptake of this element is also essential (Khan et al., 2009; Schmidt et al., 2010). Those microorganisms are able to mobilize hardly-available P forms (Bashan et al., 2013) e.g. they can solubilize natural rock phosphates to be available for plants, improving the growth of roots, shoots and plant biomass or fruits. Due to those beneficial properties, bioeffector organisms are successfully involved in ecological farming (Hariprasad and Niranjana, 2009; Biró et al., 2012).

Among bioeffector (BE) strains, those with spore-forming abilities are more tolerant and survivor at serious environmental stress conditions, therefore their application is highly expanded.

The *Bacillus amyloliquefaciens* is a non-pathogenic soil bacterium. Similar to other *Bacillus* species it is capable of producing endospores. The species also shows some antifungal properties which are influenced by several environmental factors, including nitrogen availability (Caldeira et al., 2008; Choudry et al., 2015). *Bacillus* spp., i.e. *B. subtilis* is known as efficient in cellulose degradation, amylase production and can block phytopathogenic micro-organisms.

Tomato is one of the most frequent vegetable grown worldwide (Helyes et al., 2015). Tomato might contain significant amounts of natural health-protecting compounds, such as the organic acids, sugars and antioxidant materials, such as the lycopene, C-vitamins and others (Devies et Hobson, 1981). Soluble sugars play crucial role in tomato quality and food taste values. Tomato contains 5.0–7.5 % of dry matter that is mainly constituted of fructose, glucose and other organic compounds (Salles et al., 2003). Levels of soluble sugars are highly dependent on the ripeness and also on the certain cultivars (Baldwin et al., 1991). P-supply of tomato plants influences highly the quantity and quality of biologically active compounds in the fruits (Di Cesare et al., 2010).

Effect of some commercially available microbial fertilizer (bioeffector) inoculations, including spore-forming bacteria was studied on the fruit quality of tomato among light-chamber conditions. Main focus of the study was to answer: Is *Bacillus* spp. inoculation increasing the soil microbial counts and available P for plants? Is some of inside tomato fruit quality parameters improved by the inoculations? Is there any correlation between bioeffector treatments and fruit quality?

Materials and methods

Pot experiment with tomato test plant

Experiments were carried out in pots. There were 4 different treatments used of applying 2500 g air-dried soil in 4 replicates in slightly hummus sandy soils (vertisols), originating from SZIU experimental field for organic agriculture (Soroksár, Hungary). Characteristics are: pH_{H2O}=7.9; C:N (A horizon): 9.92; NO_2+NO_3-Nitrogen: 8.5 mg kg^{-1} soil; P (CAL): 43 mg (100 g^{-1} soil), K (CAL): 17 (100 g^{-1} soil); Mg ($CaCl_2$); 10 (100 g^{-1} soil).

For nutrient supplementation we applied: Viano (13% N; 660 kg ha^{-1}), Patentkali (30% K_2O; 10% MgO; 17% S; 1200 kg ha^{-1}). Light-period of pot experiment was 14/10 hours in a daily basis in a controlled room, with 17 °C to 28 °C temperature. Tomato (*Solanum lycopersicon* Mill.) Hungarian cultivar of 'Mobil' were used.

Bioeffector inoculations and P-analysis

Commercially available bioeffectors (BE biofertilizers) were used on the basis of supplier suggestions. Bioeffector (BE) strains were used in the rates of 1.33 % (v/v), i.e. 0.2 ml for each plants Hungarian BR1 and BR2 products, as a 2-component-biological fertilizer was also applied, BR1: 0.1875 ml $plant^{-1}$; BR2: 0.375 ml $plant^{-1}$, shown in *Table 1*. Ammonium lactate method (P-AL) was used to estimate available phosphorus content colorimetrically (Egner et al., 1960)

Table 1. *Treatments and density of bioeffector inoculums, used in the pot experiment*

Treatments	Bioeffector strains	Colony forming units (CFU g^{-1})	Inoculation rates
C (Control)	No inoculums, only water	-	-
BE (Bioeffector)	*Bacillus amyloliquefaciens* FZB42	2.5×10^{10}	1.33 % (v/v), i.e. 0.2 ml $plant^{-1}$
BR1 (Biorex-1)	*Bacillus subtilis; B. thüringiensis; B. megaterium*	2×10^{10}	5 l ha^{-1}, 0.1875 ml $plant^{-1}$
BE+BR1	Combination of 2 products	$1.25\times10^{10}+1\times10^{10}$	0.2+0.375 ml $plants^{-1}$

Note: producers of BE-FZB42 strain is RhizoVital (Germany); BR1 is produced by Biorex Ltd. (Hungary).

Microbial counts and tomato fruit quality assessments

Most probable number (MPN) method of sporeforming bacteria in soil capable of growing in nutrient broth was used by a microplate method from 10-fold serial soil dilutions (Libisch et al., 2010). The statistical method of Cochran (1950) was applied to calculate MPN values.

Soluble solids concentration (Brix or TSS) was determined by Atago® PAL-3 refractometer device (Cavalcanti et al., 2013).

The fruits (skins and fruit-pulp) were homogenized with a blender and homogenates were stored at -25 °C until analysis. Two g from each fruit samples were diluted in 2 ml deionized water, shake for 1 hour in dark and centrifuged for 10 min at 10000 rpm (Hettich Mikro 22R). One ml supernatant was then pipetted off and filtered through a 0.45 µm MILLEX®-HV Syringe Driven Filter Unit (SLHV 013 NL, PVDF

Durapore), purchased from Millipore Co. (Bedford, MA, USA). High Performance Liquid Chromatograph (HPLC, Waters Co., 34 Maple Street, Milford, MA, USA) were used for sugar content analysis. Two replicates were used. Concentrations were calculated from areas of corresponding peaks and expressed in mg 100^{-1} g.

Statistical analysis

For evaluation of the results one-way ANOVA test was applied. Normality assumption was proven by Kolmogorov-Smirnov test ($p>0.05$; $p=0.200$) or Shapiro-Wilk test ($p>0.05$) and the homogeneity of variances was checked by Levene's test ($p>0.05$). As the estimation was proven we applied Tukey HSD post hoc test. If it was not proven the Games-Howell post hoc test was applied (Juhos et al., 2010). Pearson correlation analysis was used to estimate the interrelation among soil and tomato fruit quality parameters.

Results

Soil parameters and fruit yield of tomato with bioeffector application

Table 2 is showing the most probable number (MPN) of cultivable microorganisms in the soils, treated or non-treated by bioeffectors.

Table 2. *Most probable number counts (MPN) of bacteria and phosphorous (P_2O_5) content of soil in the pot experiment with bioeffector inoculations (n=4)*

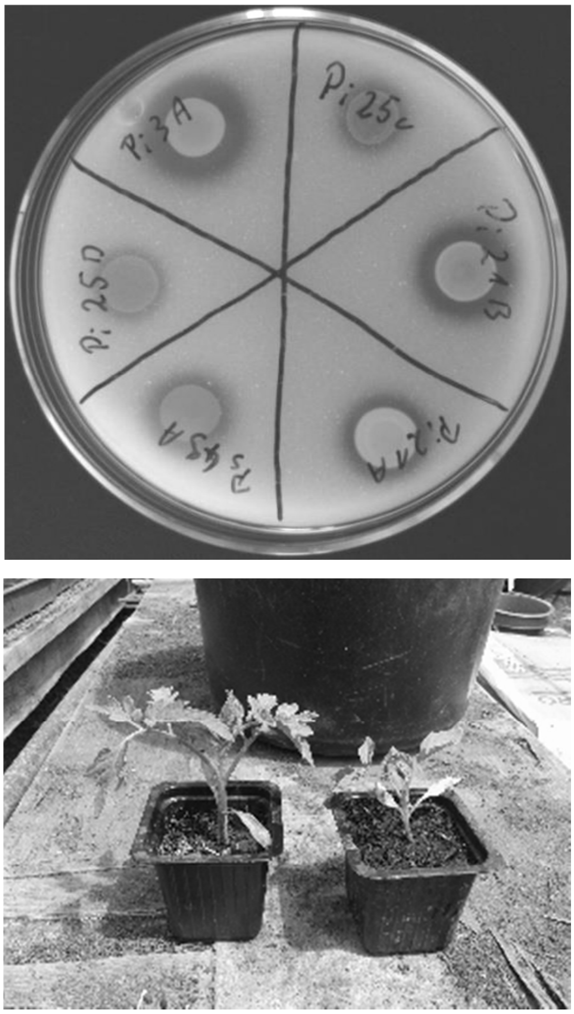
Treatments	MPN	Phosphorus (mg 100 g soil^{-1})	Fruit number pot^{-1}	Fruit yield pot^{-1}
Initial	5.74±0.76a	59.2±6.5a	-	-.
Control	5.42±0.61a	67.4±19.1ab	3.25±1.16a	13.49±-8.2a
BE	6.07±0.66a	10.9±7.3c	3.75±0.92ab	24.81±9.1b
BR1-2	8.05±1.83b	90.4±12.7bc	4.00±2.7ab	n.d.

Note: treatments – BE-*Bacillus amyloliquefaciens* FZB42 strain (RhizoVital); BR1-mixture of *Bacillus subtilis, B. thüringiensis, B. megaterium* strains (Biorex); BR2-mixture of *Azotobacter chroococcum, Azospirillum lipoferum* and *Pseudomonas putida* strains (Biorex)

Differences among the treatments were not appeared significantly. However, it is apparent that the number (logMPN) of microorganisms in the BE-treated soils was enhanced tendentiously during plant growth.

Prior to the pot-trial a preliminary soil analysis was performed. A specific focus was given for the availability of phosphor (P) doses, due to the fact, that used bioeffector products were predicted of containing specific P-mobilizing microorganisms. It was found, that phosphor content was increased by the used soil inoculums. Treatment with the phosphorous-mobilizer *Bacillus* (BE, BR1) strains could increase the available P-content in the soil significantly (*Table 2, Figure 1*).

Figure 1. *Calcium- and phosphor- (Ca-P)-solubilizing ability of various bacterium isolates and tomato plants inoculated or not by phosphor-mobilizing bioeffector (BE) bacterium, (Bacillus amyloliquefaciens, FZB 42)*



Source: www.biofector.info project. Note: the greater the halo around the colony, the higher of P-solubilizing ability of isolates and higher growth of tomato plant below.

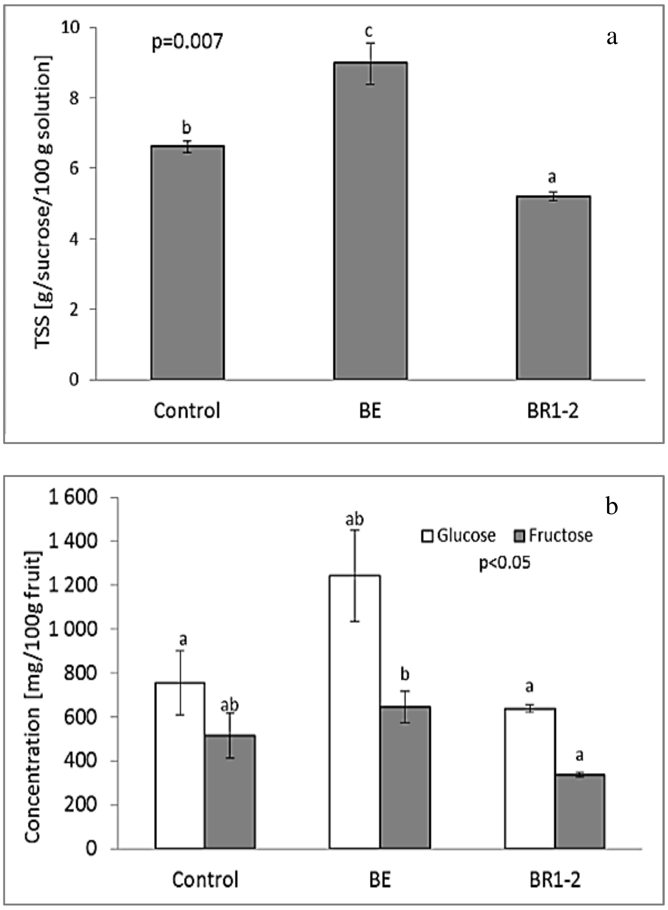
Fruit quality parameters of tomato with bioeffector application

Tomato’s fruit quality parameters were examined, by assessing the total soluble solid content (TSS). Measured value was significantly higher through the effect of used BE industrial product; while in case of BR1-2 treatments TSS value was significantly lower compared to the control.

Post hoc comparisons using Games-Howell test indicated, that the mean score of BE (M=9.00; SD=0.608) and the BR1-2 treatment (M=5.2;

SD=0.122) were significantly different from the control (M=6.62; SD=0.16) (*Figure 2a*).

Figure 2. *Total soluble solid (TSS) content of tomato fruits and sugar (glucose, fructose) content of tomato fruits, grown in pot experiment (n=4)*



Note: treatments – BE-*Bacillus amyloliquefaciens* FZB42 strain (RhizoVital); BR1-mixture of *Bacillus subtilis, B. thüringiensis, B. megaterium* strains (Biorex); BR2-mixture of *Azotobacter chroococcum, Azospirillum lipoferum* and *Pseudomonas putida* strains (Biorex).

Among inside fruit quality parameters some of the sugar- (glucose, fructose) components were assessed. Regarding the sugar content in the pot experiment there were no significant differences found in comparison with the control but differences were significantly supported between the two treated groups. TSS value was found to correlate slightly with the concentration of sugar component.

Correlation among soil characteristics and tomato fruit quality parameters

Correlation analysis was performed among total cultivable sporeforming microorganisms (*Bacillus* sp.), estimated by MPN method and some of the tomato fruit quality parameters both at pot- and at field experiments. Correlation results of MPN, measured TSS values and estimated inside fruit quality parameters are shown in *Table 3*. Interrelation of inside fruit quality with the used biofertilizer application was found.

Table 3. *Correlation among abundance of sporeforming bacteria (MPN), the total soluble sugars (TSS) and inside fruit quality parameters (glucose, fructose) in tomato fruits, grown in pot experiment (n=4)*

	MPN	TSS	Glucose	Fructose
MPN	1	**0.788****	0.666	0.643
TSS	**0.788****	1	**0.945****	**0.936****
Glucose	0.666	**0.945****	1	**0.910****
Fructose	0.643	**0.936****	**0.910****	1

Note: ** correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level.

Discussion

"Biofector" is an integrated international project with the aim to reduce input of mineral fertilizers in European agriculture by development of specific bio-effector microorganisms (BEs) to improve the efficiency of alternative fertilization strategies, such as organic and low-input farming, use of fertilizers based on waste recycling products and fertilizer placement technologies. Bioeffectors addressed comprise fungal and bacterial strains (e.g. *Bacillus*, *Trichoderma*, *Pseudomonas,* etc.) with well-characterized root growth promoting and nutrient solubilizing potential.

In Biofector international project one of the test plants is tomato, which is important in many ways in human consumption. Significant industrial and commercial use is known, exceeding all of the vegetables in terms of both cultivation and consumption in World. The aim of Biofector research project is the development of viable alternatives to conventional mineral fertilization, and contribution to a more efficient management of non-renewable resources of mineral nutrients, energy and water, to preserve soil fertility and to counteract the adverse environmental impact of agricultural production.

Tomato is very sensitive to the P supply. It is required relatively high amount of phosphorus, especially at the beginning of its growth (Schmidt et al., 2010). For this reason, two of the marketed bioeffector products were used in this study, which are known to solubilize and mobilize the highly available phosphorus content in the soil. The general use of *Bacillus* spp. strains as P-mobilizing microorganisms are supported by the fact that due to its spore forming ability the cells of the *Bacillus* spp. can survive long in the soils and at any severe environmental conditions. It is why the individual species and *Bacillus* spp. genus are considered as one of the microbial groups, with specific food-quality and safety importance (Kocsis and Biró, 2015; Kocsis et al., 2017).

Among the marketed products, which are containing spore forming bacteria there were single and combined inoculation treatments used in this study. It was hypothesized that the more types of bacteria, including more than one particular species could produce better performance for the plant growth. This idea was already supported by several authors of using different combinations of bacteria or fungal-bacterial consortium.

Among sustainable agricultural practice the synergism of symbiont type of microbes is also demonstrated between arbuscular mycorrhiza fungi and the nitrogen-fixing bacteria. The behaviour of microbes in a consortium is dependent on several environmental factors. It has been reported, that even a beneficial symbiosis is diminished in a short time of periods when the environmental condition, such as the drought, high temperature or reduction of light intensity or heavy metals toxicity become non-tolerable to the macro- and micro-symbionts (the higher plants and the microbes) (Biró et al., 2000, 2015; Hayat et al., 2010).

This fact indicates the potential of fast microbial changes in the rhizosphere and the interrelation among microbial colonization with plant growth parameters. Significantly increasing of the microbial abundance is necessary to use soil improvers (e.g. biochar) or organic fertilizer, compost application, which might enhance soil microbial status in one step. We found in the pot experiment that the abundance of microorganisms is increasing with the plant age for a certain points. The importance of microbial inoculation is highlighted at early seedling status, when the PGR hormone-effect of inoculated microbes might enhance root-volume, indirectly improve water- and nutrient-uptake of plants (Biró et al., 2000, Jakab and Kátai, 2013; Choudry et al., 2015).

Testing the MPN abundance of specific sporeforming microorganisms in the tomato plant rhizosphere, differences among the used treatments were not found significantly in our study. It was apparent, however that the number (logMPN) of studied microbes in the BE treated soils was enhanced tendentiously with plant age (from April to July) in the pot experiment. It was also demonstrated in this study, that the abundance was found parallel with the available phosphorus concentrations in the soil. In connection with this finding Fekete et al. (2011), Kotroczó et al. (2010) have found, that more organic P can enhance the alkaline phosphatase activities, which finally increase the P-availability in the soil.

The alkaline phosphatase is mainly produced by the soil microorganisms and the extent of its synthesis and excretion of it can be coupled to the microbial activity and/or the population size (Veres et al., 2015). Due to the presence of BE *Bacillus amyloliquefaciens* FZB42 an increased phosphorus content was found in the treated pots.

Regarding the size and number of fruits and fruit quantity there was also an increase in treatment BE and BR1-2 in the pot experiment. Idriss et al. (2002) made a similar finding in their experiments in laboratory and field conditions with the same *Bacillus amyloliquefaciens* species. They found that improved phosphorus nutrition is achievable by mobilization of phosphorus fixed as insoluble inorganic polyphosphates which accounts for 20–50% of the total soil organic phosphorus. It is generally accepted that higher microbial counts can result higher nutrient availability in soil (Rodriguez and Faga, 1999).

Considering of the fruit quality there are also several study of showing the importance of biofertilizer application. Phosphorous fertilization is a key component in the metabolism and regulation of several pathways involved in the biosynthesis of secondary plant metabolites. Many of those materials are biologically active compounds. P may increase the level of some acids such as ascorbic acid, although interaction with climatic factors and the growing season growing area may occur. Oke et al. (2005) studied the effect of P fertilizer on the quality of tomato under field conditions for three consecutive years, by evaluating the pH, the acidity, the lycopene, the vitamin C content and also the flavour volatiles. They noted that the influence of P application on several of the quality parameters mentioned above was marginal, while climatic conditions had a more predominant effect. Organic acids and sugar comprise the majority of the total dry matter content of tomatoes (Malundo et al., 1999). The "reducing type" of sugars and organic acids are significant components of fruits, determining the sweet- and the sour taste of the tomatoes, respectively. Their concentration may also affect flavour acceptability (Salles et al., 2003). Tomato fruits harvested at the same time from the bioeffector-treated plots had more balanced quality parameters, increasing of the food tasty value of the tomato fruits.

Conclusion

On the bases of this study we found that phosphorus mobilizer microorganisms can be successfully and bioeffectively used as bio-fertilizers, since through applying them soluble phosphorus of the soil might be increased. Phosphorus content of the soil was higher as a result of the used bioeffector inoculation, capable for phosphorus mobilization.

As a consequence, the nutritional and food quality and tasty value of tomato fruits (the TSS and sugar content) have changed to a more favourable, tasteful direction. A tastier, more marketable food was produced. Governed by the principles of sustainable farming methods, the application of artificial agrochemicals can be reduced, including also the inorganic phosphorus fertilizer use. Eco-friendly, natural bio-fertilizers and combined bioeffective solutions might play important role in the sustainable agri-/horticultural practices.

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