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# FRUIT QUALITY OF TOMATO AFFECTED BY SINGLE AND COMBINED BIOEFFECTORS IN ORGANICALLY SYSTEM

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Sugars, acids, and antioxidants as biologically active compounds, are significant elements of healthy tomato foods. The question arises if beneficial bioeffector microbes are able to improve the food quality of the tomato up scaled, among organic environmental conditions? Spore-forming bacteria used in the industry of *Bacillus amyloliquefaciens* FZB42 as single inoculums and its combinations with other *Bacillus* strains and N-fixing siderophore bacteria were used on tomato test plant in pots and in an organic field experiment. Soil nutrients, yield, and inside fruit quality, the content of some essential organic acids and sugars were measured during the growth period. The abundance of aerob heterotroph soil microbes was determined by the Most Probable Number method. A single industrial inoculum of used bioeffector had a positive effect on P-availability and yield in the pots. Among field conditions, the greater variability in environmental parameters could diminish the final significant differences of soluble P in soil and fruit yield. The soil available P-content and the fruit Total Soluble Solid content (TSS) and soluble sugar and organic acid content were significantly improved ($p<0.05$) both in pots and in the organic field conditions. Combination of different microbial treatments did not give further enhancement. There were seasonal differences also recorded in sugar-acid ratio, mainly at the field experiment. Appropriate bioeffector applications are the part of sustainable, organic and healthier tomato food production.
**Keywords:** Biofertilizer, phosphate solubilization, *Bacillus* spp., sugar-acid ratio, tomato, sustainable agriculture, soil biology

## INTRODUCTION

Sustainable agriculture and food production become one of the most important aspects nowadays. An increasing demand exists for fruits and vegetables, which come from ecological farming systems and their growth without chemical fertilizers and pesticides, xenobiotics, which are harmful also for the human health (FIBL 2001; Hesammi *et al.,* 2014). These healthy products can be provided more particularly by sustainable farming systems. As a consequence of intensive agrochemical application, environmental impacts on soils are increasing parallel with loss of abundance and diversity of living organisms (Johnston, 1986; Varga *et al.*, 2007). One of the most important requirements, therefore, is the sustainable farming practices and food production with the need to reduce the use of artificial chemicals and stimulants (Juhos, 2014; Juhos *et al.,* 2015, 2016; Tariq *et al.*, 2016). With those conditions consumers' highlighted expectation for healthy and chemical-free foods are also required.

Depending on the variability of environmental conditions 3-15 t microorganisms can be found in each hectare of the soils (Fekete *et al.,* 2008; Kotroczo *et al.*, 2008; Veres *et al.,* 2013). The biological activity of soil organisms might play a crucial role in the mobilization of soil nutrients and make them available for plants. Several organisms are known of supporting the degradation of soil organic matters (Kotroczo *et al.,* 2014a; Fekete *et al.,* 2014; Veres *et al.*, 2015), hereby giving an opportunity for the plants to get available nutrients (Toth *et al.,* 2013). There are several microbial groups in the soils, which can promote plant's uptake through mobilization of nutrients and make them water-soluble (Schweitzer *et al.,* 2008; Kotroczo *et al.,* 2014b). In recent decades chemical fertilization was the most dominant in agricultural production and active microbial soil characteristics, which can be key-important for successful growing, were largely ignored.

Tomato is one of the most important vegetable grown worldwide, consumed both in fresh and in processed forms. Nowadays, this tasty vegetable has more important role in healthy lifestyle hereby there is an increasing demand for the sustainable tomato production. In order to achieve the highest possible yield, unreasonable tillage and overuse of agrochemicals (pesticides, chemical fertilizers) are applied at the intensive growing systems (Glendining *et al.,* 2009). This can reduce the nutritional value of crops, vegetables, and fruits, as well. Tomato fruits might contain significant amounts of natural health-protecting compounds, such as the organic acids, sugars, and antioxidant materials, as the lycopene, etc. (Devi *et al.*, 2008, Mohacsi-Farkas *et al.*, 2014).

Concern for the healthy environment, eco-friendly production methods, and chemical-free farming systems are increasing tremendously (den Hollander *et al.*, 2007). One of the alternative methods of intensive agricultural practices is the application of living and non-living bioeffectors, which might include one or more beneficial microorganisms. Those selected microbes are known to support plant-growth and development, nutrient and water uptake of plants and might suppress the damage of harmful pathogen microorganisms. Those bioeffectors therefore can be successfully integrated into any of the environmental-friendly plant-growth production and/or any remediation technologies. The symbiotic connection between Plant Growth Promoting Bacteria (PGPB) or Rhizobacteria (PGPR) and their host plants was first described and nominated by Kloepper and Schroth (1978). PGPB or PGPR microorganisms are considered as beneficial soil organisms, having a favorable impact on plant growth and yield. Those organisms can protect the plants from soil-borne plant pathogens, in addition, they enhance the soil chemical and physical characteristics (Glick *et al.*, 2007). By using PGPB the nitrogen and phosphorus content and other useful component in the soil can be raised, therefore a number of synthetic fertilizers might be reduced (Hayat *et al.*, 2010).

The plant-soil-microbe systems have many distinct effects, mechanisms and their combinations, and different bacteria or fungi species might have more than one of these. Schippers (1985) described three effect mechanisms in plant-soil-microbe systems: a) increasing the nutrient supply, nitrogen fixation, nutrient exploration or nutrient transport; b) increasing defense mechanisms against plant pathogens and microorganisms that inhibit plant growth; competition for space and nutrients, antibiosis, parasitism, degradation of inhibitors, induced systemic resistance; c) direct enhancement of plant growth, production of several hormone-like substances.

These protected effects can overlap in many cases and different microorganisms can also have more than one efficient plant growth promoting (PGPR) or plant growth regulating (PGR) mechanisms. In several occasions not all these effects might be developed, due to the fact, that they can be modified by several biotic and abiotic environmental (stress) factors (Carvalhais *et al.*, 2013). Those particular effects are especially specific for combined “second-generation” of microbial inoculums where microorganisms were used as specifically adapted to a certain environment and for a host-plant. Adapted microorganisms can confer their tolerance towards the higher plants and also used therefore more efficiently at the agri-/viti-/silvi- and horti-cultural practices (Taczmann-Brückner *et al.*, 2004; Biro *et al.*, 2000, 2012).

Phosphorus supply is a key issue for an optimal tomato production. The necessity of P-elements is especially crucial at the beginning of tomato growth. Presence of phosphor-mobilizing microorganisms as *Bacillus* spp., the fluorescent-putida type of *Pseudomonas* which can help the uptake of this element is also essential (Khan *et al.*, 2009). Those microorganisms are able to mobilize the hardly available phosphor forms (Bashan *et al.*, 2013) e.g. they can solubilize the natural rock phosphate to be available for the plants, helping the growth of roots and shoots and the plant biomass. Due to those beneficial properties, the bioeffector organisms can be successfully involved in ecological farming practices (Hariprasad and Niranjana, 2009). Among the bioeffector (BE) strains, those which are having spores are known to be more tolerant to the serious environmental stress factors, therefore their use is highly suggested in industrial microbial inoculum products.

The *Bacillus amyloliquefaciens* is a non-pathogenic soil bacterium. Similar to other *Bacillus* species it is capable of producing endospores allowing it to survive for extended periods of time. The species also shows some antifungal properties which are influenced by environmental nitrogen availability (Caldeira *et al.*, 2008). *Bacillus subtilis* is known as efficient in cellulose degradation, amylase production and can block phytopathogenic micro-organisms (Yang *et al.*, 2009). *Bacillus thuringiensis* is generally used against soil pests, by creating an alkaline reaction and leading to the serious pest destruction. *Bacillus megaterium* is efficient in phosphate mobilization, the production in growth substances and vitamin B12 and it is also possible to modify the plant residues into humus. *Azotobacter chroococcum* bacteria are free-living nitrogen-fixing microbes, with drought and cold tolerance (3-4 °C) ability. *Azospirillum lipoferum* is an associative nitrogen-fixing bacteria, which secondary interact with the roots of monocots and also known as auxins, gibberellins, cytokinin plant hormone producer (Diamantidis *et al.*, 2000). *Pseudomonas putida* might produce siderophore-like compounds which use of iron ions in front of pathogenic organisms (competitive inhibition), pesticides decomposed, and disposal action and in addition, macro-and micro-nutrients and other compound (Timmis *et al.*, 2002). In case of ecological farming production these properties are highly appreciable; therefore, bioeffector treatments may be even more feasible (Rodriguez and Fraga, 1999).

Soluble sugars and organic acids play an important role in the characterization of the tomato quality and food taste value. This vegetable contains 5.0-7.5 % of dry matter that is mainly constituted of fructose, glucose, citric acid, malic acid, and other organic compounds (Salles *et al.*, 2003; Sariyer and Oztokat, 2015). The major organic acids in tomatoes are citric- and malic acids, with mainly citric-acid predominating (Davies and Hobson, 1981). Their levels are highly depending on the ripeness and/or the cultivars, beside the crucial soil/environmental factors (Baldwin *et al.*, 1991; Beni *et al.*, 2014). As phosphorus supply influences the quantity of biologically active compounds in the fruit (Cesare *et al.*, 2010) the most important organic acid and sugar components by High Performance Liquid Chromatography (HPLC) and the Total Soluble Solid content (TSS) were also evaluated.

Effect of some commercially available microbial fertilizers was studied under controlled light-chamber conditions and also in the fields of organic agricultural practices. The influence of some bioeffector microorganisms was studied on the shoot growth, yield, fruit, and food quality of the tomato. Our hypothesis was to demonstrate the facts: i) through the BE treatment a larger vegetative production and more valuable of nutrient content, higher yield can be expected; ii) the effect of microbial fertilizers increasing the amount of available P for plants; iii) the tomato yield will be more healthy and balanced regarding the sugar-acid ratio.

## MATERIALS AND METHODS

***Experimental conditions*:** The experiment was carried out in pots and also among organic field conditions in 2014. There were 4 different treatments with 4 replicates at the pot (3 $dm^3$/pot) experiment (Table 1). On the other hand, there were 12, of 4×5m plots used at the Research and Experimental Farm of the Szent Istvan University in Soroksar, Hungary (N 47°40´; E 19°15´ at 111 m altitude).

The ecological farming practice is carried out for more than ten years now on the experimental field. For nutrient supplementation, we apply authorized substances used in organic farming: Viano (13% N), Patentkali (30% $K_2O$, 10% MgO, 17% S). For plant protection, we used a copper-containing fungicide (Cuproxat FW-1). The experimental area was watering by drip irrigation system. Climatic conditions of the area are shown in Table 2. In 2014 it was an excessively rainy growing season with almost 1.5-times higher precipitation than the usual average annual value.

The pot experiment was performed in the light-chamber of Department of Soil Science and Water Management of SZIU, Budapest, Hungary. Soil originated from the field experimental station of Soroksar, was used. Lighting of the pot experiment was 14 hours on a daily basis in accordance with the requirements of the tomato plants. Pots were placed in a controlled-temperature light room, programmed at a temperature ranging from 17°C to 28°C, while the light intensity (Lux) had been 25000 lx (Herrera *et al.*, 2008). Parameters of the applied soil are shown in Table 2.

**Table 2. Main characteristics of soil at Soroksar site, Szent Istvan University, Hungary.**

| Soil parameters | Soil content |
|---|---|
| $pH_{H2O}$ | 7.9 |
| C:N (A horizon) | 9.92 |
| $NO_2^+$-$NO_3^-$N | 8.5 mg/kg |
| P (CAL) | 43 mg (100 $g^{-1}$ soil) |
| K (CAL) | 17 (100 $g^{-1}$ soil) |
| Mg ($CaCl_2$) | 10 (100 $g^{-1}$ soil) |
| Soil type | slightly humous sandy soil |
| Mean annual temperature (°C) | 11°C |
| Mean annual precipitation (mm) | 500 mm |

In both pot and field experiments, the applied plant material was the tomato (*Solanum lycopersicon* Mill.) Hungarian cultivar of 'Mobil', of which seedlings were grown under controlled conditions and planted into their final place after $14^{th}$ day at the same stage of maturity. The pot experiment was carried out between 31 March 2015 and June 16, 2015. In the case of field trials, the seeding was 25 March 2015 in cold plastic house, the transplant of the seedling to small pots on $9^{th}$ of May 2015. The seedlings transplantation was between 20-26 of May 2015.

Examination of P contents of soil samples was measured by ammonium-lactate (AL) method (Egner *et al.*, 1960). Total Soluble Solid content (TSS) of tomato fruits was determined by Atago® PAL-3 Refractometer device.

***Soil treatment methods*:** Commercially available microbiological fertilizers were used for both the pot and the field experiment. On the basis of the results achieved in the pot experiment, the combinations of BE and BR treatments were also tested among the field conditions according to the Table 1.

During the general set-up of the pot experiment, there was 2500 g air-dried soil to put in each pot and one tomato seedling was planted into. The microorganism fertilizers were applied as it was suggested by the provider. The Bioeffector BE strains were used in concentration of 1.33 % (v/v), i.e. 0.2 $ml.plant^{-1}$, the Hungarian product BR-1 and BR-2, as a 2-component biological fertilizer was applied jointly by the

**Table 1. Treatments and density of biofector inoculums at the pot and field experiment.**

| Treatments | Biofector strains | Bioeffector cell concentration in the product ($cfu.g^{-1}$) |
|---|---|---|
| C (Control) | Without any treatment | - |
| BE (Biofector 1) | *Bacillus amyloliquefaciens* FZB42 | $2.5\times10^{10}$ |
| BR1 (Biorex-1) | *Bacillus subtilis; Bacillus thüringiensis; Bacillus megaterium* | $2\times10^{10}$ |
| BR2 (Biorex-2) | *Azotobacter chroococcum: Azospirillum lipoferum; Pseudomonas putida* | $2\times10^{10}$ |
| BE+BR2 | Combination of 2 different products | $1.25\times10^{10} + 1\times10^{10}$ |

supplier's protocol in concentration as follows: BR-1: 0.1875 $ml.plant^{-1}$; BR-2: 0.375 $ml.plant^{-1}$ (Table 1).

Most probable number (MPN) method of soil microorganisms capable of growing in nutrient broth (meat extract 3 g; peptone 5 g; pH 7±0.2) (Downes and Ito, 2001) was used by a microplate method. 10-fold serial soil dilutions were made from $10^{-1}$ to $10^{-8}$ dilutions as described (Libisch *et al.,* 2010). From each sample three parallel 20 µl aliquots from each dilution were transferred to sterile polystyrene 96-well microplates. After incubation at 22°C for 1 week, the plates were tested for growth using the respiration indicator iodonitrotetrazolium violet (INT) (Sigma-Aldrich) (Johnsen and Henriksen, 2009). 50µl INT solution (3g/L INT dissolved in water) was added to each well, and the plates were incubated overnight at 22°C. Metabolically active bacteria reduce INT to the corresponding formazan forming a purple precipitate. The number of growth-positive wells at each dilution was determined by visual inspection of the plates. The statistical method of Cochran was applied to calculate MPN values using the MPN calculator VB6 (Cohran, 1950). One-way ANOVA Tukey test with logMPN values and standard logMPN errors was used to determine whether MPN values of various pot experiment and field experiment soil samples were not significantly different at the 95% confidence level (<0.05) (Mukherjee, 2006; Vladar, 2008).

***Fruit quality measurements*:** The fruits (skins and fruit-pulp) were homogenized with a blender and homogenates were stored at -25°C until analysis. After thawing, 2 g from each fruit puree 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). WATERS High Performance Liquid Chromatograph (HPLC, Waters Co., 34 Maple Street, Milford, MA, USA) equipped with 2487 Dual λ Absorbance Detector (for determination of organic acids), 2414 Refractive Index Detector (for the determination of sugars) 1525 Binary HPLC Pump, In-Line Degasser, Column Thermostat (set at 40°C) and 717 plus Autosampler controlled with EMPOWER™2 software was used for HPLC analysis. For separation of organic acids Shodex KC-811 column (8 mm × 300 mm) with Shodex RSpak KC-G guard column were used. The mobile phase was a 0.1% aqueous solution of phosphoric acid. The flow rate was adjusted to 1 $ml.min^{-1}$, giving a pressure of 600±25 psi on the column at 40°C. The volume injected on the column was 20 µl, the detection time was adjusted to 20 min. Detection was carried out at an analytical wavelength of 220 nm. The retention times (in min) of the standards were 7.19 for citric and 9.10 for malic acid. For separation of sugars, a Sugar-PakTM column was used placed in a thermostat at 90°C. The mobile phase was a 0.0001 M aqueous solution of Ca-EDTA. The flow rate was 0.5 ml $min^{-1}$, resulting in a pressure of 450±10 psi on the column. The injected volume was 20 µl, the detection took 30 min for each sample. Retention times of standards were as follows: 8.32 for sucrose, 10.93 for glucose and 11.77 for fructose. Two replicates were used. Concentrations were calculated from the areas of the corresponding peaks and expressed in $mg.100^{-1}$ g.

***Statistical analysis*:** For the 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; p=0.244) and the homogeneity of variances was checked by Levene's test. As the estimation was proven we applied Tukey HSD post hoc test or Games-Howell post hoc tests.

## RESULTS

***Most probable number of microorganisms in pot and in field experiments*:** Figure 1 is showing the most probable number (MPN) of microorganisms in the soils, treated or non-treated by the used bioeffectors in case of the pot and of the field experiments. Days after inoculation (DAI) 2 and 10 weeks of growth in the pot experiment and 7 and 14 of growth in the field experiment. According to the total plate count results of PE and FE both normality assumption was proved by Shapiro-Wilk test (p>0.05; $p_1$= 0.715; $p_2$=0.883) and the homogeneity of variances was checked by Levene's test. As the assumption was proved, [F(7;32)=2.151; p1>0.05; p=0.066] we applied Tukey HSD post hoc test [F1(7;8)=0.015; F2(7;19)=0.057; p>0.05] we applied Tukey post hoc test. Differences among the treatments were not appeared significantly either any growing conditions. However, it is apparent that the number (logMPN) of microorganisms in the BE-treated soils was enhanced tendentiously from April to July in the pot experiment. The same on the other hand was not proven among the field conditions. No differences among the various bioeffector treatments could be found (Fig. 1a, b).

***Available phosphor content in soils with and without the bioeffector treatments:*** Prior to the trial, an entire soil analysis was carried out in the experimental field. A specific focus was given mainly for the phosphor (P) doses, as the used bioeffectors were predicted as P-mobilizing microorganisms. Among the macro elements, examined the phosphor content was found to be increased mainly in the pot experiment. The treatment with the presumable phosphorus mobilizer *Bacillus* (BE, BR-1) strains could increase the available P-content in the soil. A greater P-mobilizing efficiency was found on the BE industrial product, among the two treatments (BE and BR 1-2), however, no significant differences were recorded. Even though the F-value was higher than that was shown in case of the field experiment [F(3;8) = 8.198; p = 0.008] the found differences was not statistically approved (Fig. 2a, b).

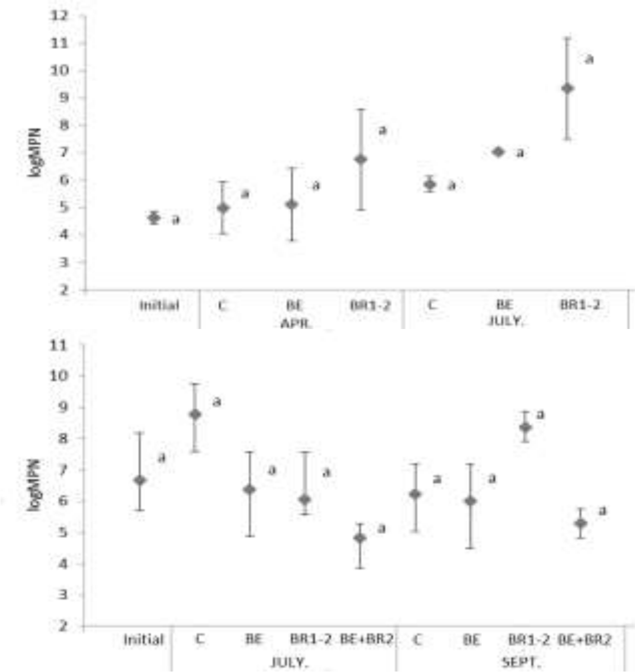


**Figure 1a, b. Abundance, most probable number of microorganisms (logMPN) in soils treated or not by various bioeffector products in the pot experiment (a) and among the field conditions (b) after certain sampling periods. Further details of the assessments are in the text.**

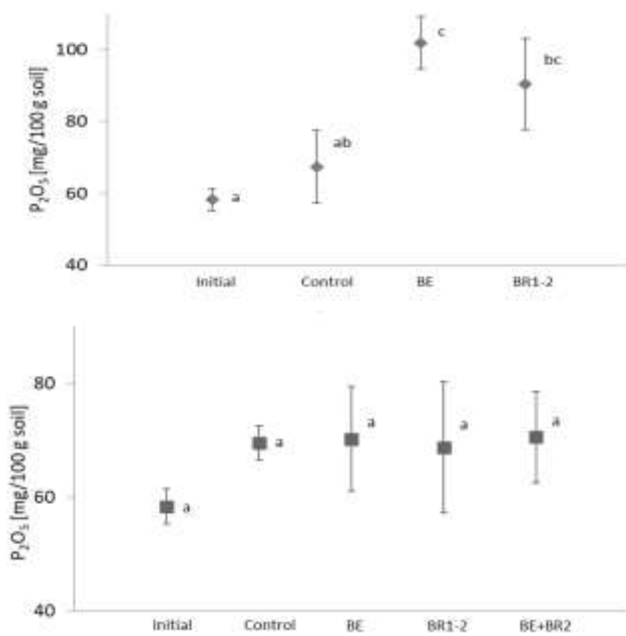


**Figure 2a, b. The $P_2O_5$ concentration of soil samples in the pot (a) and in the field (b) experiment after 10 or 14 weeks of vegetative growth of tomato, respectively.**

***Total soluble solid content of tomato fruits*:** Tomato's fruit quality parameters were also examined, by assessing the total Soluble Solid content (TSS). In case of the pot experiment, the TSS value was significantly higher through the effect of the BE industrial product while in case of the BR1-2 treatments the TSS value was significantly lower compared to the control. Post hoc comparisons using the 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 than the control (M=6.62; SD=0.16) (Fig. 3a, b).

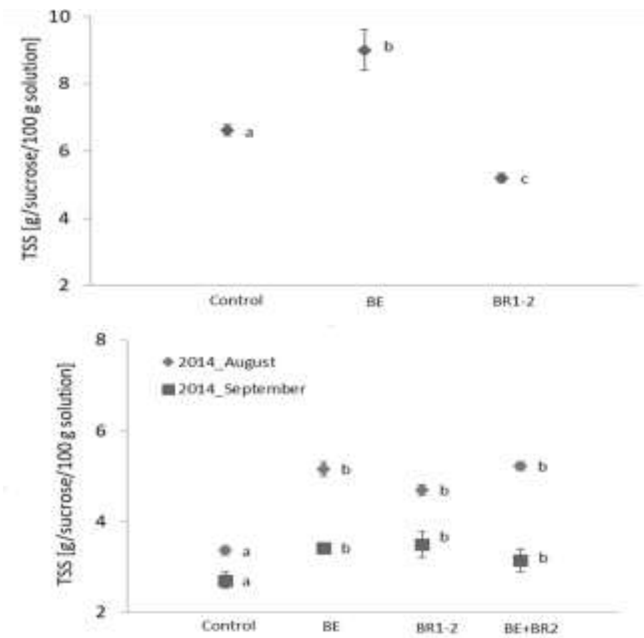


**Figure 3a, b. Brix value of tomato fruits in pot experiment (a) after 10 weeks of growth and among the field conditions (b) after 10- (August) and 14- (September) weeks of growth.**

Experiments were also performed under field conditions. Based on the results of the pot experiment besides the single BE treatment, the combined effect of bioeffectors (BR 1-2 and BE + BR-2) was also examined. Among the field conditions, the fruits were harvested two times during the season (after 10 weeks, in August 2014, and after 14 weeks in September 2014). The fruit quality analysis was carried out after both harvests. In case of the field experiment, the TSS value of fruits from the treated plots was significantly higher than the control at both harvests. Post hoc comparisons using the Tukey HSD test indicated that the mean score of BE (M=5.16, SD=0.15), BR1-2 treatment (M=4.7; SD=0.02) and the BE3 + BR2 combination (M=5.22; SD=0.08) were significantly different than the control (M=3.36; SD=0.089). In September the TSS value decreased but the differences have remained similar (Fig. 3a, b).

***Soluble sugar and organic acid content of tomato fruits*:** Some of the sugar- (glucose, fructose) and the organic acids (citric-, malic-) components of tomato fruits were assessed by High Performance Liquid Chromatography (HPLC). In comparison with the total soluble solid (TSS) values, similar result appeared with the HPLC-measured sugar components, however, those results were found to be lower. In case of 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. (Fig. 4a, b) The TSS value was found to correlate slightly with the concentration of sugar component measured by HPLC. There were no significant differences found in the sugar concentration among the used BE treatments assessed by HPLC. No significant differences were found between the two harvesting time regarding sugar content, not in fructose either in glucose components. Both normality assumption was proven by Kolmogorov-Smirnov test ($p>0.05$; $p_1= 0.200$; $p_2=0.117$) and the homogeneity of variances was estimated by Levene's test As the assumption was not proven [$F_1=F_2(3;4)=0.005$; $p<0.05$] a Games-Howell post hoc test was applied. Post hoc comparisons using the Games-Howell test indicated that the mean score of BE, BR1-2 treatment, and the BE + BR2 combination were no significantly different than the control (Fig. 4a, b).

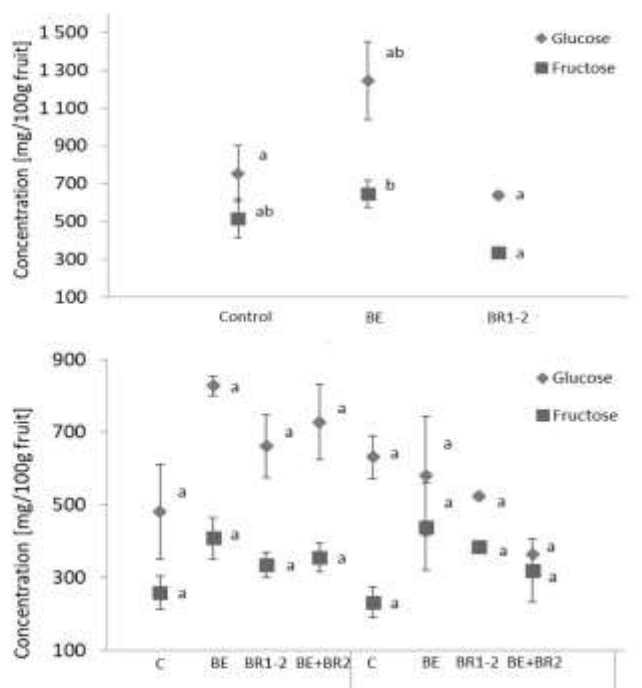


**Figure 4a, b. Sugar (glucose and fructose) concentration of tomato fruits after 10 weeks of growth in the pot experiment (a) and after 10 (Aug) or 14 (Sept) weeks of growth in the field experiment (b).**

The organic acids (citric- and malic ones) were also assessed by HPLC analysis. Regarding the pot experiment, those organic acid content was significantly higher in case of control (M=565.79; SD=3.46) than the BE (M=525.39; SD=2.79) and BR1-2 treatments (M=261.03; SD=3.25). In the field experiment, two sampling periods and measurement of the organic acids were applied. In September there was an increasing amount of citric acid in fruits of plants grown in the bioeffector treated soil. The same for malic acid was only tendentiously supported by the statistical analysis (Fig. 5a, b).

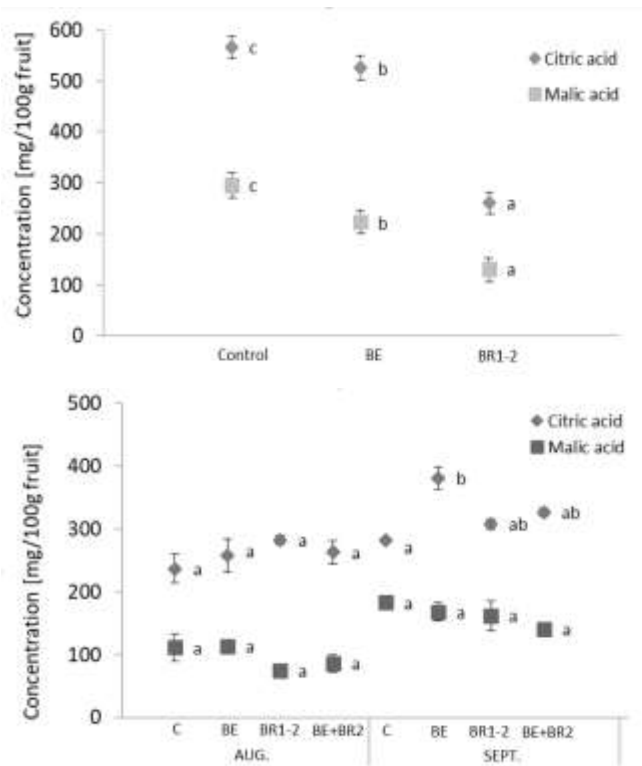


**Figure 5a, b. Organic acid (citric-, malic-) concentration of tomato fruits after 10 weeks in pot experiment (a) and after 10 (Aug) or 14 (Sept) months of growth in the field experiment (b).**

## DISCUSSION

Various marketed bioeffector products were performed on organically grown tomato, both in pot experiment and field conditions. Tomato is one of the most important vegetables, generally consumed worldwide either as fresh or in processed food forms. Bioeffectors might efficiently improve the nutritional value of the tomato. The biologically active compounds, sugars, acids and antioxidants are important elements of all healthy tomato products. The aim of the experiments was to apply beneficial microorganisms, which might support the growth and development of the tomato plants among the organic conditions.

Tomato is very sensitive to the P supply. It is required a relatively high amount of phosphor, especially at the beginning of its growth (Schmidt *et al.*, 2010). For this reason some of the marketed bioeffector products were used in this study, which is known to solubilize and mobilize the highly available phosphor content in the soil. The general use of *Bacillus* spp. strains as P-mobilizing microorganisms are also supported by the fact that those microbes can survive in the soils among several, even at the most serious environmental stress conditions. Due to its spore-forming ability the cells of the *Bacillus* spp. can survive long in the soils and at any other environmental conditions. It is why the individual species and *Bacillus* spp. genus is considered as one of the microbial groups, which might be considered as food-safety important bacteria (Beczner *et al.*, 2004; Dudas *et al.*, 2014; Kocsis and Biro, 2015; Kocsis *et al.*, 2017). Among the marketed products, which are containing spore-forming bacteria there were single and combined inoculation treatments used. It was hypothesised that the more types of bacteria, including more than one particular species, could produce better performance for the plant growth promotion (PGPR activity). The abundance of inoculated microorganisms was followed by most probable number (MPN) method. Although the presence or absence of those inoculated microorganism was assessed only occasionally at the beginning and at 2-times during the vegetation periods, there were not really greater abundance recorded as the total countable number in the tomato rhizosphere. It was found only in the pot experiment that the abundance of microorganisms is increasing with the plant age. The connection of microbial presence and activity with plant-necessity was already demonstrated among serious environmental stress conditions in several previous publications (Vivas *et al.*, 2006; Panwar *et al.*, 2011; Domonkos *et al.*, 2013).

Testing the MPN abundance of 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 microorganisms 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) 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, as it was shown by Garg (2008) and Kaleeswari (2007). 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, there was also an increase in treatment BE and BR1-2 in the pot experiment. Contrarily, in case of the combined treatments, where beside the *Bacillus* spp. strains some other microorganisms, as the Nitrogen-fixing *Azospirillum* and *Azotobacter* furthermore the siderophore-producing *Pseudomonas* bacteria (BE+BR-2) were given, no further improved growth parameters were recorded.

According to Bruulsema *et al.* (2004) 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. The high level of citric acid concentration was found at a high level of phosphorus concentration by Cesare *et al.* (2010). Toth *et al.* (2007) and 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 flavor 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). Reducing 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 flavor acceptability (Salles *et al.*, 2003). Tomato fruits harvested at the same time from the bioeffector-treated plots had a more balanced acid-sugar ratio and the control had a higher level of organic acid concentration. The well-balanced acid-sugar ratio appreciably increases the food quality and tasty value of the tomato fruits.

***Conclusion*:** On the bases of this study, we found that phosphorus mobilizer microorganisms can be successfully used as bio-fertilizers in the ecological farming system since through applying them soluble phosphorus of the soil might be increased under certain environmental conditions. The phosphorus content of the soil was higher as a result of the used bioeffector inoculation, presumably capable for phosphor-mobilization. As a consequence, the nutritional and food quality and tasty value of tomato fruits (the acid-TSS ratio) has changed to a more favorable, tasteful direction. A tastier, more marketable food was produced by an eco-friendly organic way. Governed by the principles of sustainable farming methods, the application of artificial agrochemicals can be reduced, including also the inorganic phosphorus fertilizer application. Eco-friendly, natural bio-fertilizers might play important role in the sustainable agricultural and horticultural practices.

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