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PRELIMINARY RESULTS ON THE EFFECT OF PLANT PROBIOTIC BACTERIA ON THE MACRO- AND MICRONUTRIENT CONTENT OF STRAWBERRY

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Abstract
In recent years there has been a growing interest in food with preventive and therapeutic effects on human health (functional food). A series of experiments were started to identify different factors and treatments influencing the level of health protection compounds in strawberry. In the present paper preliminary results on the effect of PGPR on the macro- and micronutrient content of strawberry are presented.

1 Introduction

Recently, there has been a growing interest in healthy foods, including fresh vegetables and fruits, containing high levels of nutrients, antioxidants, etc. and therefore having a positive effect on human health.

Based on literature data, strawberry is an excellent source of nutrients, vitamins, antioxidants, fiber etc. (for review see Basu and co-workers, 2014) [1]. There are strong evidences that consumption of strawberry fruits has a positive physiological effect (for review see Basu and co-workers, 2014) [1].

At the same time, it should be noted that the nutrient, vitamin and antioxidant composition of fruits are highly dependent on environmental factors. The conditions of agricultural practices, cultivation, nutrient supply, application of chemicals etc. affect the quality of the fruits. Therefore we started a series of experiment in order to determine some of the conditions and treatments resulting in production of fruits rich in valuable ingredients, which -as functional food-, could contribute to human health.

In order to produce health promoting food, the first step is to reduce the level of applied chemicals such as high levels of synthetic fertilizers, herbicides, pesticides. Intensive agriculture resulted in shortage of soil nutrients and the upset of soil life. Application of beneficial bacteria as plant probiotics came in forefront, as a solution for increasing crop production while avoiding the application of chemicals [3] [4] [13]. We are looking for an efficient probiotic formula applicable in organic strawberry farming. In the first series of experiments we investigate the effect of different microbiological preparations on the macro- and microelement content of strawberry (*Fragaria* x *ananassa* 'Joly').

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PPB (Plant Probiotic Bacteria) are microorganisms providing benefits to plants. The term has been used first by Haas and Keel in 2003 [10]. PGPR (Plant Growth Promoting Rhizobacteria) are the subset of PPB living in the soil and rhizosphere. Different genera have different traits, resulting in plant growth promotion. They can help plant nutrition trough nitrogen fixation [2] [21], phosphate solubilization [5] [19] [22], potassium solubilization [12] siderophore production [9] [20] in different crops. Therefore we can expect that application of the appropriate beneficial strains leads to better nutrition of strawberry. Several reports discuss increases in nutrition of strawberry as a result of PGPR inoculation especially under stress conditions [6] [7] [11].

Furthermore, there are some reports suggesting that the application of some beneficial bacteria can result in the increase of antioxidant level [8] [15] [18]. In that case, certain microbes can help us producing high-value functional strawberry fruits.

In the present study, macro- and microelement content of strawberry, treated with Bactofil B-10 microbial formula and non-treated (control) were compared.

2 Material and Methods

2.1 Experimental site, planting, culture conditions

Our experiments were set up in the Experimental Garden of John von Neumann University, Kecskemét (46 ° 54' N; 19 ° 40' E) in Hungary.

Planting took place on the 27th of March 2017. Frigo transplants (*Fragaria* x *ananassa* 'Joly') of 9-13 mm in diameter were planted with a planting density of 70+40*30 cm, in tween-rows.

Based on the results of preliminary soil analysis, the sandy soil of our experimental area is slightly alkaline, with low nitrogen and organic matter content. Nutrient supply was planned based on the results of soil analysis, in accordance with national and EU regulations on organic farming.

In the year of planting, 600 g /m^2 of Itallpollina (granulated chicken manure fertilizer), 500 g /m^2 of Greensoil Natural (Dudar brown coal) and 80 g /m^2 of potassium sulphate were applied in total.

At the beginning of the second growing season 200 g /m^2 Italpollina, 100 g /m^2 Greensoil Natural és 60 g /m^2 potassium sulphate was spread.

All culture practices (fertilization, irrigation, weed control) were performed according to the national and EU regulations of organic farming.

2.2 Bacterial inoculations

The applied microbial product (Bactofil B-10) contains cold tolerant strains of seven different species (*Azospirillum lipoferum*, *Azotobacter vinelandii*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus subtilis*, *Pseudomonas fluororescens*, *Micrococcus roseus*) in a concentration of 5*10^9 CFU /cm^3.

Bactofil B-10 was diluted with tap water, sprayed on the soil and worked in immediately. One plot was treated with 2 l /ha of microbial inoculant (treated plot) while the other was sprayed with tap water (control plot). Inoculation was executed directly before planting, and repeated in next March. Besides, during the planting process, 100 ml of 5000 x diluted Bactofil B-10 was poured in every planting hole, before planting the transplants.

Leaf analysis

In the second growing season 3-3 groups of treated and control plants were selected. Selection was performed by simple random sampling during the fruit harvesting period. Three times 50-50 leaves were collected from the treated and the control plants (6 times 50 leaves). Petiole was removed, and then leaf blade samples were thoroughly washed. Leaf samples were dried at 70 °C. The air-dry samples were thoroughly minced. For elemental studies powdered samples were digested in a microwave device by means of concentrated nitric acid and hydrogen peroxide (Milestone Ethos Plus). Main macro element content was measured by optical emission spectrometer (ICP-AES method). Nitrogen content in leaf blades was determined using the

Kjeldahl method [14] after sulphuric acid digestion (FOSS Kjeltec 2300). Macro element (N, P, K, Ca, Mg) contents were calculated in m/m% dry matter, whereas micro-element (Mn, Zn, Cu, B, Mo) contents were given in mg/kg dry matter.

3 Results and Discussion

Nutrient concentrations of leaf bled samples are given in *Table 1*. At first glance, *Table 1.* shows, that there were no significant differences in the concentrations of the nutrients in the treated and control plants.

Table 1. Macro- and microelement content of leaves of treated and control plants (2018)

(m/m %)

Treatment	*N*	*P*	*K*	*Ca*	*Mg*
control	1.88	0.279	1	1.62	0.484
control	2.01	0.303	1.21	1.71	0.489
control	2.02	0.303	1.2	1.61	0.483
treated	1.73	0.363	1.26	1.19	0.428
treated	1.6	0.307	1.19	1.11	0.382
treated	1.62	0.301	1.11	1.13	0.382

Level of nutrient supply
optimal
moderate
insufficient

(mg/kg)

Treatment	*Mn*	*Zn*	*Cu*	*B*	*Mo*
control	68.3	22.4	5.77	24.4	< 0.5
control	56.4	19.4	6.57	25.9	< 0.5
control	75.8	19.9	6.35	23,.6	0.505
treated	69	20.6	7.54	23.1	0.902
treated	64	16.6	6.6	19.5	< 0.5
treated	59.6	20.6	6.28	19.5	0.512

Favorable nutrient content of strawberry leaves during the harvest is summarized in *Table 2.,* based on Bould (1964) in Papp and Porpáczy (1999) [16].

We compared our leaf analysis results with the data of *Table 2*., and found, that for most elements, both control and treated plants show favorable supply. Both control and treated plants have moderate nitrogen supply, which needs improvement by extra N supply in future experiments, because strawberries are very sensitive to N deficiency [16]. In the case of Zn and Mo supply, the treated plants show better results, but in the case of boron concentration, the treated plants tend to be moderate. (*Table 1*).

Table 2. Favorable macro- and microelement content of leaves during harvesting based on Bould (1964) in Papp and Porpáczy (1999) [16].

Macronutrient content (%)					
N	P	K	Ca	Mg	
2.5-3.0	0.2-0.3	1.0-1.5	0.8-1.4	0.3-0.4	
Micronutrient content (ppm)					
Fe	Mn	B	Zn	Cu	Mo
50-100	50-100	20-50	20-30	5-8	0.5

Treatment with Plant Growth Promoting Rhizobacteria (PGPR) is known to increase the uptake of various macro- and microelements in plants [17]. According to studies by Esitken and co-workers [6] there were significant increases in P and Zn concentrations after PGPR treatment under organic farming conditions. The level of increase strongly depended on the bacterial strains used (the combination of strains used), and on the treatment method. In our present experiment P, Zn, Mo, Cu levels of leaves of treated plants were slightly higher than controls, but the difference is not remarkable and its statistical significance cannot be proved. Based on the report of Ipek and co-workers [11], under calcareous soil conditions inoculation of strawberry with Alcaligenes 637Ca resulted in an increase of concentrations of all plant tissue nutrients [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), copper (Cu), manganese (Mn), boron (B)] except for magnesium (Mg) and zinc (Zn) in the leaf.

Flores-Félix and co-workers [8] found that inoculation of strawberry with selected *Rhizobium* strains, resulted in higher concentrations of Fe, Zn, Mn and Mo.

4 Conclusion

Based on our preliminary results, no significant increases in the nutrient content of leaves were detected as a result of treatment with Bactofil B-10. Experiments have to be repeated.

There are limited data in scientific literature about the correlation between the nutrient composition of vegetative organs and the content of macro- and micronutrients in fruit, and how nutrient composition of plant affects the composition of other metabolites in fruit. Complex processes determine the quality of the fruit. There are papers indicating that treatment of strawberry with plant probiotic non-pathogenic bacterial strains result in an increase in nutrients and phytochemicals of importance to human health [7] [8] [11]. Therefore, it is definitely worthwhile to investigate which treatments will lead to a rise in health protection components.

The direction of our research is investigation of effects of the different treatments (different PGPR and microalgae strains, conventional and organic farming conditions, different mulching methods etc.) on the macro- and micronutrient content of the plant and fruit and on the amounts of other metabolic products (antioxidants, polyphenols, etc.).

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