# The role of biofertilizers in improving vegetative growth, yield and fruit quality of apple

Walid Fediala Abd El-Gleel Mosa $^{1,2,*}$ , Lidia Sas Paszt $^1$ , Mateusz Frąc $^1$ , Paweł Trzciński $^1$ , Waldemar Treder $^1$ , Krzysztof Klamkowski $^1$ 

#### Abstract

Mosa W.F.A.E-G., Sas Paszt L., Frąc M., Trzciński P., Treder W., Klamkowski K. (2018): The role of biofertilizers in improving vegetative growth, yield and fruit quality of apple. Hort. Sci. (Prague), 45: 173–180.

Apple trees which grafted on M.M. 106 stocks were planted at the beginning of spring 2014 in pots at the Research Institute of Horticulture in Skierniewice, Poland. The used treatments in the experiment were: NPK fertilization and bioproducts: Fertigo (Manure), Micosat, Humus UP, Humus Active + Aktywit PM, BioFeed Quality, BioFeed Amin, Vinassa, Florovit Natura and Florovit Eko. In the spring, these treatments were applied to apple trees alone or enriched with *Pantoea* sp., *Pseudomonas fluorescens, Klebsiella oxytoca* and *Rhizobium* sp. bacterial strains. Growth, yield and fruit quality parameters were evaluated. The results revealed that photosynthetic rate was greatly improved by the addition of bacteria to Fertigo (Manure), Micosat, Humus UP and Humus Active + Aktywit PM as compared to NPK. Tree trunk thickness was significantly enhanced with Vinassa, Florovit Natura and Florovit Eko enriched with bacteria. The application of bacterial strains increased the effectiveness of Fertigo (Manure), Humus UP, Humus Active + Aktywit PM, Biofeed Amin and Yeast in increasing significantly number and weight of fruits as compared to NPK.

Keywords: beneficial bacteria; bioproducts; apple; growth tree; productivity

Application of native mycorrhizal fungi and beneficial strains of bacteria and fungi incorporated in new bioproducts ensures their better adaptation and survival in the prevailing environmental conditions, which is an extremely important factor for their long-term effects on plants (Regvar et al. 2003). One of the proposed solutions to environmental and human health protection issues is the implementation of natural technologies of plant cultivation and fertilization through the applications of biofertilizers. Products of this kind have a positive influence on the growth and yielding of crop plants as well as on the soil fauna, including the development of arbuscular mycorrhizal fungi

(AMF) (Kuwada et al. 2005). Enriching fertilizers with beneficial strains of bacteria and fungi can increase their effectiveness in crop production (Chen 2006) by enhancing the physiology of crop plants, stimulating their growth and yielding, as well as by increasing their resistance to environmental and biotic stresses (Corte et al. 2013). Von-Bennewitz and Hlusek (2006) found that the biofertilization was beneficial in stimulating the growth and fruiting of pome and stone fruits. Moreover, the applications of biofertilizers containing beneficial microorganisms instead of synthetic chemicals are known to improve fixation of nutrients in the rhizosphere, and produce growth

<sup>&</sup>lt;sup>1</sup>Research Institute of Horticulture, Skierniewice, Poland

<sup>&</sup>lt;sup>2</sup>Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt

<sup>\*</sup>Corresponding author: walidbreeder@yahoo.com

stimulants for plants. Furthermore, they can also improve soil stability, provide biological control, biodegrade substances, recycle nutrients, and promote mycorrhiza symbiosis, (RIVERA-CRUZ et al. 2008). KARAKURT and ASLANTAS (2010) stated that the use of more sustainable technologies, such as biofertilization, is inevitable for the mitigation of environmental damage. Sas PASZT et al. (2015) mentioned that microbiological enrichment of organic fertilizers, composts, and liquid plant growth promoters with consortia of beneficial microorganisms are modern and environmentally-friendly agriculture fertilizers.

This study was designed to evaluate the effects of some bioproducts, used alone or enriched with four bacterial strains, on the growth, yield and fruit quality of apple trees cv. 'Topaz'.

#### MATERIAL AND METHODS

The experiment was carried out in pots at the Research Institute of Horticulture in Skierniewice, Poland, on apple trees cv. 'Topaz' which were planted in the beginning of spring 2014 and were grafted on MM.106 rootstock. The experiment comprised twenty-two treatments and each one contained 8 trees/replicates. In the spring, during 2014–2016, NPK fertilization, Fertigo, Micosat, Yeast, Florovit Natura and Florovit Eko were added to the soil at two times: at the end of April and in the middle of June. Humus UP, Humus Active + Aktywit PM, BioFeed Quality, BioFeed Amin and Vinassa were applied in a liquid form to the soil at the end of May and in the middle of July.

The following are the fertilization combinations used in this experiment in 2014–2016:

- 1. Chemical NPK fertilization (control):  $17.64~\rm g/m^2$  NH<sub>4</sub>NO<sub>3</sub>,  $6.52~\rm g/m^2$  triple super phosphate, and  $16.0~\rm g/m^2~\rm K_2SO_4$ . It was applied as a  $60~\rm kg/ha$  N,  $30~\rm kg/ha$  P, and  $80~\rm kg/ha$  K.
- Fertigo (Manure) (Ferm-O-Feed, The Netherlands): Granulated bovine manure containing 55% C, 1% N, 0.3% P and 1% K and microelements. Applied at 150 g/m² (1.500 kg/ha).
- 3. Micosat (CCS Aosta Srl, Italy): Microbial inoculum containing mycorrhizal fungi (*Glomus mosseae* and *G. intraradices*), and plant growth promoting bacteria (*Pseudomonas fluorescens* and *Bacillus subtilis*). The product contained 40% C, 0.15% N, 43.1% P and 0.96% K. Micosat F12

- WP was applied to the soil at a dose of  $10 \text{ g/m}^2$  (100 kg/ha).
- 4. Humus UP (Ekodarpol, Poland): An extract from vermicomposts containing 0.65% C, 0.03% N, 3.08 % P and 0.45 % K. Applied to the soil as a 2% solution at  $2 \text{ ml/m}^2$  (20 l/ha).
- 5. Humus Active + Aktywit PM (Ekodarpol, Poland): An extract from vermicomposts based on a product derived from molasses. Humus Active is a soil improver and contains 0.78% C, 0.03% N, 0.105% P and 0.412% K. Aktywit PM is a soil improver and contains 20.5% C, 0.92% N, 8.12% P and 4.30% K. Humus Active was applied to the soil as a 2% solution (20 l/ha), and Aktywit PM was applied to the soil as a 1% solution (10 l/ha).
- 6. BioFeed Quality (Agrobio Products (Koppert) B.V., the Netherlands): An extract from several seaweed species reinforced with humic and fulvic acids, containing 0.6% C, 0.07% N, 3.26% P. It was applied to the soil as a 0.5% solution at 0.5 ml/m<sup>2</sup> (5 l/ha).
- 7. BioFeed Amin (Agrobio Products (Koppert) B.V., the Netherlands): An extract reinforced with amino acids an extract of vegetal amino acids contains 1.12% C, 0.14% N and 34.7% P. The product was applied to the soil as a 0.5% solution at 0.5 ml/m<sup>2</sup> (5 l/ha).
- 8. Yeast (Biopuls Start-up of Micro Life Company). Applied to the soil at 112.5 kg/ha.
- 9. Vinassa (Józefów Sp. z o.o., Poland): molasses residue from yeast production containing 12.0% C, 1.86% N, 94.9% P, 1.761 K. Applied to the soil as a 0.5% solution at (5 l/ha).
- 10. Florovit Natura (NPK) (Inco, Poland) containing 5% N, 3% P<sub>2</sub>O<sub>5</sub>, 2% K<sub>2</sub>O, and 30% of organic matter. It was applied at 468.75 kg/ha.
- 11. Florovit Eko (PK) (Inco, Poland) containing 3%  $P_2O_5$ , 5%  $K_2O$ , and of 30% organic matter. It was applied at 468.75 kg/ha.

Four bacterial strains: Pi22C Pantoea sp. with  $0.9 \times 10^9$  CFU/ml, Ps49A Pseudomonas fluorescens with  $0.5 \times 10^9$  CFU/ml, NAzot2 Klebsiella oxytoca with  $2.8 \times 10^9$  CFU/ml, and N65AB Rhizobium sp. with  $0.3 \times 10^9$  CFU/ml were added together in a mixture in 240 ml per each tree/replicate to the soil via the irrigation system at two times: in May and in July 2014–2016. These bacterial strains were bred in a nutrient broth which contained pepton 5 g, beef extract 3 g, distilled water 1,000 ml and was supplemented with glucose (1 g/l) and incubated at  $28^{\circ}$ C on a horizontal shaker at 100 rpm

(revolutions per minute) for 48 hours. The bacterial biomass was separated from the nutrient broth in a centrifuge at 6,000 rpm and then suspended in sterile tap water.

The effects of the treatments were studied by evaluating their influence on the following parameters:

Gas exchange measurements (net photosynthesis, transpiration and stomatal conductance) were recorded by using the LCpro + (ADC BioScientific, UK) portable system. The measurements of gas exchange were performed in the morning from 10–12 o'clock, in July and in August 2016, during the vegetative period.

**Trunk cross-sectional area** (TCSA) was measured at two times, in July and in November 2015, during the vegetative period, using a Vernier calliper.

**Yield per tree** was estimated by measuring the weight of all fruit in kg and the number of all fruits per tree, replicated in each treatment at harvest time (third week in October).

Fruit quality: Apple fruits were stored in a cold storage room in the normal atmosphere at 1°C and 80% air humidity for one month. The quality of apples was assessed one day after removing them from cold storage: weight of individual fruits (g), percentage of blush, flesh firmness (FF), total soluble solids content (TSS) and titratable acidity (TA). Weight of fruit was measured using WPS 2100/C/2 balance (Radwag, Poland). Flesh firmness (kg) was measured by the penetrometer method on two opposite sides of each fruit (on blush and on background colour) using an EPT-1R Pressure Tester (Kelowna, Canada) equipped with Magness-Taylor probe of 11 mm diameter. Total soluble solids content and titratable acidity were measured in freshly prepared juice. TSS (%) was determined using ATAGO PR-101 digital refractometer (ATAGO, Japan). Titratable acidity (malic acid, %) was determined by standard titration method using automatic titrator DL 50 Graphix (Mettler Toledo, Switzerland), by titration of juice with 0.1N NaOH to the end point at pH = 8.1.

**Determination of shoot, main stem and root growth characteristics**: Apple plants were removed from pots after harvest time in November 2016, to determine their morphological features. After washing with tap water, they were scanned with an Epson Expression 10000 XL root scanner. Surface area and volume of shoots, main stem and root were measured with WinRhizo software (Arsenault et al. 1995) and expressed with cm.

The obtained data were subjected to the analysis of variance (ANOVA) using Statistica 13.1.336.0 64-bit (PL). Least significant difference (LSD) at 0.05% level of significance was used to compare the means for the treatments.

#### **RESULTS**

Data in Table 1 indicate that photosynthetic rate was improved by the application of Yeast and Vinassa to the soil over NPK. Moreover, it was greatly increased by the addition of bacteria to Fertigo, Micosat, Humus UP, and Humus Active + Aktywit PM as compared to NPK. Vinassa, Micosat, Fertigo, and Humus UP enriched with bacteria significantly improved stomatal conductance over NPK. It was also enhaced with Fertigo, Micosat, Yeast, Vinassa and Florovit Natura treatments.

Tree thickness was improved by BioFeed Quality, Yeast, Vinassa, Florovit Natura and Florovit Eko as compared to NPK. Additionally, it was greatly enhanced with Vinassa, Florovit Natura and Florovit Eko enriched with bacteria.

Data in Table 2 shows that yield in terms of the number of fruits and fruit weight was significantly increased by the addition of bacteria to Fertigo, Humus UP, Humus Active + Aktywit PM, Biofeed Amin, and Yeast as compared to NPK. Fruit firmness was increased by Micosat, Humus Active + Aktywit PM + bacteria, BioFeed Quality, BioFeed Quality + bacteria and Yeast + bacteria. TSS% was appreciably increased by BioFeed Quality + bacteria, BioFeed Amin and Yeast.

Table 3 shows that bacteria with Vinassa, Florovit Natura and Florovit Eko substantially increased the surface area and the volume of the roots, main stem and shoots of plants over NPK. Besides, surface area and volume of roots was also increased by Fertigo, BioFeed Quality, BioFeed Amin, Yeast, Vinassa and Florovit Natura as compared to NPK. Furthermore, the surface area and volume of shoots was improved with Florovit Natura. Main stem surface and volume was enhanced with Humus UP and Yeast treatments.

Results presented in Table 4 demonstrate that adding bacteria to Humus Active + Aktywit PM, Yeast and Biofeed Amin significantly improved the nitrogen content as compared to NPK. Phosphorus content was statistically enhanced by Biofeed Amin, Florovit Eko, Florovit Natura, Vinassa, and

Table 1. Effect of NPK fertilization and application of bioproducts on photosynthetic activities and tree trunk thickness of apple trees cv "Topaz" in 2016

Treatment	Photosynthesis (μmol CO <sub>2</sub> /m²·s)		Stomatal conductance (mol/m²·s)		Tree thickness (mm)	
	June	August	June	August	May	October
NPK	7.40 <sup>e-g</sup>	7.06 <sup>e-g</sup>	0.112 <sup>i</sup>	0.110 <sup>de</sup>	21.1 <sup>d</sup>	23.6 <sup>de</sup>
NPK+ bacteria	9.30 <sup>a-e</sup>	6.43 <sup>g</sup>	$0.177^{c-f}$	0.110 <sup>de</sup>	$21.9^{\rm cd}$	$24.4^{c-e}$
Fertigo	$7.62^{d-g}$	6.28 <sup>g</sup>	$0.150^{f-h}$	$0.120^{b-e}$	20.6 <sup>d</sup>	23.3 <sup>e</sup>
Fertigo + bacteria	11.12 <sup>a</sup>	9.32 <sup>a-c</sup>	0.203 <sup>bc</sup>	$0.140^{a-c}$	$21.4^{\mathrm{cd}}$	$25.0^{c-e}$
Micosat	$7.12^{\mathrm{fg}}$	6.69 <sup>fg</sup>	$0.153^{f-h}$	$0.130^{a-e}$	$21.2^{d}$	23.9 <sup>de</sup>
Micosat + bacteria	9.86 <sup>a-c</sup>	$9.74^{ab}$	$0.198^{b-d}$	0.150 <sup>a</sup>	$21.3^{\rm cd}$	25.1 <sup>c-e</sup>
Humus UP	$9.10^{a-f}$	$7.02^{e-g}$	$0.162^{e-g}$	0.115 <sup>c-e</sup>	$21.5^{\rm cd}$	$24.4^{c-e}$
Humus UP + bacteria	10.88 <sup>ab</sup>	9.18 <sup>a-c</sup>	0.262 <sup>a</sup>	0.143 <sup>ab</sup>	$22.1^{\rm cd}$	$25.4^{c-e}$
Humus Active + Aktywit PM	$8.05^{c-g}$	6.76 <sup>fg</sup>	0.168 <sup>c-g</sup>	$0.105^{\rm ef}$	$21.5^{\rm cd}$	24.7 <sup>c-e</sup>
Humus Active + Aktywit PM + bacteria	9.66 <sup>a-d</sup>	$9.07^{a-d}$	$0.222^{b}$	$0.123^{b-e}$	22.3 <sup>a-d</sup>	$25.7^{b-d}$
BioFeed Quality	6.70 <sup>g</sup>	$6.71^{\mathrm{fg}}$	$0.120^{hi}$	$0.083^{f}$	22.2 <sup>a-d</sup>	24.3 <sup>c-e</sup>
BioFeed Quality + bacteria	$9.22^{a-f}$	$8.49^{b-f}$	$0.150^{f-h}$	$0.137^{a-c}$	$21.3^{\rm cd}$	$24.2^{c-e}$
BioFeed Amin	8.09 <sup>c-g</sup>	6.54 <sup>g</sup>	$0.142^{f-i}$	$0.083^{f}$	$21.1^{d}$	23.5 <sup>de</sup>
BioFeed Amin + bacteria	$9.12^{a-f}$	$9.06^{a-d}$	$0.135^{g-i}$	0.133 <sup>a-d</sup>	$23.0^{a-d}$	26.3 <sup>a-c</sup>
Yeast	8.00 <sup>c-g</sup>	$7.30^{d-g}$	$0.152^{f-h}$	$0.137^{a-c}$	$21.5^{\rm cd}$	24.4 <sup>c-e</sup>
Yeast + bacteria	$8.96^{b-f}$	10.52ª	$0.140^{g-i}$	0.135 <sup>a-d</sup>	$21.5^{\rm cd}$	24.9 <sup>c-e</sup>
Vinassa	$8.20^{c-g}$	$7.59^{c-g}$	$0.158^{e-g}$	$0.125^{a-e}$	$22.0^{\rm cd}$	$24.6^{c-e}$
Vinassa + bacteria	9.31 <sup>a–e</sup>	$8.80^{a-e}$	$0.192^{b-e}$	0.138 <sup>a-c</sup>	24.8ª	27.9ª
Florovit Natura	7.39 <sup>e-g</sup>	6.61 <sup>g</sup>	$0.163^{d-g}$	$0.120^{b-e}$	22.4 <sup>a-d</sup>	24.7 <sup>c-e</sup>
Florovit Natura + bacteria	9.81 <sup>a-c</sup>	9.30 <sup>a-c</sup>	$0.170^{c-g}$	$0.140^{\mathrm{abc}}$	$24.7^{ab}$	27.8 <sup>ab</sup>
Florovit Eko	6.30 <sup>g</sup>	6.69 <sup>fg</sup>	$0.108^{i}$	$0.127^{a-e}$	$22.1^{b-d}$	$24.8^{c-e}$
Florovit Eko + bacteria	$7.93^{c-g}$	$8.05^{b-g}$	$0.162^{e-g}$	$0.127^{a-e}$	23.8 <sup>a-c</sup>	26.2 <sup>a-c</sup>

means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability

BioFeed Quality enriched with bacteria. Potassium content was increased by adding bacteria to Fertigo, Humus Active + Aktywit PM, BioFeed Quality, BioFeed Amin and Florovit Natura, and also by Humus Active + Aktywit PM, BioFeed Quality. The combination of bacterial strains with Humus UP, Micosat, Humus Active + Aktywit PM and Florovit Eko over NPK raised Magnesium content in the roots. Calcium content was evidently improved by the combination of bacteria to Humus Active + Aktywit PM, Biofeed Amin, Yeast, Micosat, Fertigo, BioFeed Quality and Humus UP and also by Micosat, Fertigo , BioFeed Quality, and BioFeed Amin comparing with NPK control.

## **DISCUSSION**

The obtained results clearly showed that photosynthetic rate and stomatal conductance were greatly improved by the addition of bacteria to Fertigo, Micosat, Humus UP, and Humus Active + Aktywit PM as compared to NPK. These results are consistent with the findings of NARDI et al. (2002). They found a positive effect of humic substances on the chlorophyll content of the leaves, and thereby on the intensity of photosynthesis. Moreover, Game and Navale (2006) reported treating the custard apple with VAM increased the phosphorus uptake. Moreover, OJHA et al. (2008)

Table 2. Effect of NPK fertilization and application of bioproducts on fruit yield and fruit chemical characteristics of apple trees cv "Topaz" in 2016

Treatments	Fruit yield/tree		Fruit chemical characteristics				
	No.*	weight (kg)	blush (%)	TSS (%)	acidity (malic acid, %)	firmness (kg)	
NPK	15.5 <sup>f-h</sup>	3.2 <sup>e-g</sup>	74.0 <sup>g</sup>	12.7 <sup>c-f</sup>	0.93 <sup>a-d</sup>	8.1 <sup>ab</sup>	
NPK+ bacteria	$17.2^{e-g}$	$2.2^{l}$	$74.0^{g}$	12.5 <sup>e-g</sup>	$0.89^{d-g}$	7.9 <sup>ab</sup>	
Fertigo	$13.2^{h-j}$	$2.4^{jk}$	91.7 <sup>a-c</sup>	$12.6^{c-f}$	$0.86^{g-j}$	7.9 <sup>ab</sup>	
Fertigo + bacteria	$23.5^{\mathrm{bc}}$	4.6 <sup>a</sup>	86.6 <sup>b-e</sup>	$12.6^{d-g}$	$0.87^{f-i}$	8.2 <sup>ab</sup>	
Micosat	$14.7^{f-i}$	2.7 <sup>hi</sup>	$80.0^{e-g}$	$12.7^{c-f}$	0.83 <sup>J</sup>	8.5ª	
Micosat + bacteria	$14.5^{g-i}$	$2.9^{\mathrm{gh}}$	89.3 <sup>a-d</sup>	$13.0^{a-e}$	$0.89^{e-h}$	8.1 <sup>ab</sup>	
Humus UP	$16.0^{f-h}$	3.3 <sup>de</sup>	$84.7^{b-f}$	$12.6^{c-f}$	$0.78^{k}$	$7.8^{\mathrm{b}}$	
Humus UP + bacteria	$20.7^{\rm cd}$	3.8°	88.7 <sup>a-d</sup>	$12.7^{c-f}$	0.95 <sup>a</sup>	7.9 <sup>ab</sup>	
Humus Active + Aktywit PM	17.5 <sup>ef</sup>	$3.0^{\mathrm{fg}}$	87.3 <sup>a-e</sup>	$12.3^{fg}$	$0.78^{k}$	7.9 <sup>ab</sup>	
Humus Active + Aktywit PM + bacteria	$21.5^{\rm cd}$	$3.8^{\rm c}$	92.7 <sup>ab</sup>	13.2 <sup>a-d</sup>	$0.95^{a}$	8.3 <sup>ab</sup>	
BioFeed Quality	$12.0^{i-k}$	$2.4^{jk}$	$89.0^{a-d}$	$12.8^{c-f}$	$0.90^{c-f}$	8.2 <sup>ab</sup>	
BioFeed Quality + bacteria	$12.2^{i-k}$	$2.3^{kl}$	$95.0^{a}$	13.5 <sup>ab</sup>	0.96 <sup>a</sup>	8.2 <sup>ab</sup>	
BioFeed Amin	$13.7^{hi}$	$2.6^{ij}$	86.7 <sup>a-e</sup>	13.5 <sup>a</sup>	$0.88^{\mathrm{f-i}}$	8.2 <sup>ab</sup>	
BioFeed Amin + bacteria	26.5 <sup>a</sup>	$4.3^{\rm b}$	$90.0^{a-d}$	$12.9^{b-f}$	$0.89^{d-g}$	8.1 <sup>ab</sup>	
Yeast	19.5 <sup>de</sup>	$3.1^{e-g}$	57.7 <sup>h</sup>	13.5ª	$0.85^{h-j}$	7.9 <sup>ab</sup>	
Yeast + bacteria	$24.5^{\mathrm{ab}}$	3.9 <sup>c</sup>	$84.7^{b-f}$	13.2 <sup>a-c</sup>	$0.79^{k}$	8.3 <sup>ab</sup>	
Vinassa	$12.2^{i-k}$	$2.4^{jk}$	$82.3^{d-g}$	13.1 <sup>a-e</sup>	$0.92^{a-d}$	8.1 <sup>ab</sup>	
Vinassa + bacteria	19.7 <sup>de</sup>	$3.5^{d}$	83.3 <sup>c-f</sup>	$12.5^{d-g}$	$0.95^{a}$	8.0 <sup>ab</sup>	
Florovit Natura	$10.5^{jk}$	1.9 <sup>m</sup>	$76.7^{\mathrm{fg}}$	12.5 <sup>e-g</sup>	$0.85^{ij}$	7.9 <sup>ab</sup>	
Florovit Natura + bacteria	$12.5^{i-k}$	3.3 <sup>de</sup>	$84.0^{c-f}$	12.5 <sup>e-g</sup>	0.94 <sup>ab</sup>	8.1 <sup>ab</sup>	
Florovit Eko	9.7 <sup>k</sup>	$3.2^{\rm ef}$	88.3 <sup>a-e</sup>	$12.6^{c-f}$	$0.94^{a-c}$	8.1 <sup>ab</sup>	
Florovit Eko + bacteria	$16.0^{f-h}$	$4.2^{b}$	91.3 <sup>a-c</sup>	$12.0^{g}$	$0.90^{b-f}$	8.0 <sup>ab</sup>	

means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability; \* number of fruits per tree

found that symbiotic association of mycorrhizal fungus amounts to greater uptake of phosphorus and increased chlorophyll content in VAM treated custard-apple plants than non mycorrhizal plants. Furthermore, phosphorus is known to play an indispensable biochemical role in photosynthesis in the living plant (SAGERVANSHI et al. 2012).

Our results showed that Vinassa, Florovit Natura and Florovit Eko combined with the bacteria greatly increased the surface area and volume of the roots, main stem, and shoots, and tree trunk thickness over NPK chemical fertilization. These results are in line with the findings of Grzyb et al. (2012).

They had found that Vinassa improved the trunk diameter, tree height, number of branched trees, number of lateral shoots and total length of lateral shoots of 'Topaz' maiden apple trees. Moreover, Grzyb et al. (2015) found that the treatments with Florovit Eko + Micosat and Vinassa + Micosat improved trunk diameter, tree height and number of lateral shoots of maiden trees of apple cv. Topaz and of sour cherry cv. Debreceni Bötermö. Mosa et al. (2016) reported that the tree trunk cross-sectional area of 'Topaz' apple trees was increased with Vinassa, Florovit Natura and Florovit Eko combined with *Pantoea* spp., *Pseudomonas fluorescens, Kleb*-

Table 3. Effect of NPK fertilization and application of bioproducts on the surface area and volume of root, main stem and shoot of apple trees cv "Topaz" in 2016

	Root		Shoo	ot	Main s	tem
Treatment	surface area (cm²)	volume (cm³)	surface area (cm²)	volume (cm³)	surface area (cm²)	volume (cm³)
NPK	1,256.95 <sup>kl</sup>	46.65 <sup>c-f</sup>	745.48 <sup>f</sup>	80.93 <sup>ef</sup>	885.43 <sup>h-k</sup>	465.42 <sup>jk</sup>
NPK + bacteria	$1,441.31^{g-i}$	$45.03^{c-f}$	$748.64^{\rm f}$	$83.83^{d-f}$	1,144.99 <sup>bc</sup>	898.10 <sup>c</sup>
Fertigo	1,478.78 f-h	54.62 <sup>bc</sup>	$495.05^{jk}$	$51.47^{\rm f}$	$812.20^{jk}$	$443.67^{jk}$
Fertigo + bacteria	1,377.35 <sup>ij</sup>	53.77 <sup>bc</sup>	621.93 <sup>gh</sup>	68.73 <sup>ef</sup>	836.63 <sup>h-k</sup>	473.95 <sup>j</sup>
Micosat	1,295.95 <sup>jk</sup>	39.02 <sup>ef</sup>	$431.51^{k}$	44.59 <sup>f</sup>	$852.94^{h-k}$	$365.47^{l}$
Micosat + bacteria	$1,431.92^{g-i}$	$43.67^{d-f}$	698.91 <sup>f</sup>	79.13 <sup>ef</sup>	1,040.16 <sup>c-f</sup>	565.14 <sup>gh</sup>
Humus UP	1,498.67 <sup>e-g</sup>	$45.43^{c-f}$	$581.42^{\rm hi}$	63.00 <sup>f</sup>	$900.67^{g-j}$	522.25 <sup>i</sup>
Humus UP + bacteria	1,184.07 <sup>l</sup>	$40.35^{d-f}$	$704.05^{\rm f}$	75.30 <sup>ef</sup>	$912.05^{g-j}$	639.30 <sup>f</sup>
Humus Active + Aktywit PM	1,436.15 <sup>g-i</sup>	$41.08^{d-f}$	$465.49^{jk}$	$52.85^{\rm f}$	909.63 <sup>g-j</sup>	$433.51^{k}$
Humus Active + Aktywit PM + + bacteria	1,307.65 <sup>jk</sup>	38.87 <sup>f</sup>	$742.82^{\rm f}$	82.52 <sup>d-f</sup>	$904.71^{g-j}$	386.20 <sup>l</sup>
BioFeed Quality	1,570.08 <sup>de</sup>	$58.42^{b}$	$520.15^{ij}$	56.78 <sup>f</sup>	777.69 <sup>k</sup>	281.663 <sup>m</sup>
BioFeed Quality + bacteria	1,488.81 <sup>e-h</sup>	$46.53^{c-f}$	$680.18^{fg}$	73.71 <sup>ef</sup>	$823.21^{i-k}$	$384.07^{l}$
BioFeed Amin	1,480.95 <sup>e-h</sup>	$48.83^{b-e}$	$467.74^{jk}$	52.39 <sup>f</sup>	$872.94^{h-k}$	312.65 <sup>m</sup>
BioFeed Amin + bacteria	1,403.48 <sup>hi</sup>	$47.05^{c-f}$	1,036.41 <sup>d</sup>	$128.17^{bc}$	$1,044.34^{b-f}$	761.03 <sup>e</sup>
Yeast	1,479.95 <sup>e-h</sup>	49.39 b-d	$699.20^{\rm f}$	$88.22^{c-f}$	1,004.94 <sup>d-g</sup>	589.18 <sup>g</sup>
Yeast + bacteria	1,370.18 <sup>ij</sup>	53.88 <sup>bc</sup>	1,008.79 <sup>d</sup>	$126.48^{b-d}$	1,064.29 <sup>b-e</sup>	532.76 <sup>hi</sup>
Vinassa	1,589.14 <sup>d</sup>	$54.13^{bc}$	735.67 <sup>f</sup>	$86.26^{c-f}$	$932.15^{\rm f-i}$	$433.96^{k}$
Vinassa + bacteria	2,093.95 <sup>a</sup>	77.57 <sup>a</sup>	1,644.30 <sup>a</sup>	255.47ª	1,364.74ª	1,046.16 <sup>a</sup>
Florovit Natura	1,546.21 <sup>d-f</sup>	58.64 <sup>b</sup>	905.36 <sup>e</sup>	111.41 <sup>b-e</sup>	948.94 <sup>e-h</sup>	373.03 <sup>l</sup>
Florovit Natura + bacteria	1,820.08 <sup>b</sup>	69.04ª	1,499.88 <sup>b</sup>	213.73 <sup>a</sup>	1,157.94 <sup>b</sup>	865.06 <sup>cd</sup>
Florovit Eko	1,450.36 <sup>g-i</sup>	44.77 <sup>c-f</sup>	$713.98^{\rm f}$	77.16 <sup>ef</sup>	857.49 <sup>h-k</sup>	856.24 <sup>d</sup>
Florovit Eko + bacteria	1,720.76 <sup>c</sup>	$70.87^{a}$	1,138.02 <sup>c</sup>	138.07 <sup>b</sup>	1,114.81 <sup>b-d</sup>	988.91 <sup>b</sup>

means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability.

*siella oxytoca* and *Rhizobium* spp. bacterial strains comparing with NPK.

Obtained results in our experiment showed also that fruit yield expressed as the number of fruits and fruit weight was greatly increased by the addition of bacteria to Fertigo (Manure), Humus UP, Humus Active + Aktywit PM, Biofeed Amin and Yeast as compared to NPK and this may be because these treatments increased the amounts of N, K, Mg, and Ca, in the roots. Moreover, these results are in accordance with the findings of ABDEL-NASSER and HARHASH (2002). They stated that organic manures increased the solubility and availability of P,

K, Ca and Mg to the plant and consequently, influence the growth and fruit production of the plant. Shamseldin et al. (2010) mentioned that the inoculation with Strain 843 of *Pseudomonas fluorescence* growth promoting rhizobacteria significantly improved fruit quality as well as increased fruit yield, fruit weight, fruit length, and TSS percentage of Washington navel orange. Mansour et al. (2011) noticed that using yeast via soil, via foliage, or via both methods at different concentrations on "Kelsey" plum trees greatly improved fruit yield and fruit quality in terms of fruit weight. Furthermore, Mosa et al. (2016) found that the addition of

Table 4. Effect of NPK fertilization and application of bioproducts on N, P, K, Mg and Ca root content of apple trees cv "Topaz" in 2016

Treatments	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
NPK	0.64 <sup>de</sup>	0.12ª	0.47 <sup>ab</sup>	0.13 <sup>ab</sup>	0.85 <sup>gh</sup>
NPK+ bacteria	$0.67^{c-e}$	$0.14^{a}$	$0.49^{ab}$	$0.10^{b}$	0.93 <sup>e-h</sup>
Fertigo	$0.69^{c-e}$	$0.13^{a}$	$0.49^{ab}$	0.13 <sup>ab</sup>	$0.98^{c-g}$
Fertigo + bacteria	$0.69^{c-e}$	0.13 <sup>a</sup>	$0.55^{a}$	$0.14^{ab}$	$1.07^{b-e}$
Micosat	$0.67^{c-e}$	0.12 <sup>a</sup>	$0.42^{b}$	0.13 <sup>ab</sup>	$1.02^{b-e}$
Micosat + bacteria	$0.78^{a-d}$	0.13 <sup>a</sup>	$0.49^{ab}$	$0.15^{ab}$	$1.09^{b-d}$
Humus UP	$0.64^{\mathrm{de}}$	0.12 <sup>a</sup>	$0.42^{b}$	0.12 <sup>ab</sup>	$0.95^{d-g}$
Humus UP + bacteria	$0.69^{c-e}$	0.13 <sup>a</sup>	$0.48^{ab}$	0.17 <sup>a</sup>	$1.02^{b-f}$
Humus Active + Aktywit PM	$0.60^{e}$	0.11 <sup>a</sup>	$0.51^{ab}$	0.12 <sup>ab</sup>	$0.84^{\mathrm{gh}}$
Humus Active + Aktywit PM + bacteria	0.91 <sup>a</sup>	0.13 <sup>a</sup>	$0.52^{ab}$	0.15 <sup>ab</sup>	1.26 <sup>a</sup>
BioFeed Quality	$0.69^{c-e}$	0.13 <sup>a</sup>	$0.50^{ab}$	0.13 <sup>ab</sup>	$0.99^{c-g}$
BioFeed Quality + bacteria	$0.72^{b-e}$	0.14 <sup>a</sup>	$0.52^{ab}$	$0.14^{ab}$	$1.06^{b-e}$
BioFeed Amin	$0.66^{c-e}$	0.11 <sup>a</sup>	$0.45^{ab}$	0.13 <sup>ab</sup>	$1.01^{c-f}$
BioFeed Amin + bacteria	$0.79^{a-c}$	0.15 <sup>a</sup>	$0.52^{ab}$	$0.14^{ab}$	1.16 <sup>ab</sup>
Yeast	$0.65^{c-e}$	0.13 <sup>a</sup>	$0.42^{b}$	$0.10^{b}$	0.80 <sup>h</sup>
Yeast + bacteria	$0.86^{ab}$	0.13 <sup>a</sup>	$0.48^{ab}$	$0.14^{ab}$	1.11 <sup>a-c</sup>
Vinassa	$0.67^{c-e}$	0.12 <sup>a</sup>	$0.49^{ab}$	0.12 <sup>ab</sup>	$0.93^{e-h}$
Vinassa + bacteria	$0.74^{b-e}$	0.14 <sup>a</sup>	$0.48^{ab}$	0.13 <sup>ab</sup>	0.86gh
Florovit Natura	$0.66^{c-e}$	0.12 <sup>a</sup>	$0.45^{ab}$	0.13 <sup>ab</sup>	$0.79^{h}$
Florovit Natura + bacteria	$0.71^{c-e}$	0.14 <sup>a</sup>	0.52 <sup>ab</sup>	$0.14^{ab}$	$0.87^{f-h}$
Florovit Eko	0.63 <sup>e</sup>	0.11 <sup>a</sup>	$0.49^{ab}$	0.13 <sup>ab</sup>	$0.96^{d-g}$
Florovit Eko + bacteria	$0.74^{b-e}$	0.15 <sup>a</sup>	$0.47^{ab}$	0.16 <sup>ab</sup>	$0.95^{d-g}$

means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability

bacteria to Fertigo (Manure), Humus UP, Biofeed Amin and Yeast improved the yield in terms of the number of fruits and fruit weight over NPK chemical fertilization.

and Yeast after enrichment with bacteria compared to NPK increased fruit number and apple weight.

# CONCLUSIONS

- The addition of bacteria to Fertigo, Micosat, Humus UP, and Florovit Natura improved significantly the Photosynthetic rate and stomatal conductance.
- Beneficial bacteria combined with Vinassa, Florovit Natura and Florovit Eko comparing with NPK increased evidently the tree thickness.
- Fertilized apple trees with Fertigo, Humus UP,
  Humus Active + Aktywit PM, Biofeed Amin,

# References

Abdel-Nasser G., Harhash M.M. (2002): The effect of organic manure in combination with elemental sulfur on soil physical and chemical characters, yield, fruit quality, leaf water contents and nutritional status contents and nutritional status of Flame seedless grapevines. 1 – Soil physical and chemical characteristics. Journal of Agricultural Science, Mansoura University, Egypt, 25: 3541–3558.

Arsenault J.-L., Poulcur S., Messier C., Guay R. (1995): Win-RHIZO<sup>TM</sup>, a root-measuring system with a unique overlap correction method. HortScience, 30: 906.

- Chen J. (2006): The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. In: International workshop on sustained management of the soil rhizosphere system for efficient crop production and fertilizer use, Bangkok: 1–11.
- Corte L., Dell'Abate M.T., Magini A., Migliore M., Felici B., Roscini L., Sardella R., Tancini B., Emiliani C., Cardinali G., Benedetti A. (2013): Assessment of safety and efficiency of nitrogen organic fertilizers from animal-based protein hydrolysates A Laboratory Multidisciplinary Approach 2013. Journal of the Science of Food and Agriculture, 94: 235–245.
- Game B.C., Navale A.M. (2006): Effect of VAM inoculation on nitrogen and phosphorus uptake by custard-apple seedlings. International Journal of Agriculture Sciences, 2: 354–355.
- Grzyb S.Z., Piotrowski W., Bielicki P., Sas Paszt L. (2012): Quality of apple maidens as influenced by the frequency of application of different fertilizers in the organic nursery preliminary results. Journal of Fruit and Ornamental Plant Research, 20: 41–49.
- Grzyb S.Z., Sas Paszt L., Piotrowski W., Malusa E. (2015): The influence of mycorrhizal fungi on the growth of apple and sour cherry maidens fertilized with different bioproducts in the organic nursery. Journal of Life Sciences, 9: 221–228.
- Karakurt H., Aslantas R. (2010): Effects of some plant growth promoting rhizobacteria (PGPR) strains on plant growth and leaf nutrient content of apple. Journal of Fruit and Ornamental Plant Research, 18: 101–110.
- Kuwada K., Kuramoto M., Utamura M., Matsushita I., Shibata Y., Ishii T. (2005): Effect of mannitol from *Laminaria japonica*, other sugar alcohols, and marine alga polysaccharides on *in vitro* hyphal growth of *Gigaspora margarita* and root colonization of trifoliate orange. Plant and Soil, 276: 279–286.
- Mansour A.E.M., Ahmed F.F., Abdelaal A.M.K., Eissa R.A.R., Fouad A.A. (2011): Selecting the best method and dose of yeast for "Kelsey" plum trees. Journal of Applied Sciences Research, 7: 1218–1221.

- Mosa W.F.A.E-G., Sas Paszt L., Frąc M., Trzciński P., Przybył M., Treder W., Klamkowski K. (2016): The influence of biofertilization on the growth, yield and fruit quality of cv. Topaz apple trees. Horticultural Science, 43: 105–111.
- Nardi S., Pizzeghello D., Muscolo A, Vianello A. (2002): Physiological effects of humic substances in plant growth. Soil Biology and Biochemistry, 34: 1527–1536.
- Ojha S., Chakraborty M.R., Dutta S., Chatterjee N.C. (2008): Influence of VAM on nutrient uptake and growth of custard-apple. Asian Journal of Experimental Sciences, 22: 221–224.
- Regvar M., Vogel-Mikuš K., Ševerkar T. (2003): Effect of AMF inoculums from field isolates on the yield of green pepper, parsley, carrot and tomato. Folia Geobotanica, 38: 223–234.
- Rivera-Cruz M., Trujillo A., Córdova G., Kohler J., Caravaca F., Roldán A. (2008): Poultry manure and banana waste are effective bio-fertilizer carriers for promoting plant growth and soil sustainability in banana crops. Soil Biology and Biochemistry, 40: 3092–3095.
- Sagervanshi A., Priyanka K., Anju N., Ashwani K. (2012): Isolation and characterization of phosphate solubilizing bacteria from agriculture soil. International Journal of Life Science and Pharma Research, 23: 256–266.
- Sas Paszt L., Malusá E., Sumorok B., Canfora L., Derkowska E., Głuszek S. (2015): The influence of bioproducts on mycorrhizal occurrence and diversity in the rhizosphere of strawberry plants under controlled conditions. Advances in Microbiology, 5: 40–53.
- Shamseldin A., El-Sheikh M.H., Hassan H.S.A., Kabeil S.S. (2010): Microbial bio-fertilization approaches to improve yield and quality of washington navel orange and reducing the survival of nematode in the soil. Journal of American Science, 6: 264–271.
- Von-Bennewitz E., Hlusek J. (2006): Effect of the application of two bio-preparations on the nutritional status, vegetative and generative behavior of "Jonagold" apple trees. Acta Horticulturae (ISHS), 721: 129–136.

Received for publication June 8, 2017 Accepted after correctons December 7, 2017