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ARTICLE

Effects of foliar biofertilization on the quality parameters of apple *(Malus domestica* Borkh.*)*

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Abstract - Nowadays the main task of scientists is to find natural ways of improving plant productivity that lead to environmentally friendly agriculture. Biofertilizers have a great potential to achieve this aim but unfortunately there is little information about its application in fruit growing mostly in Hungary. For this reason, a foliar nutrition experiment was conducted to investigate the effect of two biofertilizers (an algae product and a biostimulator, containing amino acids) on leaf nutrient concentration and quality parameters of apple (*Malus domestica* Borkh.) fruits. The study was conducted in 2014 at Újfehértó in East Hungary in a 14 year-old organic apple plantation on cv. 'Remo'. Treatments (application time and doses) were adjusted to the phenological phases of apple. Effect of treatments was monitored by soil and leaf analysis, apple quality measurements and field observations. Usage of biofertilizers resulted greener and healthier leaves. Treatments had no significant effect on leaf N, P, Mg and micronutrients but using algae suspension increased leaf K and Ca content significantly.

Biofertilizers increased size and weight of apples but did not affect the fruit quality compared to the control. Treatments decreased the fruit firmness which may be explained by a diluting effect in fruits. Higher apple size resulted softer tissues.

Keywords – apple, biofertilizer, nutrition, plant and soil analysis

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Introduction

Apple (*Malus domestica* Borkh.) is suggested to be the most important fruit in Hungary, especially in the East Hungary which is a traditional apple growing territory. Fortunately, the importance of environmental friendly growing technology, site oriented fertilization and ecological approaching of apple-growing is continuously increasing nowadays (Nagy 2012). Therefore, the role and impact of biofertilizers and bioregulators in apple nutrition are increasing in this region.

Biostimulators are natural growth regulators or chemicals, most of them contain plant hormones as auxin, gibberellic acid, cytokinins and aminoacids. Some of them contain additive macro and micro nutrients. Their ability to regulate aspects of plant growth and development and to improve yield, quality, stress tolerance and postharvest life makes them to play important role in fruit nutrition (Greene 2010).

Algae suspensions from green algae (*Chlorella vulgaris)* have been regarded as excellent bio-fertilizer containing macro and micro nutrients some growth regulators, polyamines and vitamins applied to improve nutritional status, vegetative growth, yield and fruit quality in different orchards (Eman et al. 2008).

Similar results are published by Khan et al. (2012) who reported that foliar applications of amino acids and seaweed extract improved growth and physico-chemical berry quality of grapevine cv. '*Perlette*'.

Amino acids are considered as precursors and constituents of proteins (Rai 2002), which are important for stimulation of cell growth. They contain both acid and basic groups and act as buffers, which help to maintain favourable pH value within the plant cell (Davies 1982). Amino acids can directly or indirectly influence the physiological activities in plant growth and development (Shiraishi et al. 2010; Khan et al. 2012).

According to Basak and Mikos-Bielak (2008) biostimulators cause development of unusually strong flowers on apple trees. Their application result in production of large fruits in the year of application and in the abundant flowering in the following year.

On pear, Błlaszczyk (2008) pointed out that the biostimulator used had beneficial influence on some internal fruit quality traits, as fruit firmness and titratable acidity, during the storage and maturation.

In our experiment, the effects of two natural fertilizers (algae suspension and a mixture of amino acids) were

investigated on yield, nutrient status of leaves and quality parameters of an apple variety: *'Remo'*.

Materials and methods

Foliar nutrition experiment was made to investigate the effect of different biofertilizers on yield, nutrient uptake and quality parameters of apple.

The trial conducted in 2014 at Újfehértó (47º 47' N; 21º 40'E) in East Hungary in a 14 years old organic apple plantation on cv. '*Remo*'. The uniform in vigor trees space at 5.0 m and 2.5 m used M26 rootstock. Trees in the fourteen years old organic apple orchard having uniform vigor and health were selected for the experiment.

All trees received uniform management practices including pruning, insecticide and pesticide applications without irrigation. Treatments (application times and doses) were adjusted to the phenological phases of apple and the orders of manufacturer and the control was used as a check.

In our trial the apple trees were sprayed with an aqueous solutions of Activator Plus (AP) (a mixture of amino acids, applied dose: 1 l/ha) and Organic Green Gold (OGG, green, colour free algae suspension; *Chlorella vulgaris*), applied dose: 10 l/ha). The trees are not received other fertilizers. The control trees were sprayed with the same volume of clean water.

Times of application: at full blooming stage (May 06, 2014); two weeks after full blooming (May 20, 2014); and finally (June 05, 2014). All the apple trees were thoroughly sprayed using a hand held backpack sprayer.

Three rows of approximately 0.5 hectare were taken as experimental unit to record the data with three replicates. One replicate is consisted from 120 trees.

In our trial leaf diagnostic and apple quality measurements and field observations were made to study the effectiveness of applied products in very drought conditions of 2013.

Leaf and fruit samples were taken to the laboratory (Institute of Environmental Sciences, Károly Róbert College, Gyöngyös) for chemical analysis.

Soil sampling and preparation
To establish the actual nutrient supply of the soil of examined area some chemical soil properties were measured.

Soil samples were collected with an auger from 0-30 cm and 30-60 cm layers from each treatment in October following the Hungarian sampling guidelines (MSZ-08 0202-77). After collection, the soil samples were dried outdoors. Before grinding, samples were cleaned from plant remains and other possible extraneous material and the soil was sieved through a 2 mm screen, homogenized and stored in plastic boxes in dry place until the examination.

Soil parameters were measured according to the Hungarian Standard Method (MSZ 20135:1999):

Soil pH was measured in 1 M KCl solution (1:2.5). For extracting the available phosphorus (P) and potassium (K) content of soils, ammonium-lactate solution (AL) was used. For determining magnesium (Mg), manganese (Mn), copper (Cu) and zinc (Zn) contents of soil KCl and EDTA solution was used. Soil P, K, Mg, Mn, Cu and Zn contents were quantified by Induced Coupled Plasma Optical Emission Spectrometry (ICP-OES, Labtam 8840 M Australia). Mineral nitrogen (sum of nitrate- and ammonium-N) of soil was quantified according to Houba et al. (1986).

Leaf sampling and preparation
Healthy, fully-developed, mature leaves (twenty leaves per plot) were taken before harvest at the end of September according to the Hungarian Standard Method (MI-08 0468-81).

Leaf samples were washed with distilled water to remove dust and possible remains of pesticide, than dried outdoors in an airy place for a week. After drying samples in a well-ventilated drying oven for 6 h at 80 °C, the whole sampled material was finely ground (< 1 mm by Kinematica, Polimix PX-MFC 90D grinder) and homogenized. Samples were then stored in paper bags in a dark and dry place until use.

Leaf mineral contents
The dried samples were digested with 5 mL concentrated H_2SO_4 and 5 mL H_2O_2 in a heating block digester, at 220 °C for 2 h. Totally digested samples were diluted with distilled water to 100 mL. Nutrients were determined in this solution and the results were calculated as percentages of the determined element relative to the dry weight basis. For reference values, dry matter analysis was also carried out on leaves after harvest.

Leaf nitrogen (N) contents were determined by using Kjeldahl method (VELP UDK 142) (Benton Jones et al. 1991). Leaf P, K, Mg, Mn, Cu and Zn contents determined by Induced Coupled Plasma Optical Emission Spectrometry (ICP-OES, Labtam 8840 M Australia).

Vegetative and reproductive growth
Leaf chlorophyll meter (SPAD-502 Meter, Minolta, Japan) was used to determine the leaf greenness of experimental apple trees and was expressed as SPAD value. Data were collecting from the middle of intensive shoot growing period to harvest, weekly. Data regarding fresh apple weight (g) were measured by a digital scale.

Fruit physico-chemical characteristics
At harvest fruits samples were taken to measure physic-chemical characteristics of apples. A digital refracto-meter ATAGO, RS-5000 (Atago, Japan) was used to determine soluble solid contents (SSC) of apple juice (MSZ EN 12143:1998) which is connection with sugar content of juice. Titratable acidity (TA) of apple juice was determined by titration with a known molarity solution of sodium hydroxide using phenolphthalein as indicator and expressed as g tartaric acid per juice L (MSZ EN 12147:1998). A digital penetrometer (FT 327) was used to measure the fruit hardness.

Statistical analysis
All the obtained data were tabulated and statistically analysed according to Sváb (1981) using the L.S.D. test at 5% level to recognize the significance of the differences between various treatment means. The effects of various treatments were assessed within ANOVA and Fisher's least significant differences were calculated following a significant ($P \leq 0.05$) F test.

Results and discussion

Results of soil analysis
To receive general information about nutrient status of orchard soil analysis was made.
Physical and chemical characteristics of the soil samples (from 0.0 to 60 cm depth) were determined according to the standard procedures of Hungarian guidelines (MSZ 20135:1999). The main properties are shown in Table 1.

Table 1. Results of soil analysis (Újfehértó, 19.10.2013.)

Parameters	Depth (cm)	
	0-30	**30-60**
pH(in water)	6.63	5.24
$CaCO_3$ %	n.d.	n.d.
water soluble salt (%)	<0.02	<0.02
Plasticity index according to Arany (K_A)	25	25
Humus content (%)	0.69	0.64
(NO_3+NO_2)-N (mg/kg)	4.7	5.2
NH_4^+-N (mg/kg)	1.5	5.4
P_2O_5 (mg/kg) (AL)	154	97
K_2O (mg/kg) (AL)	149	78
Mg (mg/kg)	57	42
Mn (mg/kg)	69	74
Cu (mg/kg)	2.1	4.5
Zn (mg/kg)	1.1	1.9

Orchard soil type was slightly acidic, non-calcareous sandy soil with very low humus content.

Water capacity of soil was low according to the soil type. The texture grade of soil was sandy according to the soil plasticity index (K_A) (Table 1). According to our results the soil pH was slightly acidic, optimal for apple growing (MÉM NAK 1981) and decreased by the depth. Carbonate content of soil was not detectable. Soil organic matter content was very low, varied between 0.69 and 0.64. Mineralized nitrogen form (NO_3-N+NH_4-N) of soil was very low according to the soil type. The rate of mineralization was slowly and the N supply of soil was weak.

Available soil P and K content (AL soluble) was low and decreased by the depth. Available soil Cu and Zn were suitable for apple growing in the examined soil layer. However, available Mg and Mn concentrations were medium in the soil of examined area. Our results pointed out that the amount of nutrients and the main soil properties were similar in the soil of all treatments. There was no difference between nutrient supplies of soil of experimental plots.

Results of plant analysis
Foliar application of algae suspension (OGG) and the mixture of amino acids (AP) significantly improved leaf greenness, measured by SPAD (Table 2). Only mean values are shown in Table 2 which calculated from the weekly data during vegetation period.

Table 2. Effect of foliar application of OGG and AP on leaf greenness of apples cv. Remo (Újfehértó, 2014)

Treatments	Leaf greenness (SPAD)*
Control	51.51a
OGG	54.04b
AP	53.78b

*-twenty leaves per sample were measured (average value calculated from five measuring points per leaf)
In each column, means followed by the same letter are not significantly different (P<0.05).

Measured values are in good connection with our visual observations. It was established that the treated leaves were greener, larger and healthier than the control leaves.

Foliar application of mixture of amino acids (AP) and algae suspension (OGG) did not significantly influence the N content of apple leaves (Table 3).

Treatments had no significant effect on leaf P and Mg but OGG treatment increased the leaf K and Ca.

It was found that the leaf micronutrient status was not significantly affected by the treatments.

This result is highly similar to the findings of Khan et al. (2012) who reported that foliar treated grapevines (mixture of amino acids and seaweed extract) showed no significant change in the leaf mineral contents.

Moreover leaf analytical results are corresponding with soil measurements. Low nutrient status of microelements in leaves can be explained by the low availability of these nutrients in soil.

Results of fruit analysis

Foliar application of mixture of amino acids and algae suspension significantly influenced the weight of apples (Table 4). Both of the tested materials increased apple weight and size. The increase of weight was 4 at OGG and 9% at AP treatment compared to the control.

Results showed that foliar application of biofertilizers had a positive effect on yield and resulted bigger and healthier therefore more marketable fruits.

Beside reproductive growth parameters, the properties of fruit quality (sugar and acid content of fruits and fruit firmness) were also measured (Table 5).

Acidity of apple juice was not affected by treatments as the value of titratable acidity (TA) showed. AP treatment resulted a slightly but not significant decreasing in acid concentration of apple juice.

SSC (Brix) value of apple juice was significantly lower when OGG was used. It means that the soluble sugar concentration was lower applying OGG.

Fruit firmness was significantly lower at treated samples than control. The lower values measured in treated samples may be explained by a diluting effect in fruits. Higher apple size resulted softer tissues and higher volume of pressed juice with lower concentrations.

Table 3. Effect of foliar application of OGG and AP on leaf macronutrient contents of apples cv. Remo (Újfehértó, 2014)

Treatments	N	P	K	Ca	Mg
Control	1.69a	0.19a	1.172a	1.395a	0.266a
OGG	1.65a	0.20a	2.649c	2.739c	0.251a
AP	1.58a	0.20a	1.296a	1.318a	0.236a

In each olumn, means followed by the same letter are not significantly different (P<0.05).

Table 4. Effect of foliar application of OGG and AP on leaf micronutrient contents of apples cv. Remo (Újfehértó, 2014)

Treatments	Zn	Fe	Cu	Mn
Control	10.0a	177a	9.1a	82a
OGG	11.2a	150a	8.9a	74a
AP	10.4a	137a	8.7a	65a

In each olumn, means followed by the same letter are not significantly different (P<0.05).

Table 5. Effect of foliar application of OGG and AP on reproductive growth of apples cv. Remo (Újfehértó, 2014)

Treatments	Fruit weight (g)*	Weight increment (%)**	SSC (Brixº)	TA	Fruit firmness (kg/cm^2)*
Control	123a	-	15.1b	14.50a	8.1b
OGG	131b	4	14.2a	14.45a	7.5a
AP	134b	9	15.0b	12.40a	7.3a

In each olumn, means followed by the same letter are not significantly different (P<0.05). *- average of 30 fruits. **- compared to the control

Conclusions

According to our field visual observations the used plant growth regulators as biofertilizers resulted in vigorous development, greener and larger leaves, despite the unfavourable, very dry climatic conditions. Foliar application of algae suspension and mixture of amino acids at different growth stages significantly improved the greenness of apple leaves.

Spraying of algae suspension significantly influenced the potassium and calcium content of apple leaves. However the concentration of the other examined nutrients was not affected by used biofertilizers.

Foliar application of mixture of amino acids and algae suspension significantly increased the weight and the size of apples.

Therefore, our results showed that foliar application of algae suspension had significant increasing effect on yield. Our data showed that foliar application of mixture of amino acids and algae suspension treatments decreased the fruit firmness significantly.

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