Acta Sci. Pol. Hortorum Cultus, 18(1) 2019, 197–205

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.1.20

ORIGINAL PAPER

Accepted: 10.01.2019

INFLUENCE OF NPK MINERALS AND BIOSTIMULANTS ON THE GROWTH, YIELD, AND FRUIT NUTRITIONAL VALUE IN CV. 'ŠAMPION' APPLE TREES GROWING ON DIFFERENT ROOTSTOCKS

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ABSTRACT

The study was conducted in 2015–2017 to assess the influence of rootstocks on the growth and fruiting of apple trees of cv.'Šampion' cultivated on rootstocks M26, P2. M9, and P22 with the following treatments: mineral fertilization (NPK), nano-concentrations of elements (Fe, Co, Al, Mg, Mn, Ni, Ag), natural chicken manure fertiliser, humus, microbial product, plant amino acids, and stillage yeasts. 'Šampion' apple trees grew vigorously on rootstocks M26 and P2 when humus and microbiological biostimulants were applied, especially in terms of the shoot diameter and TCSA. The best fruit yield and quality parameters were obtained in apple trees growing on rootstocks M9 and M26 fertilised with microbiological biostimulants and formulations containing plant amino acids. Apples with the highest concentration of nutrients, in particular minerals, were harvested from trees growing on rootstocks M9 and P22 and stimulated with nanoparticle mineral preparations and humus formulations.

Key words: biostimulants, apple cultivation, fruiting trees, fruit nutrients

INTRODUCTION

In fruit production, the choice of an appropriate rootstock can determine high productivity and, therefore, profitability. The rootstock has a fundamental influence on the growth vigour, productivity, and health status of the tree [Gainza et al. 2015, Kiczorowski et al. 2018b]. It can also determine the quality or even the nutritional value of fruit [Aka-Kacar et al. 2010, Fazio et al. 2015, McCollum and Bowman 2017, Kiczorowski et al. 2018a]. In this respect, the choice of the rootstock in fruit production can be as important as the choice of the fruit tree variety. It is equally important to adjust the apple tree rootstock to the prevailing climatic conditions, besides ensuring the appropriate height of the tree [de Carvalho et al. 2019]. In countries with severe winters, it is essential to provide fruit trees with frost resistance with maintenance of adequate growth strength. Dwarf rootstocks, which produce trees with the lowest growth strength, are recommended in orchards characterised by intense production. However, trees obtained from such rootstocks are usually less vital than those grafted on rootstocks with more potent growth, and require staking as well as a higher level of cultivation procedures. Such rootstocks are represented by M9, P59, P60, P2 and P22.

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Their advantage is the earlier onset of yielding and high yields, although the fruits have lower weight. A disadvantage is the shallow and poorly developed root system [Foster et al. 2016]. In moderately intensive orchard production, semidwarfing rootstocks are used most frequently. Trees growing on such rootstocks are more resistant to stress conditions but exhibit a stronger tendency to irregular fruiting. These rootstocks include M26, P14, M7 and MM106. They are characterised by a strongly developed root system and lower soil, water, and care requirements [Bielicki and Pąśko 2018, Schluchter et al. 2018].

To provide trees with optimal growth and production conditions, mineral fertilisers are applied in conventional fruit production [Cheng et al. 2017, Grzyb et al. 2014]. Consumers' expectations in terms of safety combined with a high nutritional value of fruit make producers search for biostimulants of natural origin, which effectively increase productivity. Such biostimulants include organic fertilisers, humus extracts, plant amino acids, seaweed extracts, or microbiological and mycorrhizal preparations. Their effectiveness has been confirmed in studies conducted on fruit trees indicating a necessity of individual selection of biostimulants with respect to not only the fruit tree species but also the cultivar or even the rootstock [Grzyb et al. 2012, Mosa et al. 2015a].

The aim of this study was to evaluate the effect of fertilisation with standard minerals (NPK) and biostimulants on the growth, yield, and fruit quality of cv. 'Šampion' apple trees growing on different rootstocks.

MATERIAL AND METHODS

The study was conducted on a private horticultural farm in Podkarpackie province (50.0303N, 22.2016E) Poland from 2015 to 2017. The investigations involved 'Šampion' apple trees cultivated on rootstocks M26, P2. M9, and P22. One-year-old whips grafted on chosen rootstocks were planted in the autumn of 2009 on a forest humus substrate (4-year-old deciduous leaf compost. The humus was applied each year 2014−2017 at the end of April. Twenty-five litres of mulch per research plot were applied within a 60-cm-wide band placed along both sides of the planted trees. The treatment was immediately incorporated into the soil to a depth of approx. 15 cm. A randomised block experiment was conducted in four replications with three trees per plot planted at 4.0 m × 1.5 m spacing, with guard trees between the plots. Each plot included one experimental treatment. The entire experiment was conducted on one field. Disease, weed, and pest control followed general recommendations developed for apple tree orchards. Soil samples were taken from the depths of 0−20, 20−40, and 40−60 cm in November 2011 and 2012. The content of minerals in soil before the treatments is shown in Table 1.

Table 1. The mineral content and pH in soil before experimental treatments (d.w.)

Minerals	Content in soil
pH	5.3
	Macroelements (g kg^{-1})
N	9.3
P	16.0
K	15.3
Mg	5.6
	Microelements (mg kg^{-1})
B	3.1
Cu	13.7
Fe	1171
Mn	96.4
Zn	12.1

The first crop was harvested from the trees in 2010. For the determination of available forms of phosphorus and potassium in the mineral soil, the Egner-Riehm method was used. The Schachtschabel method was employed for the determination of available forms of magnesium in the mineral soil [Domagała-Świątkiewicz 2005]. Available forms of microelements in the soil were determined using the method of extraction in 1 M HCl [Merck Poland 2002]. Total nitrogen and organic carbon content in both soil and plant material was determined with the conductometric method using a TruSpec CNS analyser.

The following eight treatments were applied in the experiment: 1) control (C) – no treatment; 2) mineral fertilisation (NPK) – 176.3 kg ha^{-1} NH_4NO_3, 65.3 kg ha^{-1} triple superphosphate, and 160 kg ha^{-1} K_2SO_4; 3) nano-minerals (nM) – nano-concentrations of Fe, Co, Al, Mg, Mn, Ni, and Ag in the form of sulphates – 10 granules 500 l ha^{-1} with sugar purified with methanol (1000 g sugar, 20 ml^{-1} alcohol) (NANO-GRO; Agrarius, Krasiczyn, Poland); chicken manure fertilizer (NF) – 1500 kg ha^{-1}; humus (HU) – 2% solution at a dose of 20 l ha^{-1} (Humus UP, Przedsiebiorstwo Produkcyjno-Handlowe Ekodarpol, Dębno, Poland), microbial product (MI) – *Glomus mosseae*, *Glomus intraradiaces*, *Pseudomonas fluorescence*, and *Bacillus subtilis*: 10 kg ha^{-1} (Microsat, CCS Aosta Sr, Italy); plant amino acids (PA) – seaweed extract: 0.5% solution at a dose of 20 l ha^{-1} (BioFeed Amin, Agro Bio Products B.V., Wageningen, Nederland); stillage from yeast production (SY) – 0.5% solution at a dose of 5 l ha^{-1} (Lesaffre Polska, SA, Wołczyn, Poland). Application of the biopreparations was carried out twice during the growing season – the first time in early May and the second time in the first ten days of June. The preparations were applied by hand to the soil around the tree trunk within a radius equal to the radius of the tree crown.

Soil cultivation and protection of trees against pests and diseases were in line with measures commonly employed in commercial apple orchards. The experiment assessed tree growth vigour measured by the shoot diameter (mm) and cross-sectional area of the tree trunk (TCSA in cm^2), one-year-old shoot length (cm), leaf area (cm^2), fruit yield, productivity index (kg $(cm^{-2})^{-1}$), and fruit weight (kg), length (cm), and diameter (cm).

Five one-year-old shoots and 20 leaves from the middle parts of the 10 tagged shoots were selected from each tree for the aforementioned measurements during the last week of September [Liu et al 2008, Karlıdağ et al. 2014]. The growth of the trees (the tree height in centimetres) was evaluated annually after completion of the growing season; the trunk diameter was measured at 30 cm above the soil level annually after completion of the growing season.

Total yield (kg) was evaluated as the weight of all fruits picked from the trees at harvest, when the fruits reached harvest maturity (8 Brix, iodine test; refractometer Rudolph J-157, Rudolph Research Analytical, NJ). The fruit size was evaluated as the fruit diameter. The data for the above-mentioned parameters and fruit yield were obtained from 16 trees in four replications (each replication represented by four trees) of each treatment. The given values are means of the data collected in 2015–2017.

The basic chemical composition of apples, n = 15 from each plot from every experimental harvest year, was determined in compliance with standard procedures (AOAC 2000): dry matter (method 985.14), total minerals (method 920.153), easily hydrolysable sugars (calculated on the total basic chemical composition), and L-ascorbic acid using an enzymatic test kit (No. 10 409 677 035 – test – combination for 21 determinations) from Boehringer Mannheim/r-Biopharm [Czerwiecki and Wilczyńska 1999]. The total phenolic content was determined using the spectrophotometric method [Singleton and Rossi 1965].

The results of the experiments were analysed with the Shapiro-Wilk test for determination of normality of the distribution and Levene's test for assessment of the equality of variances using Statistica 10 software (Statsoft Inc. 2010). The results were analysed with the ANOVA one-way analysis of variance. The significance of the differences was determined with the Tukey HSD (honestly significant distance) test ($P < 0.05$). This test was employed to verify whether the tested fertilizations had a significant influence on the analyzed parameters of the trees and apples and the chemical composition of the fruits.

RESULTS AND DISCUSSION

The application of the biostimulant-based fertilisation of cv. 'Šampion' apple trees growing on rootstocks M26, P2, M9, and P22 exerted a positive effect on the growth parameters (Tab. 2). The greatest impact on the apple growth parameters was noted in the MI and PA treatments in comparison with the control. The fertilisation with the MI and PA biostimulants increased ($P < 0.05$) the shoot diameter in the range of 58–66% (M26), 53–49% (P2), 51–47% (M9), and 37–55% (P22); leaf area by 31–33% (M26), 18–24% (P2), 24–27% (M9), and 37–31% (P22); and TCSA by 22–26% (M26) and 17–15% (M9). The TCSA value of apple trees growing on rootstock M9 was significantly ($P < 0.05$) influenced by the HU treatment, compared with the control (about 14.9%). The most beneficial ($P < 0.05$) effect on the TCSA value in apple trees growing on rootstock P2 was exerted by the MI treatment (about 28%) as well as nM, PA, and NF (17.2, 17.2, and 20.5%, respectively) in comparison with the C treatment. In turn, the TCSA value in apple trees growing on rootstock P22 increased ($P < 0.05$) in the NF, MI, and PA treatments (18.7, 25.2, and 20.5%, respectively), compared with the TCSA of the control trees (C). There was no clear effect of the experimental treatments on the one-year-old shoot length.

Table 2. Shoot and trunk diameter, length shoot, and leaf area of 'Šampion' trees growing on different rootstocks depending on applied biostimulants

Parameters	Treatment[a]								Statistical parameters	
	C	NPK	nM	NF	HU	MI	PA	SY	HDS Tukey's[b]	ANOVA[c]
M26										
Shoot diameter (mm)	5.33	6.51	4.12	6.71	7.35	8.43	8.87	5.95	0.028	0.034
TCSA [d] (cm^2)	14.3	16.7	15.7	16.7	15.7	17.4	18.01	15.9	0.024	0.028
One-year-old shoot length (cm)	43.7	46.8	41.4	38.2	49.8	45.4	42.8	48.3	0.143	0.108
Leaf area (cm^2)	28.9	39.4	36.1	29.3	35.6	37.8	38.6	26.34	0.041	0.036
P2										
Shoot diameter (mm)	4.23	5.81	3.29	5.22	5.98	6.47	6.31	4.25	0.011	0.016
TCSA [d] (cm^2)	12.2	13.8	14.3	14.7	13.8	15.6	14.3	13.8	0.047	0.039
One-year-old shoot length (cm)	35.7	38.6	36.1	37.3	39.1	42.3	41.7	36.3	0.209	0.137
Leaf area (cm^2)	24.7	22.4	27.9	26.1	28.4	29.1	30.63	26.1	0.127	0.105
M9										
Shoot diameter (mm)	4.13	5.14	4.09	4.87	5.16	6.24	6.08	5.13	0.025	0.018
TCSA [d] (cm^2)	12.7	14.3	13.8	14.1	14.6	14.9	14.6	13.5	0.038	0.041
One-year-old shoot length (cm)	36.1	37.5	39.6	37.3	39.7	46.2	42.7	39.9	0.042	0.039
Leaf area (cm^2)	24.0	25.1	26.98	31.1	27.2	29.7	30.5	28.7	0.108	0.073
P22										
Shoot diameter (mm)	3.15	4.28	4.03	3.75	3.87	4.31	4.89	3.67	0.059	0.041
TCSA [d] (cm^2)	10.7	12.8	13.5	12.7	13.1	13.4	12.9	11.7	0.042	0.031
One-year-old shoot length (cm)	36.7	37.9	38.1	37.8	39.4	37.8	39.9	38.4	0.245	0.173
Leaf area (cm^2)	19.2	20.4	19.8	21.7	22.4	26.4	25.1	20.7	0.051	0.041

[a] C – control, NPK – mineral fertilisation, nM – nano minerals, NF – natural fertiliser, HU – humus, MI – microbial product, PA – plant amino acids, SY – stillage yeasts
[b] HSD (honestly significant distance) Tukey's test, P-value, $P < 0.05$
[c] ANOVA, analysis of variance, P-value, $P < 0.05$
[d] Trunk cross-sectional area

The investigations confirmed the beneficial effects of the microbiological preparations and seaweed extracts on the parameters of apple tree growth. As reported by Khan et al. [2009], seaweed extracts, which are rich in amino acids, can protect plants against pathogens, weeds, and pests and can mitigate the adverse effects of environmental factors such as drought or temperature by maintenance of an appropriate moisture level. Seaweed extracts can also improve nutrient uptake by roots [Crouch et al. 1990]. In contrast, application of microbial biostimulants was found to contribute to a significant increase in the population of rhizospheric mycorrhizal fungi and plant growth promoting bacteria (PGPR), which led to enhanced availability of mineral nutrients as well [Bardi and Malusà 2012]. A beneficial effect of microbiological bioformulations on the increase in the thickness and height of apple trees growing on rootstocks M26 was observed by Grzyb et al. [2012]. This was also confirmed by Wang et al. [2017], who recorded a more intensive growth of apple trees and increased yields in apple orchards where bioorganic fertiliser was applied. The authors emphasise that this procedure has particular benefits in apple orchard soils whose biological properties have been degraded by the long-term use of chemical fertilisers

In the first years of fruiting, the highest ($P < 0.05$) yields were harvested from trees growing on rootstocks M26 and M9 in the MI, PA, and HU treatments (average 8.26 kg $tree^{-1}$ and average 5.86 kg $tree^{-1}$, respectively) (Tab. 3). Apple trees growing on rootstock P2 achieved the highest ($P < 0.05$) yields in the MI, PA, and NF treatments (average 5.57 kg tree^{-1}), and those growing on rootstock P22 produced the highest yields in the MI, PA, and nM treatments (average 4.39 kg tree^{-1}). Regardless of the type of the biostimulants used, the highest yield was observed in apple trees growing on rootstock M26 and the lowest values were noted in trees growing on rootstocks P2 and P22. The lowest yields in the entire experiment were provided by the control trees (C) growing on rootstocks M26, M9, P2, and P22. The highest ($P < 0.05$) productivity index was calculated for trees fertilised with HU, PA, and MI (M26) as well as MI and PA (P2). The values of this index in the case of the other trees did not differ significantly from the control. The largest ($P < 0.05$) fruits with an average weight of approx. 216 g were collected from an apple

Table 3. Tree size, fruit yield, and quality of 'Šampion' cv. apples from trees growing on different rootstocks depending on applied biostimulants

Parameter	Treatment[a]								Statistical parameters	
	C	NPK	nM	NF	HU	MI	PA	SY	HDS Tukey's[b]	ANOVA[c]
				M26						
Yield (kg tree^{-1})	5.26	6.12	6.45	5.93	7.84	8.12	8.83	6.16	0.047	0.035
Productivity index (kg (cm^2)$^{-1}$)	0.37	0.37	0.41	0.36	0.50	0.47	0.49	0.39	0.036	0.023
Fruit length (cm)	5.27	5.31	5.19	5.48	5.67	5.67	5.46	5.15	0.189	0.237
Fruit diameter >8.01 cm (%)	54.4	55.7	57.6	53.4	52.7	55.3	54.9	55.1	0.197	0.167
>8.50 cm (%)	16.2	18.3	17.6	15.7	16.9	17.3	17.6	15.9	0.043	0.0.59
Weight of 100 fruits (kg)	20.5	21.9	21.3	19.8	19.4	21.6	20.4	18.8	0.038	0.056
				P2						
Yield (kg tree^{-1})	3.48	4.56	3.98	4.91	4.32	5.78	6.03	4.57	0.041	0.038
Productivity index (kg (cm^2)$^{-1}$)	0.29	0.33	0.28	0.33	0.31	0.37	0.42	0.33	0.024	0.018
Fruit length (cm)	4.39	4.57	4.31	4.63	4.49	4.89	4.97	4.73	0.166	0.267
Fruit diameter >8.01 cm (%)	49.2	49.7	50.3	47.8	46.6	49.7	47.6	46.8	0.183	0.203
>8.50 cm (%)	14.8	15.6	15.3	14.2	14.1	15.2	14.9	14.3	0.097	0.067
Weight of 100 fruits (kg)	18.7	19.6	19.8	18.6	17.9	18.2	18.3	17.2	0.024	0.019
				M9						
Yield (kg tree^{-1})	4.12	5.89	5.32	4.78	5.69	5.73	6.17	5.09	0.051	0.043
Productivity index (kg (cm^2)$^{-1}$)	0.32	0.41	0.39	0.34	0.32	0.41	0.45	0.38	0.294	0.187
Fruit length (cm)	4.46	5.02	4.98	4.59	4.72	5.03	4.97	4.62	0.204	0.193
Fruit diameter >8.01 cm (%)	48.7	49.3	49.6	48.6	49.3	49.7	49.3	48.5	0.193	0.209
>8.50 cm (%)	16.9	17.2	17.5	16.8	17.6	18.0	17.9	16.4	0.026	0.037
Weight of 100 fruits (kg)	18.3	18.7	17.9	17.7	17.6	18.1	17.8	17.9	0.094	0.195
				P22						
Yield (kg tree^{-1})	3.31	4.12	4.26	3.97	3.76	4.54	4.38	3.81	0.306	0.257
Productivity index (kg (cm^2)$^{-1}$)	0.31	0.32	0.32	0.31	0.29	0.34	0.34	0.33	0.193	0.183
Fruit length (cm)	3.76	4.19	4.43	3.97	4.16	4.98	5.12	3.97	0.145	0.154
Fruit diameter >8.01 cm (%)	43.9	44.8	45.2	43.7	42.6	44.9	43.6	41.3	0.233	0.278
>8.50 cm (%)	14.2	14.1	13.9	12.8	12.9	13.6	14.5	13.7	0.026	0.045
Weight of 100 fruits (kg)	17.4	17.3	17.1	16.8	16.9	17.6	17.2	17.4	0.135	0.216

[a] C – control, NPK – mineral fertilisation, nM – nano minerals, NF – natural fertiliser, HU – humus, MI – microbial product, PA – plant amino acids, SY – stillage yeasts

[b] HSD (honestly significant distance) Tukey's test, P-value, $P < 0.05$

[c] ANOVA, analysis of variance, P-value, $P < 0.05$

Table 4. Chemical contents in 'Šampion' cv. apples from trees growing on different rootstocks depending on applied biostimulants (n.m.)[a]

Parameters	Treatment[a]								Statistical parameters	
	C	NPK	nM	NF	HU	MI	PA	SY	HDS Tukey's[b]	ANOVA[c]
M26										
Dry matter (g 100 g^{-1})	17.26	17.54	17.89	17.13	17.98	18.52	18.47	18.03	0.184	0.157
Sugars (g 100 g^{-1})	15.42	15.59	15.49	15.39	15.97	16.03	15.89	15.81	0.167	0.153
Minerals (g 100 g^{-1})	0.36	0.42	0.45	0.35	0.47	0.38	0.36	0.37	0.021	0.033
Phenols (mg kg^{-1})	273	269	275	289	294	308	289	315	0.053	0.047
Vitamin C (mg 100 g^{-1})	8.47	8.51	8.78	8.94	8.34	8.54	8.48	8.60	0.149	0.136
P2										
Dry matter (g 100 g^{-1})	16.29	16.38	16.54	16.35	16.77	16.89	17.03	16.73	0.209	0.155
Sugars (g 100 g^{-1})	13.27	13.64	13.58	13.49	13.52	13.78	13.65	13.82	0.183	0.167
Minerals (g 100 g^{-1})	0.32	0.35	0.39	0.31	0.38	0.33	0.34	0.31	0.047	0.029
Phenols (mg kg^{-1})	254	271	268	276	298	267	278	294	0.106	0.094
Vitamin C (mg 100 g^{-1})	8.12	8.23	8.08	8.16	8.32	8.19	8.31	8.24	0.167	0.171
M9										
Dry matter (g 100 g^{-1})	17.31	17.45	17.64	17.42	17.65	17.89	17.67	17.62	0.184	0.254
Sugars (g 100 g^{-1})	14.13	14.37	14.15	14.37	14.52	14.64	14.49	14.56	0.343	0.167
Minerals (g 100 g^{-1})	0.31	0.35	0.35	0.34	0.38	0.35	0.34	0.32	0.037	0.026
Phenols (mg kg^{-1})	267	273	266	259	284	291	287	269	0.344	0.163
Vitamin C (mg 100 g^{-1})	7.98	8.13	8.19	7.78	7.92	8.34	8.23	8.15	0.023	0.019
P22										
Dry matter (g 100 g^{-1})	18.11	18.21	18.09	18.34	18.26	18.47	18.39	18.16	0.221	0.177
Sugars (g 100 g^{-1})	14.66	14.81	14.89	14.74	14.67	14.89	14.77	14.83	0.147	0.239
Minerals (g 100 g^{-1})	0.39	0.43	0.45	0.40	0.46	0.44	0.43	0.44	0.016	0.027
Phenols (mg kg^{-1})	256	267	275	281	264	289	287	269	0.106	0.073
Vitamin C (mg 100 g^{-1})	8.34	8.51	8.43	8.67	8.71	8.64	8.59	8.43	0.117	0.215

[a] n.m. – natural matter
[b] Treatment: C –control, NPK – mineral fertilisation, nM – nano minerals, NF – natural fertiliser, HU – humus, MI – microbial product, PA – plant amino acids, SY – stillage yeasts
[c] HSD (honestly significant distance) Tukey's test, P-value, $P < 0.05$
[d] ANOVA, analysis of variance, P-value, $P < 0.05$

tree fertilised with MI (M26) and fruits with a weight of 197 g were harvested from NPK- and nM-treated trees (P2). The weight of fruits collected from trees growing on rootstocks M9 and P22 was in the range of 176–187 g (M9) and 169–176 g (P22), which was not significantly different from the weight of the control fruits (183 and 174 g). The highest percentage ($P < 0.05$) of apples with a diameter larger than 8.5 cm was collected from apple trees M26 in the nM, MI, and PA treatments (average 17.5%) and on rootstock M9 in the HU, MI, and PA treatments (average 17.8%).

Similarly, the yielding of cv. 'Šampion' trees was most effectively stimulated in the MI, PA, and HU treatments. Microbiological formulations containing mycorrhizal fungi are recommended for use not only in so-called hardly reclaimable areas but also in production orchards. Due to the strong intensification of plant production, plant roots are unable to fulfil the growth and production needs of trees without cooperation with mycorrhizal fungi. They increase the efficiency of water and biogenic element uptake, which positively stimulates plant growth and development [Müller et al. 2007]. Tree growth vigour is enhanced by humic compounds via improvement of the soil structure. They increase the humus fractions and provide so-called growth-promoting substances such as vitamins, auxins, organic acids, and antibiotic substances, which intensify the physiological processes in plants. Fertilisation with humic acids increases the productivity of fruit trees by 10 to 20% [Milosevic and Milosevic 2009]. This positive production effect was confirmed in investigations conducted by Rozpara et al. [2014] on cv. 'Ariwa' apple trees in organic farming conditions, where there was a nearly 2-fold higher yield than in the case of microbiological fertilisation and an almost 3-fold higher yield upon application of humus formulations and their combinations with microbiological fertilisation. A positive effect of biostimulants was reported by Mosa et al. [2015a] in their investigations of cv. 'Anna' apple trees. Furthermore, Fathi et al. [2002] observed that biostimulants improved not only the yield but also the weight and quality of apples. The beneficial effect of bioorganic fertilisers on the increase in apple yields was also reported by Wang et al. [2017]. The improvement of apple yields was explained by the increase in soil total nitrogen and altered bacterial community by enrichment with *Rhodospirillaceae*, *Alphaproteobateria*, and *Proteobacteria*. In turn, Meyer et al. [2015] found that application of organic fertilisers in a 'Cripp's Pink'/M7 apple orchard increased the presence of mycorrhizal fungi in the soil, but this colonisation was negatively correlated with yield.

The largest concentration of nutrients was determined in apples collected from trees growing on rootstocks P22 and M9 (Tab. 4). The biostimulants applied improved ($P < 0.05$) their nutritional value mainly in terms of their content of minerals (M26, P2, M9, P22) and the levels of phenols (M26) and vitamin C (M9). Improvement ($P < 0.05$) of the mineral composition of the analysed apples was noted upon the nM and HU treatments (average M26: 0.46; P2: 0.38; M9: 0.36; P22: 0.45 g 100 g^{-1} n.m.). As reported by Aka-Kacar et al. [2010], humus biostimulants applied already in the fruit tree nursery contribute to substantial improvement of the environmental conditions of tree growth, stimulation of the intensity of physiological processes, and activation of the biodistribution of nutrients and minerals in the plant. They have a favourable impact on productivity in the early production stages on the yield, quality, and nutritional value of fruits [Grzyb et al. 2014, Kiczorowski et al. 2018b]. Interesting results were obtained upon fertilisation with a complex formulation containing mineral nanoparticles. Jędrszczyk and Ambroszczyk [2016] as well as Doležal [2010] reported that the biostimulant triggers the plant defence system against stress, inducing metabolic processes. In the present study, no beneficial effect of the biostimulants on the tree vigour was observed. Nevertheless, the improvement of photosynthesis processes and the regulation of water metabolism reflected in elevated levels of organic and mineral compounds [Doležal 2010] resulted in increased accumulation of minerals in the apples. The beneficial effect of biofertilisation in apple orchards on the chemical composition of fruit was reported by Mosa et al. [2015b] as well. Particularly positive effects were observed upon humus and animal manure fertilisation.

CONCLUSIONS

'Šampion' apple trees grow vigorously on rootstocks M26 and P2, especially in terms of the shoot diameter and TCSA, upon application of humus extracts and microbiological biostimulants. The highest yields and fruit quality parameters were obtained from apples trees growing on rootstocks M26 and M9 and fertilised with microbiological biostimulants and formulations containing plant amino acids. Apples with the highest concentration of nutrients, especially minerals, were produced by trees growing on rootstocks M9 and P22 and stimulated with formulations of mineral nanoparticles and humus extracts.

ACKNOWLEDGEMENTS

The research was financed by the Ministry of Science and Higher Education of the Republic of Poland (TBK-DS/1).

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