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The influence of biofertilization on the vegetative growth, yielding and nutritional status of 'Sampion' apple trees in first years after planting

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Abstract: The effects of biofertilizers on the growth, yielding, and nutritional status of apple trees (in comparison with the standard mineral fertilization) was evaluated in 3-years experiment (2018–2020). The experiment included the following treatments: (*i*) Super Fos Dar, (*ii*) Polifoska 6, (*iii*) Super Fos Dar + fungi (*Aspergillus niger*, *Purpureocillium lilacinum*), (*iv*) Urea enriched with fungi, (*v*) Urea dose reduced to 60% enriched with fungi, (*vi*) Super Fos Dar + bacteria applied on their own to the soil (*Bacillus* sp., *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa*), (*vii*) Super Fos Dar enriched with bacteria, (*viii*) Super Fos Dar 60% enriched with bacteria, (*ix*) Polifoska 6 enriched with bacteria, (*x*) Polifoska 6 60% enriched with bacteria. The application of mineral fertilizers (even in reduced doses) enriched with beneficial microorganisms enhanced the growth of apple trees. The most abundant flowering was recorded when fertilization (with Super Fos Dar) was supplemented with beneficial microorganisms applied to the soil. No significant differences in plant yielding were noted (due to damages caused by spring frosts), but the highest fruit weight was recorded in the case of the trees fertilized with microbiologically enriched Super Fos Dar.

Keywords: beneficial microorganisms; tree vigour; *Malus domestica*; fruit quality

Due to its economic importance, apple cultivation has a significant impact on the development of Polish agriculture. Poland is the number one producer of apples in Europe and the third in the world (GUS 2017). In 2019, apple orchards accounted for 72.3% of the fruit tree cultivation area, and apples accounted for 78.8% of the fruit harvest in orchards in Poland. The World apple production has increased from 17.05 to 89.33 million tonnes during the period 1961–2016, showing a rapid growth of global apple consumption (FAO 2018).

In the past years, very intensive fruit-growing systems have been developed in all fruit-growing centres. Such a method of farming requires appli-

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cation of excessive amounts of chemical fertilizers, pesticides and herbicides, which could be harmful to soil microorganisms, human beings, animals, and the entire natural environment (Boy, Arcad 2013; Mosa et al. 2016; Garima 2019). In recent years, farmers' awareness of the need to limit the use of chemicals in crop production has been growing (Sas Paszt et al. 2011). A high level of mineral fertilization does not only increase production costs but is also a potential cause of eutrophication and pollution of the soil environment, drinking water and air (Boy, Arcad 2013). Modern agriculture is still looking for environmentally friendly fertilizers. The availability of traditional means of agricultural production, such as farmyard manure and composts, is being reduced. One of the proposed solutions in the area of environmental and human health protection is the implementation of natural plant cultivation technologies and plant fertilization through the use of biofertilizers (Khan et al. 2009). Products of this type exert a positive influence on the growth and yield of arable crops, as well as on soil fauna, including the development of arbuscular mycorrhizal fungi and filamentous fungi (Kuwada et al. 2005; Smith, Read 2008). Mineral fertilizers and beneficial microorganisms can be added to the soil separately or in combination as biofertilizers.

Results of numerous experiments have revealed that biofertilizers enriched with arbuscular mycorrhizal fungi and filamentous fungi produce stimulating effects on the growth and reproduction of various plant species (Santhos et al. 2018; Sas Paszt et al. 2019). Beneficial microorganisms assist the roots in absorbing minerals from the soil and strengthen plant physiology (Mahdi et al. 2010; Garima 2019). Microorganisms play a vital role in maintaining long-term soil fertility and sustainability by fixing atmospheric nitrogen, mobilizing fixed macro- and micro-nutrients, or by converting insoluble P in the soil into forms available to plants (Mahdi et al. 2010).

Knowledge of mineral nutrition, due to its expressiveness, is of great importance when recommending a certain amount of fertilizers. Leaf analysis is a good way to enable diagnosis of potential insufficiency or excess and offers the possibility of determining the nutritional status of crops and correcting deficiencies, if necessary (Montañés, Heras 1991). The assessment of the mineral nutritional status of plants is based on comparing the nutrient concentration in plant tissues with reference concentrations obtained from a population with an optimal nutrition status.

The present study was conducted to compare the impact of mineral fertilisation (NPK) with the impact of some biofertilizers on the growth, yielding, fruit quality and nutritional status of 'Sampion' apple trees.

MATERIAL AND METHODS

The experiment was established in the spring of 2018 in the Experimental Orchard of the National Institute of Horticultural Research (Dąbrowice, Central Poland, 51°54'51.1"N 20°06'41.0"E (51.914188, 20.111389), 145 m a.s.l.) and was run for three consecutive years. The subjects of the research were dwarf apple trees of the cultivar 'Sampion' grafted on M9 rootstock. They were planted in early April on a podzolic soil underlaid by sandy loam, rated as soil quality class 3b. At planting time, the soil pH was slightly acidic at pH 6.2 (in KCl), and the average humus content of the soil was 1.2%. The levels of minerals in the soil, including macroelements, were as follows: P – 7.5, K – 12.4, Mg – 5.8 mg/100 g, and microelements: B – 2.4, Cu – 4.8, Fe – 862, Mn – 75.5, Zn – 3.7 mg/1 000 g. Soil samples for the analyses were taken from the surface layer (0–20 cm) of herbicide strips, 30 cm from a dripper. The reaction (pH) was measured potentiometrically. The available P and K were determined using a solution of calcium-lactate (at a pH of 3.6) and the available magnesium by means of a solution of 0.0125 M Ca-chloride. Available Fe, Mn, Zn, Cu and B were determined by means of 0.03 M acetic acid. K, Mg, Fe, Mn, Cu, Zn and B were determined by inductively coupled plasma spectroscopy (ICP Perkin-Elmer model Optima 2000 DV, Boston, USA). Phosphorus was determined using a spectrophotometer (Cintra 916, GBC, Dandenong, Australia) to quantify the molybdenum blue reaction. The details of used soil analysis methods were presented in the paper by Wójcik and Filipczak (2015).

The trees were spaced 4 m between rows and 2 m in the row. The experiment was established in a random block design in four replications. Each experimental combination was represented by 12 trees.

The experiment included the following treatments:

1. NPK(SFD) – soil fertilization before planting with a dose of 20 g of Super Fos Dar 40 of granulated fertilizer, 160 g of potassium salt per plot (12 m^2) and 55 g of urea applied under each tree (6 m^2).

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2. NPK(P6) – soil fertilization before planting with a dose of 40 g of Polifoska 6. Urea, in the amount of 48 g, was applied once before planting; potassium salt was used in the amount of 100 g.
3. NPK(SFD) + beneficial fungi – NPK(SFD)+F – soil fertilization as in point 1 with the beneficial soil fungi on their own applied in the amount of 5.25 g per plot (by thoroughly mixing them with the soil). The mixture of beneficial soil fungi contained two species: *Aspergillus niger* and *Purpureocillium lilacinum*.
4. NPK(SFD) with urea and fungi – NPK(SFD-U+f) – urea enriched with strains of filamentous fungi (5.25 g) of the species as in point 3. For each plot before planting, 160 g of potassium salt, 55 g of urea, and 20 g of Super Fos Dar 40 fertilizer were used.
5. NPK(0.6 SFD) with urea and fungi – NPK(SFD-0.6U+f) – 60% of the number 4 dose variant. Urea enriched with strains of filamentous fungi of the species as in point 3. Applied to each plot before planting; 96 g of potassium salt, 35 g of urea, and 12 g of Super Fos Dar 40.
6. NPK(SFD) + beneficial bacteria – NPK(SFD)+B – soil fertilization as in point 1 with the addition of beneficial soil bacteria on their own applied in the amount of 3.83 g (by thoroughly mixing them with the soil). The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* spp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*).
7. NPK(SFD) with bacteria – NPK (SFD+b) – 20 g of Super Fos Dar 40 enriched with three strains of *Bacillus* bacteria applied in the amount of 3.83 g per plot. In addition, before planting the trees, the soil was fertilized with 100 g of potassium salt and 55 g of urea.
8. NPK(0.6SFD) with bacteria – NPK(0.6SDF+b) 60% of the number 7 dose variant. Super Fos Dar enriched with three *Bacillus* bacterial strains was used in the same way as in point 7. Before planting, 12 g of Super Fos Dar 40, 60 g of potassium salt, and 33 g of urea were applied per plot.
9. NPK(P6) with bacteria – NPK(P6+b) – Polifoska 6 enriched with three strains of *Bacillus* bacteria of the species composition as in point 6. The fertilizers were applied before planting in a dose of 40 g of Polifoska 6, 100 g of potassium salt, and 48 g of urea per plot.
10. NPK(0.6P6) with bacteria – NPK(0.6P6+b) 60% of the number 9 dose variant. Polifoska enriched with three strains of bacteria of the genus *Bacillus* as specified in point 6. Before planting, 14 g of Polifoska 6, 60 g of potassium salt, and 30 g of urea were used for each plot.

Characteristics of the fertilizers used in the experiment:

(*i*) Polifoska 6 – 6% nitrogen (NH_4), 20% phosphorus (P_2O_5); 30% potassium (K_2O); 7% sulphur trioxide (SO_3).

(*ii*) Super Fos Dar 40 – 40% phosphorus pentoxide (P_2O_5) soluble in mineral acids and 25% P_2O_5 soluble in a neutral citrate solution, 10% calcium oxide (CaO), and microelements (Cu, Ca, Fe, Mn, Zn).

(*iii*) Urea – 46% nitrogen in the amide form.

The Urea mineral fertilizer was enriched with selected strains of the fungi: *Aspergillus niger* and *Purpureocillium lilacinum*. Polifoska 6 and Super Fos Dar 40 were enriched with strains of the bacteria: *Bacillus* spp., *Bacillus amyloliquefaciens*, and *Paenibacillus polymyxa*. The selected microorganism isolates came from the collection of the Department of Microbiology and Rhizosphere, The National Institute of Horticultural Research, Skierniewice, Poland. Dry formulations of the bacteria, in which maltodextrin was the carrier, were prepared by the production company Skotan S.A. (Czechowice-Dziedzice). The product containing conidial spores of *A. niger* and *P. lilacinum* fungi was prepared in the Department of Microbiology and Rhizosphere of the Institute.

All the treatments were repeated in the second and third year after planting (2019, 2020). In the first year of the experiment, the mineral fertilizers were applied to the soil surface around trees at planting time. The microorganisms (if applied on their own) were mixed with the soil in the planting hole. In the second and third year, the fertilizers were applied to the soil surface, whereas the microorganisms were mixed with the topsoil. The experimental field was fitted with concrete poles reinforced in tree rows, with wires stretched between the poles and bamboo canes tied to the wires and the trees. Drip irrigation was established after planting. The trees were irrigated in a dry season from May to September. In the first year, the soil was kept in clean cultivation with rotating mechanical implements from the spring to August, the alleyways were grassed down and frequently mowed. From the second year, 'Basta' herbicide was applied within tree rows to control weeds. To control pathogens and pests,

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pesticides were used according to up-to-date recommendations for integrated apple production (Bryk et al. 2018). In the first year after planting, the flower buds that appeared on some trees were removed so that the fruit would not inhibit the growth of the young plants. No flower or fruit thinning were done in the subsequent years.

Meteorological data. During the experiment, meteorological data was collected by the weather station installed in Dąbrowice (iMETOS, Pessl Instruments, Austria). The average air temperature in the growing season (April–October) during the measurement period was at the level of the 30-year average. The highest average temperatures were recorded in 2018, with the values 1.97 °C higher than the multi-year average, while the lowest values were recorded in 2020 (0.52 °C lower than the multi-year average). The highest total rainfall was recorded in 2020, 176.8 mm higher than the 30-year average. The lowest rainfall occurred in 2019, 74.2 mm lower than the multi-year average.

Measurements

The influence of the microbiologically enriched fertilizers and microorganisms applied to the soil was assessed in the first year on the basis of annual shoot growth, trunk diameter, and mineral composition of leaves. In the second and third year, these measurements were repeated, and the records extended to include: number of flower clusters, yield per tree, mean fruit weight, fruit firmness, and percentage of soluble solids (TSS).

Assessment of tree vigour. To evaluate tree growth, the stem diameter and the total length of shoot growth produced per tree were measured. Trunk diameter was measured 30 cm above the ground using a slide calliper.

Assessment of fruit quality. To estimate fruit quality samples of 30 fruits from each treatment were taken and sorted for mean fruit mass, firmness and soluble solids content (TSS). Flesh firmness was measured with the hand-held penetrometer, TSS with the digital refractometer (PAL-1, Atago, Tokyo, Japan).

Assessment of mineral nutrition. Samples of twenty leaves from the middle part of shoots were selected at random from each replicate in early August to measure leaf mineral content. The concentrations of elements (N, P, K, Mg, Ca) in leaf tissue were analysed by the Chemical Laboratory of the National Institute of Horticultural Research, Skierniewice, Poland. Determination of the mineral content of plant material was done following the process of mineralization (combustion). Wet combustion of vegetable matter consists in complete oxidation with liquid oxidants such as concentrated sulphuric acid, nitric acid or perchloric acid, used individually or in various combinations and proportions. For the determination of mineral content of the solutions obtained by the analytical methods mentioned above, measurements were carried out using the technique of atomic emission spectrometry with excitation in inductively coupled plasma (ICP-OES). The N content in plant samples was analysed using the Kjeldahl method (Latimer 2012) (the Kjeldahl apparatus Vapodest, Königswinter, Germany). All the nutrients were determined in three repetitions.

Data analysis. The results were statistically analysed using two-way analyses of variance with the Duncan test, $\alpha = 0.05$, using the statistical program Statistica 13.1. Due to the significant influence of the years, the analyses were statistically conducted separately for each study year. Data not significantly different from each other were marked with the same letters.

RESULTS

Tree growth. After planting, the trees took root very well. In the first year of cultivation, no differentiation in the growth (length) of annual shoots was evident in individual variants of the experiment (Figure 1). In 2019, the increases in annual shoot growth were already much stronger, but also without any significant differentiation between the combinations. Significant differences in this respect were evident in the third year of cultivation, in which the average total growth of annual shoots of the trees fertilized with the mixture containing Super Fos Dar was almost half of the average total annual growth of the trees fertilized with the fertilizers containing Polifoska 6 with the addition of bacteria.

Another parameter describing tree growth vigour is trunk diameter and the increase in this diameter (Figures 2, 3). As in the case of the total shoot growth, the highest increases in tree trunk diameter were found in the third year of cultivation. That season saw the strongest growth in trunk diameter of the trees fertilized with reduced (by 40%)

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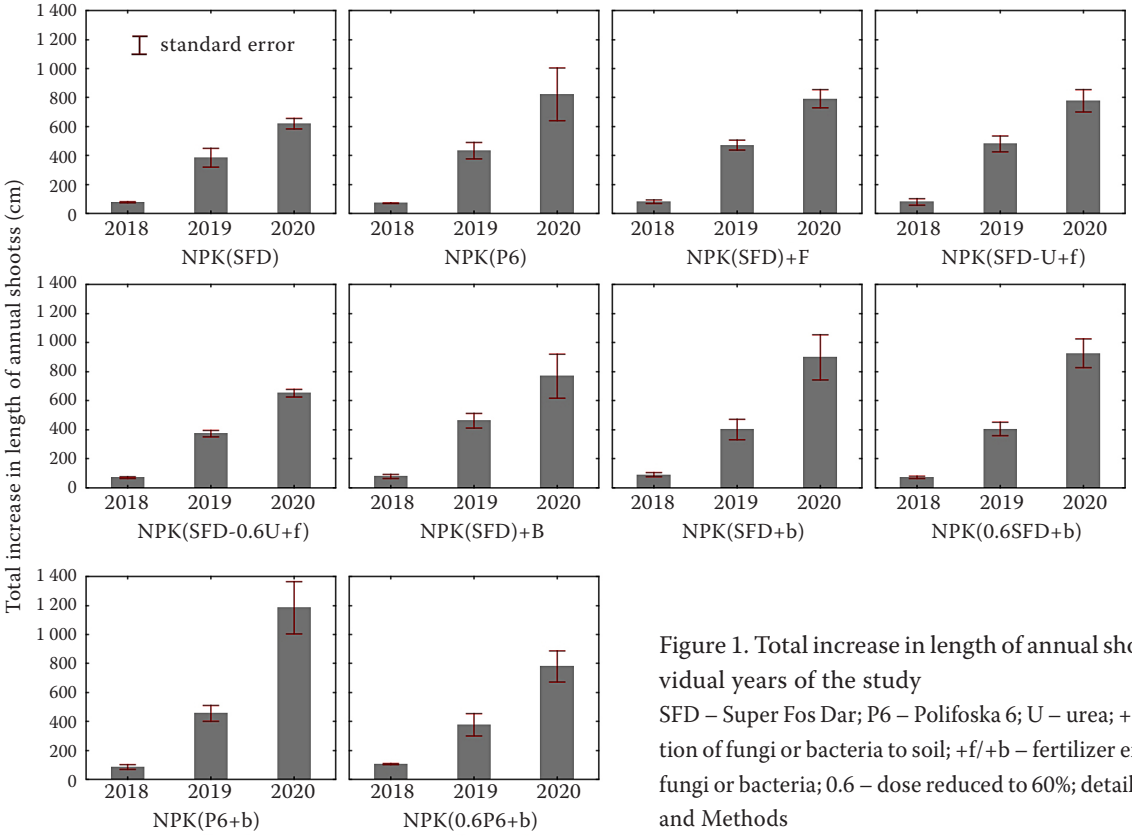

Figure 1. Total increase in length of annual shoots in individual years of the study

SFD – Super Fos Dar; P6 – Polifoska 6; U – urea; +F/+B – addition of fungi or bacteria to soil; +f/+b – fertilizer enriched with fungi or bacteria; 0.6 – dose reduced to 60%; details in Material and Methods

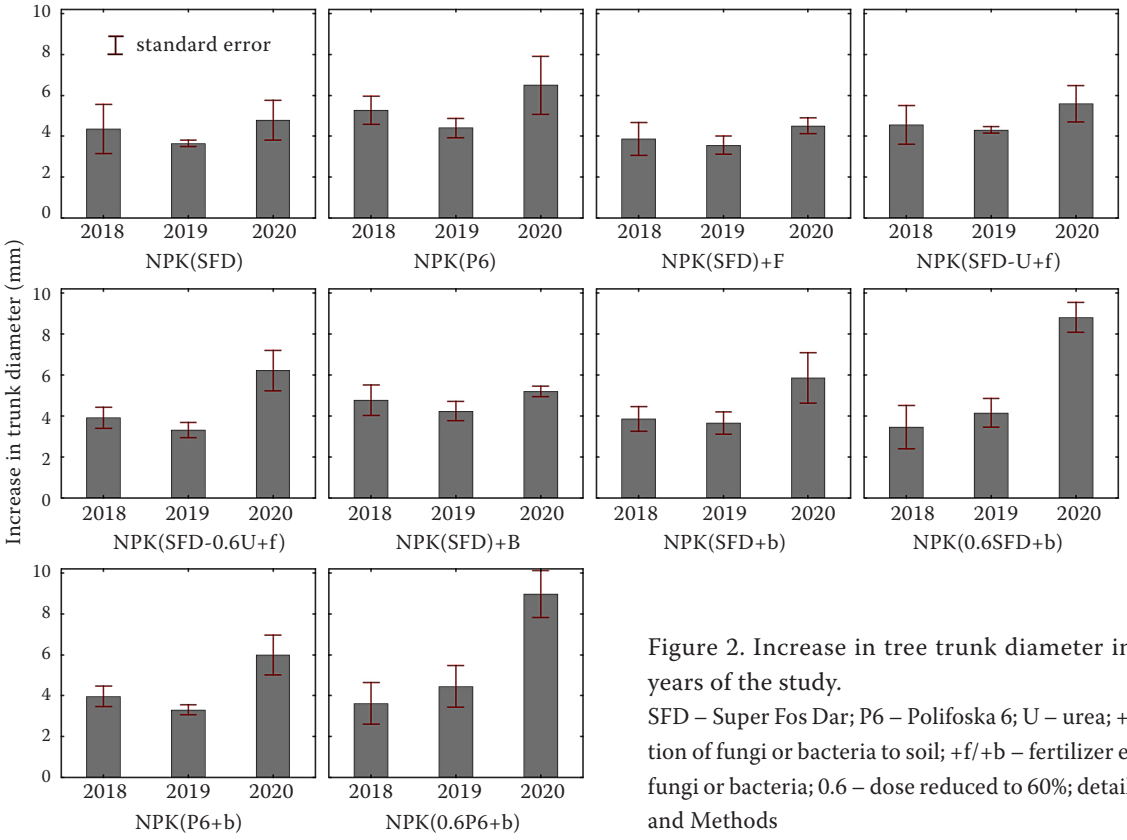

Figure 2. Increase in tree trunk diameter in individual years of the study.

SFD – Super Fos Dar; P6 – Polifoska 6; U – urea; +F/+B – addition of fungi or bacteria to soil; +f/+b – fertilizer enriched with fungi or bacteria; 0.6 – dose reduced to 60%; details in Material and Methods

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Table 1. Flowering and fruiting

Treatment	No. of flower clusters (per tree)		Yield (kg/tree)		Average fruit weight (g)	
	2019	2020	2019	2020	2019	2020
NPK(SFD)	27.7^{a}	39.50^{a}	4.17^{a}	8.17^{a}	173.0^{a}	171.8^{ab}
NPK(P6)	28.8^{a}	57.25^{ab}	3.67^{a}	9.45^{a}	207.3^{a}	164.3^{ab}
NPK(SFD)+F	31.6^{a}	77.67^{b}	4.46^{a}	11.99^{a}	189.3^{a}	156.0^{ab}
NPK(SFD-U+f)	28.8^{a}	61.0^{ab}	4.25^{a}	10.69^{a}	199.3^{a}	171.0^{ab}
NPK(SFD-0.6U+f)	29.7^{a}	50.08^{ab}	3.58^{a}	8.50^{a}	191.0^{a}	168.8^{ab}
NPK(SFD)+B	31. 3^{a}	81.25^{b}	4.4^{a}	11.58^{a}	200.8^{a}	140.3^{a}
NPK(SFD+b)	28.4^{a}	67.89^{ab}	3.39^{a}	11.56^{a}	227.0^{a}	168.3^{ab}
NPK(0.6SFD+b)	30.2^{a}	57.80^{ab}	4.30^{a}	9.79^{a}	201.4^{a}	183.8^{b}
NPK(P6+b)	30.7^{a}	60.92^{ab}	3.54^{a}	10.14^{a}	177.5^{a}	170.0^{ab}
NPK(0.6P6+b)	32.3^{a}	60.17^{ab}	3.79^{a}	10.18^{a}	181.8^{a}	167.3^{ab}

SFD – Super Fos Dar; P6 – Polifoska 6; U – urea; +F/+B – addition of fungi or bacteria to soil; +f/+b – fertilizer enriched with fungi or bacteria; 0.6 – dose reduced to 60%; details in Material and Methods; mean values in columns followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

doses of microbiologically enriched fertilizers. After three years of cultivation, the thickest trunks (measured at a height of 30 cm from the graft union) were those of the trees fertilized with reduced doses of microbiologically enriched fertilizers (both Super Fos Dar + bacteria, and Polifoska 6 + bacteria) and the trees fertilized with a full dose of fertilizer mixture the main component of which was Polifoska 6.

Flowering and yielding. The trees bloomed for the first time in the second year of cultivation (2019). The flowering of trees on individual plots

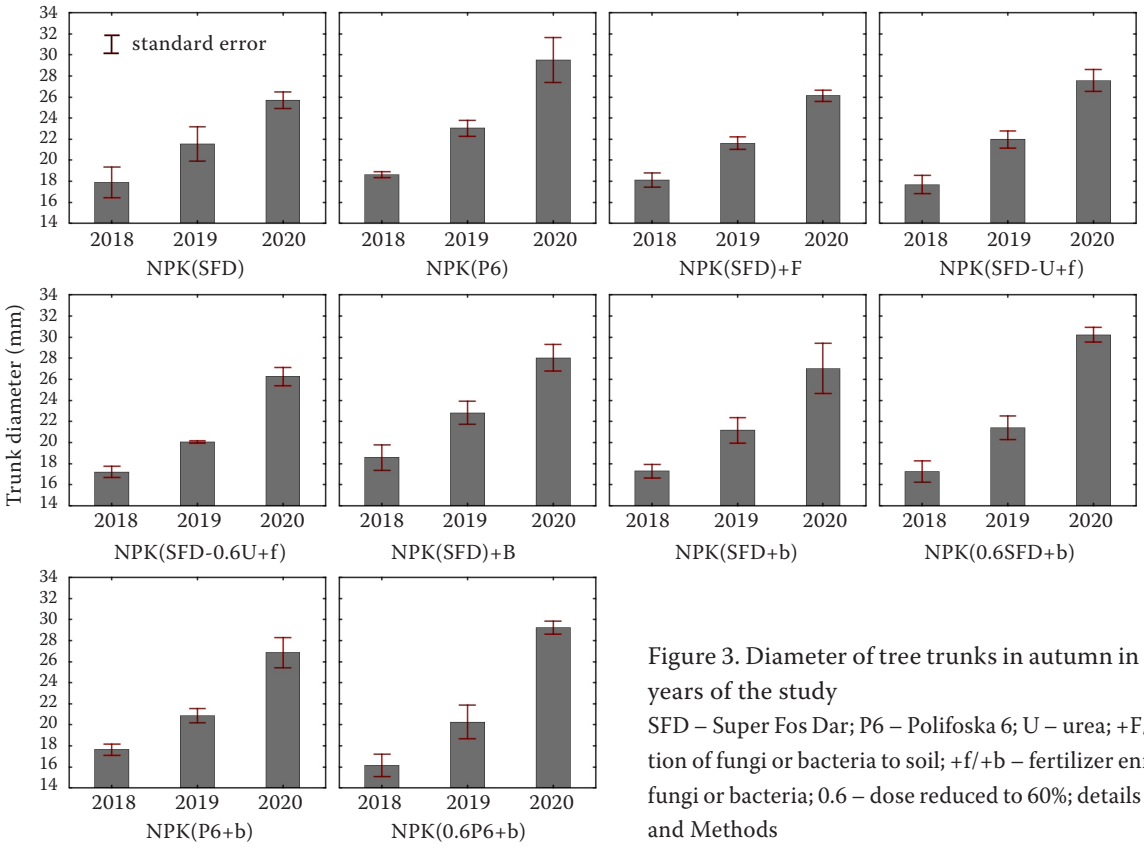

Figure 3. Diameter of tree trunks in autumn in individual years of the study

SFD – Super Fos Dar; P6 – Polifoska 6; U – urea; +F/+B – addition of fungi or bacteria to soil; +f/+b – fertilizer enriched with fungi or bacteria; 0.6 – dose reduced to 60%; details in Material and Methods

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was very uniform (Table 1). There were also no differences in the size and quality of the yield (Tables 1, 2). In the following year (2020), the trees fertilized with the mixture containing Super Fos Dar produced the least flowers, whereas those that were fertilized in the same way, but their soil was inoculated with beneficial fungi or bacteria, bloomed the most profusely. There was no significant influence of the experimental variants on yield and fruit refraction. However, significance of differences between means was demonstrated for mean fruit weight and fruit firmness. The lowest average weight was shown by the fruits of the trees fertilized with a mixture of mineral fertilizers containing Super Fos Dar, the soil was enriched with a nutrient solution containing bacteria. In contrast, the highest average weight was shown by apples from the plots fertilized with a reduced dose of fertilizers containing biologically enriched Super Fos Dar. The highest firmness was shown by apples from the trees fertilized with the mixture containing Super Fos Dar, whereas significantly lower than that fruit firmness was recorded for apples from the trees fertilized in the same way but where the soil was enriched with the bacterial inoculum.

Chemical analysis showed high levels of nitrogen in the leaves in all the years and variants of the experiment. The significance of differences between the means for individual variants was proved only in the first year of the study (Table 3). Significantly the highest nitrogen content was found in the leaves of the trees fertilized with a mixture containing the biofertilizer with bacteria that was based on Super Fos Dar. Significantly lower nitrogen levels were in the leaves of the trees fertilized with a similar dose of fertilizers containing Super Fos Dar. The fertilization variants did not have a significant effect on the levels of phosphorus and calcium in apple tree leaves (Table 3).

In all the years of the study, the potassium content of apple leaves was very high (Table 3). The significance of differences between the means was proved in the first and third year of cultivation. In 2018, the lowest values of this component were found in the leaves of the trees fertilized with a mixture containing Super Fos Dar without any addition of beneficial microorganisms. The use of the mixture with Super Fos Dar enriched with bacteria resulted in a significant increase in the level of potassium in apple leaves. In the third year of the study, the lowest potassium content was found in the leaves of both the trees fertilized with a full dose and those fertilized with a reduced dose of fertilizers containing microbiologically enriched Super Fos Dar. Significantly higher potassium levels were found in the leaves of the trees fertilized with mixtures containing either Super Fos Dar or Polifoska.

Also, the magnesium content in apple leaves was very high (Table 3). The significance of differences between the average magnesium levels in the leaves was demonstrated only in the first year of the study. The lowest concentration of magnesium was found in the leaves of the trees fertilized with a mixture containing Super Fos Dar and where beneficial bac-

Table 2. Fruit quality parameters

Treatment	Fruit firmness (kgf)		Total soluble solid content (%)	
	2019	2020	2019	2020
NPK(SFD)	3.69^{a}	4.90^{c}	13.41^{a}	14.59^{a}
NPK(P6)	3.75^{a}	4.55^{abc}	13.48^{a}	13.79^{a}
NPK(SFD)+F	3.61^{a}	4.77^{bc}	13.26^{a}	13.93^{a}
NPK(SFD-U+f)	3.75^{a}	4.57^{abc}	13.65^{a}	13.69^{a}
NPK(SFD-0.6U+f)	3.60^{a}	4.31^{ab}	13.03^{a}	13.79^{a}
NPK(SFD)+B	3.63^{a}	4.22^{a}	13.25^{a}	13.42^{a}
NPK(SFD+b)	3.97^{a}	4.53^{abc}	13.36^{a}	13.57^{a}
NPK(0.6SFD+b)	3.56^{a}	4.32^{ab}	12.93^{a}	14.18^{a}
NPK(P6+b)	3.74^{a}	4.6^{abc}	12.90^{a}	13.64^{a}
NPK(0.6P6+b)	3.90^{a}	4.71^{abc}	13.10^{a}	14.34^{a}

SFD – Super Fos Dar; P6 – Polifoska 6; U – urea; +F/+B – addition of fungi or bacteria to soil; +f/+b – fertilizer enriched with fungi or bacteria; 0.6 – dose reduced to 60%; details in Material and Methods; mean values in columns followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

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Table 3. Assessment of the effect of fertilization on the mineral composition of apple leaves (in % DW)

Treatment	N			P			K			Mg			Ca		
	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020
NPK(SFD)	2.51^{ab}	2.84^{a}	2.56^{a}	0.15^{a}	0.17^{a}	0.18^{a}	2.16^{a}	1.55^{a}	1.61^{b}	0.23^{abc}	0.16^{a}	0.18^{a}	0.97^{a}	1.38^{a}	1.62^{a}
NPK(P6)	2.50^{ab}	2.87^{a}	2.73^{a}	0.14^{a}	0.16^{a}	0.17^{a}	2.55^{b}	1.63^{a}	1.62^{b}	0.24^{abcd}	0.16^{a}	0.17^{a}	0.94^{a}	1.34^{a}	1.53^{a}
NPK(SFD)+F	2.49^{ab}	2.90^{a}	2.73^{a}	0.14^{a}	0.16^{a}	0.16^{a}	2.52^{b}	1.53^{a}	1.46^{ab}	0.24^{abcd}	0.15^{a}	0.18^{a}	0.82^{a}	1.19^{a}	1.65^{a}
NPK(SFD-U+f)	2.45^{ab}	2.89^{a}	2.64^{a}	0.13^{a}	0.17^{a}	0.17^{a}	2.31^{ab}	1.57^{a}	1.58^{ab}	0.24^{abcd}	0.16^{a}	0.15^{a}	0.99^{a}	1.24^{a}	1.50^{a}
NPK(SFD-0.6U+f)	2.59^{ab}	2.710^{a}	2.62^{a}	0.12^{a}	0.17^{a}	0.16^{a}	2.42^{ab}	1.62^{a}	1.42^{ab}	0.25^{bcd}	0.14^{a}	0.18^{a}	0.92^{a}	1.26^{a}	1.60^{a}
NPK(SFD)+B	2.41^{a}	2.88^{a}	2.71^{a}	0.14^{a}	0.16^{a}	0.16^{a}	2.33^{ab}	1.55^{a}	1.45^{ab}	0.20^{a}	0.15^{a}	0.17^{a}	0.91^{a}	1.27^{a}	1.58^{a}
NPK(SFD+b)	2.62^{b}	2.77^{a}	2.79^{a}	0.15^{a}	0.16^{a}	0.16^{a}	2.52^{b}	1.53^{a}	1.38^{a}	0.28^{d}	0.15^{a}	0.18^{a}	0.89^{a}	1.20^{a}	1.57^{a}
NPK(0.6SFD+b)	2.53^{ab}	2.90^{a}	2.56^{a}	0.14^{a}	0.16^{a}	0.16^{a}	2.27^{ab}	1.49^{a}	1.38^{a}	0.27^{cd}	0.17^{a}	0.18^{a}	0.97^{a}	1.31^{a}	1.48^{a}
NPK(P6+b)	2.54^{ab}	2.87^{a}	2.71^{a}	0.15^{a}	0.17^{a}	0.16^{a}	2.56^{b}	1.61^{a}	1.56^{ab}	0.26^{bcd}	0.15^{a}	0.16^{a}	0.94^{a}	1.29^{a}	1.56^{a}
NPK(0.6P6+b)	2.53^{ab}	2.88^{a}	2.69^{a}	0.14^{a}	0.16^{a}	0.16^{a}	2.39^{ab}	1.65^{a}	1.50^{ab}	0.22^{ab}	0.17^{a}	0.15^{a}	0.86^{a}	1.38^{a}	1.46^{a}

SFD – Super Fos Dar; P6 – Polifoska 6; U – urea; +F/+B – addition of fungi or bacteria to soil; +f/+b – fertilizer enriched with fungi or bacteria; 0.6 – dose reduced to 60%; details in Material and Methods

Mean values in columns followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

teria were added to the soil, whereas the highest Mg content was found in the leaves of the trees fertilized with the bio-fertilizer based on Super Fos Dar.

DISCUSSION

Results of many experiments have revealed that biofertilizers enriched with filamentous fungi produce stimulatory effects on the growth and reproduction of several plant species, including apple trees (Mahdi et al. 2010; Mosa et al. 2016). In the experiment carried out in this study, urea was enriched with *Aspergillus niger* and *Purpureocillium lilacinum*, while the fertilizers Polifoska 6 and Super Fos Dar 40 with strains of *Bacillus*. Many studies have found that isolates belonging to these species can colonize plant roots, increase yields and, with the ability to produce toxic compounds, control pathogens (Yadav et al. 2011; Yin et al. 2015). Soil microorganisms enhance plant growth by mobilizing nutrients in the soil, producing numerous plant growth regulators and improving soil structure (Mosa et al. 2016; Garima 2019). Beneficial soil microorganisms have an ability to convert nutrients from unavailable (insoluble) to available forms through biological processes, which in turn may limit the negative environmental effects of frequent use of chemical fertilizers (Vessey 2003).

In our study the trees fertilized (with Super Fos Dar) with the addition of beneficial fungi or bacteria to the soil, bloomed the most intensely (in the third year after cultivation). Unfortunately, ground frosts damaged some of the flowers and caused a reduction in yield and a very large differences in the fruiting of individual trees. Due to this differentiation, the statistical analysis did not show any significant differences between the mean values of the yield obtained in individual variants of the experiment.

Fruit trees, including apple trees, grow and bear fruit best in areas that have not been used for orchards before. A particular problem for fruit growers is the phenomenon known as the replant disease. It is related to the deterioration in plant growth and yielding caused by a disturbance in the functioning of soil biocenosis (Čatská et al. 1982). Previous studies have shown that the replant disease is caused by a set of biotic and abiotic factors present in the soil, including unfavourable for plant growth accumulation of pathogenic and harmful microorganisms and phytotoxic compounds, in-

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adequate soil pH and poor soil structure (Hoestra 1988; Blok, Bollen 1993).

Numerous studies have shown that increasing the number of some groups of microorganisms in the soil, e.g. from the genera *Pseudomonas* or *Bacillus*, may contribute to reducing the effects of this disease (Utkhede, Smith 2000; Schoor et al. 2009). The beneficial effects of *Bacillus* spp. on plant growth and yielding have been demonstrated in several agricultural crops including wheat, maize, soybean, sunflower, common bean, tomato, pepper, potato, cucumber, and many others (Aloo et al. 2019).

Regardless of the experimental variant, the level of macroelements in apple tree leaves was within the optimal or high range. The influence of the bacteria on tree growth observed in the experiment could be related not only to the nutritional status of the trees, but also to their overcoming the replant disease, which significantly reduces plant growth and yielding. Despite the applied crop rotation, ours was yet another apple tree planting in such a long period of time, and our earlier study showed occurrence of replant problems in the orchard (Tryngiel-Gać et al. 2015).

Proper fertilization is important for plant growth, development, and yielding. It affects the extent of flowering and the size, colour, and taste of the fruit. In addition to nitrogen, phosphorus is a very important element as it affects the growth of roots and the firmness and storability of fruit. Phosphorus has difficulty in penetrating deep into the soil; therefore, it is not easy to increase its amount in a growing orchard by spreading fertilizers on the surface. Phosphorus and potassium fertilizers are not effectively used at low soil pH. Sharma et al. (2017) studied the bacteria of the genus *Pseudomonas* inhabiting the roots of apple trees in terms of their ability to dissolve insoluble phosphorus compounds. They believe that organic compounds secreted by apple roots can stimulate the multiplication of phosphorolytic (fluorescing) *Pseudomonas* bacteria and *Bacillus* bacteria.

As mentioned earlier, the presence of beneficial microorganisms plays an essential role in apple cultivation. They can, for example, compete for nutrients with pathogenic and/or unfavourable fungi or bacteria, and prevent them from multiplying. Other mechanisms of action of beneficial microorganisms include the induction of defence mechanisms against pathogens inside plants, the formation of a biofilm on the roots and aboveground parts of plants, the production of phytohormones, antibiotics and other biocidal compounds, and hyperparasitism (Gupta et al. 2015; Olanrewaju et al. 2017).

In recent years, research has been intensified to limit the use of mineral fertilizers (due to environmental costs) and to improve the fertility of arable soils through the use of various types of biofertilizers. Derkowska et al. (2017) have observed that the use of Micosat F, a preparation containing microorganisms, has a beneficial effect on the root system of apple trees. In the cultivation of 'Berkutovskoye' apple trees, Kuzin et al. (2020), using half the dose of NPK fertilizers ($N_{45}P_{15}K_{60}$) together with a consortium of microorganisms (*Azotobacter chroococcum*, *Bacillus subtilis*, *B. megaterium*, *Trichoderma harzianum*), obtained an apple yield of the same amount as that obtained with the full NPK dose ($N_{90}P_{30}K_{120}$). The positive effect of microorganisms applied together with mineral fertilizers in full and reduced doses has also been noted in other cultivated species (Zafar-ul-Hye et al. 2015).

CONCLUSION

The application of mineral fertilizers (even in reduced doses) enriched with beneficial microorganisms enhanced the growth of apple trees.

The most abundant flowering was recorded when fertilization (with Super Fos Dar) was supplemented with beneficial microorganisms applied to the soil.

No significant differences in plant yielding were noted (though spring frosts might have affected tree yielding). The highest fruit weight was recorded in the case of the trees fertilized with microbiologically enriched Super Fos Dar.

REFERENCES

Aloo B.N., Makumba B.A., Mbega E.R. (2019): The potential of *Bacilli rhizobacteria* for sustainable crop production and environmental sustainability. Microbiological Research, 219: 26–39.

Blok W.J., Bollen G.J. (1993): The role of autotoxins from root residues of the previous crop in the replant disease of asparagus. Netherlands Journal of Plant Pathology, 99: 29–40.

Boy J., Arcad Y. (2013): Current trends in green technologies in food production and processing. Food Engineering Reviews, 5: 1–17.

https://doi.org/10.17221/101/2021-HORTSCI

Bryk M., Lisek J., Łabanowska H., Sobiczewski P. (eds.) (2018): Programs of Protection of Fruit Crops. Praca zbiorowa. Warsaw, Hortpress. (in Polish)

Čatská V., Vančura V., Hudská G., Přikryl Z. (1982): Rhizosphere micro-organisms in relation to the apple replant problem. Plant and Soil, 69: 187–197.

Derkowska E., Sas-Paszt L., Głuszek S., Trzciński P., Przybył M., Frąc M. (2017): Effects of treatment of apple trees with various bioproducts on tree growth and occurrence of mycorrhizal fungi in the roots. Acta Scientiarum Polonorum, Hortorum Cultus, 16: 75–83.

FAO (2018): FAOSTAT. Available at http://www.fao.org/faostat/en/#data/QC

Garima J. (2019): Biofertilizers – a way to organic agriculture. Journal of Pharmacognosy and Phytochemistry, 4 (Special Issue): 49–52.

Gupta G., Parihar S.S., Ahirwar N.K., Snehi S.K., Singh V. (2015): Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. Journal of Microbial & Biochemical Technology, 7: 96–102.

GUS (2017): Statistical Yearbook of Poland. Available at https://stat.gov.pl (in Polish)

Hoestra H. (1988): General remarks on replant disease. Acta Horticulturae, 233: 11–16.

Khan W., Rayirath U.P., Subramanian S., Jithesh M.N., Rayorath P., Hodges D.M., Critchley A.T., Craigie J.S., Norie J., Prithiviraj B. (2009): Seewead extracts as biostimulants of plant growth and development. Journal of Plant Growth Regulation, 28: 386–399.

Kuwada K., Kuramoto M., Utamura M., Matsusita 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.

Kuzin A., Solovchenko A., Stepantsova L., Pugachev G. (2020): Soil fertility management in apple orchard with microbial biofertilizers. E3S Web of Conferences, 222: 03020.

Latimer G. (2012): Official Methods of Analysis. 19th Ed. Gaithersburg, AOAC International.

Mahdi S.S., Hassan G.I., Samoon S.A., Rather H.A., Dar S.A., Zehra B. (2010): Biofertilizers in organic agriculture. Journal of Phytology, 2: 42–54.

Montañés L., Heras L. (1991): Desviación del Optimo Porcentual (DOP): nuevo índice para la interpretación del análisis vegetal. Anales de la Estación Experimental de Aula Die, 20: 93–108. (in Spanish)

Mosa W.F.A.E.-G., Sas-Paszt L., Frąc M. Trzciński P. (2016): Microbial products and biofertilizers in improving growth and productivity of apple – A review. Polish Journal of Microbiology, 65: 243–251.

Olanrewaju O.S., Glick B.R., Babalola O.O. (2017): Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology, 33: 197.

Santhos M., Kumar G., Chandra Mohan R., Phogmt M., Santosh K. (2018): Role of biofertilizers towards sustainable agricultural development: A review. Journal of Pharmacognosy and Phytochemistry, 7: 1915–1921.

Sas-Paszt L., Sumorok B., Malusa E., Głuszek S., Derkowska E. (2011): The influence of bioproducts on root growth and mycorrhizal occurrence in the rhizosphere of strawberry plants 'Elsanta'. Journal of Fruit and Ornamental Plant Research, 19: 13–33.

Sas-Paszt L., Sumorok B., Derkowska E., Trzciński P., Lisek A., Grzyb S.Z., Sitarek M., Przybył M., Frąc M. (2019): Effect of microbiologically enriched fertilizers on the vegetative growth of strawberry plants under field conditions in the first year of plantation. Journal of Research and Applications in Agricultural Engineering, 64: 29–37.

Schoor L., Denman S., Cook N.C. (2009): Characterisation of apple replant disease under South African conditions and potential biological management strategies. Scientia Horticulturae, 119: 153–162.

Sharma R., Pal J., Kaur M. (2017): Isolation of phosphate solubilizing pseudomonas strains from apple rhizosphere in the Trans Himalayan Region of Himachal Pradesh, India. bioRxiv, 193672.

Smith S.E., Read D.J. (2008): Mycorrhizal Symbiosis. 3rd Ed. New York, London, Burlington, San Diego, Elsevier and Academic.

Tryngiel-Gać A., Treder W., Wójcik K., Klamkowski K. (2015): The efficiency of irrigation in replanted apple orchard. Infrastructure and Ecology of Rural Areas, II/1: 257–267.

Utkhede R.S., Smith E.M. (2000): Impact of chemical, biological and cultural treatments on the growth and yield of apple in replant-disease soil. Australasian Plant Pathology, 29: 129–136.

Vessey J.K. (2003): Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 255: 571–586.

Wójcik P., Filipczak J. (2015): Growth and early fruit production of 'Tiben' blackcurrants fertilised with pre and post-planting applications of mineral fertilisers and swine manure. Scientia Horticulturae, 185: 90–97.

Yadav J., Verma J.P., Tiwari K.N. (2011): Plant growth promoting activities of fungi and their effect on chickpea plant growth. Asian Journal of Biological Sciences, 4: 291–299.

Yin Z., Shi F., Jiang H., Roberts D.P., Chen S., Fan B. (2015): Phosphate solubilization and promotion of maize growth by *Penicillium oxalicum* P4 and *Aspergillus niger* P85 in a calcareous soil. Canadian Journal of Microbiology, 61: 913–923.

Zafar-ul-Hye M., Farooq H.M., Mubshar H. (2015): Bacteria in combination with fertilizers promote root and shoot growth of maize in saline-sodic soil. Brazilian Journal of Microbiology, 46: 97–102.

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