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Influence of microbially enriched mineral fertilizers on the composition of rhizosphere microorganisms of *Thuja occidentalis*

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Abstract. Rhizosphere is a region of the strongest interactions between plants, soil and microorganisms, which play an important role in plant development. Due to a number of interdependencies, they are main factors determining the health and proper growth of plants. All methods used in agriculture that promote the growth of microbial populations and their biodiversity are beneficial both for cultivated plants and for the environment. *Thuja occidentalis* cv. Brabant, is a very popular plant in Poland. In the study the effect of mineral fertilizers: Urea, Polifoska 6, Super Fos Dar 40, enriched with beneficial strains of fungi and bacteria, on selected groups of soil microorganisms was evaluated. The fungi *Aspergillus niger* and *Purpureocillium lilacinum* and bacteria *Bacillus* sp.*, Bacillus amyloliquefaciens, Paenibacillus polymyxa*, which have been shown to have beneficial effects on plant growth in previous works, have been used as additives to bio-fertilizers. The use of mineral fertilizers Urea, Polifoska 6 and Super Fos Dar 40, enriched with selected strains of fungi and bacteria had a beneficial effect on the development of microorganisms in the rhizosphere of the *Thuja occidentalis*, especially in the second year of research.

Keywords: *Thuja occidentalis* cv. Brabant, rhizosphere, microorganisms, biofertilizers

INTRODUCTION

In recent years, white cedars, conifers from the cypress family, commonly called thuyas, have become very popular in home gardens. One of the most popular is *Thuja occidentalis* cv. Brabant. It is a shrub with a columnar habit, well bushy, with relatively low agrotechnical requirements, fast growth, with annual increments of up to approx. 30–40 cm. It has flattened, light green twigs and scale-like leaves with an intensive light green color. These shrubs are resistant to low temperatures and quite tolerant to water shortages in the ground. Brabant grows best in sunny or partial shade on fertile, slightly acidic soils. In gardens and green areas it is usually grown as a hedge.

Although this shrub is not very demanding in relation to soil fertility, proper fertilization promotes growth, reduces diseases and improves the color of the scales. Usually in spring and summer, fertilizers containing significant amounts of nitrogen and macro- and microelements are used. Nutrient deficiencies inhibit weight gain, plant branching and coloration (Pronk, 2004). Especially important are nitrogen and magnesium, because their lack causes a slowdown in growth, yellowing of needles, and in extreme cases even the death of the bush. Nitrogen fertilization should be completed at the beginning of August. In the autumn period it is important that the plants have adequate availability of phosphorus and potassium in the substrate, because these elements play an important role in building up resistance to low temperatures in plants (Aendekerk, 1997). Excessive use of mineral fertilizers is inadvisable, because *Thuja occidentalis* is not tolerant of excessive salinity of the soil. It impedes the uptake of mineral nutrients from the soil, adversely affects the growth of plants and their resistance to pathogens. It is recommended to periodically apply organic fertilizers (compost, manure), which not only enrich the soil with organic matter, but also provide minerals and beneficial microorganisms. Unfortunately, the availability of these fertilizers is limited. For this reason, all methods to reduce the amount of fertilizer applied, e.g. by increasing nutrient uptake through the use of selected microorganisms, are valuable. Mycorrhizal inoculations are available on the market, and their role in facilitating nutrient uptake and growth stimulation is well known (Krupa, 2010).

Synthetic fertilizers and pesticides used in agriculture contribute to environmental pollution and reduce the biodiversity of soil organisms, whose key role in mineralizing organic matter and making it available to plants is indisput-

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able (Bakker et al., 2012). In recent years, attempts have been made to use selected microorganisms to, among other things, reduce the application of synthetic fertilizers (Adesemoye et al., 2009; Aghai et al., 2019). Indeed, it has been found that the introduction of suitable microorganisms can increase the efficiency of synthetic fertilizer use by plants (Liang et al., 2020). Wang et al. (2020) showed that the use of a consortium of different microorganisms in a wheat crop increased the amount of available N, P, and K and allowed a 25% reduction in fertilization, without negative effects on yield. Unfortunately, research on the introduction of selected microorganisms is difficult because their activity in the soil and the final effect (protective or growth stimulating) depends on many abiotic and biotic factors, especially the native (autochthonous) microflora. In addition to the factors mentioned above, the composition of microorganisms in the soil is influenced by the changes induced by the plants growing in the soil (Galus-Barchan, Chmiel, 2019). It is also proven that the greater the biodiversity of microorganisms, the greater the resistance of plants to adverse environmental conditions. Particularly important in this case is the so-called functional diversity, that is, the ability of microorganisms to carry out a variety of bio-chemical reactions (Olanrewaju et al., 2019). It is associated with the ability of bacteria or fungi to produce compounds that facilitate the breakdown of nutrients (e.g., solubilization of phosphorus compounds), induction of systemic resistance, or compounds with toxic properties against plant pathogens.

Works on the role of plant root secretions in shaping rhizosphere microbial populations has been particularly intense (Canarini et al., 2019). Indeed, it has been shown that a plant can selectively influence the development (stimulation or inhibition) of specific groups of microorganisms because root secretions can "attract" beneficial microorganisms (Guyonnet et al., 2018; Olanrewaju et al., 2019). Some compounds secreted by plant roots have been found to be signals, affecting interactions in the rhizobiom in a targeted manner (Canarini et al., 2019; Olanrewaju et al., 2019). In addition to the most well-known ones, such as strigolactones, which are involved in the formation of symbiosis between plants and mycorrhizal fungi, volatile and non-volatile organic and inorganic compounds are being discovered that affect the composition and activity of the rhizosphere microbiome. This communication occurs in both directions because many soil microorganisms (including those of the genera *Bacillus* or *Pseudomonas*) produce small-molecule signaling compounds, known as QS (quorum sensing), regulating their density in the soil. These compounds may regulate the expression of certain plant genes, inducing systemic resistance and/or affecting plant growth and development (Venturi, Keel, 2016). It follows that each plant (species/cultivar) forms a unique set of microorganisms around it in the soil determined by a number of biotic (e.g. indigenous microorganisms) and abiotic (root secretions, soil type, moisture, temperature et al.) factors that influence its development. The knowledge of these processes is very important also from a practical point of view, because the introduction of selected microorganisms into the soil can have, depending on the plant species and the factors mentioned above, different efficiency (Ishag, 2017; Jacoby, 2017; Jankowska, Swędrzyńska, 2016; Klimek et al., 2010).

In the study described in this paper, the abundance of microorganisms groups that play an important role in soil fertility were analyzed in the soil taken from the root zone of *Thuja occidentalis*. Prominent among them were Actinomycetes, producing a large number of compounds with bactericidal and fungicidal activity, as well as enzymes (e.g. cellulases, chitinases, and others) that enable decomposition and mineralization of organic matter (Gohel et al., 2006; Lenart-Boroń, Banach, 2014). Another analyzed group were bacteria of the genus *Pseudomonas*, which are commonly found in soils. They play an important role in plant development as they stimulate plant growth by producing phytohormones. They produce a large number of biologically active compounds, such as antibiotics or lytic enzymes, and can induce systemic resistance in plants. Due to their rapid growth, they compete strongly with pathogens for nutrients (Pathama et al., 2011; Pociejewska et al., 2014).

One of the most important elements in plant cultivation is phosphorus, which often occurs in insoluble forms in soil and is not available to plants. This element is supplied with fertilizers, and is also made available to plants by microorganisms (e.g. *Pseudomonas, Bacillus, Streptomyces* and others), having the ability to release phosphorus from insoluble phosphorus compounds (Kurek, Ozimek, 2008). The bacteria can carry out this process with varying efficiency, depending on the strain or abiotic conditions (e.g. pH) in the soil (Ciopińska, Bezak-Mazur, 2018). The presence of phospholytic bacteria in soil has a beneficial effect on plant growth (Kaur, Reddy, 2015; Bargaz et al., 2018).

In the experiments described in this paper, synthetic fertilizers were enriched with selected strains of fungi (*Aspergillus niger, Purpureocillium lilacinum*) and bacteria (*Bacillus* sp.*, Bacillus amyloliquefaciens, Paenibacillus polymyxa*). The aim of the presented studies was to determine what effect the mineral fertilizers enriched with selected microorganisms had on the microorganisms living in the rhizosphere of *Thuja occidentalis*.

MATERIALS AND METHODS

Thuja occidentalis cv. Brabant was grown in 2018–2019 at the open Experimental Field of SGGW in Skierniewice. The experiment was conducted in ceramic containers, completely sunk into the soil, 0.50 m in diameter and 1.20 m high, containing about 120 liters of podzolic soil with pH 6.2 and organic matter content of about 1.2%.

Table 1. Scheme of experiment.

1	C	Control: without fertilizer.
2	CF	Control: without fertilizer + **strains of fungi**[1].
3	SC	Control: standard – 100 g/m^2 Yara Mila Complex; 50 g/m^2 before sowing plants; 50 g/m^2 top dressing (VI/VII).
4	U100	Urea (100%) – 26.0 g/m^2 + Fos Dar 40, 28.0 g/m^2 + potassium sulphate 36.0 g/m^2; urea applied in 2 doses (before sowing and VI/VII).
5	U100F	Urea (100%) – 26.0 g/m^2 + Fos Dar 40, 28.0 g/m^2 + potassium sulphate 36.0 g/m^2; urea applied as top dressing in II doses (before sowing and VI/VII) + **strains of fungi**[1].
6	U60F	Urea (60%) – 16.0 g/m^2 + Fos Dar 40 (60%) 17.0 g/m^2 + potassium sulphate (60%) 22.0 g/m^2; urea applied as top dressing in II doses (before sowing and VI/VII) + **strains of fungi**[1].
7	P100	Polifoska 6 (100%) – 60 g/m^2 before sowing + calcium nitrate 54 g/m^2 (or ammonium nitrate 25.0 g/m^2), calcium nitrate or ammonium nitrate applied as dressing in II doses (before sowing and V/VI).
8	P100B	Polifoska 6 (100%) – 60 g/m^2 before sowing + calcium nitrate 54 g/m^2 (or ammonium nitrate 25.0 g/m^2), calcium nitrate or ammonium nitrate applied as dressing in II doses (before sowing and V/VI) + **strains of bacteria**[2].
9	P60B	Polifoska 6 (60%) – 36 g/m^2 before sowing + calcium nitrate (60%) 32.0 g/m^2 (or ammonium nitrate (100%) 15.0 g/m^2), calcium nitrate or ammonium nitrate applied as dressing in II doses (before sowing and V/VI) + **strains of bacteria**[2].
10	SFD100	Super Fos Dar 40 (100%) – 28.0 g/m^2 before sowing + calcium nitrate (100%) 77.0 g/m^2 (or ammonium nitrate 35.0 g/m^2) + potassium sulphate 36.0 g/m^2; calcium nitrate or ammonium nitrate applied as dressing in II doses (before sowing and V/VI).
11	SFD100B	Super Fos Dar 40 (100%) – 28.0 g/m^2 before sowing + calcium nitrate (100%) 77.0 g/m^2 (or ammonium nitrate (100%) 35.0 g/m^2) + potassium sulphate (100%) 36.0 g/m^2; calcium nitrate or ammonium nitrate applied as dressing in II doses (before sowing and VI/VII) + **strains of bacteria**[2]**.**
12	SFD60B	Super Fos Dar 40 (60%) – 17.0 g/m^2 before sowing + calcium nitrate (60%) 46.0 g/m^2 (or ammonium nitrate 21.0 g/m^2) + potassium sulphate (60%) 22.0 g/m^2; calcium nitrate or ammonium nitrate applied as dressing in II doses (before sowing and VI/VII) + **strains of bacteria**[2]**.**
13	CB	Control: without fertilizer + **strains of bacteria**[2]**.**

[1] Strains of fungi: *Aspergillus niger, Purpureocillium lilacinum.*

[2] Strains of bacteria: *Bacillus* sp.*, Bacillus amyloliquefaciens, Paenibacillus polymyxa*

The surface area of one container was approx. 0,2 m^2. The top layer of soil was enriched with deacidified peat in the amount of about 8 liters per container. Three 20–25 cm high *Thuja occidentalis* plants were planted per container. The volume of the rooting zone in the first year of cultivation was about 40 liters for 3 plants, for each about 13 liters of substrate. Soil moisture in the containers was monitored using Decagon capacitance probes and irrigated as needed using drip irrigation. The experiment was conducted in 13 fertilizer combinations (Table 1), with 3 containers (9 plants) per combination.

Three commercial fertilizers were used in the experiment: 1. Polifoska 6 – 6% nitrogen in the form of NH_4; 20% phosphorus (P_2O_5); 30% potassium (K_2O); 7% sulphur trioxide (SO_3). 2. Super Fos Dar 40 – 40% phosphorus pentoxide (P_2O_5) soluble in mineral acids and 25% P_2O_5 soluble in neutral citrate solution; 10% calcium oxide (CaO); microelements (Cu, Ca, Fe, Mn, Zn). 3. Urea – 46% nitrogen in the form of amide. Fertilizers were applied in full dose (100%) and reduced to 60%. In the control combination, granular multicomponent fertilizer Yara Mila Complex (content N:P:K in proportion 12:11:18; Mg – 2.7%; S – 20% and microelements), was also applied. This fertilizer is used in the large-scale cultivation of *Thuja occidentalis* in ornamental and fruit plant nurseries.

In the first year of the experiment (2018), the fertilizer rates shown in Scheme 1 were applied. In 2019, due to the increased fertilizer requirements of the plants, the rate of established fertilizers was doubled compared to 2018. Each time the fertilizers were applied, the topsoil around the plants was stirred and then the plants were watered.

Synthetic fertilizers: Urea, Polifoska 6 and Super Fos Dar 40 were enriched with selected strains of fungi or bacteria. Microorganisms were applied about two weeks after the first dose of mineral fertilizers. Fungi (*Aspergillus niger*, *Purpureocillium lilacinum*) and bacteria (*Bacillus* sp., *Bacillus amyloliquefaciens, Paenibacillus polymyxa*) were obtained from the collection of the Department of Microbiology of the Institute of Horticulture in Skierniewice. Dry bacterial formulations with maltodextrin as a carrier were prepared at Skotan S.A. (Czechowice-Dziedzice) and used in a 1:1:1 ratio, whereas the product containing fungal conidial spores of *A. niger* and *P. lilacinum* were prepared at the Department of Microbiology, Institute of Horticulture. Fungi were propagated on maize flour (*P. lilacinum*) and rice flour (*A. niger*), and mixed at a ratio of 1:8 in 2018 and 1:10 in 2019. In combinations where selected isolates were applied, the following doses of fungal inoculum per container were used: 1.3 g of medium containing ca. 2×10^8 cfu g^{-1} (in 2018) and 1×10^7 cfu g^{-1} (in 2019). For the

bacterial strains, 3 g of dry formulation was used per container with a bacterial population density of approx. 1–2 × 10^8 cfu × g^{-1}. After microorganism application, the soil was thoroughly mixed.

Microbial surveys in the rhizosphere of *Thuja* were conducted in 2018 and 2019 in late September and early October. Soil from the rhizosphere was collected with a 1.5 cm diameter stick, making 3–4 punctures as close to the plant as possible, to a depth of about 20 cm. Soil samples were stored in a cold room at 5°C for about 24 hours. Before microbiological analysis, the soil was thoroughly mixed, ground in a mortar, and four weights of 10 g each were weighed. Three were poured into flasks containing 100 ml of saline with glass beads each, the fourth weighing was placed in a thermostat and dried for 24 hr at temp. 104°C. The soil in the flasks was shaken on a shaker for 20 min, then the appropriately diluted suspensions were inoculated onto selective media and incubated in thermostats at 28°C (bacteria) and 25°C (fungi) for 7–14 days.

The abundance of selected groups of microorganisms in soil samples was determined by inoculating the soil suspension on selective media. The total bacterial population was estimated on soybean medium (TSA10%). The number of bacteria of the genus *Pseudomonas* was determined on Gould's medium, while of *Pseudomonas* secreting fluorescent dyes, on the same medium under UV light (Gould et al., 1985). Fungal colonies were analyzed on commercial medium produced by BTL (Rose Bengal Chloramphenicol Agar).

Pikovska's medium, after pasteurization of the soil suspension (Picovska, 1948), was used to determine the population of spore-forming bacteria having the ability to solubilize calcium phosphate. Colonies of bacteria exhibiting these properties were counted separately, depending on the presence of the "halo" zone. We determined: phosphorus bacteria A – bacterial colonies forming clear transparent 'halo' zones – with strong calcium phosphate solubilising ability; phosphorus bacteria B – all bacterial colonies that can grow on Pikovska's medium, including bacteria with poor ability to dissolve calcium phosphate.

Actinomycetes were analyzed on colloidal chitin agar after 7 days of incubation of the plates at 28°C (Hsu, Lockwood, 1975). Colonies of these bacteria, depending on the intensity of chitin degradation, were divided into two groups: *Actinomycetes* A – *Actinomycetes* forming a distinct 'halo', and *Actinomycetes* B – colonies, which, although they did not form a distinct 'halo', exhibited morphological characteristics like the *Actinomycetes*. As in the case of phosphorus bacteria, both groups of *Actinomycetes* were counted separately.

The results obtained were statistically processed using analysis of variance for one-factor experiments. The Newman-Keuls test using Statistica 13.1 statistical program was used to evaluate differences between means.

RESULTS

The total abundance of bacteria present in the soil of the rhizosphere zone of *Thuja occidentalis* was little different between the combinations in the first year of the 2018 study (Table 2). Only in two cases, in the combination fertilized with Polifoska 6 (P100), and in combination CB, where bacteria alone were used, the number of bacteria was significantly higher than in the other combinations. The highest number of bacteria of the genus *Pseudomonas* was observed in soil fertilized with Urea (U100), and in combination with Super Fos Dar 40 (SFD60B), in which fertilizer amounts reduced by 40% were enriched with selected bacteria. The amount of *Pseudomonas* bacteria, secreting fluorescent dyes was elevated in the same combinations as *Pseudomonas* in total.

The content in the soil of *Actinomycetes*, an important bacterial group from the point of view of soil fertility, developed differently depending on their chitinolytic properties (Table 3). No differences were observed in the abundance of *Actinomycetes* with strong chitinolytic properties (*Actinomycetes* A). Slightly greater differences were among the group of *Actinomycetes* B. In this group, the highest amounts were found in soil fertilized with Polifoska 6 (P100B) and Urea (U100). Three times fewer *Actinomycetes* were present in the combination of SFD60B.

The highest number of filamentous fungi, nearly 7 times more than in the standard control, was in the combination U100F, fertilized with a full dose of Urea fertilizers enriched with selected fungi, and in a combination U100, containing the same fertilizers but no fungi (Table 3). The least amount of filamentous fungi was in control 0, without fertilization (C).

The abundance of bacteria with the ability to degrade insoluble phosphorus compounds was also analyzed in 2018 (Table 4). The highest abundance of phosphorus bacteria with strong lytic properties, those whose colonies produced the largest "halo" on selective media, was observed in the combination CB, to which only selected bacteria and SFD100, were added. The highest number of all phosphorus bacteria was observed in the combination of P100B and SFD100, while less in U100F, with addition of selected fungi.

In 2019, the study of microbial populations in the rhizosphere zone of *Thuja* was repeated. The total bacterial abundance varied more between combinations compared to the previous year (Table 5). In the second year of cultivation, the effect of the addition of selected bacteria became apparent, as the highest results were obtained in the combinations in which they were applied. The highest amount of bacteria was in the soil taken from the combination P60B, in which a lower dose of Polifoska 6 was applied and in the combination CB, with the addition of bacteria alone. High bacterial counts were also in combinations with Super Fos

Table 2. Total bacteria and *Pseudomonas* genus abundance in the rhizosphere of *Thuja occidentalis* in 2018.

Combinations#		Total number of bacteria	Total number of *Pseudomonas*	Fluorescent *Pseudomonas*
		cfu × 10^6/g d.m of soil	cfu × 10^3/g d.m of soil	cfu × 10^3/g d.m. of soil
1.	C	7.7 a	35.0 a	9.5 a
2.	CF	11.3 a	11.2 a	7.3 a
3.	SC	10.1 a	25.4 a	15.2 a
4.	U100	14.7 a	125.3 b	93.9 c
5.	U100F	13.4 a	17.7 a	13.8 a
6.	U60F	9.0 a	41.2 a	16.7 a
7.	P100	48.4 b	7.8 a	10.0 a
8.	P100B	10.1 a	44.7 a	31.7 a
9.	P60B	10.3 a	17.5 a	12.6 a
10.	SFD100	14.4 a	37.4 a	14.2 a
11.	SFD100B	16.2 a	35.3 a	20.6 a
12.	SFD60B	10.7 a	109.0 b	57.5 b
13.	CB	42.0 b	37.3 a	17.1 a

see Table 1

Values marked with the same letter in columns are not significantly different according to the Newman-Keuls test (p=0.05).

Table 3. Abundance of *Actinomycetes* and filamentous fungi in the rhizosphere of *Thuja occidentalis* in 2018.

Combinations#		*Actinomycetes* (A)	*Actinomycetes* (B)	Filamentous fungi
		cfu × 10^5/g d.m. soil		cfu × 10^4/g d.m. of soil
1.	C	24.9 a	42.8 abcd	3.2 a
2.	CF	25.7 a	33.7 abcd	28.4 bc
3.	SC	16.2 a	19.8 ab	10.9 ab
4.	U100	29.7 a	51.9 cd	44.0 c
5.	U100F	20.7 a	24.3 abc	71.3 d
6.	U60F	25.2 a	29.4 abcd	27.3 abc
7.	P100	28.6 a	35.1 abcd	31.1 bc
8.	P100B	42.6 a	52.3 d	35.1 bc
9.	P60B	31.3 a	36.9 abcd	22.0 abc
10.	SFD100	38.9 a	47.8 bcd	25.0 abc
11.	SFD100B	34.4 a	41.3 abcd	31.5 bc
12.	SFD60B	31.2 a	16.0 a	17.9 ab
13.	CB	16.7 a	22.0 ab	15.7 ab

see Table 1

Values marked with the same letter in columns are not significantly different according to New-man-Keuls test (p=0.05)

Table 4. Abundance of phosphorus bacteria in the rhizosphere zone of *Thuja occidentalis* in 2018.

Combinations#		Phosphorus bacteria (A)	Phosphorus bacteria (B)
		cfu × 10^4/g d.m. soil	
1.	C	16.7 bcd	31.5 ab
2.	CF	8.6 ab	21.2 ab
3.	SC	17.4 cd	33.9 ab
4.	U100	8.4 ab	36.6 ab
5.	U100F	10.5 abc	4.7 a
6.	U60F	12.1 abc	18.0 ab
7.	P100	9.0 abc	27.0 ab
8.	P100B	16.4 bcd	48.2 b
9.	P60B	7.1 a	37.8 ab
10.	SFD100	20.2 d	47.4 b
11.	SFD100B	7.1 a	29.4 ab
12.	SFD60B	9.7 abc	39.6 ab
13.	CB	21.1 d	37.7 ab

see Table 1

see Table 1

Values marked with the same letter in columns are not significantly different according to Newman-Keuls test (p=0.05)

Table 5. Total abundance of bacteria and Pseudomonas bacteria in the rhizosphere of *Thuja occidentalis* in 2019.

Combinations#		Total number of bacetria	Total number of *Pseudomonas*	Fluorescent *Pseudomonas*
		cfu × 10^6 /g d.m. of soil	cfu × 10^3 /g d.m. of soil	
1.	C	13.8 bcd	17.8 ab	6.4 ab
2.	CF	9.8 abc	21.6 ab	13.4 ab
3.	SC	3.6 a	32.5 b	15.0 bc
4.	U100	1.8 a	7.5 a	4.8 a
5.	U100F	2.4 a	53.2 c	30.9 d
6.	U60F	3.4 a	4.1 a	2.9 a
7.	P100	11.2 abcd	9.8 a	3.6 a
8.	P100B	4.6 a	6.3 a	4.7 a
9.	P60B	28.7 e	26.1 b	7.5 ab
10.	SFD100	5.7 ab	7.3 a	7.9 ab
11.	SFD100B	16.0 cd	27.2 b	15.7 bc
12.	SFD60B	19.4 d	7.6 a	3.8 a
13.	CB	26.4 e	31.9 b	22.7 c

see Table 1

Values marked with the same letter in columns are not significantly different according to New-man-Keuls test (p=0.05)

Table 6. Abundance of *Actinomycetes* and filamentous fungi in the rhizosphere of *Thuja occidentalis* in 2019.

Combinations#		*Actinomycetes* (A)	*Actinomycetes* (B)	Filamentous fungi [cfu × 10^4/g d.m. of soil]
		cfu × 10^5/g d.m. of soil		
1.	C	14.3 a	21.4 a	9.2 a
2.	CF	9.5 a	16.7 a	20.9 a
3.	SC	13.6 a	22.7 a	79.2 cd
4.	U100	16.2 a	26.6 ab	61.8 bc
5.	U100F	24.4 ab	35.1 abc	27.4 a
6.	U60F	27.2 ab	42.9 abc	30.0 a
7.	P100	16.7 a	26.2 ab	25.1 a
8.	P100B	35.5 bc	53.8 c	51.9 b
9.	P60B	36.6 bc	58.3 c	66.5 bcd
10.	SFD100	38.5 bc	57.3 c	19.7 a
11.	SFD100B	50.1 c	61.6 c	84.4 d
12.	SFD60B	36.8 bc	49.6 bc	8.1 a
13.	CB	46.3 c	58.8 c	8.4 a

see Table 1

Values marked with the same letter in columns are not significantly different according to Newman-Keuls test (p=0.05)

Table 7. The number of phosphorus bacteria in the rhizosphere of *Thuja occidentalis* in 2019.

Combinations#		Phosphorus bacteria (A)	Phosphorus bacteria (B)
		cfu × 10^4/g d.m. of soil	
1.	C	5.6 a	14.5 a
2.	CF	10.0 a	18.8 ab
3.	SC	6.4 a	14.9 a
4.	U100	6.0 a	18.0 ab
5.	U100F	21.6 b	34.4 abc
6.	U60F	13.8 ab	26.6 abc
7.	P100	7.0 a	26.7 abc
8.	P100B	34.9 c	76.9 e
9.	P60B	20.8 b	47.1 cd
10.	SFD100	34.4 c	60.8 d
11.	SFD100B	16.4 ab	41.3 bcd
12.	SFD60B	11.0 a	22.0 ab
13.	CB	29.8 c	47.3 cd

see Table 1

Values marked with the same letter in the columns are not significantly different according to the Newman-Keuls test (p=0.05).

Dar 40, SFD60B and SFD100B. The soil treated with Urea had the lowest total bacterial abundance.

The situation was different for bacteria of the genus *Pseudomonas*, as the highest number of these bacteria was found in the treatments fertilized with Urea U100F. Many fluorescent *Pseudomonas* occurred also in combination CB, in soil enriched with selected bacteria (Table 5).

A higher abundance of *Actinomycetes*, as compared to the control combination, was observed, similarly to the total bacterial abundance, in the combinations to which selected bacteria were added (Table 6). The population of these bacteria was significantly higher in the soil fertilized with Super Fos Dar 40 (SFD100B, SFD100 and SFD60B) and Polifoska 6 (P100B and P60B). The abundance of *Actinomycetes* was also high in soil with the addition of selected bacteria (CB).

No increased abundance of filamentous fungi was observed in combinations enriched in these microorganisms (Table 6). The lowest number of fungi was in control 0, without fertilization (C), in combination SFD60B and in the control with selected bacteria (CB). They were isolated in the highest number from the soil in the combination SFD100B and standard control SC.

The application of selected bacteria to the soil in which the *Thuja occidentalis* plants were grown increased, in most cases, the populations of bacteria degrading insoluble phosphorus compounds (Table 7). The greatest number of phosphorus bacteria with strong lytic properties was isolated from the soil of combinations fertilized with Polifoska 6 P100B, with Super Fos Dar 40 SFD100 and with CB. Significantly more group B phosphorus bacteria were also observed in soil from the combination of P100B, SFD100, as well as P60B and CB.

DISCUSSION

The beneficial effects of most soil microorganisms on plant growth are indisputable (Timmusk, Wagner, 1999; Borris, 2011; Yadav et al. ,2011; Chowdhury et al., 2015; Yin et al., 2015; Ishaq, 2017; Lan et al., 2017; Yi et al., 2019). In recent years, due to the more ecological approach to agriculture, intensive research has been conducted on the interaction among plants, soil, and soil microorganisms. Microorganisms in the root zone of plants influence the efficiency of mineral nutrient uptake by plants, thereby increasing the utilization rate of fertilizers applied to the soil (Bakker et al., 2012). It follows that the type of microorganisms and their abundance, determined by many biotic and abiotic factors, is of great importance both economically (allowing reduced fertilizer application rates) and environmentally.

The study of selected groups of microorganisms living in the root zone of *Thuja occidentalis* presented in this paper is the result of analyses conducted over a period of two years. As it is known, one of the factors strongly affecting the populations of microorganisms in the

rhizosphere is the amount and type of root secretions. Their chemical composition changes with the age of the plant, because younger plants excrete more root secretions, on the other hand, they usually have less developed root system (Olanrewaju et al., 2019).

When analyzing the total bacterial counts, it can be seen that in 2018 there were significantly more bacteria in the combination with Polifoska 6 (P100) and in CB. In the first year of the study, only in these two cases an increased level of these microorganisms was observed. Most likely, the fertilization applied in these combinations affected the development of the root system and may have stimulated an increase in soil bacteria abundance. In 2019, there was more variation in total bacterial abundance between combinations. The application of bacteria *Bacillus* sp.*, Bacillus amyloliquefaciens, Paenibacillus polymyxa* positively influenced the total number of bacteria in soil, as high abundances of these microorganisms were found in combinations P60B, SFD60B, SFD100B and CB, where selected strains of bacteria were applied. However, on the basis of the study, it cannot be determined whether this was a direct effect (i.e. applied strains) or an indirect effect as a result of improved root growth or increased release of nutrients into the soil by the plants.

One group of bacteria whose population is particularly correlated with the quantity and quality of root secretions are bacteria of the genus *Pseudomonas*. Many bacteria belonging to this genus were identified as PGPR, bacteria that benefit plant growth. In both years, the highest abundance of these bacteria was observed in combinations with Urea (U100 in 2018 and U100F in 2019). Reduction of Super Fos Dar 40 to 60% and application of selected bacteria (treatment SDF60B) also significantly increased their population in 2018 compared to the control combinations. *Pseudomonas* bacteria are a group of microorganisms characterized by rapid growth, in part because of their ability to utilize a wide range of organic compounds (McSpadden Gardener, 2007). It is most likely that nutrients secreted by the roots of *Thuja occidentalis* growing in soil fertilized with these fertilizers stimulated the growth of this group of bacteria.

Actinomycetes commonly occurring in soils play a key role in the cycling of many elements (Lenart-Boroń, Banach, 2014). In the first year of the study, differences between the combinations were small, especially among the *Actinomycetes* with strong chitinolytic properties (group A). Similarly as *Pseudomonas*, many *Actinomycetes* were observed in soil from the combination with Urea U100 and with Polifoska 6 P100B. In 2019, there was more variation between combinations. It can be observed that elevated abundance of *Actinomycetes* often occurred in microbially enriched combinations, especially with strains of selected bacteria. This could be due to the effect of applied fertilization on the quality or quantity of root secretions, which are the food of these microorganisms.

As it is known, the interaction between plant and microorganisms, and between microorganisms among themselves, can be direct (e.g. parasitism, antagonism, competition) and indirect, due to changes in the surrounding environment (Jankowska, Swędrzyńska, 2016). This means that if a fertilizer with microorganisms (fungi or bacteria) is added to the substrate, it can positively or negatively affect the plants, the development of the root system, or the quantity and quality of root secretions. These changes can result in an increase in the population of e.g. *Pseudomonas* bacteria in the soil, despite the fact that selected fungi were added, which, having a beneficial effect on plants, increased the secretion of compounds affecting the development of this group of microorganisms. This phenomenon was also observed in the case of fungi of the genus *Trichoderma*, which, when added to soil, favourably influenced the development of other groups of microorganisms, e.g. *Pseudomonas* bacteria, or *Actinomycetes* (Smolinska et al., 2014). A significant effect of soil enrichment with selected fungal strains on the total abundance of this group was observed in 2018 in the combination with Urea U100F. In the second year of the study, the highest number of fungal colonies was isolated from the combination fertilized with Super Fos Dar 40 (SFD100B).

Rhizosphere microorganisms significantly affect the efficiency of mineral nutrient uptake, influencing the utilization rate of applied fertilizers (Liang et al., 2020). Several studies have shown that a significant number of soil microorganisms, including bacteria belonging to the genera *Pseudomonas* or *Bacillus*, have the ability to dissolve phosphorus compounds and release plant-available forms of phosphorus into the soil (Wang et al., 2020). Analyzing the abundance of phosphorus bacteria in the soil from different fertilizer combinations in the cultivation of *Thuja*, it can be observed that in 2018, increased abundance of these bacteria was observed in the combination with SFD100, while in 2019 in treatments fertilized with Polifoska **6** P100B and Super Fos Dar 40 SFD100. Enrichment of soil with selected bacteria alone (without fertilizers) also had a beneficial effect on the growth of this group of microorganisms.

CONCLUSIONS

1. For the first time it was demonstrated that it is possible to increase the number of beneficial microorganisms in the rhizosphere of *Thuja occidentalis* by using mineral fertilizers enriched with selected microorganisms.
2. The enrichment of fertilizers Urea, Polifoska 6 and Super Fos Dar 40 with selected strains of fungi and bacteria in many combinations had a beneficial effect on the development of microorganisms in the rhizosphere of *Thuja occidentalis*.

3. The growth of *Pseudomonas* bacteria was most favorably affected by the application of Urea (U100 and SFD60B in 2018 and U100F in 2019).

4. Soil fertilization with Polifoska 6 (P100B) and Super Fos Dar 40 (SFD100) favoured the multiplication of phosphorus bacteria.

5. Based on the research conducted to date, it cannot be determined whether the fungi and bacteria added to the fertilizers interacted with soil microorganisms directly or indirectly by stimulating root growth and plant secretion of nutrients into the soil.

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