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COMMUNITIES OF BACTERIA AND FUNGI IN THE SOIL AFTER RUNNER BEAN (*Phaseolus coccineus* L.) CULTIVATION

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ABSTRACT

The object of the studies was the soil after one-year, two-year, three-year and four-year cultivations of runner bean and the soil from black fallow. The microbiological analysis showed that particular soil samples varied in quantitative and qualitative composition. The smallest number of bacteria and fungi colonies characterized the black fallow soil. On the other hand, the greatest number of microorganism colonies was found in the soil after a three-year-long cultivation of runner bean. In the soil after a four-year-long cultivation of this plant the studies observed a decreased total number of bacteria, *Bacillus* spp., *Pseudomonas* spp. as well as the total number of fungi. The mycological analysis showed that after a one-year-long cultivation of bean pathogenic fungi constituted 25%, while after four years of cultivation the pathogens made up 82% of all isolations. The proportion of saprotrophic fungi was reverse. After a four-year-long cultivation of runner bean the species diversity was smaller and *Fusarium oxysporum* dominated among the isolated fungi.

Runner bean, due to its rich composition of organic compounds, especially aminoacids in the root exudates, had a significant influence on the quantitative and qualitative composition of microorganisms.

Key words: *Bacillus* spp., *Pseudomonas* spp., runner bean, saprotrophic fungi, pathogenic fungi

INTRODUCTION

The soil is the habitat of both bacteria and fungi, which have a negative or a positive effect on the growth, and development of plants [9, 16]. Both biotic and abiotic factors affect the quantitative and qualitative composition of the population of microorganisms. One of the main factors having a stimulating or inhibiting effect on the

communities of bacteria and fungi is the plant. Each, plant, including runner bean, secretes various organic compounds to the soil through its roots, and after the harvest leaves crop residues with the proper chemical composition. Root exudates, which are the main source of aminoacids, sugars, vitamins, phenols, organic acids and metal ions, affect the composition of microorganism populations in the soil, especially in the rhizosphere [3, 4, 5, 17, 18, 22]. According to Darcy [4], Pięta [17], and Vancur and Stanko [29] the greatest amount of aminoacids is secreted by papilionaceous plants, which stimulate the development of microorganisms. A high level of sugars, especially glucose as the major source of C, has a similar effect [5, 20, 21]. Phenols and their derivatives occurring in root exudates inhibit the development of microorganisms, since these compounds are considered to be bacterio- and fungistatic [1, 4, 5, 6, 23].

The competitiveness of some microorganisms towards others is determined mainly by such properties as the ability of fast colonization of the root zone of cultivated plants and utilization of the compounds secreted by the roots [8].

The purpose of the present studies was to determine the effect of runner bean on the quantitative and qualitative composition of bacteria and fungi communities in the soil.

MATERIALS AND METHODS

The object of the studies was the soil after one-year, two-year, three-year and four-year cultivation of runner bean, Eureka cv. and the soil from a belt of black fallow – as the control. Soil samples were taken after the harvest, which was at the end of September. A mycological analysis was conducted in the laboratory in accordance with the method described by Martyniuk et al. [13] and Pięta [19]. The media Pseudomonas agar F and Triptic soy agar were used in order to isolate *Pseudomonas* spp. and *Bacillus* spp, respectively, while Martin's agar medium [12] was used in order to isolate the fungi colonies. Fungi from the genus *Fusarium* were marked within the species using SNA medium, while a maltose medium and Czapek Dox were used for *Penicillium* spp. The other fungi were marked within the species using Malt-agar medium.

In the growth chamber experiment after 30 days of bean growth in containers with sterile distilled water, a water solution of root exudates was obtained. The manner of setting and performing this experiment was described in the paper concerning the studies on *Phaseolus vulgaris* L. [18]. Dehydrated root exudates were subjected to chemical analysis through the proper preparation of the solution according to the method described by Wierciński [30]. The solutions were introduced into an automatic analyzer of aminoacids so that the quantitative and qualitative composition of aminoacids could be determined.

RESULTS

Results of a laboratory microbiological analysis of particular soil samples showed differentiated numbers of bacteria and fungi (tab. 1). The total number of bacteria in 1 g of soil d.w. ranged from $8.36 \cdot 10^6$ to $30.11 \cdot 10^6$ colonies. The smallest number of bacteria colonies was found in the soil from black fallow considered as the control, while the greatest, i.e. e. $30.11 \cdot 10^6$ of bacteria was observed in 1 g of the d.w. of the soil taken from the field after a three-year-long cultivation of runner bean. A gradual increase of the total number of bacteria, *Bacillus* spp., *Pseudomonas* spp., and fungi took place during the first three years of bean cultivation (tab. 1). On the other hand, the total number of bacteria decreased by 30%, *Bacillus* spp. by 40% and *Pseudomonas* spp. by 37% in the soil after a four-year-long bean cultivation, while the total number of fungi decreased only by 7% colonies in comparison to the soil after a three-year-long cultivation of bean.

Table 1. Number of bacteria and fungi after cultivation of runner bean

Experimental combination	Total number of bacteria (mln·g^{-1} d.w. of soil)	Number of *Bacillus* spp. (mln·g^{-1} d.w. of soil)	Number of *Pseudomonas* spp. (mln·g^{-1} d.w. of soil)	Total number of fungi (thous.·g^{-1} d.w. of soil)
Soil after a one-year-long cultivation	12.79^{b*}	2.96^{b}	3.92^{b}	93.17^{b}
Soil after a two-year-long cultivation	24.27^{c}	4.71^{c}	6.83^{c}	139.08^{c}
Soil after a three-year-long cultivation	30.11^{d}	6.63^{d}	9.14^{d}	191.03^{e}
Soil after a four-year-long cultivation	21.07^{c}	3.98^{c}	5.76^{bc}	178.17^{d}
Soil from black fallow	8.36^{a}	1.99^{a}	1.13^{a}	33.86^{a}

*Means in columns differ significantly ($P \leq 0.05$), if they are not marked with the same letter

The mycological analysis showed that after a one-year-long cultivation of bean there occurred a considerable, almost 4-fold increase of the number of colonies of *Trichoderma* spp. and *Gliocladium* spp. as compared to the number of colonies isolated from the black fallow soil. On the other hand, the colonies of these fungi were isolated only sporadically or were not isolated at all from the soil after three- and four-year-long cultivation of bean

Table 2. Fungi isolated from investigated samples of soil

Fungus species	Experimental combination					Total
	1	2	3	4	C	
Alternaria alternata (Fr.) Keissler	3	5	18	6	2	34
Botryotrichum pilluliferum Sacc. et March.	2	1	-	1	-	4
Botrytis cinerea Pers.	2	16	12	6	-	36
Cladosporium cladosporioides (Fres.) de Vries	4	3	-	-	-	7
Cladosporium herbarum (Pers.) Link	3	4	2	3	2	14
Epicoccum purpurascens Ehr. ex Schl.	3	2	1	-	-	6
Fusarium avenaceum Sacc.	3	4	16	4	3	30
Fusarium culmorum (W.G.Sm.) Sacc.	3	6	10	16	2	37
Fusarium equiseti (Corda) Sacc.	6	18	11	13	2	50
Fusarium oxysporum Schl.	2	16	29	83	1	131
Fusarium solani (Mart.) Sacc.	4	8	10	15	1	38
Gliocladium catenulatum Gil. et Abb.	6	2	1	-	2	11
Gliocladium roseum Bainier	2	-	-	-	-	2
Humicola fuscoatra Traaen	-	2	14	-	2	18
Humicola grisea Domsch	-	-	1	-	1	2
Mucor hiemalis Wehmer	2	6	-	2	3	13
Mucor mucedo Fres.	4	3	11	8	-	26
Penicillium canescens Sopp	-	2	-	-	2	4
Penicillium chrysogenum Thom	3	1	-	3	-	7
Penicillium frequentans Westling	3	-	1	-	1	5
Penicillium meleagrinum Biourge	-	1	-	4	-	5
Penicillium nigricans Bainier ex Thom	4	2	13	-	3	22
Penicillium paxilli Bainier	-	1	-	-	1	2
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	5	3	12	10	2	32
Periconia macrospinosa Lef. et Johnson	1	-	1	-	-	2
Rhizopus nigricans Ehr.	3	10	12	-	-	25
Rhizoctonia solani Kühn	2	16	14	6	-	38
Scopulariopsis brevicaulis (Sacc.) Bainier	3	-	-	-	1	4
Trichoderma hamatum (Bon.) Bain	4	1	-	-	1	6
Trichoderma harzianum Rifai	3	-	-	-	-	3
Trichoderma koningii Oud.	5	3	1	1	2	12
Trichoderma viride Pers. ex S.F.Gray	7	2	1	1	2	13
Total	92	138	191	182	36	639

1 – soil after a one-year-long cultivation
2 – soil after a two-year-long cultivation
3 – soil after a three-year-long cultivation
4 – soil after a four-year-long cultivation
C – soil from black fallow as the control

The pathogenic fungi isolated from particular soil samples included *Botrytis cinerea, Rhizoctonia solani* and *Fusarium* spp. After a one-year-long cultivation of bean the number of *Fusarium* spp. in the soil was twice as big, after a two-year-long cultivation six times as big, after a three-year-long cultivation more than eight times as big and after a four-year-long cultivation almost 15 times as big as the number of colonies isolated from black fallow. The dominating species from the genus *Fusarium* in the soil after a four-year-long cultivation of bean was *F. oxysporum*, and its isolates constituted 45.6% of all the fungi. Results of the mycological analysis showed that the number of *B. cinerea* and *R. solani* colonies decreased in this soil (tab. 2).

The quantitative and qualitative composition of fungi isolated from particular soil samples varied (tab. 2). The smallest number of fungi was isolated from the soil taken from a belt of black fallow, since 36 colonies from 20 species were obtained. Almost three times as many fungi colonies were obtained from the soil after a one-year-long cultivation of bean as from the soil taken from black fallow. The mycological analysis of soil samples after two- and three-year-long cultivation of bean showed that a further increase of the number of fungi colonies took place in those years. On the other hand, after the fourth year of bean cultivation the number of fungi colonies decreased (tab. 2). Among the colonies isolated from black fallow, 75% were saprotrophic and 25% pathogenic fungi (fig. 1). Throughout the following years of bean cultivation the proportion of saprotrophic fungi decreased, while the number of pathogenic fungi increased. After the fourth year of bean cultivation the proportion of saprotrophic fungi in the soil constituted only 18%, while pathogenic fungi turned out to be dominating (fig. 1).

Fig. 1. The proportion of saprotrophic and pathogenic fungi in particular soil samples: 1 – soil after a one-year-long cultivation, 2 – soil after a two-year-long cultivation, 3 – soil after a three-year-long cultivation, 4 – soil after a four-year-long cultivation, C – soil from black fallow as the control

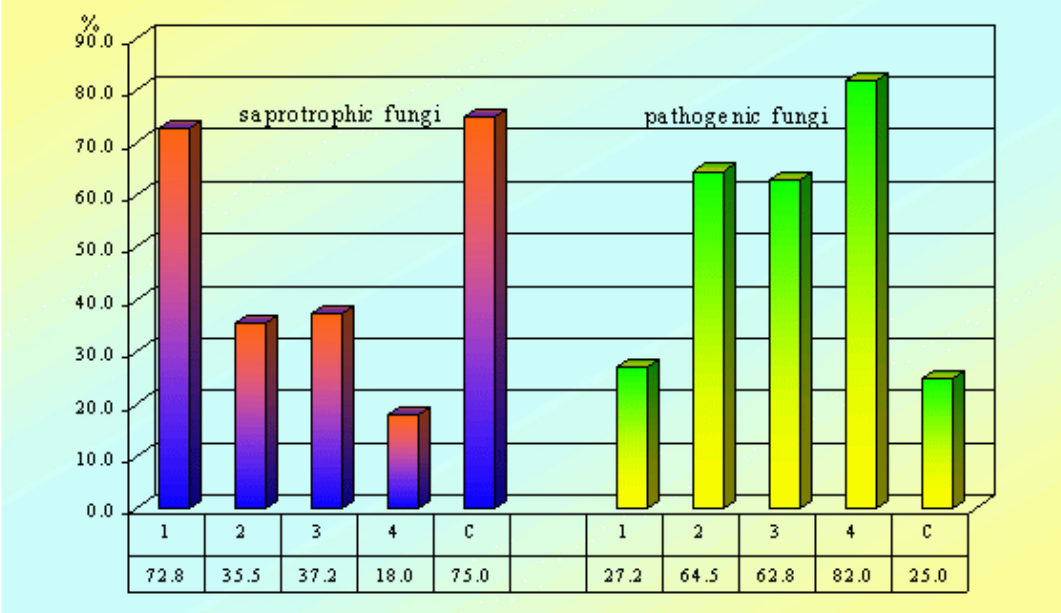

Table 3. The contents of free amino acids (mg·ml^{-1}) in the exudates of runner bean roots

Plant	Sour amino acids		Aromatic amino acids		Alkaline amino acids			treonine	serine	glycine	alanine	valine	metionine	isoleucine	leucine
	aspartic acid	glutam. acid	tyrosine	phenyla-lannine	lisine	histidine	arginine								
Runner bean	0.367	0.308	0.159	0.049	0.043	0.041	0.049	0.103	0.017	0.008	0.006	0.020	0.056	0.019	0.020
	0.675		0.208		0.133			0.249							

It was found out after the chemical analysis of root exudates of runner bean that they contained considerable quantities of free aminoacids which constituted 1.265 mg·ml^{-1} (tab. 3). Aspartic acid and glutamic acid, which are acidic aminoacids, dominated among 15 free aminoacids secreted by the roots of the examined plant (tab. 3). The proportion of this group of aminoacids was 53.5% of the total number of free aminoacids. The roots of runner bean also secreted aromatic acids (tyrosine, phenylalanine) and alkaline aminoacids (lysine, histidine, arginine). The proportion of these groups of aminoacids in the root exudates constituted 16.6% and 10.5% of the total quantity of free aminoacids, respectively. The other aminoacids constituted 19.6% of the total number, and treonine (8.1%) and metinine (4.4%) were the most frequently secreted compounds.

DISCUSSION

The studies showed that runner bean had a significant influence on the increase of the number and bacteria and fungi colonies in the soil. Introduction of bean to the cultivation in the first year brought about a 50% increase of

the total number of bacteria and *Bacillus* spp., besides, there were 3 times as many colonies of *Pseudomonas* spp. and fungi colonies as the number of these microorganisms in the soil of black fallow. The following years of bean cultivation had an effect on the further increase of the number of microorganisms in the soil, with a greater proportion of pathogenic fungi and a smaller number of saprotrophic fungi, including antagonistic ones. The greatest increase of the number of saprotrophic fungi such as *Trichoderma* spp. and *Gliocladium* spp. considered to be antagonists [2, 10, 11, 14, 15, 25, 26, 28] occurred in the first year of bean cultivation. On the other hand, a negative phenomenon took place in the following years of bean cultivation, i.e. e. the population of *Trichoderma* spp. and *Gliocladium* spp. decreased and the number of pathogenic fungi colonies increased.

The dynamic increase of the number of microorganisms in the soil after runner bean cultivation can be explained by the favourable quantitative and qualitative composition of organic compounds provided in the form of root exudates and crop residues. This fact is confirmed by earlier information from the Polish and foreign literature [5, 7, 20, 22, 23, 24, 27, 31].

According to Pięta [17, 18, 19], Piotrowski [21] and Ślusarczykowa [28], acidic aminoacids found in root exudates had an especially positive effect on the increase and development of various species of fungi species. There were a big proportion of these compounds in the water solution of root exudates of runner bean. Alkaline and aromatic aminoacids that had a negative effect on the growth and development of fungi were found in small quantities. Hence, it should be supposed that runner bean root exudates had a stimulating effect on the growth and development of microorganisms, especially pathogens.

CONCLUSIONS

1. Runner bean modifies the quantitative and qualitative composition of bacteria and fungi populations in the soil through the root exudates.
2. The stimulating effect of aminoacids secreted by the roots on soil microorganisms was observed during the first three years of runner bean cultivation.
3. The fourth year of monoculture bean cultivation proved unfavourable for saprotrophic fungi, including antagonistic ones, since their number dropped more than four times as compared to the number of fungi obtained from the soil after single cultivation of this plant.
4. Many years' cultivation of runner bean leads to the accumulation of pathogens in the soil, therefore, frequent cultivation of this plant is not recommended in the same field.

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