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CHANGES IN POPULATION OF SELECTED BACTERIA IN THE RHIZOSPHERE OF POTATO UNDER DIFFERENT FARMING SYSTEMS

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ABSTRACT

The research was carried out on farms of the Bory Tucholskie Landscape Park near Tuchola. The aim of the research was to define the quantitative content of bacteria of *Pseudomonas* (the fluorescent subgroup), *Arthrobacter*, *Azotobacter* and the coryneform group in the rhizosphere of potato in different stages of its development. The "Aster" potato was grown in two farming systems: ecological and conventional. Microbiological analysis indicated that the bacteria of the coryneform group were the most numerous, and the next was *Arthrobacter* spp. More of investigated bacteria were isolated from the rhizosphere of potatoes grown in ecological farming system than in the conventional one. From the results obtained during three year's study it is evident that the number of potentially antagonistic bacteria increased with the development of vegetation and was the highest in root zone area of the plants in harvest-mature tubers.

Key words: rhizosphere, non-rhizosphere soil, antagonistic bacteria, potato, farming system

INTRODUCTION

The rhizosphere is a specific habitat for microbial growth. Its physicochemical properties create different growing conditions for microorganisms in comparison with root-free soil. To emphasize the separate character of the population of bacteria in the rhizosphere Suslow et al. [28] defined them as rhizobacteria. As early as during germination of seeds and the growth of plants in the developing root zone the number of microorganisms in root area increases (10-1000 times) and they differ in respect of quality comparing with root-free soil [13,22]. The increased number of microorganisms remains relatively constant in this area during the whole period of plant development [13]. A plant is a partner in the biocenotic system and all the physiological changes it undergoes during vegetation are reflected in the features of coexisting microorganisms. The inseparable plant-microorganism system is set up which undergoes short and long-term fluctuations, depending on plant development stage as well as agroecological conditions [1]. The roots of plants excrete various nutrients and auxo-substances to soil. They bring about a specific selection of both useful and pathogenic microorganisms [18]. Biological reduction in bacterial and fungal pathogens using the antagonism between microorganisms is becoming an alternative way of plant preservation [4]. Antagonistic bacteria can inhibit the activity and decrease the number of pathogens, and thus reduce plant diseases [18].

Some of the best known antagonists are fluorescent *Pseudomonas* spp. and *Arthrobacter* spp. Bacteria of *Pseudomonas* genus compete successfully with fitopathogens owing to production of antibiotics, siderefores and hydrocyanic acid [7,24]. The antagonism of *Arthrobacter* spp. is based mostly on their lytic abilities [9]. Bacteria of *Azotobacter* genus are famous mainly for providing plants with nitro-compounds, but they also proved to have inhibitory abilities in relation to many pathogenic fungi [2]. Beneficial impact of above-mentioned bacteria on plants is also connected with their excretion of amino acids, vitamins, organic acids, and their ability to induction of the state of plant systemic resistance. Additional factors enhancing the action of antagonistic bacteria are their mobility, the fast and stable colonization of the plant surface as well as the bacteria viability on plants in changing environmental conditions [8].

The man can partially control the quantitative and qualitative composition of microorganisms as well as the intensity of processes catalyzed by them through the use of a farming system and fertilization, pesticides etc. [20]. Ecological farming, which is based on the protection of agricultural production environment, tends to use only organic fertilizers and avoid chemical inputs [14]. In the ecological farming system, the whole technique of field-crop production should enable to obtain high fertility of soil and its favorable sanitary state. Plant protection is formulated as prevention, not control. Therefore preventive actions are of great importance. The treatments applied should in the first place create the proper conditions stimulating the development of antagonistic microorganisms which decrease the survival and activity of soil pathogens.

The working hypothesis assumes that the two compared farming systems, ecological and conventional ones, exert a various influence on the number of the researched populations of rhizobacteria. It was assumed that the microbial development during vegetation is more stimulated in the ecological farming system than in the conventional one.

The aim of this study then was to estimate the quantitative content of potentially antagonistic bacteria of *Pseudomonas* (fluorescent subgroup), *Arthrobacter*, *Azotobacter* and bacteria of the coryneform group in the rhizosphere of potato (*Solanum tuberosum* L.) grown in ecological and conventional farming system. The investigations were also supposed to define the influence of a farming system on the dynamics of bacteria development during vegetation.

MATERIAL AND METHODS

The research was carried out over 1997-1999 on the farms of the Bory Tucholskie Landscape Park in the vicinity of Tuchola. 'Aster' cultivar potato cultivated on lessive soils, the good rye soil agricultural suitability complex, was used in the experiment. Different rotations of crops and technologies of production were used in compared systems. The ecological farm, from which the material to the research was taken, has been run according to the international standards since 1991 and it is a member of the Association of Ecological Food Producers "EKOLAND". In the system according to the rules of ecological agriculture mineral fertilizers and chemical pesticides were not used.

The potato plantation was fertilized only with compost in the single dose of 20 $t \cdot ha^{-1}$, and peat (5-15 $t \cdot ha^{-1}$) before the spring tillage (ploughing). The pesticides used included only a biological preparation against *Colorado potato-beetle* – Novodor (4 $l \cdot ha^{-1}$). The conventional farming included organic-and-mineral fertilization with: manure (25 $t \cdot ha^{-1}$), and mineral fertilizers NPK applying 70-30-120 $kg \cdot ha^{-1}$, respectively. Over the potato vegetation the following pesticides were used against potato blight: Sandofan Manco 64 WP and Curzate M 72.5 WP in the doses of 2 $kg \cdot ha^{-1}$ and preparation Bancol –0.4 $kg \cdot ha^{-1}$ to fight *Colorado potato-beetle*. More detailed information concerning the elements of the technique of field-crop production of potato and the tuber yield obtained are presented in Table 1.

Table 1. Yield and selected elements of potato field-crop production in different farming systems (1997-1999)

Specification	Farming system	
	Ecological	Conventional
Forecrop 1997	winter rye + aftercrop (mustard)	winter triticale
1998	winter rye + aftercrop	winter rye
1999	winter rye + aftercrop	winter wheat
Mineral and organic fertilization	1997	1997-1999
	compost – 20 t·ha^{-1}	manure – 25 t·ha^{-1}
	basalt dust – 300 kg·ha^{-1}	
	1998	mineral fertilization NPK – 70:30:120
	Compost – 15 t·ha^{-1}	(ammonium nitrate, ordinary
	peat – 5 t·ha^{-1}	superphosphate, potassium salt 50%)
	1999	
	Compost – 15 t·ha^{-1}	foliar fertilization with Ekolist
	peat – 30 t·ha^{-1}	3.5-5l·ha^{-1}
Sowing date 1997	3rd decade of April	1st decade of May
1998	3rd decade of April	3rd decade of April
1999	2nd decade of April	2nd decade of April
Cultivar	Aster (very early)	Aster (very early)
Fungicides	–	Sandofan Manco 64 WP (2 kg·ha^{-1}) Curzate M 72.5 WP (2 kg·ha^{-1})
Potato beetle control	Novodor (4 l·ha^{-1})	Bancol (0.4 kg·ha^{-1})
Weed control	mechanical (3 x earthing + 2 x harrowing)	mechanical
Tuber yield, t·ha^{-1}	14.3	21.1

The weather changes (the air temperatures and precipitation) during vegetation period over the years examined are presented in Figs 1-2. During the research period the highest precipitation sum in comparison to multi-year mean was observed in 1998. The highest air temperature was noted in 1999, also in that year very low precipitation means were noted from June until August.

Fig. 1. Air temperature distribution from April till September (1997-1999) in the area of Tuchola according to the Chojnice Meteorological Station

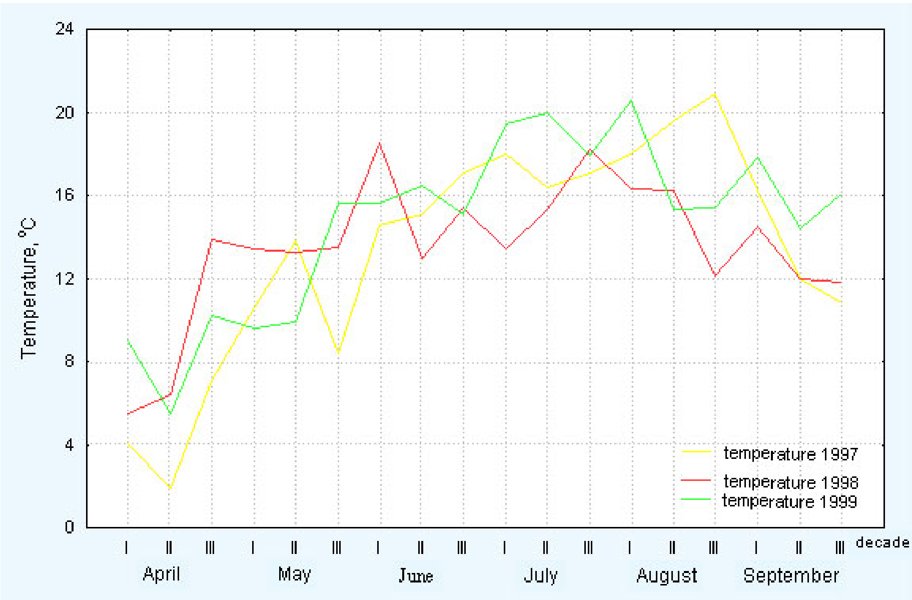
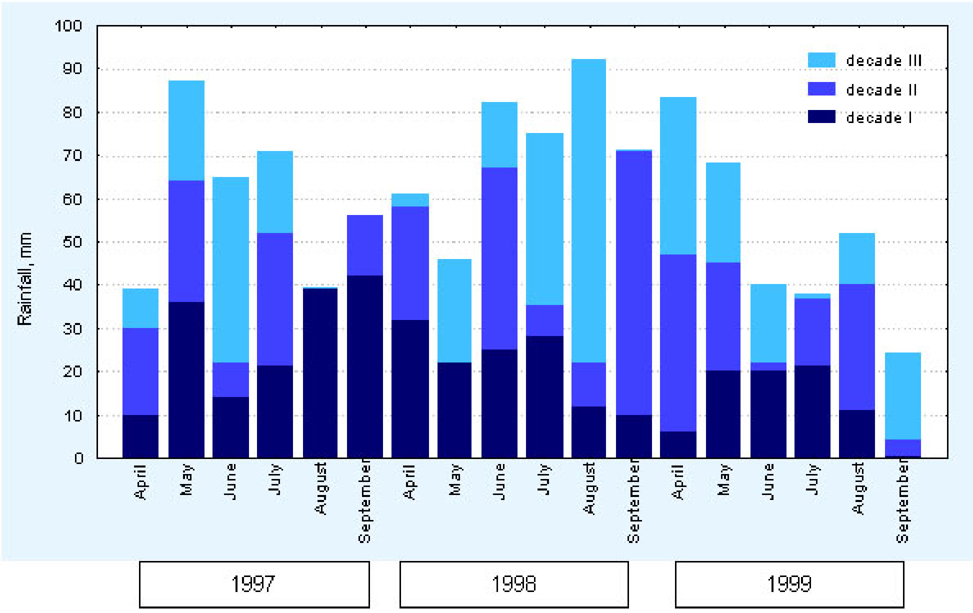


Fig. 2. Total precipitation in decades from April till September (1997-1999) in the area of Tuchola according to the Chojnice Meteorological Station



The material for analysis was sampled three times over the vegetation period at the time of emergence, flowering and tuber harvest maturity. The same number of tubers was dug out (along the field diagonal) from compared facilities so as not to disturb the roots together with their rhizosphere. The several millimetres' layer of soil directly adjacent to roots was considered as rhizosphere soil. Afterwards 1 g of rhizosphere soil was weighed out from each summary sample and shaken in 99 ml of Ringer fluid. For the determination of bacteria researched a plate method of microbiological inoculation following Koch was applied.

To define the count of bacterial groups researched a method of surface seeding was applied on selective media:

- fluorescent bacteria of *Pseudomonas* genus on the Simon & Ridge medium [26],
- bacteria of *Arthrobacte*r genus on the Hagedorn & Holt medium [10],
- bacteria of the *Azotobacter* genus on agar medium without nitrogen following Harrigan & McCance [11],
- bacteria of the coryneform group on the Seiler & Kammerbauer medium [25].

The mean values of four replications obtained from quantitative analysis cfu were variance analysed with the Student t-test (p = 0.95) using STATISTICA.

RESULTS

The count of investigated microorganisms during the study proved to be significantly differentiated according to a cropping system and potato development stage. The results concerning the counts of groups of bacteria analyzed for particular years of the research are presented in Tables 2-5, while the dynamics of their development based on the mean results of three years' observation (1997-1999) are presented in Figs 3-6.

Table 2. Number of bacteria of *Pseudomonas* genus (fluorescent) in the rhizosphere and non-rhizosphere soil of potato cultivated in the ecological and conventional farming systems

1997				1998				1999			
Date of analysis*											
I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
Rhizosphere, cfu $10^4 \cdot g^{-1}$ of dry soil											
Ecological farming											
11.2^{b**}	22.0^a	127.2^a	53.5^a	34.4^a	64.7^a	77.6^a	58.9^a	11.4^a	42.0^a	41.9^a	31.7^a
Conventional farming											
32.3^a	11.0^b	45.6^b	29.7^b	19.0^b	49.5^a	57.9^a	42.1^a	7.9^a	33.8^a	60.5^a	34.1^a
Non-rhizosphere soil, cfu $10^4 \cdot g^{-1}$ of dry soil											
Ecological farming											
1.1^a	1.1^a	11.2^a	4.5^a	7.2^a	8.6^a	9.3^a	8.4^a	16.0^a	9.5^a	18.6^a	14.7^a
Conventional farming											
2.2^a	1.1^a	11.7^a	5.0^a	2.3^b	3.3^b	3.4^b	3.0^b	8.7^a	4.8^a	15.9^a	9.8^a

* I, II, III date of microbiological analyses: I - emergence, II - flowering, III – potato tuber maturity
** mean values followed by the same letters in columns did not differ at p = 95%

Table 3. Number of bacteria of *Arthrobacter* genus in the rhizosphere and non-rhizosphere soil of potato cultivated in the ecological and conventional farming systems

1997				1998				1999			
Date of analysis											
I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
Rhizosphere, cfu $10^5 \cdot g^{-1}$ of dry soil											
Ecological farming											
44.8^b	22.1^b	130.5^a	65.8^a	95.5^a	307.5^a	430.6^a	277.9^a	34.2^a	86.7^a	233.9^a	118.3^a
Conventional farming											
110.5^a	68.9^a	96.9^a	92.1^a	56.9^b	285.9^a	301.5^b	214.8^b	39.8^a	84.5^a	147.7^a	90.7^a
Non-rhizosphere soil, cfu $10^5 \cdot g^{-1}$ of dry soil											
Ecological farming											
18.2^a	20.9^a	8.9^a	16.0^a	21.8^a	50.4^a	23.9^a	32.0^a	18.8^a	30.6^a	45.1^a	31.5^a
Conventional farming											
19.9^a	28.7^a	3.4^a	17.3^a	18.9^a	50.2^a	14.5^a	27.9^a	14.2^a	19.4^a	1.7^b	11.8^b

Table 4. Number of bacteria of *Azotobacter* genus in the rhizosphere and non-rhizosphere soil of potato cultivated in the ecological and conventional farming systems

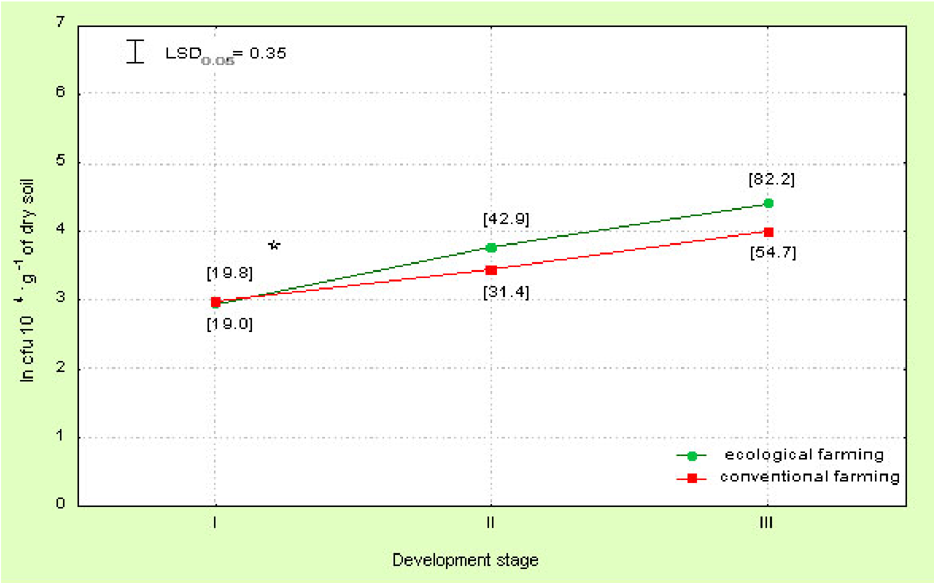
1997				1998				1999			
Date of analysis											
I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
Rhizosphere, cfu $10^{1}\cdot g^{-1}$ of dry soil											
Ecological farming											
0.0^{a}	6.0^{b}	45.0^{a}	17.0^{a}	0.0^{a}	116.0^{a}	0.0^{b}	38.7^{a}	0.0^{a}	0.0^{a}	57.0^{a}	19.0^{a}
Conventional farming											
0.0^{a}	45.0^{a}	9.0^{b}	18.0^{a}	0.0^{a}	8.0^{b}	46.0^{a}	18.0^{b}	0.0^{a}	0.0^{a}	9.0^{b}	3.0^{b}
Non-rhizosphere soil, cfu $10^{1}\cdot g^{-1}$ of dry soil											
Ecological farming											
0.0^{a}	8.0^{b}	0.0^{a}	2.7^{b}	11.0^{a}	5.0^{a}	27.0^{a}	14.3^{a}	0.0^{b}	0.0^{b}	0.0^{a}	0.0^{b}
Conventional farming											
0.0^{a}	27.0^{a}	0.0^{a}	9.0^{a}	14.0^{a}	3.0^{a}	3.0^{b}	6.6^{a}	19.0^{a}	11.0^{a}	0.0^{a}	10.0^{a}

Table 5. Number of bacteria of coryneform group in the rhizosphere and non-rhizosphere soil of potato cultivated in the ecological and conventional farming systems

1997				1998				1999			
Date of analysis											
I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
Rhizosphere, cfu $10^{5}\cdot g^{-1}$ of dry soil											
Ecological farming											
11.2^{b}	11.0^{a}	89.9^{a}	37.4^{b}	384.2^{a}	229.3^{a}	465.5^{a}	359.7^{a}	274.0^{a}	149.2^{a}	576.2^{a}	333.1^{a}
Conventional farming											
64.7^{a}	14.6^{a}	69.0^{b}	49.4^{a}	188.0^{b}	283.2^{a}	336.3^{a}	269.2^{b}	102.3^{b}	211.2^{a}	255.6^{b}	189.7^{b}
Non-rhizosphere soil, cfu $10^{5}\cdot g^{-1}$ of dry soil											
Ecological farming											
11.2^{a}	5.5^{a}	3.1^{a}	6.6^{b}	91.5^{a}	40.5^{a}	40.7^{a}	57.6^{a}	89.6^{a}	40.9^{a}	50.2^{a}	60.2^{a}
Conventional farming											
35.8^{b}	14.6^{a}	8.3^{a}	19.6^{a}	41.6^{b}	25.3^{b}	11.6^{b}	26.2^{b}	39.2^{b}	18.0^{b}	28.9^{b}	28.7^{b}

The count of bacteria of *Pseudomonas* genus (fluorescent subgroup) in investigated facilities varied from 11.2 to 127.2 x 10^{4} cfu·g^{-1} of soil dry matter (Table 2). Generally more fluorescent P*seudomonas* spp. were isolated from the rhizosphere of potato cultivated in the ecological farming system than in the conventional one. Only in two analyses over three year's observation the higher count of the bacteria was determined in the conventional farming system (at the stage of emergence in 1997 and tuber harvest maturity in 1999). A potato development stage appeared to be a significant factor affecting the count of fluorescent *Pseudomonas* spp. On the base of the mean results of three years' observation (1997-1999) dynamics of bacteria population proved to increase together with the development of vegetation, reaching the maximum in the period of tuber harvest maturity (Fig. 3). The synthesis of results also confirms that the ecological farming system enhances the development of *Pseudomonas* population in comparison with conventional farming. Significantly higher count was noted down in this system at the stage of flowering and plant maturity.

Fig. 3. Population dynamics of fluorescent *Pseudomonas* spp. in the rhizosphere of potato cultivated in the ecological and conventional farming systems



Development stage (1997-1999): I - emergence , II - flowering , III - potato tuber maturity
* the values given in brackets show actual data

Fig. 4. Population dynamics of *Arthrobacter* spp. in the rhizosphere of potato cultivated in the ecological and conventional farming systems (1997-1999)

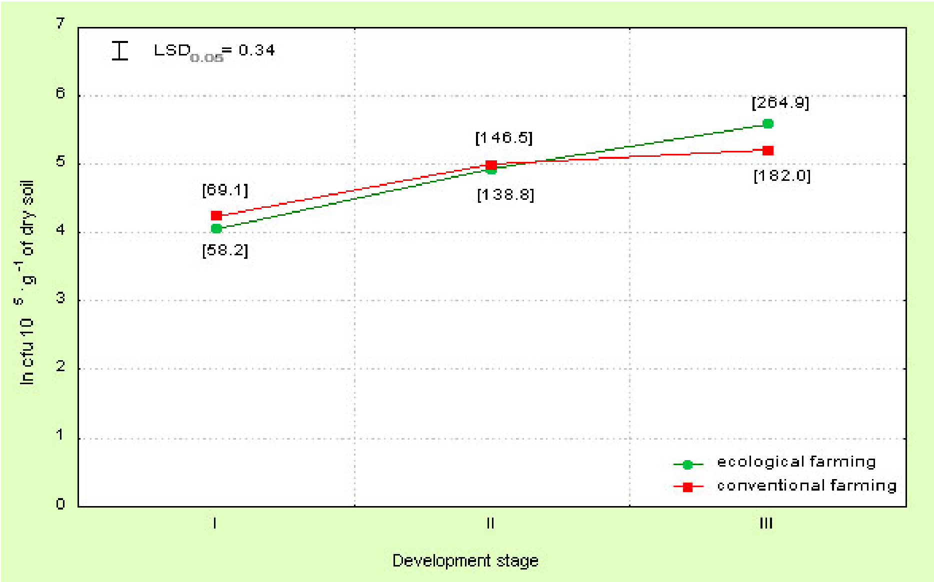


Fig. 5. Population dynamics of *Azotobacter* spp. in the rhizosphere of potato cultivated in the ecological and conventional farming systems (1997-1999)

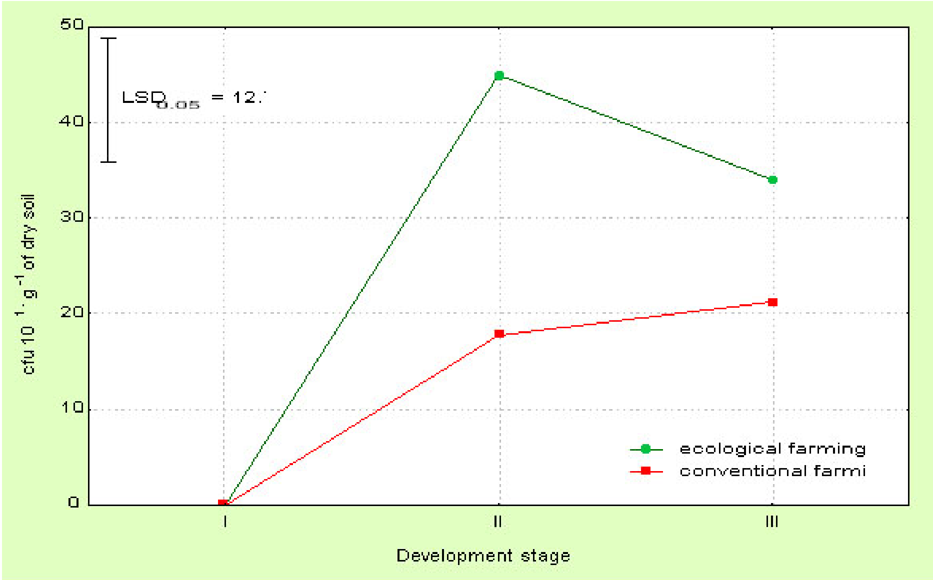
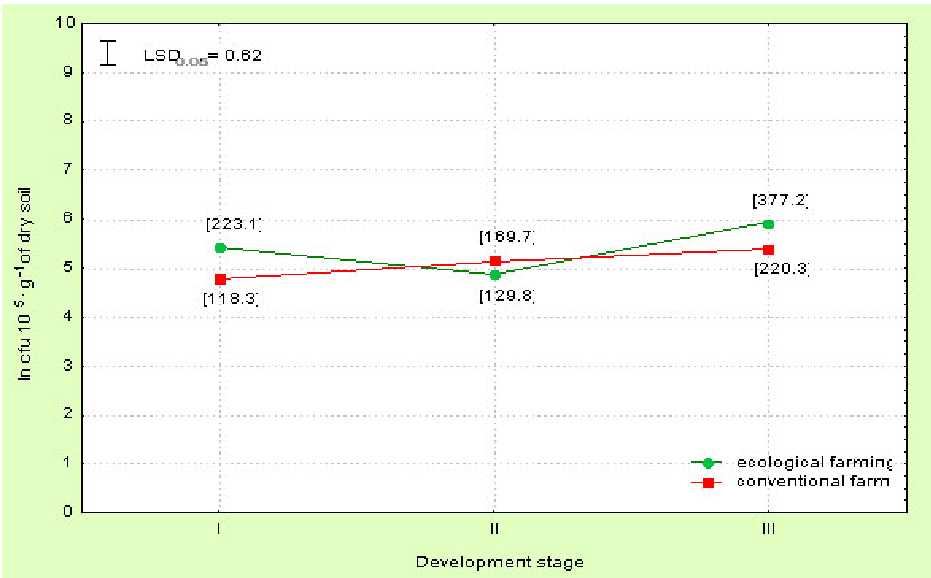


Fig. 6. Population dynamics of coryneform group in the rhizosphere of potato cultivated in the ecological and conventional farming systems (1997-1999)



Seasonal fluctuations of the number of *Arthrobacter* spp. ranged from 22.0 to 430.0 x 10^5 cfu·g^{-1} of soil dry matter (Table 3). In the first year of investigation significantly more *Arthrobacter* spp. were isolated from the conventional farming system at the stage of emergence and flowering. In 1998 and 1999, however, (except for the first date) this group of bacteria was found in greater number in the rhizosphere of potato from the ecological farm. The most favorable conditions for the development of *Arthrobacter* spp. were observed in 1998, when four times more bacteria were obtained from the ecological farm and over twice more from the conventional one than in 1997. The synthesis of results obtained during three years of the experiment shows a little higher number of *Arthrobacter* spp. in potato rhizosphere in the period from emergence till flowering in conventional farming system, while at the end of vegetation there were significantly more of them in the ecological farm (Fig. 4). Vegetation period appeared to be a significant factor affecting the count of *Arthrobacter* spp. population, just as the dynamics of *Pseudomonas* spp. The gradual increase in bacteria count was observed from potato emergence till plant maturity in both investigated farming systems.

The count of the population of *Azotobacter* spp. isolated in the soils investigated was very low and it ranged between 0 to 116 x 10^1 cfu·$g^{-1}$of soil dry matter (Table 4). The dynamics of bacteria development was characterized by a big variability and was different in each year of study. Despite using low dilutions they were not detected in several stages. In three years' study no *Azotobacter* cells were isolated during emergence period, nor in 1999 during the flowering period, irrespective of the farming system. The highest count of *Azotobacter* – 116 x 10^1 cfu – was noted down at flowering stage in 1998 in the ecological farming system. However, in spite of such a low participation of these bacteria in settling the root zone, the synthesis of the three years' study results indicates more favorable conditions for their development under the ecological farming system in comparison with the conventional one (Fig. 5).

The highest count of rhizobacteria was obtained in the case of *Corynebacterium* genus. Their amount varied from 11 to 576.2 x 10^5 cfu·g^{-1} of soil dry matter on the ecological farm and from 14.6 to 336.3 x 10^5 cfu·g^{-1} of soil dry matter on the conventional one (Table 5). The dynamics of the bacteria development was similar throughout the research period. In the ecological farming, a high amount of *Corynebacterium* spp. was noticed at the beginning of the vegetation, a strong decrease was observed during the flowering period, and then the population grew rapidly in the tuber maturity period. Gradual growth in number from the beginning till the end of the plant vegetation was typical in conventional farming in years 1998-1999. The synthesis of the 1997-1999 results confirms a significantly higher amount of investigated bacteria under ecological farming at the time of emergence and at the end of vegetation, while during the flowering period significantly more bacteria were isolated in the rhizosphere of potato grown in the conventional system (Fig. 6).

In order to show more favorable conditions for the bacteria development in rhizosphere than in the soil beyond roots, the results of the count of the groups researched in non-rhizosphere soil were also presented in Tables 2-5. The count of all the bacteria was approximately from once or twice to several times higher within the root zone than out of it.

Climatic conditions during the vegetation of potato are also essential. The most favorable conditions for the development of investigated groups of bacteria were in 1998, which is confirmed by the results shown. Total precipitation in this year was decidedly the biggest and also sufficient for the proper development of potato. The weather conditions were also reflected by the height of tuber yield obtained. The big differences in yield between the systems were the consequences of the infection time and the degree of infection of plants by potato blight. Chemical control of the fungus *Phytophthora infestans* in the conventional system extended the plant vegetation and considerably increased the yield.

DISCUSSION

The results gathered during three years' study indicate that more favorable conditions for the bacteria development were created in the rhizosphere of potato grown under the ecological system than under the conventional one. The use of a very correct technique of field-crop production in the ecological farming system resulted in a growing count of the investigated groups of bacteria. The results obtained are difficult to compare with other authors' studies. Besides the commonly prevailing opinion about positive aspects of the ecological farming system, little is known about the quantitative composition of particular groups of microorganisms.

The development of C*orynebacterium* spp. was the most strongly stimulated of all the investigated bacteria in potato rhizosphere. The results obtained did not confirm the predominant role of fluorescent *Pseudomonas* among rhizobacteria in respect of the count. Buyer and Kaufman [3] investigating the effect of cereal cropping system (conventional one and the one based only on organic fertilizing) found that bacteria of *Pseudomonas* genus were predominant among the isolated microorganisms, and the second group was *Arthrobacter*. *Arthrobacter* spp. was also strongly represented in personal studies at the level of 10^5-10^6 cfu. The population of fluorescent *Pseudomonas* in the environment researched was at the level of 10^4 cells. In their experiments, Pięta and Patkowska [22] isolated more of those bacteria from potato rhizosphere - over 10^6 cells·g^{-1} of soil. According to the literature *Pseudomonas* and *Arthrobacter* are able to colonize plant roots actively, then compete effectively with pathogens for nutrients available in root excretions, and become an essential factor for biological protection of plants [12,17,21].

The bacteria of *Azotobacter* genus were the least numerous of all groups of investigated microorganisms. The susceptibility of *Azotobacter* spp. and negative impact of mineral fertilization NPK in conventional farms can be noted on the ground of the results presented by Mazur [19]. The author reports that fertilizing with NPK resulted in the decrease in the bacteria count, while many years' use of manure influenced a big growth of the count of free nitrogen assimilators from the air. Other authors' studies indicated that the development of *Azotobacter* is influenced by physicochemical and biotic soil properties as well as pesticides applied [27,31]. In present research, the small occurrence of these bacteria in the conventional farming system may be explained by the use of unfavorably pesticides and mineral fertilization. However, not much higher count of *Azotobacter* spp. in the ecological farming system first of all confirms the impact of the soil fertility and properties. Piramowicz et al. [23] also reported a lack or very small amounts of *Azotobacter* bacteria in potato cultivation in the light soil (good rye complex), so with similar soil conditions to the ones in present research.

The results presented in the study indicate the significant effect of a potato development stage on the dynamics of development of investigated bacteria. The count of the bacteria usually increased together with the development of vegetation and reached the maximum values during tuber harvest maturity. The results of Lambert et al. [16] indicate that the bacteria of *Pseudomonas* genus predominated in the rhizosphere of cereals as well as root crops at the beginning of the vegetation. Also Vančura and Kunca [29] noticed that the development of *Pseudomonas* spp. was stimulated in rhizosphere of seedlings, and not in older plants, while *Arthrobacter* spp. developed only in the later stages of plant development. The results of our studies also show that the population of *Arthrobacter* spp. and the coryneform group occurred the most numerously in potato rhizosphere at the end of vegetation. Increasing count of bacteria together with the development of vegetation might have been caused by the changes in quantitative and qualitative composition of root excretions during plant development. Amino-acids excreted by potato roots, especially glutaminic acid and asparagine acid, as well as a vast amount of sugars, make the suitable conditions for rhizobacteria [22].

Most authors both home and abroad confirm an advantageous influence of ecological system on development of soil microorganisms [5,6,15,30]. Using the phenomenon of the antagonistic interaction between microorganisms as an alternative way of protection of plants in comparison with the chemical control is of particular importance in ecological agriculture. Therefore it seems that the microorganisms commonly recognized as potential antagonists, which have been an object of our study, are favorable indeed. The higher the count of antagonistic bacteria of *Pseudomonas, Arthrobacter, Azotobacter, Corynebacterium* due to farming conditions, the less occurrence of pathogens and weaker infection of plants we can expect. As for the phytosanitary aspect, the presence of bacteria investigated in groups of microorganisms is thought to be the measure of favorable changes taking place in soil [18].

CONCLUSIONS

1. The occurrence of investigated groups of potentially antagonistic bacteria in the potato rhizosphere appeared to differ according to the farming system. Generally more bacteria were isolated from the potato grown in the ecological system than in the conventional one.
2. The biggest differences in the dynamics of development and the count between the two compared farming systems were reported in the bacteria of *Azotobacter* genus and *Corynebacterium* genus.
3. A significant factor affecting the amount of populations of the investigated groups of bacteria was a period of vegetation and fenological stage of plant development. The considerably highest level of the count was noticed at the end of the vegetation, except for *Azotobacter* spp. which highest count was obtained in the ecological farming system during flowering.
4. The predominant group among the rhizobacteria researched was the coryneform group, and then *Arthrobacter* spp. Fluorescent bacteria of *Pseudomonas* genus were less numerous. The rhizosphere of potato made the least favorable conditions for the development of bacteria of *Azotobacter* spp.

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