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Impact of biofertilizers on the rhizosphere microflora in pepper cultivated as organic farming system

Veselka Vlahova and Vladislav Popov*

Agricultural University, Faculty of Plant Protection and Agroecology,4000 Plovdiv, Bulgaria
**Corresponding author:* vpopov_bg@yahoo.com

Abstract

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Organic fertilizers have a positive effect on soil biogenicity, its physicochemical properties, as they increase the content of soil microorganisms. This paper aims to investigate the effect of selected biofertilizers on one of the agroecological parameters, namely the changes in the population of the rhizosphere microflora. The experiment was conducted in 2009-2011 at the Agroecological Center at the Agricultural University – Plovdiv with the variety of pepper Sofiiska Kapia. The above was observed for the combination of biofertilizers with the two basic fertilizations, as well as for the independent application in optimum concentration. The applied biofertilizers as nutrition additionally stimulated the development of microorganisms, which was expressed best for Emosan, Baikal, and Bio One. The change in the development of microbial populations may affect the quantity of accessible nutrients absorbable by pepper and may enrich the soil cenosis by improving soil fertility.

Key words: biofertilizers; organic agriculture; pepper; rhizosphere microflora

Introduction

In recent decades, there has been a growing interest in organic farming as an alternative to intensive fertilization with mineral fertilizers. Furthermore, it has been considered a prerequisite for soil protection and a logical consequence for ensuring safe and healthy food. One of the directions of organic farming is the need to preserve the local biological variety and obtain optimal production, with a low environmental risk and upon using environmentally friendly technologies. One of the solutions proposed for dealing with environmental and human health protection issues is the implementation of natural technologies of plant cultivation and fertilization through the applications of biofertilizers (Mosa et al., 2018). Biofertilizers are considered to be an important alternative source of plant nutrition and an essential component of organic farming (Kawalekar, 2013; Roychowdhury et al., 2014), as they have been gaining importance in sustainable agriculture (Brahmaprakash & Sahu, 2012). Biofertilizers refurbish the natural nutrient cycles in the soil, build soil organic matter (Raffi et al., 2018) and improve soil microbial properties (Araújo et al., 2014).

Pepper is highly demanding in regards of the nutrition and water regime, as well as in having optimal agroecological conditions ensured in the agroecosystem. Organic fertilizers have a positive effect on soil biogenicity, its physicochemical properties, as they increase the content of soil microorganisms (Anonymous, 2007; Kostadinova & Popov, 2012) that improve the microbiological properties of the soil (Ayoola & Makinde, 2007). The amount and distribution of the organic matter in the soil depends on its migration (Dimitrov, 1971; Vessey, 2003) and is a key element in the distribution and depletion of nutrients (Daudu et al., 2006). Organic matter improves the physico-chemical characteristics of the soil, as it gives a better structure to the soil particles and improves the water retention capacity (Srinivasan et al., 2005; Araújo et al., 2009).

Microorganisms are an integral part of ecological systems in nature (Denchev & Tsekova, 2001; Kuzmanova &

Sapundzhieva, 2011). There is a wide variety of soil microorganisms that thrive in the soil environment, especially in the plant rhizosphere (Wu et al., 2005). A significant number of bacteria and fungi are known to have functional connections and interactions with plants, forming a holistic system and being able to exert beneficial effects on the soil and plant growth (Karem et al., 2000; Ghosh, 2009; Mostafa & Abo-Baker, 2010). The role that soil microorganisms play in the decomposition of organic matter and in the food cycle is important for the management of soil fertility in agriculture (Bing- Ru et al., 2006; Elbanna & Atalla, 2010). Microbial biomass is also a supply of nutrients for plants and determines the soil structure. The interaction between microorganisms in the rhizosphere, roots and soil microorganisms has a significant role in plant growth and development (Tringovska & Naydenov, 2003). The development of rich rhizosphere microflora helps for the release of mineral elements from the solid phase of the soil (Kirchmann & Bergström, 2008; Maggio et al., 2008).

The interest in the Associative Nitrogen Fixation (ANF) with non-leguminous species is explained by the desire to absorb atmospheric nitrogen in return for the use of polluting nitrogen fertilizers (Stancheva, 2000). The microbial mobilization of phosphorus in the soil plays a role in the root nutrition of plants and has real significance for the rhizosphere zone (Nedyalkova & Taleva, 1995; Malusa & Sas, 2009). Fertilization is a factor that affects the productivity of plants and the efficiency of photosynthetic apparatus. Intensified photosynthetic and metabolic processes in plants contribute to soil enrichment with root exudates that serve as nutrient elements for soil microorganisms. There is a constant growth of the share of biologically managed agricultural land, and an increasing number of organic farms apply the principles and methods applicable for organic farming. Therefore, scientific researches focus on the application of biofertilizers and their impact on the vegetative growth of pepper plants (Vlahova et al., 2014; Vlahova & Popov, 2014b), higher yields (Vermany, 2007; Vlahova & Popov, 2014a), improvement of fruit quality (Altieri and Nicholls, 2003; Kuzmanova et al., 2003), higher levels of Vitamin C (Vlahova & Popov, 2013; Vlahova, 2014), and stabilization of soil fertility in the agroecosystem (Vlahova & Popov, 2014a; Vlahova et al., 2015), as well as sustainable development in rural areas (Popova, 2019a; Popova, 2019b).

Many authors point out that biofertilizers have a positive effect on crops and act as a tool for agroecosystem stabilization, and that their impact has a slow (Karem et al., 2000; Kartalska et al., 2003; Tringovska & Naydenov, 2003; Boteva & Cholakov, 2011; Mosa et al., 2014; Raffi, 2018) but yet explicit effect with regard to soil fertility management due to its effect on the separate groups of soil microorganisms living in the rhizosphere zone of plants.

This paper aim was to investigate the effect of selected biofertilizers on one of the agroecological parameters, namely the changes in the population of the rhizosphere microflora.

Material and Methods

The experiment was carried out in 2009-2011 at the Agroecological Center at the Agricultural University – Plovdiv with the variety of pepper Sofiiska Kapia. The studied biofertilizers were selected on the basis of the permitted substances for soil fertilization according to Commission Regulation (EC) No 889/2008. The experiment was carried out according to the method of long plots, in four replicates, with a size of the experimental plot of 9.6 m^2 with a scheme of 120 + 60x15 cm. Biofertilizers – Boneprot and Lumbrical, were used as the main fertilizer and were introduced into the rows by incorporation, before planting the plants in two concentrations – optimal and reduced by 50%. The optimum concentrations corresponded to: 70 kg/da for basic fertilization with Boneprot, 400 l/da for basic fertilization with Lumbrical. During vegetation the biofertilizers Baikal, Seasol, Emosan, and Bio One were imported as soil fertilization, twice – in the phenophase 'flower bud' and 'after mass formation of flowering', in the following concentrations: a solution at a concentration of 1:1000 (Baikal); a solution at a concentration of 1: 500 – 0.3-0.4 l/da (Seasol); a solution of 15 l/da (Emosan); a solution at a concentration of 165 ml/da (Bio One). The Demonstration experimental field at the Agroecological Center of the Agricultural University-Plovdiv is located at the eastern end of the city of Plovdiv. In terms of climate, Plovdiv (24°45´) belongs to the Transitional- Continental Climate Subregion of Bulgaria and is 160 m above sea level.

Study Parameters: *Microbiological analysis*

Soil samples were taken from the rhizosphere of plants and analyzed for up to 24 hours. The microbiological analysis was performed three times: the first sample was taken before the application of Boneprot and Lumbrical, as basic fertilization, to determine the initial state of the microbiological activity; the second sample was taken 10 days after application of liquid biofertilizers; and the third sample was taken in the beginning of September. Soil microbial activity included the determination of: a total number of bacteria; cellulose decomposing bacteria and microscopic fungi in 1g soil. Soil microflora quantity was established according to the classic method of decimal dilutions, through cultures on their relevant growing-friendly nutrient medium – a total number of bacteria of TSA; microscopic soil fungi in Chapek's nutrient medium; cellulose decomposing bacteria in Hutchinson's nutrient medium (Kuzmanova et al., 1995; Sapundzhieva et al., 2010).

Characteristics of the biofertilizers

Boneprot is pellet organic fertilizers, and has following composition: N – 4.5 %; P_2O_5 – 3.5 %; K_2O – 3.5 %; C of biological origin- 30 %; humidity – 13-15%; pH in water – 6- 8.

Lumbrical is a product obtained from processing natural organic manure and other organic waste by the *Lumbricus rubellus* and *Eisenia foetida* and consists of their excrements. It has humidity – 45-55 %; organic matter content – 45-50 %; NH_4N-33.0 ppm; P_2O_5 – 1410 ppm.

Baikal has the following content: effective microorganisms, mixed cultures of useful microorganisms, which are antagonists with respect to the pathogenic and conditionally pathogenic microflora. It has the composition: Organic C – 0.15%; N – 0.01%; P_2O_2 – 0.001; pH – 3.2.

Seasol is an extract of brown algae *Durvillaea potatorum*. It contains 60% of alginic acids; raw protein (2.5 ± 0.1% w/w); alginates (6 ± 2% w/w); N (0.10 ± 0.05% w/w), P (0.05 ± 0.02% w/w), K (2.0 ± 0.5% w/w), pH (10.5 ± 0.5% w/w).

Emosan contains total nitrogen – 5 %; organic N – 5 %; organic C – 14 %; protein – 34 p/p; humidity- 65 p/p; P – 0.06 p/p; pH – 7.0-10.0.

Bio One consists of living organisms (aerobic – *Azotobacter vinelandii*, and anaerobic – *Clostridium pasteurianum*) and is 100% natural liquid concentrated microbiological product.

The statistical processing of the experimental data was performed using SPSS V. 9.4 for Microsoft Windows, by Duncan, Anova. A Duncan multiple – range test was also performed to identify the homogeneous type of the data sets among the different treatments at P < 0.05 level (Duncan, 1955).

Results and Discussion

As regards the variety of pepper Sofiiska Kapia, the data for the total number of bacteria in July 2009 showed that upon combined application of a biofertilizer and basic fertilization with Boneprot, the highest value was reported for the variant with Emosan followed by Baikal, which was confirmed in 2010 and 2011. The stimulating effect of these two biofertilizers increased the quantity of the total number of bacteria and intensified the biological activity of the soil complex (Table 1). Upon testing biofertilizers on basic fer-

Table 1. Data on the total number of bacteria (Number CFU/g soil x 10^6), 2009- 2011

Variants	2009			2010			2011		
	June (First reporting)	July (Second reporting)	September (Third reporting)	June (First reporting)	July (Second reporting)	September (Third reporting)	June (First reporting)	July (Second reporting)	September (Third reporting)
		Average ± St.dev			Average ± St.dev			Average ± St.dev	
Control	1.5	5.0 ± 0.265 ^{f}	4.8 ± 0.503 ^{d}	1.8	5.7 ± 0.569 ^{d}	5.5 ± 0.207 ^{d}	1.7	5.6 ± 0.208 ^{e}	3.1 ± 0.208 ^{e}
Boneprot (opt.)	1.5	7.9 ± 0.208 ^{d}	7.4 ± 0.757 ^{bc}	1.8	8.4 ± 0.206 ^{c}	8.0 ± 0.208 ^{c}	1.7	8.2 ± 0.252 ^{d}	7.8 ± 0.208 ^{c}
Boneprot (50%) + Baikal	1.5	9.5 ± 0.404 ^{c}	9.8 ± 0.231 ^{a}	1.8	10.4 ± 0.100 ^{b}	10.9 ± 0.153 ^{ab}	1.7	10.0 ± 0.208 ^{c}	10.8 ± 0.208 ^{a}
Boneprot (50%) + Seasol	1.5	5.3 ± 0.351 ^{f}	5.1 ± 0.874 ^{d}	1.8	6.1 ± 0.265 ^{d}	5.5 ± 0.513 ^{d}	1.7	5.8 ± 0.321 ^{e}	5.2 ± 0.208 ^{d}
Boneprot (50%) + Emosan	1.5	10.8 ± 0.153 ^{a}	9.8 ± 0.808 ^{a}	1.8	11.8 ± 0.557 ^{a}	10.2 ± 0.624 ^{b}	1.7	11.6 ± 0.208 ^{a}	10.9 ± 0.153 ^{a}
Boneprot (50%) + Bio One	1.5	8.6 ± 0.100 ^{d}	7.2 ± 0.379 ^{bc}	1.8	9.1 ± 0.252 ^{ab}	8.7 ± 0.115 ^{b}	1.7	8.9 ± 0.351 ^{cd}	8.3 ± 0.200 ^{b}
Lumbrical (opt.)	1.5	7.0 ± 0.265 ^{e}	6.8 ± 0.264 ^{c}	1.8	8.2 ± 0.624 ^{c}	7.9 ± 0.265 ^{c}	1.7	8.0 ± 0.100 ^{d}	7.7 ± 0.321 ^{c}
Lumbrical (50%) + Baikal	1.5	8.0 ± 0.208 ^{d}	7.0 ± 0.306 ^{bc}	1.8	9.6 ± 0.100 ^{ab}	9.0 ± 0.300 ^{b}	1.7	9.0 ± 0.200 ^{cd}	8.2 ± 0.115 ^{b}
Lumbrical (50%) + Seasol	1.5	9.4 ± 0.586 ^{c}	8.0 ± 0.153 ^{b}	1.8	10.3 ± 0.265 ^{b}	10.0 ± 0.289 ^{b}	1.7	10.1 ± 0.208 ^{c}	9.7 ± 0.208 ^{ab}
Lumbrical (50%) + Emosan	1.5	10.1 ± 0.473 ^{b}	10.2 ± 0.351 ^{a}	1.8	11.8 ± 0.458 ^{a}	12.0 ± 0.061 ^{a}	1.7	10.8 ± 0.153 ^{b}	10.9 ± 0.265 ^{a}
Lumbrical (50%) + Bio One	1.5	11.0 ± 0.200 ^{a}	9.6 ± 0.289 ^{ab}	1.8	13.0 ± 0.100 ^{a}	10.7 ± 0.321 ^{ab}	1.7	12.0 ± 0.200 ^{a}	10.5 ± 0.100 ^{a}

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test tilization with Lumbrical, its highest value per total number of bacteria was reported for the Emosan variant following by Bio One, which was confirmed in 2010 and 2011. The above gave us grounds to believe that combined variants provided an opportunity for a higher microbial activity, which was probably due to the contents of the introduced biofertilizers and their combination with the basic fertilization. The reporting in September revealed higher microbial activity in the end of the vegetation with Baikal with basic fertilization Boneprot and Emosan with basic fertilization Lumbrical, which was applicable for the three-year period of the study.

The data about the number of microscopic fungi in July 2009 showed a maximum value reported for the location of Seasol upon basic fertilization with Lumbrical – 4.0x10^3 CFU g^{-1}, which was confirmed in 2010 and 2011 (Table 2). Very good values were also reported upon application of Baikal combined with Lumbrical, which has been of confirmative nature throughout the years of study. Out of all tested fertilizers on basic fertilization with Boneprot, the highest values of the reviewed indicators were reported for Seasol, followed by Bio One, which was of a confirmative nature during the three-year period. The active development of microscopic fungi for the biofertilizer Seasol coincided with the lower pH value reported for the agrochemical analysis (Vlahova & Popov, 2014a). It was established that all tested biofertilizers on basic fertilization with Lumbrical were characterized with higher values of the number of microscopic fungi, which was applicable for the three-year period of the experiment. Upon comparing the results from the independent introduction of biofertilizers in optimum concentration, a higher activity was found for the number of microscopic fungi in Lumbrical (2009, 2010, 2011) as such higher microbial activity had its beneficial role for the next crops to utilize the substances synthesized by microorganisms. The results obtained in September determined a tendency towards an increase of the number of microscopic fungi in all variants on basic fertilization with Boneprot, as compared to the reported values in July, which was probably due to the contents of Boneprot and to its granular appearance, which dissolves more slowly and activates the rhizosphere microflora upon reaching the root system of plants. There is an increase observed of the indicator under review, which was expressed most clearly in Baikal and Seasol on the two types of basic fertilizations, being of confirmative nature for the three-year period.

Based on the data presented above, it becomes clear that the tested biofertilizers had a positive effect on cellulose decomposing bacteria being a material indicator of the soil microbial activity. The highest value was reported in July 2009 for Seasol (18.4x10^3 CFUg^{-1}) on basic fertilization with

Table 2. Data on the number of microscopic fungi (Number CFU/g soil x 10^3), 2009- 2011

Variants	2009			2010			2011		
	June (First reporting)	July (Second reporting)	September (Third reporting)	June (First reporting)	July (Second reporting)	September (Third reporting)	June (First reporting)	July (Second reporting)	September (Third reporting)
		Average ± St.dev			Average ± St.dev			Average ± St.dev	
Control	0.9	1.3 ± 0.208 ^{d}	1.1 ± 0.153 ^{e}	1.3	1.6 ± 0.208 ^{d}	1.3 ± 0.173 ^{f}	1.1	1.4 ± 0.058 ^{f}	1.2 ± 0.058 ^{f}
Boneprot (opt.)	0.9	1.6 ± 0.173 ^{d}	1.7 ± 0.115 ^{d}	1.3	1.9 ± 0.208 ^{d}	2.1 ± 0.208 ^{e}	1.1	1.8 ± 0.115 ^{e}	1.9 ± 0.058 ^{e}
Boneprot (50%) + Baikal	0.9	1.8 ± 0.153 ^{d}	2.4 ± 0.200 ^{c}	1.3	2.1 ± 0.300 ^{c}	2.7 ± 0.208 ^{d}	1.1	2.0 ± 0.153 ^{d}	2.6 ± 0.100 ^{d}
Boneprot (50%) + Seasol	0.9	2.5 ± 0.173 ^{c}	2.6 ± 0.153 ^{c}	1.3	2.8 ± 0.208 ^{c}	3.1 ± 0.115 ^{d}	1.1	2.6 ± 0.208 ^{d}	2.9 ± 0.115 ^{d}
Boneprot (50%) + Emosan	0.9	1.5 ± 0.100 ^{d}	1.6 ± 0.208 ^{d}	1.3	1.8 ± 0.200 ^{d}	2.0 ± 0.115 ^{e}	1.1	1.6 ± 0.115 ^{ef}	1.8 ± 0.115 ^{e}
Boneprot (50%) + Bio One	0.9	2.2 ± 0.153 ^{c}	2.4 ± 0.058 ^{c}	1.3	2.5 ± 0.100 ^{c}	2.6 ± 0.173 ^{d}	1.1	2.4 ± 0.153 ^{d}	2.5 ± 0.058 ^{d}
Lumbrical (opt.)	0.9	3.4 ± 0.208 ^{b}	3.6 ± 0.208 ^{b}	1.3	3.8 ± 0.208 ^{b}	3.9 ± 0.100 ^{b}	1.1	3.7 ± 0.115 ^{b}	3.8 ± 0.058 ^{b}
Lumbrical (50%) + Baikal	0.9	3.8 ± 0.100 ^{a}	4.4 ± 0.153 ^{a}	1.3	4.0 ± 0.153 ^{a}	4.6 ± 0.115 ^{a}	1.1	3.9 ± 0.231 ^{a}	4.2 ± 0.200 ^{a}
Lumbrical (50%) + Seasol	0.9	4.0 ± 0.208 ^{a}	4.4 ± 0.100 ^{a}	1.3	4.3 ± 0.208 ^{a}	4.7 ± 0.100 ^{a}	1.1	4.2 ± 0.058 ^{a}	4.5 ± 0.100 ^{a}
Lumbrical (50%) + Emosan	0.9	2.8 ± 0.208 ^{c}	2.9 ± 0.100 ^{c}	1.3	3.5 ± 0.153 ^{b}	3.6 ± 0.115 ^{c}	1.1	3.3 ± 0.115 ^{c}	3.5 ± 0.115 ^{c}
Lumbrical (50%) + Bio One	0.9	3.1 ± 0.264 ^{b}	3.0 ± 0.242 ^{b}	1.3	3.6 ± 0.115 ^{b}	3.4 ± 0.153 ^{c}	1.1	3.5 ± 0.200 ^{b}	4.0 ± 0.153 ^{a}

*Means followed by the same letter are not statistically different ($P<0.05$) by Duncan's multiple range test

Table 3. Data on the cellulose decomposing bacteria- (Number CFU/g soil x 10 ^{3}), 2009- 2011

Variants	2009			2010			2011		
	June (First reporting)	July (Second reporting)	September (Third reporting)	June (First reporting)	July (Second reporting)	September (Third reporting)	June (First reporting)	July (Second reporting)	September (Third reporting)
		Average ± St.dev			Average ± St.dev			Average ± St.dev	
Control	3.0	5.7 ± 0.153 ^{g}	5.5 ± 0.115 ^{f}	3.2	5.9 ± 0.100 ^{f}	5.7 ± 0.173 ^{f}	3.4	6.0 ± 0.100 ^{g}	5.6 ± 0.173 ^{f}
Boneprot (opt.)	3.0	8.0 ± 0.115 ^{e}	8.1 ± 0.100 ^{d}	3.2	8.3 ± 0.100 ^{d}	8.3 ± 0.153 ^{d}	3.4	8.3 ± 0.100 ^{e}	8.4 ± 0.058 ^{d}
Boneprot (50%) + Baikal	3.0	9.1 ± 0.208 ^{de}	11.6 ± 0.404 ^{c}	3.2	9.2 ± 0.208 ^{d}	11.2 ± 0.529 ^{c}	3.4	9.4 ± 0.265 ^{de}	11.7 ± 0.252 ^{c}
Boneprot (50%) + Seasol	3.0	12.5 ± 0.115 ^{c}	12.4 ± 0.208 ^{c}	3.2	12.6 ± 0.115 ^{c}	12.5 ± 0.153 ^{c}	3.4	12.6 ± 0.115 ^{d}	12.5 ± 0.100 ^{c}
Boneprot (50%) + Emosan	3.0	7.2 ± 0.058 ^{f}	7.6 ± 0.231 ^{e}	3.2	7.4 ± 0.115 ^{e}	7.6 ± 0.208 ^{e}	3.4	7.5 ± 0.115 ^{f}	7.8 ± 0.153 ^{e}
Boneprot (50%) + Bio One	3.0	11.2 ± 0.173 ^{d}	10.5 ± 0.300 ^{cd}	3.2	11.4 ± 0.100 ^{cd}	10.8 ± 0.200 ^{c}	3.4	11.5 ± 0.153 ^{de}	11.1 ± 0.200 ^{c}
Lumbrical (opt.)	3.0	15.5 ± 0.153 ^{b}	13.4 ± 0.153 ^{b}	3.2	15.7 ± 0.115 ^{b}	13.5 ± 0.153 ^{b}	3.4	15.8 ± 0.153 ^{b}	13.7 ± 0.100 ^{b}
Lumbrical (50%) + Baikal	3.0	14.5 ± 0.100 ^{b}	16.1 ± 0.115 ^{a}	3.2	14.8 ± 0.100 ^{b}	16.2 ± 0.379 ^{a}	3.4	14.8 ± 0.200 ^{b}	16.2 ± 0.100 ^{a}
Lumbrical (50%) + Seasol	3.0	18.4 ± 0.100 ^{a}	19.2 ± 0.173 ^{a}	3.2	18.6 ± 0.173 ^{a}	19.3 ± 0.306 ^{a}	3.4	18.8 ± 0.153 ^{a}	19.5 ± 0.100 ^{a}
Lumbrical (50%) + Emosan	3.0	11.9 ± 0.058 ^{d}	13.5 ± 0.100 ^{b}	3.2	12.8 ± 0.115 ^{c}	13.5 ± 0.100 ^{b}	3.4	13.8 ± 0.153 ^{c}	13.6 ± 0.115 ^{b}
Lumbrical (50%) + Bio One	3.0	16.1 ± 0.100 ^{a}	15.8 ±0.100 ^{a}	3.2	16.1 ± 0.208 ^{a}	15.9 ± 0.153 ^{a}	3.4	16.3 ± 0.153 ^{a}	16.1 ± 0.100 ^{a}

*Means followed by the same letter are not statistically different ($P<0.05$) by Duncan's multiple range test

Lumbrical followed by Bio One (16.14x10^3 CFUg^{-1}), which was of confirmative nature in the next couple of years (Table 3). The efficiency of these biofertilizers is leading whenever also applied on Boneprot and whenever the confirmation element is also present. All combined variants of a biofertilizer and basic fertilization have higher values whenever applied on Lumbrical. There was activation of cellulose decomposing bacteria reported in September for basic fertilization with Boneprot for the variants Baikal and Emosan (2009, 2010, 2011), and on basic fertilization with Lumbrical for the variants Baikal and Seasol (2009, 2010, 2011). Higher activity was reported for the biofertilizer Seasol on the two basic fertilizations, which was due to the contents of the fertilizer as a suitable nutrition component for cellulose decomposing bacteria, thus resulting in the increase of their number. It was found that the combined variants of biofertilizers provided an opportunity for a more active microbial activity as compared to the control. Greater activity of cellulose decomposing bacteria as found for all variants with biofertilizers on basic fertilizations with Lumbrical, which was determined as more favorable nutrient medium for them.

It was found that biofertilizers introduced as fertilization and added biofertilizers contained nutrients easily absorbable for microorganisms and stimulated the development of soil microflora. The above was observed for the combination of biofertilizers with the two basic fertilizations, as well as for the independent application in optimum concentration. The introduced biofertilizers for nutrition additionally stimulated the development of microorganisms, which was expressed best for Emosan, Baikal, and Bio One. It was found that the addition of Seasol biofertilizer stimulated the development of cellulose decomposing bacteria and microorganisms immobilizing mineral nitrogen. This was probably due to the carbon available to the microorganisms contained in the organic product, which is confirmed by the results for the amount of nitrogen in the soil. Since the nitrogen in the fertilizers used is in organic form, its absorption by plants depends entirely on the active activity of the bacteria involved in its mineralization. It was found that Boneprot used as basic fertilization, had a stimulating effect on soil bacteria.

The balance of agroecosystems is of great importance for organic agriculture. The balanced development of the different physiological groups of types of microorganisms under equal climatic conditions is determined based on the chemical composition of soil. The management of the ecological balance of microbial groups is most important to soil fertility. The conservation of fertility is achieved with the proper choice of biofertilizers of suitable contents and required ratio. The development of microorganisms is also affected by the mechanical structure of biofertilizers. Lumbrical not only introduces nutrient elements to the soil but also improves the soil structure. This effect has a more prolonged period of validity. The biofertilizers Baikal and Bio One are microbial preparations, the addition of which to the soil does not change its chemical composition. Their action is related to the development of the microorganisms contained in them. In the biofertilizer Bio One, these are the nitrogen-fixing microorganisms *Azotobacter* and *Clostridium*, which are able to fix atmospheric nitrogen and stimulate plant growth by releasing biologically active substances. In the experiment, their nitrogen-fixing ability was shown in basic fertilization with Lumbrical, where the C: N ratio was much higher than in basic fertilization with Boneprot (Vlahova & Popov, 2014a).

Conclusions

In general, there were similar trends in the influence of individual biofertilizers on different groups of microorganisms for the three-year period of the experiment, as the amount of bacteria in the soil was best affected by the application of the biofertilizer Emosan on the two basic fertilizations, as well as the biofertilizer Baikal by the basic fertilizations Boneprot and Bio One on basic fertilization with Lumbrical. The change in the development of microbial populations may affect the quantity of accessible nutrients absorbable by pepper and may enrich the soil cenosis by improving soil fertility.

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