

INFLUENCE OF BIOFERTILISERS ON THE VEGETATIVE GROWTH, MINERAL CONTENT AND PHYSIOLOGICAL PARAMETERS OF PEPPER (*Capsicum annuum* L.) CULTIVATED UNDER ORGANIC AGRICULTURE CONDITIONS

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Abstract. In recent years, biofertilisers have emerged as a promising component of an integrated nutrient supply system in agriculture. The objective of this study was to examine the influence of selected biofertilisers on the vegetative growth, the content of N, P₂O₅ and K₂O in the leaves and stems, and on the physiological parameters of pepper of the variety of ‘Sofiiska Kapiya’ cultivated under organic agriculture conditions. This experiment was carried out from 2009 to 2011 on the experimental fields of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria), situated on the territory of a certified ecological farm. The study included the following biofertilisers – Boneprot, Lumbrical, Baikal EM “Effective Microorganisms”, Emosan, and Bio One. The results of the biometric measurements of the average plant height at the end of the vegetative period showed the highest values for the variant treated with Emosan on the Boneprot basic fertilisation (62.60 cm – 2009; 64.80 cm – 2010, and 63.87 cm – 2011). Upon feeding with the biofertilisers Emosan and Baikal EM on basic fertilisation with Boneprot (2009, 2010 and 2011) at the pepper mass fruit yield stage, plants showed higher values of net photosynthesis (P_N) that were also similar to the high values observed in the flower bud stage. The highest intensity of transpiration (E) was observed for the variants treated with the biofertilisers Baikal EM (2009 and 2011) and Emosan (2010) on basic fertilisation with Boneprot. It was concluded that the feeding with Emosan stimulated the vegetative growth of the pepper plants due to the high concentrations of nutrient-providing proteins contained in this biofertiliser. The results showed that biofertilisers do not significantly impact the P content of the pepper leaves and stems, but changes were more obvious in the leaves. The fertilisation with the studied biofertilisers increased the K₂O content in leaves and stems compared to the control (non-fertilised) plants; the values were higher for the leaves.

Key words: biofertilisers, *Capsicum annuum* L., mineral content, organic agriculture, physiological parameters, vegetative growth

INTRODUCTION

In recent years, biofertilisers have emerged as a promising component of integrated nutrient supply system in agriculture [Shehata and El Khawas 2003]. Biofertilisers are ready to use formulation of beneficial microorganisms, when amended to seed, root or soil, they mobilize the availability of microorganisms and thus soil health [Topre et al. 2011]. Biofertilisers, in strict sense, are not fertilizers, which provide directly nutrients to crop plants. These are cultures of microorganisms such as bacteria, fungi, etc. packed in a carrier material. Thus, the critical inputs in biofertilisers are the microorganisms. They help plants indirectly through better Nitrogen (N) fixation or by improving the nutrient availability in the soil [Boraste et al. 2009].

Nitrogen stimulates the growth of the vegetative mass [Rankov et al. 1983]. Doykova [1996] has found that plants supplied with sufficient nutrients form more nodes and a larger number of leaves. Yacheva [1981] points out that the size of the vegetative mass is of a great significance for the productivity of vegetables. Increase of the quantity of mineral fertilisers results in an increase of the absorbing root surface, of the leaf surface, as well as of the total vegetative mass. Frederickson et al. [2007] point out that Atiyeh et al. [1999], Buckerfield et al. [1999], Edwards and Burrows [1988] have investigated the characteristics of vermicompost derived from various feedstock and the effect of vermicompost on plant growth. In order to achieve optimization of the nutritional regime of plants cultivated under the conditions of organic production, there is a necessity of new and complex information about the influence of soil supplements (organic-based biofertilisers) on the vegetative and physiological parameters of pepper. Many investigators have tried to use biofertilisers containing free living bacteria in order to increase the N content of the soil and consequently N uptake by plants to improve vegetative growth parameters [Abd El-Hakeem Saad 2003]. The Bio-N fertiliser has improved the vegetative growth, yield and quality of sweet pepper plants [Fawzy et al. 2012].

The objective of this study was to examine the influence of selected biofertilisers on the vegetative growth, the content of mineral elements (N, P₂O₅ and K₂O) in the formed vegetative mass, i.e. leaves and stems, and on the physiological parameters of pepper of the variety of 'Sofiiska Kapiya' cultivated under organic agriculture conditions.

MATERIALS AND METHODS

This experiment was carried out from 2009 to 2011 on the experimental fields of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria), situated on the territory of a certified ecological farm.

Treatments (variants). Control (non-fertilised); 2. Basic fertilisation with Boneprot (optimum concentration); 3. Basic fertilisation with Boneprot (50%) + Baikal EM; 4. Basic fertilisation with Boneprot (50%) + Emosan; 5. Basic fertilisation with Boneprot (50%) + Bio One; 6. Basic fertilisation with Lumbrical (optimum concentration); 7. Basic fertilisation with Lumbrical (50%) + Baikal EM; 8. Basic fertilisation with Lumbrical (50%) + Emosan; 9. Basic fertilisation with Lumbrical (50%) + Bio One.

Vegetable tested. The research examined the pepper variety of ‘Sofiiska Kapiya’ grown as mid-early field production according to the principles of organic agriculture [Panayotov 2000]. The plants were grown from seedlings in a polyethylene greenhouse. The seedlings were planted on a permanent place during the third decade of May, on a high-levelled seed-bed, according to the planting scheme 120 + 60 × 15 cm. The experiment was done according to the method of long plots, into four replications, with a size of the test plot of 9.6 m². Irrigation was carried out via a drip-irrigation installation.

Fertilisation. Two basic fertilisations were used, namely Boneprot and Lumbrical. They were applied into the soil through incorporation prior to planting of the seedlings on the field. They were applied in two concentrations – optimum (corresponded to 700 kg·ha⁻¹ for the basic fertilisation with Boneprot and 4000 l·ha for the basic fertilisation with Lumbrical) and reduced by 50%. During the vegetation the liquid biofertilisers Baikal EM, Emosan and Bio One was introduced as a soil amendment at the plant growing stages of ‘flower bud’ and ‘mass fruitset’ stage in concentrations of 1:1000 for Baikal EM, 150 l·ha for Emosan and 1.65 l·ha for Bio One.

Characteristics of the biofertilisers included into the study. The treatments were done with biofertilisers Boneprot (Arkobaleno), Lumbrical, Baikal EM, Emosan (Hemozym Bio N5) and Bio One, included in the list of the permitted substances for soil maintaining fertility according to Regulation (EC) No. 889/2008.

Boneprot (Arkobaleno, Italy) is a pellet organic fertiliser and has the following composition: (organic nitrogen (N) – 4.5%; phosphorus anhydride (P₂O₅) total – 3.5%; potassium (K₂O) – 3.5%; calcium (CaO) – 5–8%; magnesium (MgO) – 0.8–1%; organic carbon (C) of biological origin – 30%; humification rate (HR) – 10–13%; degree of humification (DH) – 40–42%; humification index (HI) – 1.3–1.4%; humidity – 13–15%; pH in water – 6–8. Boneprot is an entirely organic fertiliser consisting solely of cattle manure. These materials are collected from farms, which do not use antibiotics and are subject to controlled fermentation for a period of about one year.

Lumbrical (private producer from village Kostievo, Bulgaria) is a product obtained from processing natural organic manure and other organic waste by the Californian red worms (*Lumbricus rubellus* and *Eisenia foetida*) and consists of their excrements. The commercial product has humidity of 45–55% and organic matter content of 45–50%. Ammonium nitrogen (NH₄N) – 33.0 ppm; nitrate nitrogen (NO₃N) – 30.5 ppm; P₂O₅ – 1410 ppm, K₂O – 1910 ppm and MgO – 1.8%. It contains useful microflora 2 × 10¹² pce·g, humic and fulvic acids, nutritional matters. The product activity is 6.5–7.0 (pH in water).

Baikal EM-1Y (Ukraine) has the following content: effective microorganisms (EM), mixed cultures of useful microorganisms, which are antagonists with respect to the pathogenic and conditionally pathogenic microflora. This is a large group of microorganisms living under a regime of activity upon interaction with the nutritional environment, etc. Bacterial inoculation includes *Lactobacillus casei*, *Lactobacillus lactis*, *Rhodopseudomonas palustris* and *Saccharomyces cerevisiae*. It is used for activation of microbiological processes in the soil and for increasing of yield. The product has the following chemical composition: Organic carbon (C) – 0.15%; total nitrogen – 0.01%; total phosphorus (such as P₂O₂) – 0.001%; total potassium (K₂O) – 0.02%; chaminic acids – 0.015%; pH – 3.2 and secondary microflora, a total titer of 10⁶–10⁷.

Emosan (HemoZym NK, Hemozym Bio N5, Arkobaleno, Italy) contains total nitrogen (N) – 5%; organic nitrogen (N) – 5%; organic carbon (C) of biological origin – 14%; protein – 34 p/p; humidity – 65 p/p; K – 0.4 p/p; P – 0.06 p/p, etc.; pH – 7.0–10.0.

Bio One (USA) consists of living organisms and is 100% natural liquid concentrated microbiological product. Bacterial inoculation includes two types of microorganisms – aerobic (*Azotobacter vinelandii*) and anaerobic (*Clostridium pasteurianum*). It is recommended for increasing the nitrogen fixation in the soil. It is applied in soil.

Study parameters

Vegetative growth. Biometric parameters are: plant height (cm), number of leaves, stem diameter (mm), number of internodes, and root length (cm). at the end of the vegetation (at the mass fruit yield stage) there were 10 plants per variant analyzed.

Mineral content. In the end of the vegetation of an average sample of vegetable material (leaves and stems) a chemical analysis was carried out according to the following methods: mineral N – via wet burning with sulphuric acid according to Keldal; P₂O₅ and K₂O – via covering with ashes at 550°C; phosphorus– via formation of a molybdenum vanadate phosphoric complex; potassium – via flame photometry.

Physiological parameters. Parameters of Leaf gas exchange are: Net photosynthetic rate – P_N (μmol m⁻²s⁻¹), Transpiration intensity – E (mmol m⁻²s⁻¹) and Stomatal conductance – g_s (mol m⁻²s⁻¹), using a portable infrared gas analyser LCA-4 (ADC, Hoddesdon, England). Measurements were made under a light intensity of 800 μmol m⁻²s⁻¹ PAR, at a temperature of 24 ± 2°C, an external CO₂ concentration of 390 μmol mol⁻¹ and relative air humidity of 70%. Measurements were taken on well-developed leaves from the central floors of the plants, which were representative of each treatment. Two analyses were carried out, i.e. the first measurement was taken at the flower bud stage on the 15–20 day after the application of the liquid biofertilisers (Baikal EM-1Y, Emosan and Bio One) and the second measurement was taken at the stage of the mass fruit yield.

Statistical data – processing used Ms Office Excel 2007, SPSS [Duncan 1955] and Biostat. An analysis of variance (ANOVA) was used to analyze the differences between treatments. A Duncan multiple-range test was also performed to identify the homogeneous type of the data sets among the different treatments. BIostat was used to compare the results as treated compared to the control.

RESULTS

Vegetative growth (Biometric measurements)

The results of the biometric measurements of the average plant height at the end of the vegetative period showed the highest values for the variant treated with Emosan on the Boneprot basic fertilisation (62.60 cm – 2009; 64.80 cm – 2010 and 63.87 cm – 2011) followed by the variant treated with Baikal EM on the Lumbrical basic fertilisation (tab. 1). The difference between these two variants was not significant at P < 0.05 (2009 and 2010) and significant at P < 0.05 in 2011.

Table 1. Height of plants (cm) at the end of the vegetation, variety of 'Sofiiska Kapiya'

Treatments (variants)	2009		2010		2011	
	mean; st. dev.	GD	mean; st. dev.	GD	mean; st. dev.	GD
Control	48.90 ± 0.884 ^d	base	47.00 ± 1.195 ^f	base	47.20 ± 1.082 ^g	base
Boneprot (opt.)	57.90 ± 2.446 ^{bc}	+++	55.47 ± 3.292 ^c	+++	56.27 ± 3.348 ^f	++
Boneprot (50%) + Baikal EM	56.70 ± 1.013 ^c	+++	62.13 ± 2.587 ^{bc}	+++	57.53 ± 0.915 ^{def}	++
Boneprot (50%) + Emosan	62.60 ± 0.507 ^a	+++	64.80 ± 0.6761 ^a	+++	63.87 ± 0.743 ^a	+++
Boneprot (50%) + BioOne	57.80 ± 2.007 ^{bc}	+++	61.47 ± 1.922 ^c	+++	57.80 ± 1.373 ^{de}	+++
Lumbrical (opt.)	60.10 ± 1.168 ^{ab}	+++	56.27 ± 3.348 ^c	+++	56.73 ± 2.251 ^{ef}	++
Lumbrical (50%) + Baikal EM	62.40 ± 0.986 ^a	+++	63.87 ± 0.352 ^{ab}	+++	61.93 ± 0.884 ^b	+++
Lumbrical (50%) + Emosan	58.80 ± 0.594 ^{bc}	+++	62.87 ± 0.834 ^{abc}	+++	58.27 ± 2.120 ^d	++
Lumbrical (50%) + BioOne	57.80 ± 0.945 ^{bc}	+++	58.47 ± 3.925 ^d	+++	58.80 ± 1.373 ^d	++
GD 5%		2.89		2.05		6.49
GD 1%		3.94		2.80		8.85
GD 0.1%		5.33		3.79		11.98

Duncan's multiple range test, $P < 0.05$

The study showed that the fertilisation with Emosan provided formation of stronger and taller plants, as compared to the control (non-fertilised) and other treatments, i.e. the average for the period also had the highest number of internodes, i.e. 10.3.

Table 2. Number of internodes and stem diameter at the end of the vegetation

Treatments (variants)	Number of internodes				Diameter of stem, mm			
	2009			average	2009			average
	2009	2010	2011		2009	2010	2011	
	mean				mean			
Control	7.6 ^f	6.8 ^f	8.2 ^e	7.5	7.3 ^f	7.4 ^e	6.8 ^c	7.2
Boneprot (opt.)	8.6 ^c	7.4 ^e	10.3 ^a	8.8	8.0 ^e	8.6 ^d	8.4 ^b	8.3
Boneprot (50%) + Baikal EM	9.6 ^{bc}	9.4 ^c	9.4 ^c	9.5	9.1 ^c	9.7 ^{ab}	9.7 ^a	9.5
Boneprot (50%) + Emosan	10.2 ^a	10.4 ^a	10.3 ^a	10.3	10.3 ^a	9.9 ^a	10.1 ^a	10.1
Boneprot (50%) + BioOne	9.0 ^{de}	9.4 ^c	8.8 ^d	9.1	8.8 ^{cd}	8.9 ^{cd}	8.5 ^b	8.7
Lumbrical (opt.)	9.1 ^{cde}	8.6 ^d	8.2 ^e	8.6	8.6 ^d	8.8 ^{cd}	8.5 ^b	8.6
Lumbrical(50%) + Baikal EM	9.8 ^{ab}	9.4 ^c	9.8 ^b	9.6	9.6 ^b	9.2 ^{bc}	10.2 ^a	9.7
Lumbrical (50%) + Emosan	9.2 ^{cd}	9.8 ^b	9.6 ^{bc}	9.5	10.0 ^{ab}	9.8 ^a	9.8 ^a	9.9
Lumbrical (50%) + BioOne	8.8 ^{de}	8.6 ^d	10.4 ^a	9.3	8.8 ^{cd}	9.2 ^{bc}	8.6 ^b	8.9

Duncan's multiple range test, $P < 0.05$

The statistical analysis showed that the differences in all variants between the average and compared to the control were significant at $P_{0.1\%}$ (2009 and 2010). There were positive results observed upon the application of Baikal EM on the basic fertilisation

with Lumbrical, i.e. 62.40 cm (2009); 63.87 cm (2010) and 61.93 cm (2011) (tab. 1), which as an average for the period was shown by the larger number of internodes – 9.6 (tab. 2).

An additional application of liquid biofertilisers influenced the vegetative growth that reflected in formation of plants with strong habitus. For instance, the stem diameter showed highest average value of 10.1 mm after application with the biofertiliser Emosan on basic fertilisation with Boneprot (10.3 mm – 2009 and 9.9 mm – 2010) (tab. 2).

There was no unidirectional tendency towards changes in the number of leaves per plant for the period of the experiment in the same variant (tab. 3).

Table 3. Number of leaves per plant at the end of the vegetation, variety of ‘Sofiiska Kapiya’

Treatments (variants)	2009		2010		2011	
	mean; st. dev.	GD	mean; st. dev.	GD	mean; st. dev.	GD
Control	86.4 ±8.806 ^c	base	71.0 ±5.083 ^d	base	78.5 ±5.890 ^c	base
Boneprot (opt.)	165.2 ±9.002 ^a	+++	107.0 ±12.519 ^c	+++	131.4 ±17.920 ^b	+++
Boneprot (50%) + Baikal EM	148.0 ±12.461 ^{ab}	+++	148.6 ±7.570 ^a	+++	145.7 ±3.305 ^{ab}	+++
Boneprot (50%) + Emosan	148.8 ±12.999 ^{ab}	+++	146.0 ±15.932 ^a	+++	148.1 ±29.298 ^a	+++
Boneprot (50%) + Bio One	162.0 ±11.065 ^{ab}	+++	154.8 ±19.872 ^a	+++	151.1 ±25.524 ^a	+++
Lumbrical (opt.)	145.8 ±12.237 ^{ab}	+++	124.0 ±12.218 ^b	+++	142.9 ±4.868 ^{ab}	+++
Lumbrical (50%) + Baikal EM	163.2 ±11.188 ^a	+++	151.7 ±7.292 ^a	+++	149.0 ±15.033 ^a	+++
Lumbrical (50%) + Emosan	151.1 ±15.394 ^{ab}	+++	144.0 ±11.044 ^a	+++	144.2 ±12.813 ^{ab}	+++
Lumbrical (50%) + Bio One	148.5 ±6.254 ^{ab}	+++	117.0 ±11.698 ^{bc}	+++	145.4 ±5.578 ^{ab}	+++
GD ^{5%}		21.5		13.7		14.7
GD ^{1%}		29.3		18.7		20.1
GD ^{0.1%}		39.6		25.3		27.2

Duncan’s multiple range test, $P < 0.05$

The largest number of leaves per plant was observed for the variants treated during vegetation with Bio One on Boneprot basic fertilisation – 154.8 pcs·plant (2010); 151.1 pcs·plant (2011), respectively, followed by the variant fed with the biofertiliser Baikal EM on the Lumbrical basic fertilisation – 151.7 pcs·plant (2010); 149.0 pcs·plant (2011), respectively. The difference between these two variants was not significant for $P < 0.05$ in 2010 and 2011. The continuous effect of the biofertilisers applied in a form of basic fertilisation, plus additional vegetation feeding with liquid biofertiliser, reflected in formation of a larger number of leaves in comparison with the single application of basic fertilisation in an optimum concentration on the Lumbrical basic fertilisation (2009 and 2011) and on the Boneprot basic fertilisation (2010 and 2011). The total number of leaves for all variants was larger compared to the control variants and the differences between the average values were significant at $P_{0.1\%}$ (2009, 2010 and 2011).

Table 4. Root length at the end of vegetation, variety of 'Sofiiska Kapiya'

Treatments (variants)	2009	2010	2011	average
	mean; st. dev.	mean; st. dev.	mean; st. dev.	
Control	14.5 ±0.601 ^d	15.0 ±0.333 ^d	15.3 ±0.731 ^d	14.9
Boneprot (opt.)	16.1 ±0.285 ^{cd}	17.2 ±0.750 ^{cd}	17.1 ±0.135 ^d	16.8
Boneprot (50%) + Baikal EM	23.7 ±1.678 ^a	21.0 ±1.202 ^{bc}	23.0 ±0.333 ^b	22.6
Boneprot (50%) + Emosan	24.0 ±0.333 ^a	26.5 ±0.391 ^a	24.6 ±0.486 ^{ab}	25.0
Boneprot (50%) + Bio One	19.9 ±0.629 ^{abc}	20.4 ±0.176 ^{bcd}	20.0 ±0.441 ^c	20.1
Lumbrical (opt.)	18.7 ±0.366 ^{bcd}	17.8 ±0.550 ^{bcd}	16.2 ±0.550 ^d	17.6
Lumbrical (50%) + Baikal EM	23.3 ±1.237 ^{ab}	22.1 ±2.313 ^{abc}	22.9 ±0.473 ^b	22.7
Lumbrical (50%) + Emosan	20.7 ±0.694 ^{abc}	23.1 ±0.634 ^{ab}	26.8 ±0.677 ^a	23.5
Lumbrical (50%) + Bio One	19.8 ±0.926 ^{abc}	18.1 ±0.500 ^{bcd}	17.9 ±0.418 ^{cd}	18.6

Duncan's Multiply Range Test, ($P < 0.05$)

The results showed that the application of the biofertilisers as basic fertilisation and during vegetation increased the length of roots of pepper plants (tab. 4). The highest results by this parameter were shown after treatment with Emosan, where the average value for the experimental period was 25.0 cm on the Boneprot basic fertilisation, and 23.5 cm on the Lumbrical basic fertilisation.

Mineral content of N, P₂O₅ and K₂O in the vegetative mass, i.e leaves and stems

The results on the impact of chemical content of biofertilisers on the nutrient content (N, P₂O₅ and K₂O) of the vegetative mass (leaves and stems) showed that nitrogen content varied but it was the lowest in the control (non-fertilised) plants. Higher nitrogen content was detected in leaves compared to stems (figs 1 and 2). Similar changes were found regarding the potassium content.

The highest N content was found in plant leaves and in the plants treated with the biofertiliser Emosan on the basic fertilisation with Boneprot and treated with Emosan on the basic fertilisation with Lumbrical, i.e. 29.2 g·kg⁻¹ and 26.2 g·kg⁻¹, respectively. The stimulation effect of the biofertiliser Emosan can be attributed to the higher N content (i.e. up to 5%), which provided nutrients for the major groups of microorganisms in the plant rhizosphere [Vlahova 2013]. The higher nitrogen content shown by these treatments correlated with the detected higher fresh leaf mass, i.e. 1760.2 kg·ha⁻¹ and 1598.7 kg·ha⁻¹.

Higher N content was also detected after treatment with the biofertiliser Baikal EM on the basic fertilisation with Boneprot, i.e. 24.5 g·kg⁻¹ and with Baikal EM on the basic fertilisation with Lumbrical, i.e. 23.8 g·kg⁻¹.

The present research was in conjunction with Gorbanov et al. [2005], i.e. most of the N content was concentrated in the vegetation parts, which showed the most intensive activity, i.e. the leaves. Huett and Dettmann [1991, 1992] also maintain that plants absorb more N and K₂O, mainly in the leaves.

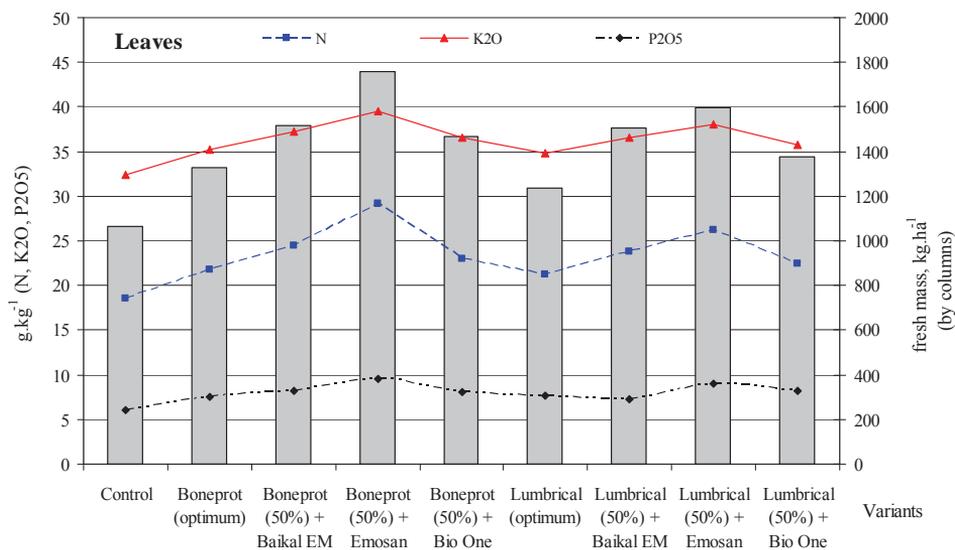


Fig. 1. Fresh mass of the leaves and content of N, P₂O₅ and K₂O (from 2009 to 2011)

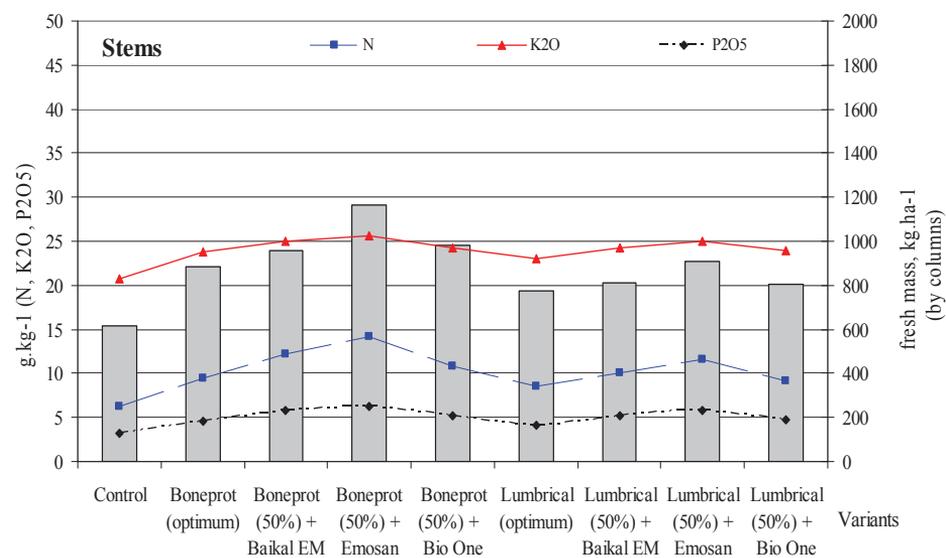


Fig. 2. Fresh mass of the stems and content of N, P₂O₅ and K₂O (from 2009 to 2011)

Physiological parameters (Leaf gas exchange)

The results showed a positive effect of fertilisation at the flower bud stage on rate of net photosynthesis (P_N) of tested plants but there were no uniform tendencies during the period of three years (tab. 5). Maximum values of P_N were observed for plants of variant treated with Emosan on the Lumbrical basic fertilisation (2009), Baikal EM on Lumbrical basic fertilisation (2010) and Baikal EM on Boneprot basic fertilisation (2011). The difference between the average values compared to the control ones was significant at $P_{0.1\%}$. Stimulating growth was also shown upon Emosan application on the Boneprot basic fertilisation (2009, 2010 and 2011). There was an increase of P_N in most variants compared to the control ones, except for those variants characterised with the optimum concentration on the Lumbrical basic fertilisation (2010) and the biofertiliser Emosan applied on the Lumbrical basic fertilisation (2010). A higher P_N value was observed for all combined variants included into the study and cultivated on both basic fertilisations, as compared to the optimum concentration of the respective basic fertilisation (2010 and 2011). In 2009 this was found upon the application of the biofertilisers Baikal EM and Emosan on the Boneprot basic fertilisation and the biofertiliser Emosan on the Lumbrical basic fertilisation. The statistical analysis showed that in all variants, as compared to the control, there was no unidirectional tendency observed during the period of three years, and that only the difference between the average ones was significant at $P_{0.1\%}$ in 2011.

Table 5. Rate of the net photosynthesis P_N ($\mu\text{mol m}^{-2}\text{s}^{-1}$) at the flower bud stage

Treatments (variants)	2009		2010		2011	
	mean; st. dev.	GD	mean; st. dev.	GD	mean;st. dev.	GD
Control	12.05 \pm 0.713 ^{ab}	base	11.10 \pm 0.850 ^b	base	9.76 \pm 0.389 ^b	base
Boneprot (opt.)	14.86 \pm 0.968 ^{ab}	ns	11.77 \pm 0.332 ^b	ns	11.55 \pm 0.099 ^f	+++
Boneprot (50%) + Baikal EM	15.26 \pm 1.051 ^a	ns	14.57 \pm 1.186 ^a	+++	18.12 \pm 0.035 ^a	+++
Boneprot (50%) + Emosan	15.23 \pm 1.992 ^a	ns	14.09 \pm 0.790 ^a	+++	15.05 \pm 0.028 ^b	+++
Boneprot (50%) + Bio One	13.15 \pm 0.083 ^{ab}	ns	11.99 \pm 0.808 ^b	ns	12.09 \pm 0.064 ^e	+++
Lumbrical (opt.)	14.76 \pm 0.833 ^{ab}	ns	10.68 \pm 0.391 ^b	ns	11.05 \pm 0.226 ^g	+++
Lumbrical(50%) + Baikal EM	14.36 \pm 1.534 ^{ab}	ns	14.74 \pm 0.092 ^a	+++	15.22 \pm 0.113 ^b	+++
Lumbrical(50%) + Emosan	15.44 \pm 2.176 ^a	+	10.93 \pm 0.486 ^b	ns	14.09 \pm 0.035 ^{cd}	+++
Lumbrical(50%) + Bio One	13.91 \pm 0.471 ^{ab}	ns	11.14 \pm 0.261 ^b	ns	11.22 \pm 0.014 ^{fg}	+++
GD 5%		3.37		1.24		0.35
GD 1%		4.60		1.69		0.50
GD 0.1%		6.22		2.29		0.72

Duncan's multiple range test, $P < 0.05$

The intensity of transpiration (E) at the flower bud stage during the three- year study showed maximum values by plants fed with Emosan on the Boneprot basic fertilisation, thus proving the positive influence of the combination of both biofertilisers (tab. 6).

Table 6. Intensity of transpiration E (mmol m⁻² s⁻¹) at the flower bud stage

Treatments (variants)	2009		2010		2011	
	mean; st. dev.	GD	mean; st. dev.	GD	mean; st. dev.	GD
Control	1.60 ± 0.053 ^d	base	1.20 ± 0.061 ^{bc}	base	1.72 ± 0.042 ^{def}	base
Boneprot (opt.)	2.59 ± 0.087 ^{ab}	++	1.47 ± 0.211 ^{ab}	+	1.80 ± 0.035 ^{bcd}	ns
Boneprot (50%) + Baikal EM	1.82 ± 1.011 ^{bcd}	ns	1.45 ± 0.105 ^{ab}	+	1.16 ± 0.014 ^h	+++
Boneprot (50%) + Emosan	2.75 ± 0.006 ^{cd}	++	1.54 ± 0.100 ^a	++	2.39 ± 0.021 ^a	+++
Boneprot (50%) + Bio one	1.61 ± 0.021 ^d	ns	1.28 ± 0.060 ^{bc}	ns	1.51 ± 0.049 ^g	++
Lumbrical (opt.)	2.47 ± 0.294 ^{abc}	+	1.25 ± 0.082 ^{bc}	ns	1.61 ± 0.092 ^{fg}	ns
Lumbrical (50%) + Baikal EM	2.30 ± 0.032 ^{abcd}	ns	1.27 ± 0.045 ^{bc}	ns	1.63 ± 0.057 ^{ef}	ns
Lumbrical (50%) + Emosan	2.35 ± 0.149 ^{abcd}	+	1.27 ± 0.111 ^{bc}	ns	1.64 ± 0.064 ^{ef}	ns
Lumbrical (50%) + Bio one	1.71 ± 0.025 ^{cd}	ns	1.12 ± 0.017 ^c	ns	1.76 ± 0.064 ^{cde}	ns
GD 5%		0.72		0.24		0.13
GD 1%		0.98		0.33		0.18
GD 0.1%		1.33		0.44		0.26

Duncan's multiple range test, P < 0.05

During the three-year experimental period, the maximum value of stomatal conductance (g_s) was found for the plants fed with the biofertiliser Emosan on the Boneprot basic fertilisation. The average value for the study period was 0.064 mol m⁻²s⁻¹. High values were also observed for Baikal EM on the Boneprot basic fertilisation (2009 and 2010), as the average one for the period was 0.055 mol m⁻²s⁻¹ (tab. 7).

Table 7. Stomatal conductance (g_s) – (mol m⁻² s⁻¹) at the flower bud stage

Treatments (variants)	2009	2010	2011
	mean; st. dev.	mean; st. dev.	mean; st. dev.
Control	0.020 ± 0.005 ^c	0.030 ± 0.001 ^c	0.030 ± 0.007 ^b
Boneprot (opt.)	0.060 ± 0.001 ^c	0.040 ± 0.004 ^b	0.035 ± 0.007 ^b
Boneprot (50%) + Baikal EM	0.080 ± 0.003 ^a	0.060 ± 0.002 ^a	0.025 ± 0.007 ^b
Boneprot (50%) + Emosan	0.083 ± 0.001 ^a	0.060 ± 0.003 ^a	0.050 ± 0.007 ^a
Boneprot (50%) + Bio One	0.033 ± 0.001 ^d	0.040 ± 0.002 ^b	0.030 ± 0.003 ^b
Lumbrical (opt.)	0.033 ± 0.006 ^d	0.030 ± 0.003 ^c	0.030 ± 0.014 ^b
Lumbrical (50%) + Baikal EM	0.033 ± 0.001 ^d	0.060 ± 0.003 ^a	0.036 ± 0.013 ^b
Lumbrical (50%) + Emosan	0.037 ± 0.006 ^d	0.040 ± 0.005 ^b	0.030 ± 0.014 ^b
Lumbrical (50%) + Bio One	0.067 ± 0.004 ^b	0.040 ± 0.003 ^b	0.030 ± 0.014 ^b

Duncan's Multiply Range Test, (P < 0.05)

At the mass fruit yield stage, the rate of the net photosynthesis (P_N) showed highest values in the plants of the variant fed with the biofertiliser Baikal EM on basic fertilisation with Boneprot (2009 and 2011) (tab. 8). The average value of P_n for the period was $15.98 \mu\text{mol m}^{-2} \text{s}^{-1}$, thus confirming the good P_N values observed for this variant at the flower bud stage.

Table 8. Rate of the net photosynthesis P_N ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at the mass fruit yield stage

Treatments(variants)	2009		2010		2011	
	mean;st. dev.	GD	mean;st. dev.	GD	mean;st. dev.	GD
Control	9.87 ±0.015 ^g	base	11.53 ±0.157 ^d	base	8.77 ±0.180 ^f	base
Boneprot (opt.)	11.85 ±0.105 ^e	+++	13.16 ±0.032 ^c	++	9.89 ±0.531 ^e	++
Boneprot (50%) + Baikal EM	16.21 ±0.091 ^a	+++	15.35 ±0.478 ^b	+++	15.73 ±0.288 ^a	++
Boneprot (50%) + Emosan	13.85 ±0.093 ^b	+++	19.55 ±0.110 ^a	+++	15.34 ±0.748 ^{ab}	+++
Boneprot (50%) + Bio One	13.35 ±0.025 ^{cd}	+++	13.25 ±0.087 ^c	+++	11.11 ±0.450 ^d	+++
Lumbrical (opt.)	10.81 ±0.044 ^f	+++	13.35 ±0.207 ^c	+++	10.12 ±0.150 ^c	+++
Lumbrical (50%) + Baikal EM	13.80 ±0.100 ^{bc}	+++	13.15 ±0.595 ^c	++	14.77 ±0.068 ^b	+++
Lumbrical (50%) + Emosan	13.64 ±0.021 ^{bcd}	+++	13.47 ±0.655 ^c	+++	14.06 ±0.075 ^c	+++
Lumbrical (50%) + Bio One	13.25 ±0.010 ^d	+++	13.46 ±1.164 ^c	+++	10.35 ±0.309 ^e	+++
GD _{5%}		0.44		0.92		0.70
GD _{1%}		0.60		1.26		0.95
GD _{0.1%}		0.81		1.70		1.28

Duncan's multiple range test, $P < 0.05$

In 2010 the maximum P_N value was observed for the Boneprot basic fertilisation upon the application of the biofertiliser Emosan. The difference between the average values compared to the control ones was significant at $P_{0.1\%}$. The combined application of the biofertilisers on both basic fertilisations showed higher values upon feeding with the biofertilisers Emosan and Baikal EM on basic fertilisation with Boneprot (2009, 2010 and 2011) that were also similar to the high values observed at the flower bud stage. It was related to the observed overall good vegetative development of the pepper upon the application of these combinations.

The plants fed with the biofertiliser Bio One on basic fertilisation with Boneprot (2010 and 2011) showed higher P_N values compared to control plants. It proves the positive effect of the basic fertilisation, which released nutrients for a longer time during vegetation thus ensuring better physiological conditions of the pepper plants for a longer period of time.

The biofertilisers applied in combination with basic fertilisation had an overall positive effect by increasing the P_N value of the plants, as compared to the non-fertilised control (2009, 2010 and 2011). The statistical analysis showed that for all variants the difference between the average values and the control ones was significant at $P_{0.1\%}$ (2009).

The maximum value of the *intensity of transpiration* (E) was observed for the variants treated with biofertilisers Baikal EM (2009 and 2011) and Emosan (2010) on basic fertilisation with Boneprot (Table 9). Upon comparison with the combined variants on both basic fertilisations, the higher E value was shown after treatment with Emosan and Baikal EM applied on the Boneprot basic fertilisation (2009, 2010 and 2011).

Table 9. Intensity of transpiration E (mmol m⁻² s⁻¹) at the mass fruit yield stage

Treatments (variants)	2009		2010		2011	
	mean; st. dev.	GD	mean; st. dev.	GD	mean; st. dev.	GD
Control	1.60 ± 0.040 ^{ab}	base	1.21 ± 0.053 ^f	base	1.33 ± 0.015 ^c	base
Boneprot (opt.)	1.35 ± 0.015 ^c	+++	1.44 ± 0.026 ^{cd}	+++	1.62 ± 0.075 ^{cd}	++
Boneprot (50%) + Baikal EM	1.68 ± 0.021 ^a	ns	1.55 ± 0.030 ^{ab}	+++	1.95 ± 0.156 ^b	+++
Boneprot (50%) + Emosan	1.68 ± 0.052 ^a	ns	1.63 ± 0.120 ^a	+++	1.55 ± 0.061 ^d	+
Boneprot (50%) + Bio One	1.34 ± 0.026 ^c	+++	1.37 ± 0.078 ^{de}	++	1.35 ± 0.050 ^e	ns
Lumbrical (opt.)	1.20 ± 0.026 ^d	+++	1.35 ± 0.017 ^{de}	++	1.94 ± 0.176 ^b	+++
Lumbrical (50%) + Baikal EM	1.53 ± 0.021 ^b	ns	1.36 ± 0.038 ^{de}	++	1.70 ± 0.040 ^{cd}	++
Lumbrical (50%) + Emosan	1.50 ± 0.070 ^{bc}	ns	1.50 ± 0.070 ^{bc}	+++	1.34 ± 0.075 ^c	ns
Lumbrical (50%) + Bio One	1.36 ± 0.026 ^c	+++	1.44 ± 0.032 ^{cd}	+++	1.92 ± 0.236 ^b	+++
GD _{5%}		0.12		0.10		0.20
GD _{1%}		0.17		0.14		0.27
GD _{0.1%}		0.23		0.19		0.37

Duncan's multiple range test, P < 0.05

Table 10. Stomatal conductance g_s (mol m⁻² s⁻¹) at the mass fruit yield stage

Treatments (variants)	2009	2010	2011
	mean; st dev.		
Control	0.025 ± 0.005 ^{cd}	0.020 ± 0.002 ^c	0.020 ± 0.002 ^c
Boneprot (opt.)	0.030 ± 0.005 ^{bc}	0.030 ± 0.003 ^b	0.030 ± 0.010 ^{cd}
Boneprot (50%) + Baikal EM	0.040 ± 0.005 ^a	0.030 ± 0.002 ^b	0.050 ± 0.010 ^a
Boneprot (50%) + Emosan	0.040 ± 0.004 ^a	0.030 ± 0.003 ^b	0.035 ± 0.009 ^{bcd}
Boneprot (50%) + Bio One	0.030 ± 0.005 ^{bc}	0.030 ± 0.002 ^b	0.025 ± 0.005 ^{de}
Lumbrical (opt.)	0.020 ± 0.005 ^d	0.030 ± 0.003 ^b	0.045 ± 0.009 ^{ab}
Lumbrical (50%) + Baikal EM	0.035 ± 0.005 ^{ab}	0.040 ± 0.002 ^a	0.035 ± 0.003 ^{bcd}
Lumbrical (50%) + Emosan	0.030 ± 0.003 ^{bc}	0.030 ± 0.003 ^b	0.030 ± 0.009 ^{cde}
Lumbrical (50%) + Bio One	0.030 ± 0.002 ^{bc}	0.030 ± 0.003 ^b	0.040 ± 0.002 ^{abc}

The stomatal conductance (g_s) (tab. 10) showed a maximum value after treatments with Baikal EM on the Boneprot basic fertilisation (2009 and 2011). This confirmed the findings at the flower bud stage of pepper. The average value of stomatal conductance (g_s) for the period was 0.055 mol m⁻² s⁻¹.

DISCUSSION

Impact on biofertilisers of vegetative growth shown by biometric data

It was found that the combined application of biofertilisers as vegetative feeding (by liquid formulation) on both basic fertilisations (by solid formulation) influenced the increase of pepper vegetative growth in comparison with the single application of basic fertilisation in an optimum concentration (2009 and 2010) (tab. 1).

The study showed that the fertilisation with Emosan provided conditions for the formation of stronger and taller plants, as compared to the control (tabs 1 and 2). The result can be explained by the high level of proteins that were inserted with the Emosan application and their respective influence on the microbiological activity of the soil [Vlahova 2013].

The study found that feeding with the biofertiliser Baikal EM impacted positively on the vegetative growth of the plants, i.e. the number of internodes (tab. 2). This can be explained with so called effective microorganisms (EM) in this biofertiliser that released nutrients in accessible forms for plants and provided biologically-active substances in the soil media [Vlahova 2013].

High values on the stem diameter were observed in the variant grown upon application of the biofertiliser Emosan on the basic fertilisation Lumbrical, as the average value for the period was 9.87 mm (tab. 2). This positive impact confirmed the combination of Emosan on Boneprot as the best among the tested biofertilisers under field conditions. The effect of the applied biofertilisers was stronger with the increase of their N-content, e.g. Emosan. Vegetation feeding with the biofertiliser Emosan stimulated the vegetative growth of the plants also due to the high concentrations of nutrient-providing proteins contained in it.

The biofertiliser Emosan influenced positively the root length thus creating conditions for better nutrition supply to plants and for development of production capacities for larger vegetative mass and standard yield [Vlahova 2013].

Overall, the tested biofertilisers improved the pepper feeding thus increasing the growth of the vegetative biomass. It confirms the findings of Rani et al. [2008] that the use of biofertilisers improves assimilation of nutrients by plants thus resulting in formation of a larger plant biomass. The study also confirms the findings of Direkvandi et al. [2008] that biofertilisers containing *Azotobacter spp.*, *Azospirillum spp.* and *Bacillus spp.* may influence the height of plants and the number of leaves. Furthermore, this study confirms the findings of Berova and Karanatsidis [2008] that biofertiliser Lumbrical has a positive effect on growth of stems and roots of pepper. This study results provide ground to state that feeding with biofertilisers during vegetation provide conditions for formation of a larger above-ground biomass with stronger and taller plants.

Mineral content of N, P₂O₅ and K₂O in the vegetative mass, i.e leaves and stems

The results show that biofertilisers do not impact significantly the P content of the leaves and stems of the pepper, but changes are more obvious in the leaves. The fertilisation with studied biofertilisers increase the K₂O content in leaves and stems compared to the control (non-fertilised) plants; the values are higher for the leaves.

The research detected significant positive correlation between the content of major nutrients and the vegetative biomass in relation to the type of bio fertilisation applied (Figure 3). Determination coefficients were from 810 g·kg⁻¹ to 930 g·kg⁻¹, which indicated that in 930 g·kg⁻¹ for K, 900 g·kg⁻¹ for N and 810 g·kg⁻¹ for P of the case the changes in nutrient content might lead to respective changes in the formed vegetative mass of pepper plants. The absolute changes in the N, P₂O₅ and K₂O content, which would occur in parallel with the changes of one unit change of the vegetative mass, were measured by using regression coefficients, i.e. B = 0.0284 for the N, B = 0.0044 for P and B = 0.0147 for K.

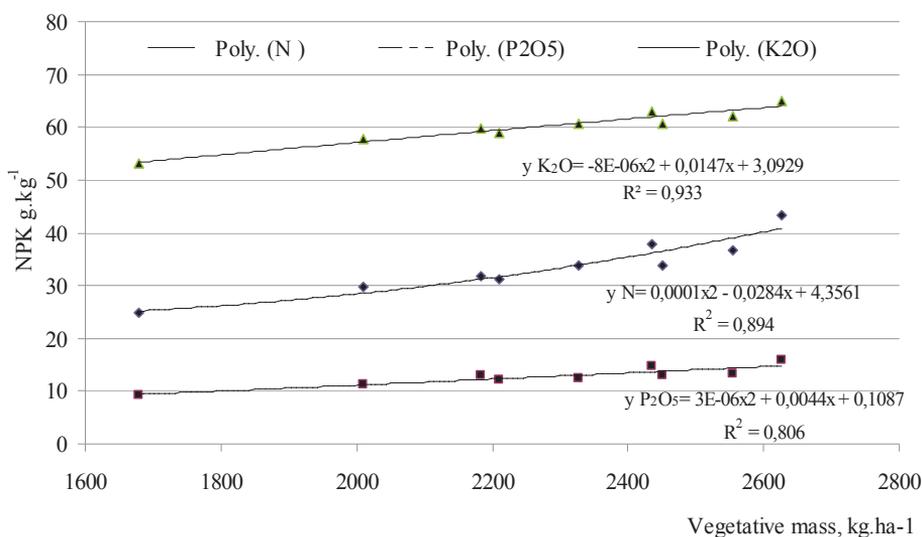


Fig. 3. Relation between the N, P₂O₅ and K₂O content and the formed vegetative mass

The results are in conjunction with the findings of other researchers, who have shown a direct relation between the amount of the vegetative mass formed per unit of land, the level of yields and biological outputs of N, P, K [Huett and Dettmann 1992, Doykova et al. 1997, Boteva 2006].

Impact on biofertilisers of physiological parameters (leaf gas exchange)

The study observed high net photosynthesis at the flower bud stage after application of the biofertiliser Baikal EM on both basic fertilisations. The results can be attributed to the effect of microbial composition of Baikal EM (2010 and 2011). The biofertiliser had the capacity to release nutrients in an accessible form and to stimulate their assimilation by the plants, thus having a positive impact on the total growth and the physiological condition of the pepper plants. During the experimental period all types of biofertilisers' applications (i.e. basic fertilisation and vegetative feeding) had an impact on the P_N (tab. 5).

Upon the single application of both basic fertilisations in optimum concentrations, the higher P_N value was observed at the stage of flower bud upon Boneprot basic fertilisation during the three-year study period. This confirmed the stimulating effect of the biofertiliser Boneprot. In addition, the positive impact of the application of Emosan after Boneprot on intensity of transpiration (E) at the stage of flower bud corresponded to the improved vegetative growth of the pepper plants.

The single applications of the basic fertilisation in optimum concentrations showed higher values of E upon treatments with Boneprot (detected during all three years). Concerning the combined variants, a positive effect on the E was observed upon the application of Emosan on Boneprot. The average value for the period was $2.23 \text{ mmol m}^{-2}\text{s}^{-1}$ (tab. 6), which was higher in comparison with the single application of basic fertilisations and can be attributed to the better vegetative development of the plants of this variant.

The present study confirmed the findings of Karanatsidis and Berova [2009] that the rate of the net photosynthesis (P_N value) and the intensity of transpiration (E) increased significantly upon the application of the organic fertiliser Emosan in the soil. Generally, feeding of the pepper plants with Lumbrical had a positive affect on the leaf gas exchange [Karanatsidis 2013].

The application of Emosan and Baikal EM on basic fertilisation with Boneprot showed the maximum stimulation impact as shown by the levels of transpiration intensity (E) and stomatal conductance (g_s). These were conditions for a better leave gas exchange of the plants at the stage of flower bud (tab. 7), which was a prerequisite for the better water status of pepper plants.

The study also found a higher rate of the net photosynthesis at the growing stage of mass fruit yield. This can be attributed to the increased assimilation of biologically active substances by the pepper plants. The substances were released upon application of microbial biofertiliser Baikal EM, which reflected in enriching the soil media. The positive effect of the biofertiliser Baikal EM and Boneprot basic fertilisation on stomatal conductance (g_s) at the mass fruit yield stage confirmed the result found at the flower bud stage. It was an evidence of the positive effect of the combination of the biofertiliser Baikal EM on the Boneprot basic fertilisation.

The observed higher values of leaves gas exchange parameters upon combined application of the biofertilisers Emosan and Baikal EM on the Boneprot basic fertilisation were determined by the longer and more profound impact of these combinations compared to the single application of basic fertilisation.

CONCLUSIONS

1. The tendency of stimulating pepper growth upon treatment with the biofertiliser Emosan on basic fertilisation with Boneprot was confirmed as the best combination showing the complex effect of biofertilisers on the ecological status of pepper plants grown under organic field conditions. The result can be explained by the high level of proteins in the biofertiliser Emosan and their influence on the microbiological activity of the soil.

2. The application of the biofertilisers Baikal EM and Emosan on basic fertilisation with Boneprot had a positive effect on a number of physiological parameters, i.e. the increase of the rate of net photosynthesis and the intensity of transpiration. Biofertilisation had a stimulating effect on the stomatal conductance as well, thus providing a better water status of the plants at the flower bud stage and at the mass fruit yield stage later on.

3. Under the impact of applied biofertilisers the N and K₂O content changed, but the P₂O₅ content did not change significantly. The impact mainly concerned the leaf growth but less the stem growth. The present research found a significant correlation between the volume of the total vegetative mass and content of the major nutrients in relation to the respective type of biofertilisation.

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WPLYW BIONAWOZÓW NA WZROST WEGETACYJNY, ZAWARTOŚĆ MINERALÓW ORAZ PARAMETRY FIZJOLOGICZNE PAPRYKI ROCZNEJ (*Capsicum annuum* L.) HODOWANEJ W WARUNKACH ROLNICTWA ORGANICZNEGO

Streszczenie. W ostatnich latach bionawozy pojawiły się w rolnictwie jako obiecujący element zintegrowanego systemu dostarczenia składników odżywczych. Celem niniejszego badania było zbadanie wpływu wybranych bionawozów na rozwój wegetacyjny i zawartość N, P₂O₅ oraz K₂O w liściach i łodygach, a także na parametry fizjologiczne papryki rocznej odmiany ‘Sofiiska Kapiya’ uprawianej w warunkach rolnictwa organicznego. Doświadczenie przeprowadzono w latach 2009–2011 na polach doświadczalnych Ośrodka Rolniczo-Ekologicznego Uniwersytetu Rolniczego Plovdiv (Bułgaria) położonych na certyfikowanej farmie ekologicznej. Badanie dotyczyło następujących bionawozów: Boneprot, Lumbrical, Baikal EM “Effective Microorganisms”, Emosan oraz Bio One. Wyniki pomiarów biometrycznych przeciętnej wysokości rośliny pod koniec okresu wegetacyjnego wykazały największe wartości dla odmiany, której aplikowano Emosan przy podstawowym nawożeniu Boneprotem (62,60 cm – 2009; 64,80 cm – 2010 oraz 63,87 cm – 2011). W przypadku podawania bionawozów Emosan i Baikal EM przy podstawowym nawożeniu Boneprotem (2009, 2010 oraz 2011) na etapie plonowania owoców papryki, rośliny wykazywały większe wartości fotosyntezy netto (P_N). Wartości te były podobne do dużych wartości zaobserwowanych na etapie pąków kwiatowych. Największą intensywność transpiracji (E) zaobserwowano u odmian, którym podawano bionawozy Baikal EM (2009 i 2011) oraz Emosan (2010) przy podstawowym nawożeniu Boneprotem. Wyciągnięto wniosek, iż zastosowanie Emosanu stymuluje wzrost wegetacyjny roślin papryki ze względu na wysokie stężenia białek dostarczających elementów odżywczych zawartych w tym bionawozie. Wyniki wykazały, że bionawozy nie wpływają w sposób istotny na zawartość P w liściach i łodygach papryki, ale zmiany były bardziej oczywiste w liściach. Nawożenie badanymi nawozami zwiększało zawartość K₂O w liściach i łodygach w porównaniu z kontrolą (rośliny nienawożone), a wartości te były większe dla liści.

Słowa kluczowe: bionawozy, *Capsicum annuum* L., zawartość minerałów, rolnictwo organiczne, parametry fizjologiczne, rozwój wegetacyjny

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