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SPECIFICS OF THE APPLICATION OF BIOFERTILISERS IN THE AGRO-ECOSYSTEM

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Abstract: Organic agriculture is a specific method of production that supports ecological balance and has a minimum negative impact on neighboring natural ecosystems and on human health. An experiment was carried out in 2009- 2011 on the territory of a certified ecological farm of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria). The experiment aims at researching the impact of applied biofertilizers on the phenological development, the biometric and physiological parameters of seedlings and the field conditions of organically cultivated pepper cv. Kurtovska Kapiya 1619, under the agroecological conditions of the region of Plovdiv. The study included biofertilizers- Lumbrical, Boneprot, Seasol, Baikal EM, Bio One, Emosan. Statistical data processing was done by Microsoft Office Excel 2017, SPSS and BIOSTAT. The stimulation effect on the growth of the vegetative organs in the seedling stage is the highest upon feeding with the biofertilizer Emosan on the Boneprot basic fertilization (2009, 2010, 2011). The biometric status of plants grown under field conditions confirms the stimulating effect of biofertilizers, as also established in seedling production. At the stage fruitfulness the application of Baikal on basic fertilization with Boneprot (2009, 2010, 2011) had the best stimulating effect on the net photosynthetic rate, and Bio One on basic fertilization with Boneprot (2009, 2011) had the highest impact on transpiration intensity and stomatal conductance, thus determining the specifics of the positive impact of the application of the biofertilizers in the agroecosystem. There is linear dependence between the temperature sum and the duration of the periods - from sprouting to the first true leaf (inclusive) at values of R=0.778, flowering (including the stages of flower bud and flowering) at values of R=0.92, and fruitfulness at values of R=0.918. The same may be used for prognostication of the duration of the separate periods of vegetation and the used variety of pepper. The application of biofertilizers aims at the optimization of the fertilization system in order to ensure the stable ecological environment in the agro-ecosystem without any environmental risk.

Key words: biofertilizers, Capsicum annuum L., leaf gas exchange, organic agriculture, phenological development, seedlings, vegetative growth.

INTRODUCTION

Agriculture has been practised for more than 10,000 years (Bellwood, 2005). For most of that period there were no synthetic fertilizers or pesticides, as this was an era of *ancien régime* for agriculture where practices were *de facto* organic (Paull, 2010). The agriculture production totally depends on the fertility level of the soil (Sharma et al., 2012). Organic farming has emerged as an important priority area globally in view of growing demand for safe and healthy food (Saini and Kumar, 2014) and concerns on environmental pollution associated with indiscriminate use of agrochemicals (Goutami et al., 2017).Organic agriculture provides high-quality production, while being at the same time a decisive element for the multi-functional development of the agricultural areas, thus ensuring sustainable development (Vlahova, 2013a; Arabska, 2014; Arabska and Velikova, 2017; Popova, 2019), with minimal impact on ecological factors such as soil fertility (Mäder et al., 2002; Zargar et al., 2017; Bozhanska et al., 2019; Guilherme et al., 2020). Organic manure plays a direct role in plant growth as a source of all necessary macro and micronutrients in available forms during mineralization, improves the physical and chemical properties of soils (Chaterjee et al., 2005; Badzhelova et al., 2016; Risal and Halim, 2020), and makes the ecosystem healthier (Mishra and Dash, 2014; Bozhanska 2018). Biofertilizers have emerged as potential environmentally friendly inputs that are supplemented for proper plant growth (Khan and Naeem, 2011; Mazid et al., 2012; Churkova and Bozhanska, 2016; Sheikh et al., 2017). A biofertilizer can be defined as formulations of live microorganisms when inoculated to seeds, on plant foliage, or to soil establishes in the rhizosphere, and promotes the growth of the host plants by increasing availability of primary nutrients (Mazid et al., 2011; Mazid and Khan, 2014; Raffi, 2018). Organic farming relies on local varieties (Kostadinova and Popov, 2012; Antonova et al., 2012a; Yakimov, 2013; Dintcheva et al., 2016; Enchev and Kokindonov, 2016; Dintcheva et al., 2020), with efforts to develop more sustainable hybrid varieties that are more resistant and suitable for organic production (Antonova, 2012; Antonova et al., 2012b; Todorova, 2013; Zorovski et al., 2018).

Studies of pepper cv. Sofiiska Kapiya have been carried out under the conditions of organic agriculture with presentation of the impact of the solid biofertilizers applied (in combination with liquid biofertilizers or applied separately as basic fertilization) on the parameters of leaf gas exchange and vegetative growth in the case of seedlings (Vlahova and Popov, 2014) and under conditions of field production (Vlahova et al., 2014). The incorporation of biofertilizers in the soil plays a major role in improving the leaf gas- exchange parameters of red pepper for grinding (Berova and Karanatsidis, 2008) and young tomato plants (Zlatev and Popov, 2013) cultivated under organic field conditions. Scientific researches in the field of phenological development of pepper upon field organic production are insufficient, as there are publications on cv. Sofiiska Kapiya (Vlahova, 2013b, Vlahova et al., 2015), but there are no publications on cv. Kurtovska Kapiya 1619, thus determining the topicality of the research.

The experiment aims at researching the impact of applied biofertilizers on the phenological development, the biometric and physiological parameters of seedlings and the field conditions of biologically cultivated pepper of the variety of Kurtovska Kapiya 1619, under the agroecological conditions of the region of Plovdiv.

MATERIAL AND METHODS

An experiment was carried out in 2009- 2011 on the territory of a certified ecological farm of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria), with the pepper variety of “Kurtovska Kapiya 1619”. The soil is alluvial, having light sandy-clay structure, good porosity (Koynov et al., 1998). The study included biofertilizers- Lumbrical, Boneprot, Baikal EM, Seasol, Emosan, Bio One, belong to the list of permitted biofertilizers in the European Union ‘Organic’ Regulation (EC) No. 889/2008. Sowing of pepper seeds took place in the second half of March. The experiment included 4 replications of 1 m^2 each. Solid biofertilisers Boneprot and Lumbrical were applied in the soil and incorporated prior to the time of sowing in two concentrations i.e. optimal (corresponding to 70 kg/da for the Boneprot and 400 L/da for the Lumbrical) and reduced by 50%. In the process of seedling production liquid biofertilizers imported twice, i.e. in the soil before sowing of seeds in concentration-1:1000 for Baikal; 1:500-0.3-0.4 L/da for Seasol; 15 L/da for Emosan; 165 ml/da for Bio One and at the end of the seedling growing in the same concentrations. The experiment was done according to the method of long plots, into four replications, with a size of the test plot of 9.6 m^2, according to the planting scheme 120+60x15 cm. Two basic fertilizations were used, namely: Boneprot and Lumbrical, applied into the soil through incorporation prior to planting of the seedlings on the field. They were applied in two concentrations, i.e. optimum (corresponded to 70 kg/da for the basic fertilization with Boneprot and 400 L/da for the basic fertilization with Lumbrical) and these concentrations reduced by 50%. The liquid biofertilizers Baikal EM, Seasol, Emosan and Bio One were introduced into the soil as feeding twice during vegetation, at the stages 'flower bud' and at the 'mass fruitfulness', in following concentrations: 1:1000 for Baikal EM; 1:500, i.e. 0.3-0.4 L/da for Seasol; 15 L/da for Emosan and 165 ml/da for Bio One (Vlahova 2013a).

Characteristics of the biofertilisers:

Boneprot (Arkobaleno, Italy) is a pellet organic fertilizer. Composition: (organic nitrogen (N)-4.5%; phosphorus anhydride (P_2O_5) total-3.5%; potassium (K_2O) - 3.5%; calcium (CaO)- 5-8%; organic carbon (C) of biological origin - 30%; humidity-13-15%; pH in water- 6- 8.

Lumbrical (Bulgaria) consists of the excrement of Californian red worms (*Lumbricus rubellus* and *Eisenia foetida*). The commercial product has humidity of 45-55% and organic substance content of 45-50%. Composition: NH_4N-33.0 ppm; NO_3N- 30.5 ppm; P_2O_5-1410 ppm; K_2O- 1910 ppm; **humic and fulvic acids,** useful microflora **$2x10^{12}$pce/g, pH of 6.5-7.0.**

Baikal EM-1Y (Ukraine) includes effective microorganisms (EM), mixed cultures of useful microorganisms, which are antagonists with respect to the pathogenic and conditionally pathogenic microflora. Composition: Organic carbon (C)- 0.15%; total nitrogen- 0.01%; total phosphorus (such as P_2O_2)- 0.001%; total potassium (K_2O)- 0.02%; pH- 3.2 and secondary microflora, a total titer of 10^6- 10^7.

Seasol, Earthcare, (Australia) is an extract of brown algae *Durvillaea potatorum* and contains 60% of alginic acids, raw protein (2.5±0.1% w/w), alginates (6±2% w/w). Has a variety of mineral elements and traces of Ca (0.05±0.03% w/w), N (0.10±0.05% w/w), P (0.05±0.02% w/w), K (2.0±0.5% w/w) and cytokines and pH (10.5± 0.5% w/w).

Emosan*,* HemoZym NK, (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.

Variants: 1.Control (non-fertilized); 2. Basic fertilization with Boneprot (optimum concentration); 3. Basic fertilization with Boneprot (50%)+ Baikal EM; 4. Basic fertilization with Boneprot (50%)+ Seasol; 5. Basic fertilization with Boneprot (50%)+ Emosan; 6. Basic fertilization with Boneprot (50%)+ Bio One; 7. Basic fertilization with Lumbrical (optimum concentration); 8. Basic fertilization with Lumbrical (50%)+ Baikal EM; 9. Basic fertilization with Lumbrical (50%)+ Seasol;10. Basic fertilization with Lumbrical (50%)+ Emosan; 11. Basic fertilization with Lumbrical (50%)+ Bio One.

Study Indicators:

Vegetative growth. At the end of the seedling stage and the end of the vegetation (at the mass fruit yield stage) there were 10 plants per variant analyzed, namely the biometric parameters: plant height (cm) and number of leaves.

Leaf gas exchange parameters are Net photosynthetic rate-P_N (μmolm^{-2}s^{-1}), Transpiration intensity- E (mmolm^{-2}s^{-1}) and Stomatal conductance- g_s (molm^{-2}s^{-1}), using a portable infrared gas analyser LCA- 4 (ADC, Hoddesdon, England). The measurements were performed in the morning from 11 o'clock. The first measurement was taken at the 'flower bud' stage on the 15-20 day after the application of the liquid biofertilizers and the second measurement was taken at the stage of the 'mass fruit yield'.

Phenological development. The occurrence of the phenophase was determined (in days): from the sowing for the sprouting phenophase and from the sprouting for the phenophases-cotyledons, first true leaf, flower bud, flowering, ripening, and botanical maturity. The beginning of each phenophase was determined at 10% of - and the mass entry was at 75% of all plants under observation. During the vegetation period 10 pre - marked plants were subject to the observations in the field condition (Ganeva, 1984).

Statistical data processing- Microsoft Office Excell 2010, ANOVA (SPSS treatment 7.5) and BIOSTAT. All data were analyzed by using Duncan's multiple range test (Duncan, 1955) at the P<0.05 level. BIOSTAT was used to compare the results as compared to the control.

RESULTS AND DISCUSSION

The maximum seedling height in 2009 was reported for the combined application of Baikal with the two basic fertilizations, as the difference between them was proven for P<0.05 (Table 1).

Table 1. Height of plants (cm) at the end of the seedling stage

Variants	2009		2010		2011	
	Mean; St. Dev.	GD	Mean; St. Dev	GD	Mean; St. Dev.	GD
Control	14.05 ± 0.243 [i]	Base	16.53 ± 0.386 [e]	Base	15.02 ± 0.012 [e]	Base
Boneprot (opt.)	15.36 ± 0.062 [f]	+++	17.00 ± 0.206 [c]	++	16.31 ± 0.441 [d]	+++
Boneprot (50%)+ Baikal	18.32 ± 0.191 [a]	+++	17.19 ± 0.234 [c]	+++	17.57 ± 0.067 [ab]	+++
Boneprot (50%)+ Seasol	16.66 ± 0.398 [e]	+++	17.00 ± 0.270 [c]	++	17.51 ± 0.035 [b]	+++
Boneprot (50%)+ Emosan	17.62 ± 0.024 [b]	+++	17.78 ± 0.036 [a]	+++	17.74 ± 0.023 [a]	+++
Boneprot (50%)+ Bio One	15.20 ± 0.147 [fg]	+++	16.79 ± 0.498 [d]	+	16.66 ± 0.051 [c]	+++
Lumbrical (opt.)	16.92 ± 0.539 [d]	+++	17.20 ± 0.217 [c]	+++	16.61 ± 0.070 [c]	+++
Lumbrical (50%)+ Baikal	17.62 ± 0.495 [b]	+++	17.69 ± 0.070 [ab]	+++	17.63 ± 0.026 [ab]	+++
Lumbrical (50%)+ Seasol	15.08 ± 0.012 [g]	+++	17.51 ± 0.064 [b]	++	17.53 ± 0.275 [ab]	+++
Lumbrical (50%)+ Emosan	17.30 ± 0.254 [c]	+++	17.70 ± 0.065 [ab]	++	17.65 ± 0.101 [ab]	+++
Lumbrical (50%)+ Bio One	14.51 ± 0.081 [h]	+++	17.54 ± 0.069 [b]	++	16.68 ± 0.334 [c]	+++
$GD_{5\%}$; $GD_{1\%}$; $GD_{0.1\%}$	*0.20; 0.27; 0.36*		*0.21; 0.29; 0.39*		*0.19; 0.26; 0.35*	

In 2010 the highest values were achieved upon the combined application of Emosan with Boneprot and the difference between the average ones, as compared to the control, was very well proven for $P_{0.1\%}$. In 2011 it was established that Emosan had a positive effect regardless of the basic fertilization, as in all variants the differences, as compared to the control, were very well proven for $P_{0.1\%}$. A positive effect of the combined application of Emosan with the two basic fertilizations was established in 2009 on a number of leaves per plant, as the difference, as compared to the control, was very well proven for $P_{0.1\%}$ (Table 2). As regards the indicator commented above, in 2010 higher values were reported for all variants cultivated on basic fertilization with Lumbrical, which was probably due to the physico-chemical properties of Lumbrical and its easier absorption by plant roots. Upon feeding with Bio One, Baikal and Emosan on basic fertilization with Lumbrical, the differences between the average ones compared to the control were very well proven for $P_{0.1\%}$. In 2011 the highest value was reported for the combined application of Emosan with the two basic fertilizations, as compared to the control the difference between the average ones was well proven for $P_{1\%}$. Feeding with a combination of biofertilizers during the seedling period resulted in an increase of the number of leaves.

Table 2. Number of leaves per plant at the end of the seedling stage

Variants	2009		2010		2011	
	Mean; St. Dev.	GD	Mean; St. Dev	GD	Mean; St. Dev.	GD
Control	5.47 ± 1.457 ^{d}	Base	5.33 ± 1.345 ^{e}	Base	5.20 ± 0.414 ^{b}	Base
Boneprot (opt.)	6.87 ± 0.640 ^{abc}	++	5.87 ± 1.246 ^{de}	ns	5.87 ± 0.640 ^{ab}	ns
Boneprot (50%)+ Baikal	5.87 ± 0.352 ^{d}	ns	6.33 ± 0.488 ^{bcd}	+	6.33 ± 1.113 ^{a}	+
Boneprot (50%)+ Seasol	6.00 ± 1.069 ^{cd}	ns	6.00 ± 0.655 ^{cde}	ns	6.20 ± 1.265 ^{ab}	+
Boneprot (50%)+ Emosan	7.20 ± 0.414 ^{a}	+++	6.33 ± 1.113 ^{bcd}	+	6.80 ± 0.414 ^{a}	++
Boneprot (50%)+ Bio One	6.20 ± 0.676 ^{bcd}	ns	6.00 ± 0.655 ^{cde}	ns	6.13 ± 0.743 ^{ab}	+
Lumbrical (opt.)	7.00 ± 0.535 ^{ab}	++	6.13 ± 0.743 ^{cde}	+	6.00 ± 0.535 ^{ab}	ns
Lumbrical (50%)+ Baikal	6.33 ± 0.488 ^{abcd}	+	7.07 ± 0.458 ^{ab}	+++	6.40 ± 1.056 ^{a}	++
Lumbrical (50%)+ Seasol	5.80 ± 0.676 ^{d}	ns	5.80 ± 0.676 ^{de}	ns	6.20 ± 0.414 ^{ab}	+
Lumbrical (50%)+ Emosan	7.13 ± 0.640 ^{ab}	+++	6.80 ± 0.414 ^{abc}	+++	6.60 ± 0.507 ^{a}	++
Lumbrical (50%)+ Bio One	6.87 ± 0.352 ^{abc}	++	7.20 ± 0.414 ^{a}	+++	6.40 ± 0.986 ^{a}	++
$GD_{5\%}$; $GD_{1\%}$; $GD_{0.1\%}$	*0.83; 1.14; 1.54*		*0.77; 1.05; 1.43*		*0.87; 1.19; 1.61*	

In 2009 the **net photosynthetic** rate- (P_N) at the **seedling stage** had a higher value in the combined variants of biofertilizers on basic fertilization with Boneprot (Table 3). In 2010 the best indicators were reported for P_N upon combined application of Emosan with the two basic fertilizations, as the difference was well proven for $P_{1\%}$. In 2011 P_N had its maximum value upon optimum concentration of basic fertilization with Boneprot, as in all variants, as compared to the control, the difference between the average ones was very well proven for $P_{0.1\%}$.

Table 3. Rate of the net photosynthetic-(P_N)- ($\mu molm^{-2}s^{-1}$) at the seedling stage

Variants	2009		2010		2011	
	Mean;	GD	Mean;	GD	Mean	GD
Control	19.00 ^{cd}	Base	18.59 ^{cd}	Base	15.90 ^{g}	Base
Boneprot (opt.)	22.04 ^{a}	++	17.68 ^{d}	ns	24.20 ^{a}	+++
Boneprot (50%)+ Baikal	21.26 ^{ab}	++	16.98 ^{d}	ns	20.76 ^{c}	+++
Boneprot (50%)+ Seasol	21.77 ^{a}	++	17.28 ^{d}	ns	22.90 ^{b}	+++
Boneprot (50%)+ Emosan	19.62 ^{c}	ns	23.11 ^{a}	++	18.10 ^{e}	+++
Boneprot (50%)+ Bio One	19.93 ^{bc}	ns	21.04 ^{abc}	ns	17.10 ^{ef}	+
Lumbrical (opt.)	19.11 ^{cd}	ns	16.84 ^{d}	ns	16.90 ^{fg}	+
Lumbrical (50%)+ Baikal	17.96 ^{d}	ns	17.72 ^{d}	ns	19.40 ^{d}	+++
Lumbrical (50%)+ Seasol	20.12 ^{bc}	ns	18.00 ^{cd}	ns	21.60 ^{c}	+++
Lumbrical (50%)+ Emosan	18.66 ^{cd}	ns	22.60 ^{ab}	++	19.18 ^{d}	+++
Lumbrical (50%)+ Bio One	18.76 ^{cd}	ns	19.81 ^{bcd}	ns	20.57 ^{c}	+++
GD 5%; GD 1%; GD 0.1%	*1.55; 2.15; 3.11*		*2.91; 3.96; 5.36*		*0.90; 1.28; 1.86*	

In 2009 the highest **transpiration intensity** was reported for the combination of Bio One and Lumbrical, as the difference between the averages ones, as compared to the control, was very well proven for $P_{0.1\%}$ (Table 4). In 2010 the best indicators of transpiration intensity were reported for basic fertilization with Boneprot in combination with Bio One, followed by Emosan, as the difference between the averages ones, as compared to the control, was very well proven for $P_{0.1\%}$. In 2011 the transpiration intensity reached its maximum rate in the case of Baikal on basic fertilization with Lumbrical, as the difference between the averages ones, as compared to the control, was very well proven for $P_{0.1\%}$. **Stomatal conductance**- (g_s) showed distinct dynamics, as the highest rate was reported for the case of feeding with Bio One on basic fertilization with Boneprot (2010, 2011) and for Emosan on basic fertilization with Lumbrical (2009, 2010) (Table 5).

Upon biological development of pepper under **field conditions in the end of the vegetative period**, the maximum value in 2009 was reported for the combination of Seasol on basic fertilization with Boneprot, which was further confirmed in 2010 and 2011 (Table 6). This stimulating effect is probably due to a seaweed extract in Seasol containing auxins and alginates that import nutrients in an easily accessible form, which are transformed by microorganisms and are easily absorbed by plants in order to accumulate organic mass. The additional vegetative feeding with biofertilizers is characterized with more distinct growth as compared to the independent application of the basic fertilization in optimum concentration (2009, 2010). In 2009 the largest number of leaves per plant was reported for the variant with application of Baikal on basic fertilization with Lumbrical-172,0 pcs/plant (Table 7). In 2010 and 2011 the best values were reported for the combined application of Emosan with the two basic fertilizations. In all variants, when compared to the control, the difference between the average ones was very well proven for $P_{0.1\%}$. The biometric status of plants grown under field conditions confirms the stimulating effect of biofertilizers, as also established in seedling production. The additional import of liquid biofertilizers provides an opportunity for more prolonged assimilation of nutrients by plants, which supports the vegetative development and creates an opportunity for shaping a strong habitus with a better assimilation apparatus.

Table 4. Intensity of transpiration-(E) ($mmolm^{-2}s^{-1}$) at the seedling stage

Variants	2009		2010		2011	
	Mean;	GD	Mean;	GD	Mean;	GD
Control	0.96^{ef}	Base	1.49^{b}	Base	1.53^{bc}	Base
Boneprot (opt.)	1.65^{b}	+++	1.69^{b}	ns	1.02^{d}	+
Boneprot (50%)+ Baikal	1.43^{c}	+++	1.74^{b}	ns	1.19^{bcd}	ns
Boneprot (50%)+ Seasol	1.39^{c}	+++	2.17^{a}	+++	1.28^{bcd}	ns
Boneprot (50%)+ Emosan	0.92^{f}	ns	2.51^{a}	+++	1.41^{bcd}	ns
Boneprot (50%)+ Bio One	1.05^{de}	ns	2.54^{a}	+++	1.98^{a}	++
Lumbrical (opt.)	1.03^{f}	ns	2.24^{a}	+++	2.00^{a}	++
Lumbrical (50%)+ Baikal	0.86^{f}	ns	2.32^{a}	+++	2.40^{a}	+++
Lumbrical (50%)+ Seasol	1.05^{de}	ns	1.79^{b}	ns	1.58^{b}	ns
Lumbrical (50%)+ Emosan	1.17^{d}	+	2.43^{a}	+++	1.13^{cd}	ns
Lumbrical (50%)+ Bio One	2.00^{a}	+++	2.31^{a}	+++	1.40^{bcd}	ns
$GD_{5\%}$; $GD_{1\%}$; $GD_{0.1\%}$	*0.17; 0.24; 0.34*		*0.32; 0.44; 0.59*		*0.12; 0.17; 0.23*	

Table 5. Stomatal conductance (gs)- (molm-2s-1) at the seedling stage and the stage fruitfulness

Variants	Seedling stage			Stage fruitfulness		
	2009	2010	2011	2009	2010	2011
Control	0.020	0.033	0.030	0.017	0.020	0.025
Boneprot (opt.)	0.040	0.040	0.030	0.035	0.030	0.035
Boneprot (50%)+ Baikal	0.040	0.037	0.035	0.040	0.040	0.040
Boneprot (50%)+ Seasol	0.040	0.043	0.040	0.030	0.030	0.030
Boneprot (50%)+ Emosan	0.040	0.047	0.040	0.030	0.040	0.030
Boneprot (50%)+ Bio One	0.020	0.057	0.045	0.045	0.020	0.050
Lumbrical (opt.)	0.030	0.047	0.040	0.030	0.030	0.035
Lumbrical (50%)+ Baikal	0.030	0.040	0.045	0.040	0.030	0.040
Lumbrical (50%)+ Seasol	0.026	0.037	0.040	0.030	0.020	0.030
Lumbrical (50%)+ Emosan	0.040	0.057	0.030	0.025	0.040	0.040
Lumbrical (50%)+ Bio One	0.040	0.050	0.030	0.030	0.020	0.025

Table 6. Height of plants (cm) at the end of the vegetation

Variants	2009		2010		2011	
	Mean; St. Dev.	GD	Mean; St. Dev	GD	Mean; St. Dev.	GD
Control	56.00 ± 0.535 [f]	Base	49.60 ± 2.640 [e]	Base	49.13 ± 1.408 [e]	Base
Boneprot (opt.)	58.01 ± 0.655 [e]	+++	57.73 ± 2.764 [d]	+++	56.80 ± 2.713 [d]	+++
Boneprot (50%)+ Baikal	62.02 ± 1.180 [bcd]	+++	62.80 ± 2.210 [bc]	+++	61.80 ± 0.400 [bc]	+++
Boneprot (50%)+ Seasol	65.20 ± 0.414 [a]	+++	65.20 ± 0.414 [a]	+++	64.40 ± 0.490 [a]	+++
Boneprot (50%)+ Emosan	60.90 ± 0.876 [cd]	+++	64.13 ± 0.834 [ab]	+++	62.60 ± 0.712 [b]	+++
Boneprot (50%)+ Bio One	61.81 ± 0.561 [bcd]	+++	57.80 ± 1.373 [d]	+++	57.00 ± 2.338 [d]	+++
Lumbrical (opt.)	63.12 ± 0.495 [bc]	+++	57.00 ± 1.414 [d]	+++	57.67 ± 0.943 [d]	+++
Lumbrical (50%)+ Baikal	62.60 ± 0.507 [bcd]	+++	62.87 ± 0.352 [bc]	+++	62.00 ± 0.730 [bc]	+++
Lumbrical (50%)+ Seasol	63.92 ± 0.743 [ab]	+++	61.20 ± 2.678 [c]	+++	62.73 ± 0.998 [b]	+++
Lumbrical (50%)+ Emosan	62.30 ± 1.280 [bcd]	+++	64.00 ± 0.926 [ab]	+++	62.00 ± 0.632 [bc]	+++
Lumbrical (50%)+ Bio One	60.42 ± 0.940 [d]	+++	57.80 ± 2.007 [d]	+++	60.80 ± 0.653 [c]	+++
$GD_{5\%}$; $GD_{1\%}$; $GD_{0.1\%}$	*2.06;2.81; 3.80*		*1.57; 2.15; 2.90*		*1.44; 1.96; 2.66*	

Table 7. Number of leaves per plant at the end of the vegetation

Variants	2009		2010		2011	
	Mean; St. Dev.	GD	Mean; St. Dev	GD	Mean; St. Dev.	GD
Control	99.0 ± 9.219 [e]	Base	79.0 ± 5.213 [e]	Base	76.9 ± 11.109 [d]	Base
Boneprot (opt.)	131.0 ± 9.672 [d]	+++	132.0 ± 15.454 [d]	+++	129.0 ± 17.497 [c]	+++
Boneprot (50%)+ Baikal	168.0 ± 25.387 [a]	+++	148.0 ± 6.761 [ab]	+++	154.7 ± 19.751 [ab]	+++
Boneprot (50%)+ Seasol	163.0 ± 18.406 [a]	+++	143.0 ± 10.518 [bc]	+++	150.7 ± 9.903 [ab]	+++
Boneprot (50%)+ Emosan	158.0 ± 9.666 [abc]	+++	154.1 ± 15.306 [a]	+++	159.3 ± 18.730 [a]	+++
Boneprot (50%)+ Bio One	159.0 ± 31.156 [ab]	+++	125.0 ± 13.537 [d]	+++	135.6 ± 23.609 [bc]	+++
Lumbrical (opt.)	146.0 ± 14.233 [bcd]	+++	130.0 ± 14.913 [d]	+++	140.8 ± 3.529 [abc]	+++
Lumbrical (50%)+ Baikal	172.0 ± 20.363 [a]	+++	149.2 ± 6.349 [ab]	+++	151.2 ± 23.346 [ab]	+++
Lumbrical (50%)+ Seasol	156.0 ± 18.731 [abc]	+++	144.7 ± 9.933 [ab]	+++	149.0 ± 21.344 [ab]	+++
Lumbrical (50%)+ Emosan	163.0 ± 11.770 [a]	+++	151.0 ± 14.248 [ab]	+++	156.3 ± 16.465 [a]	+++
Lumbrical (50%)+ Bio One	142.0 ± 10.229 [cd]	+++	134.0 ± 13.468 [cd]	+++	147.7 ± 7.218 [ab]	+++
$GD_{5\%}$; $GD_{1\%}$; $GD_{0.1\%}$	*14.30; 19.50; 26.39*		*9.35; 12.75; 17.26*		*13.10; 17.86; 24.17*	

At the **stage fruitfulness** the highest net photosynthetic (P_N) rate was reported for Baikal on basic fertilization with Boneprot (2009, 2010, 2011) (Table 8). The results give grounds to draw a conclusion that at this stage the photosynthetic activity is more stable, for the plants have reached greater vegetative mass, which is probably due to the imported biofertilizer Baikal, which provides good absorption of the nutrients necessary for the growth of the pepper plants.

Table 8. Rate of the net photosynthetic- (P_N) ($\mu molm^{-2}s^{-1}$) at the stage fruitfulness

Variants	2009		2010		2011	
	Mean;	GD	Mean;	GD	Mean	GD
Control	8.53 ^{i}	Base	12.10 ^{d}	Base	9.90 ^{d}	Base
Boneprot (opt.)	9.85 ^{h}	+++	13.50 ^{c}	+	10.72 ^{d}	ns
Boneprot (50%) + Baikal EM	15.78 ^{a}	+++	16.51 ^{a}	+++	15.35 ^{a}	+++
Boneprot (50%) + Seasol	13.84 ^{e}	+++	14.17 ^{bc}	+++	14.72 ^{ab}	+++
Boneprot (50%) + Emosan	14.80 ^{c}	+++	14.16 ^{bc}	+++	14.96 ^{a}	+++
Boneprot (50%) + Bio One	11.53 ^{g}	+++	14.33 ^{bc}	+++	13.88 ^{b}	+++
Lumbrical (opt.)	10.07 ^{h}	+++	14.40 ^{bc}	+++	10.12 ^{d}	ns
Lumbrical (50%)+ Baikal EM	15.05 ^{b}	+++	14.55 ^{bc}	+++	14.90 ^{a}	+++
Lumbrical (50%) + Seasol	13.30 ^{f}	+++	14.56 ^{bc}	+++	12.44 ^{c}	+++
Lumbrical (50%) + Emosan	14.48 ^{d}	+++	14.94 ^{b}	+++	14.57 ^{ab}	+++
Lumbrical (50%) + Bio One	13.62 ^{d}	+++	14.50 ^{bc}	+++	11.85 ^{c}	+++
$GD_{5\%}$; $GD_{1\%}$; $GD_{0.1\%}$	*0.23; 0.32; 0.43*		*1.11; 1.51; 2.05*		*0.87; 1.19; 1.61*	

Transpiration intensity in 2009 had its highest rate upon the combined application of Bio One on basic fertilization with Boneprot, which was confirmed in 2011. The difference between the averages ones, as compared to the control, was very well proven for $P_{0.1\%}$ (2009, 2011). The higher intensity of transpiration ensures better water status of the studied plants and better flow of the physiological processes within them (Table 9). The results for the **Stomatal conductance** in the stage fruitfulness show that the maximum value was reported for Bio One on basic fertilization with Boneprot (2009, 2011) (Table 5). The application of Baikal on basic fertilization with Boneprot (2009, 2010, 2011) had the best stimulating effect on the net photosynthetic rate-(P_N), and Bio One on basic fertilization with Boneprot (2009, 2011) had the highest impact on transpiration intensity- (E) and stomatal conductance- (g_s), thus determining the specifics of the positive impact of the application of the biofertilizers in the agroecosystem.

Upon following up the **phonological development** of pepper cv. Kurtovska Kapiya 1619, it was established that during the three vegetative years there was earlier entry into the pheno-stages of plants fed with the biofertilizers Baikal and Emosan on the Boneprot basic fertilization and on the Lumbrical basic fertilization, thus confirming that the combinations of introduced nutritional substances with additional feeding ensured the deployment of productive capacities of pepper (Vlahova, 2013a). It was established that the agrometeorological conditions were very suitable and, when combined with adequate agrotechnical events, contributed to the development of the crop. The specialized scientific literature presents information regarding the impact of the temperature sum on the duration of the vegetative period for the various crops, as well as its impact on time and the duration of the separate phenophases (Kirchev et al., 2010; Kirchev et al., 2014). As a result of such impact, mathematical dependences have been established on Vlahova et al., (2015) for cv. Sofiiska Kapiya.

Table 9. Intensity of transpiration- (E) ($mmolm^{-2}s^{-1}$) at the stage fruitfulness

Variants	2009		2010		2011	
	Mean;	GD	Mean;	GD	Mean;	GD
Control	1.19 [f]	Base	1.47 [c]	Base	1.63 [cd]	Base
Boneprot (opt.)	1.76 [c]	+++	1.79 [a]	+++	1.41 [e]	++
Boneprot (50%) + Baikal EM	1.80 [bc]	+++	1.54 [bc]	ns	1.82 [bc]	+
Boneprot (50%) + Seasol	1.61 [d]	+++	1.57 [bc]	ns	1.66 [cd]	ns
Boneprot (50%) + Emosan	1.46 [e]	+++	1.60 [bc]	ns	1.75 [cd]	ns
Boneprot (50%) + Bio One	1.97 [a]	+++	1.48 [bc]	ns	2.01 [a]	+++
Lumbrical (opt.)	1.44 [e]	+++	1.55 [bc]	ns	1.95 [ab]	+++
Lumbrical (50%) + Baikal EM	1.92 [ab]	+++	1.56 [bc]	ns	1.73 [cd]	ns
Lumbrical (50%) + Seasol	1.46 [e]	+++	1.63 [b]	+	1.64 [cd]	ns
Lumbrical (50%) + Emosan	1.35 [e]	+++	1.49 [bc]	ns	1.76 [cd]	ns
Lumbrical (50%) + Bio One	1.53[e]	+++	1.55 [bc]	ns	1.62 [d]	ns
$GD_{5\%}$; $GD_{1\%}$; $GD_{0.1\%}$	*1.12; 1.17; 1.22*		*0.13; 0.17; 0.24*		*0.15; 0.21; 0.28*	

Linear dependences by periods have been established as regards the conditions of the present experiment, as follows: Fig. 1 presents the dependence between the temperature sum and the duration of the period from sprouting to the first true leaf (inclusive). The same is positive and averages the empirical points at R=0.78.

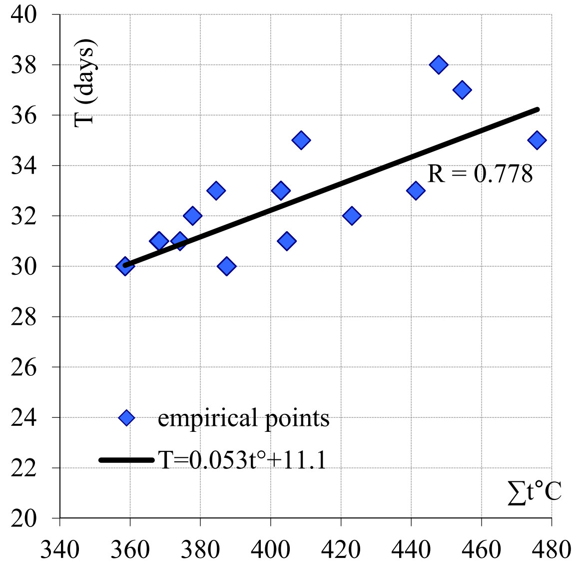

Fig. 1. Dependence between ∑T° and the duration of sprouting to the first true leaf (inclusive)

There is a closer dependence relevant to the period of flowering and the period of fruitfulness. The results are presented in Figure 2 and in Figure 3 respectively, at R>0.9.

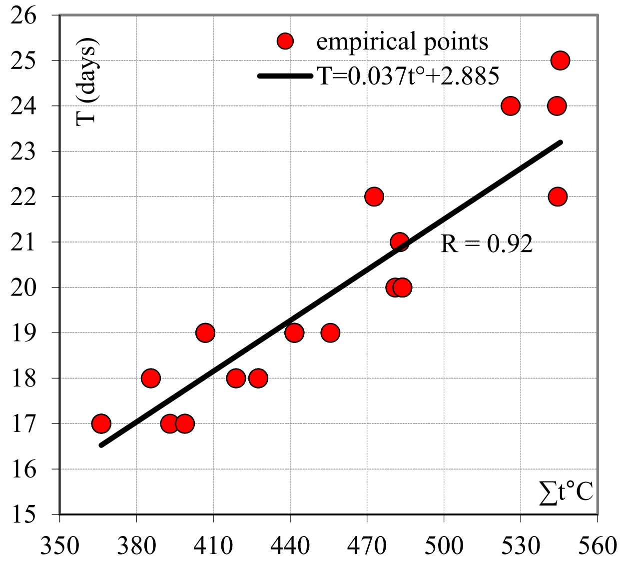

Fig. 2. Dependence between ∑T° and the duration of the flowering period (flower bud+flowering)

These dependences may be used for prognostication of the duration of the separate periods of vegetation of the used variety of pepper based on the data about the temperature sum throughout the certain vegetation or a part of it. There is linear dependence between the temperature sum and the duration of the periods - from sprouting to the first true leaf (inclusive) at values of R=0.778, flowering (including the stages of flower bud and flowering) at values of R=0.92, and fruitfulness at values of R=0.918. The same may be used for prognostication of the duration of the separate periods of vegetation and the used variety of pepper.

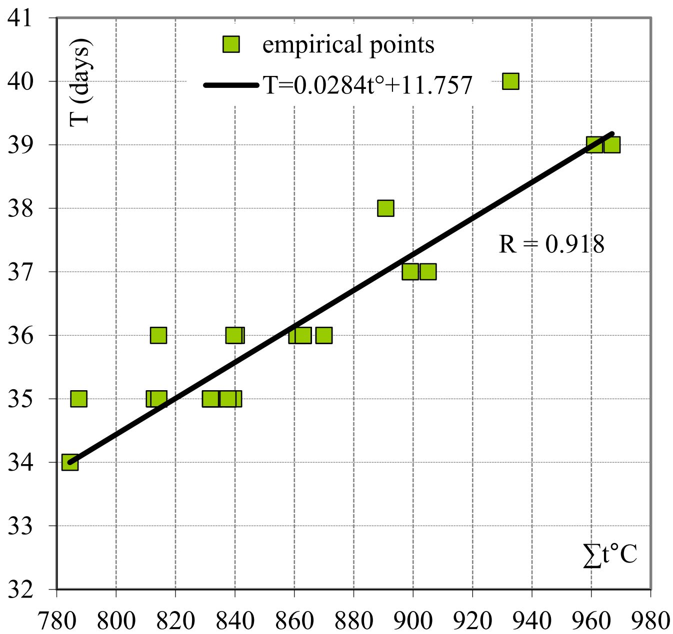

Fig. 3. Dependence between ∑T° and the duration of the period of fruitfulness

CONCLUSIONS

The combined application of biofertilizers has a stimulating effect on the height of plants and the total number of leaves per plant, which is typical upon the application of Emosan on basic fertilization with Boneprot, thus representing convincing evidence for the beneficial impact of the biofertilizers applied during seedling production. The combined application of the biofertilizers included in the experiment (as basic fertilization and additional feeding) improves the physiological status of the plants. The application of biofertilizers aims at the optimization of the fertilization system in order to ensure the stable ecological environment in the agro-ecosystem without any environmental risk.

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