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IMPACT OF BIOFERTILISERS ON VEGETATIVE GROWTH AND LEAF GAS-EXCHANGE OF PEPPER SEEDLINGS (*Capsicum annuum* L.) IN ORGANIC FARMING

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Abstract

Biofertilisers emerged as a successful alternative to mineral fertilizers in maintaining soil fertility in organic farming. The aim of the study was to examine the impact of biofertilisation on vegetative growth and indicators of leaf gas-exchange (rate of net photosynthesis, intensity of transpiration, and stomatal conductance) of pepper seedlings. An experiment was carried out in 2009-2011 in a polyethylene greenhouse at the biological farm at the Agroecological Centre at the Agricultural University-Plovdiv. It included pepper variety 'Sofiiska Kapiya' and selected biofertilizers that were tested for their effectiveness, i.e. Seasol (Earthcare), applied on two basic fertilisations- Boneprot and Lumbrical. The biofertilisers' active substances fall in the list of allowed soil fertility additives according to Regulation (EC) No. 889/2008. The results from the biometric measurements of pepper seedlings (plant height and number of leaves) showed the highest values for the variant fed with the combination of biofertiliser Seasol applied on the basic fertilisation with Boneprot (2009, 2011). The positive impact of this combined application was shown by the higher rate of net photosynthesis (2010, 2011) in comparison to the single application of the basic fertilisation with Boneprot. An overall improvement of the physiological status of the seedlings was observed. The highest value of the the intensity of the transpiration was observed for the treatment with optimum concentration of basic fertilisation with Lumbrical (2010, 2011). The stomatal conductance had a high value upon combined application of biofertiliser Seasol on the basic fertilisation with Lumbrical (2009, 2010). The highest values of stomatal conductance were observed in the treatments, which showed also a maximum intensity of transpiration that can be attributed to the supply of nutrients in easily accessible form with the liquid biofertiliser Seasol and its good combination with basic biofertiliser applications.

Keywords: *biofertilisers, Capsicum annuum L., leaf gas- exchange, organic agriculture, seedlings.*

INTRODUCTION

The research on the effectiveness of biofertilisers application in organic seedling production are not sufficient. Producing healthy and quality organic seedlings is a guarantee for producing optimal yield. Cholakov et al. (2013) point out the report of Murtazov (1980), according to whom the usage of healthy and high-quality seedling material is an important technological stage in growing the major vegetable crops.

Biofertilisers can improve the soil fertility and provide the needed plant nutrition, thus making them efficient components in organic agriculture (Vermany, 2007). Organic and inorganic fertilisers applied to the soil supply nutrients for crop growth and affect the plant's physiological processes, which serve as important instruments in yield development (Amujoyegbe et al., 2007). There is a close interdependent connection and conditionality between mineral feeding and photosynthesis of plants (Pandev et al., 1980). Photosynthesis provides the primary constructive elements and the necessary energy, but root feeding provides the remaining required nutritional substances (Aladzhadzhiyan, 2002). The additional feeding of pepper plants with vermicompost Lumbrical impacts positively the leaf gas-exchange. With the increase of the biofertiliser dose, the net photosynthesis and intensity of transpiration also increase. The higher intensity of photosynthesis can also be attributed to the decreased stomatal limitation that leads to increasing of inter- cell concentration of the CO_2 (Karanatsidis, 2013).

The aim of the study was to examine the impact of biofertilisation on vegetative growth and indicators of leaf gas-exchange (rate of net photosynthesis, intensity of transpiration, and stomatal conductance) of pepper seedlings.

MATERIALS AND METHODS

This experiment was carried out in 2009- 2011 in a polyethylene greenhouse of the Agroecological Centre at the Agricultural University of Plovdiv (Bulgaria), situated on the domain of a certified organic farm.

The research examined a pepper variety 'Sofiiska Kapiya' grown as mid-early field production according to the principles of organic agriculture (Panayotov, 2000). The seeds for seedling production were in conjunction with the EU requirements for organic plant growing. Sowing took place in the second half of March. The plot formation was followed by introduction of a basic (background) fertilisations with solid biofertilisers Boneprot and Lumbrical (g/m^2). The biofertilisers dose was in conjunction with manufacturers' recommendations. The experiment included 4 replications of 1 m^2 each.

Variants:

1. Control (non-fertilised)
2. Basic fertilisation with Boneprot (optimal concentration)
3. Basic fertilisation with Boneprot (50%) + Seasol
4. Basic fertilisation with Lumbrical (optimal concentration)
5. Basic fertilisation with Lumbrical (50%) + Seasol

Two basic fertilisations with Boneprot and Lumbrical were applied in the soil and incorporated prior to the time of sowing. The biofertilisers were applied in two concentrations i.e. optimal (corresponding to 70 kg/da for the basic fertilisation Boneprot and 400 L/da for the basic fertilisation Lumbrical) and optimal reduced by 50%.

In the process of seedling growth, the biofertiliser Seasol was introduced twice, i.e. in the soil before sowing of seeds in concentration 1:500-0.3-0.4 L/da, and at the end of the seedling growing in the same concentration (Vlahova, 2013).

The treatments were done with biofertilisers Boneprot, Lumbrical and Seasol, which are included in the list of the permitted substances for soil maintaining fertility according to Regulation (EC) No. 889/2008.

Boneprot (Arkobaleno, Italy) is pellet organic fertilisers, and has following composition: (organic nitrogen (N)-4.5%; phosphorus anhydride (P_2O_5) total-3.5%; potassium (K_2O)-3.5%; calcium (CaO)-5-8%; magnesium (MgO)-0.8-1%; organic carbon (C) of biological origin-30%; humification rate (HR)-10-13%; humidity - 13-15%; pH in water - 6-8. Boneprot is entirely organic fertiliser consisting solely of processed cattle manure.

Lumbrical (private producer from village Kostievo, Bulgaria) is a product obtained from processing 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%, organic matter content of 45-50%, Ammonium nitrogen (NH_4N) 33.0 ppm, nitrate nitrogen (NO_3N) 30.5 ppm, P_2O_5 and K_2O 1410 ppm and 1910 ppm respectively. It contains useful microflora $2x10^{12}$ pce/g, humic and fulvic acids, nutritional compounds. The product acidity is 6.5-7.0 (pH in H_2O).

Seasol (Earthcare) Seasol International Pty Ltd. (Australia) is an extraction of brown algae *Durvillaea potatorum*. Seasol is a 100% liquid natural seaweed extract. The commercial product contains raw protein (2.5±0.1% w/w), alginates (6±2% w/w), total solidity (10.0±0.5% w/w), and pH (10.5±0.5% w/w), and 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.

Study Parameters

Biometry

Ten plants per variant were analyzed at the end of the seadling period by measuring their plant height (in cm) and number of leaves.

Leaf gas- exchange parameters

Measurements on well-developed leaves from the seedlings were taken. The sampled leaves were representative of each treatment that was done 10 days after second introduction of the liquid biofertiliser Seasol and at the end of seedlings period. The analyzed parameters were: net photosynthetic rate (P_N) (μmol$m^{-2}s^{-1}$), transpiration intensity (E) (mmol $m^{-2}s^{-1}$) and stomatal conductance (g_s) (mol m^{-2} s^{-1}), using a portable infrared gas analyser LCA-4 (ADC, Hoddesdon, England).

Statistical data processing was done by Microsoft Office Excell 2007, SPSS (Duncan 1955), BIOSTAT and STATISTICA - StatSoft Treatment 9.0 (MANOVA, StatSoft). An analysis of variance (ANOVA) was used to analyse 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 at $P<0.05$ level. BIOSTAT was used to compare the results as treated compared to the control.

RESULTS AND DISCUSSIONS

Changes in biometric parameters during the vegetative growth of pepper seedlings

The results of biometric measurements on the height of the pepper seedling for the variety 'Sofiiska Kapiya' showed that in 2009 the highest value was shown by the variant treated with the biofertiliser Seasol on the basic fertilisation Boneprot, i.e. 19.32 cm, followed by the variant treated with biofertiliser Seasol on the basic fertilization Lumbrical, i.e. 19.28 cm (Table 1). The difference between these two variants was not significant (at $P<0.05$). The statistical analysis made in connection with all variants showed that the difference in comparison with the non-fertilized control was significant at $P_{0.1\%}$. When compare the variants on the two basic fertilizations, it was found that the plants are higher after the basic fertilization with Boneprot.

In 2010, the highest values of the pepper seedlings height was measured for the variant treated with biofertiliser Seasol on the basic fertilization Lumbrical, i.e. 17.50 cm. In comparison with the single application of a basic fertilization in optimal concentration, a better result was shown on the basic fertilization with Lumbrical. In all variants the difference compared to the control was significant at $P_{0.1\%}$.

The results of the biometric measurements of the height of the pepper seedlings in 2011 showed, that the liquid biofertiliser Seasol on the basic fertilization Boneprot lead to a higher value in comparison with the basic fertilization Lumbrical, thus confirming the findings made in 2009.

Table 1. Height of plants (cm) on pepper seedlings, variety of 'Sofiiska Kapiya'

No	Treatments	2009		2010		2011		Average for the period
		Mean; St. Dev.	GD	Mean; St. Dev.	GD	Mean; St. Dev.	GD	
1.	Control	16.66 ± 0.342 e	Base	16.10 ± 0.207 f	Base	15.55 ± 0.127 d	Base	16.10
2.	Boneprot (optimum)	18.50 ± 0.065 b	+++	17.20 ± 0.254 de	+++	16.21 ± 0.542 c	+++	17.30
3.	Boneprot (50%) + Seasol	19.32 ± 0.202 a	+++	17.00 ± 0.067 e	+++	16.70 ± 0.286 b	+++	17.67
4.	Lumbrical (optimum)	17.41 ± 0.586 d	+++	17.40 ± 0.312 d	+++	16.60 ± 0.046 b	+++	17.14
5.	Lumbrical (50%) + Seasol	19.28 ± 0.226 a	+++	17.50 ± 0.131 d	+++	16.62 ± 0.361 b	+++	17.80
	$GD_{5\%}$		0.22		0.30		0.33	
	$GD_{1\%}$		0.30		0.41		0.45	
	$GD_{0.1\%}$		0.41		0.56		0.61	

*Duncan's Multiply Range Test, $P<0.05$

It was found that the combined application of biofertilisers had better effect on the average height of the pepper seedlings in comparison with the single application of basic fertilization in optimum concentrations. It was detected in 2009 and confirmed in 2011. The results of biometric measurements of the average height of the pepper seedlings showed that the treated plants exceeded the controls during the three years (at $P_{0.1\%}$).

In 2009, the highest number of a leaves of a plant in the seedling phase was obtained after the combined application of the biofertiliser Seasol on the basic fertilization Boneprot, i.e. 8.87 pcs/plant, followed by treatment with the biofertiliser Seasol on the basic fertilization

Lumbrical, i.e. 8.60 pcs/plant (Table 2). The difference in these two variants, compared to the control was significant at $P_{0.1\%}$. The results showed the better effect of the combined application of biofertilisers reflecting in higher number of leaves per plant in comparison with the single application on the basic fertilizers.

In 2010 maximum value was found upon additional application of the biofertiliser Seasol on the basic fertilization Lumbrical and the single application on the basic fertilization Boneprot, i.e 7.80 pcs/plant. The difference between the average values and the non-fertilised (control) was significant ($P_{0.1\%}$).

The highest number of leaves per plant in the seedling phase in 2011, was found upon combined application of biofertiliser Seasol on the basic fertilization Boneprot, i.e. 7.40 pcs/plant, thus confirming the findings made in 2009. The best effect of influence on the formed number of leaves per plant, average for the period was reported for the combined treatment of the biofertiliser Seasol applied on the basic fertilization Boneprot, i.e 7.89 pcs/plant.

The combined application of liquid biofertiliser Seasol affected positively on the total number of leaves on the basic fertilization Boneprot (2009, 2011) and on the basic fertilization Lumbrical (2009, 2010) and had an effect on the height of pepper seedlings on the basic fertilization Boneprot (2009, 2011) and on the basic fertilization Lumbrical (2009, 2010, 2011). This can be attributed to the favorable influence on the Seasol and suitable combination with the basic fertilization, which ensured nutritional substances absorbable by seedlings.

Table 2. Number of leaves per plant on pepper seedlings, variety of 'Sofiiska Kapiya'

No.	Treatments	2009		2010		2011		Average for the period
		Mean; St. Dev.	GD	Mean; St.Dev.	GD	Mean; St.Dev.	GD	
1.	Control	6.67 ± 1.234 [e]	Base	6.47 ± 1.246 [f]	Base	5.60 ± 1.121 [e]	Base	6.25
2.	Boneprot (optimum)	7.87 ± 0.352 [d]	++	7.80 ± 0.414 [d]	+++	6.67 ± 0.488 [d]	+++	7.45
3.	Boneprot (50%) + Seasol	8.87 ± 0.352 [ab]	+++	7.40 ± 0.507 [de]	++	7.40 ± 0.507 [bc]	+++	7.89
4.	Lumbrical (optimum)	8.40 ± 0.507 [bcd]	+++	7.40 ± 0.507 [de]	++	7.20 ± 0.414 [cd]	+++	7.67
5.	Lumbrical (50%) + Seasol	8.60 ± 0.507 [abc]	+++	7.80 ± 0.414 [d]	+++	7.07 ± 0.458 [cd]	+++	7.82
	$GD_{5\%}$		0.68		0.59		0.51	
	$GD_{1\%}$		0.93		0.81		0.69	
	$GD_{0.1\%}$		1.26		1.09		0.93	

*Duncan's Multiply Range Test, P<0.05

Leaf gas-exchange analyses under seedlings

The summarized results of the rate of net photosynthesis (P_N) in the period of the experiment are presented on the Table 3 below. In the end of 2009 seedling period, the highest value was reported after applying optimal concentration Boneprot, i.e.23.40 $\mu molm^{-2}s^{-1}$. Higher P_N values were shown by the pepper seedling fed with liquid biofertiliser Seasol on the basic fertilization Lumbrical, as the difference between average values compared to the control ones was significant at $P_{0.1\%}$.

In 2010, the highest value of the rate of the net photosynthesis (P_N) was shown after application of biofertiliser Seasol on basic fertilization with Boneprot, i.e. 23.25 $\mu molm^{-2}s^{-1}$. Comparing the results of both basic fertilizations. It was found that there was a higher P_N upon the application of Boneprot, as the difference between the average compared to the control was significant at $P_{0.1\%}$.

In 2011, the highest value of the rate of the net photosynthesis (P_N) was reported for the pepper seedlings fed with liquid biofertiliser Seasol on the basic fertilization Boneprot - 24.30 $\mu molm^{-2}s^{-1}$, as the difference between the average compared to the control was significant at $P_{0.1\%}$. Combined application of the biofertiliser Seasol on the basic fertilization Boneprot in 2011, confirmed the findings in 2010. It confirmed the stimulating effect of combined application in comparison with optimum concentration on the basic fertilization Boneprot (2010, 2011) and Lumbrical (2009, 2011).

Table 3. Rate of the net photosynthesis P_N - (μmol $m^{-2}s^{-1}$) in the end of seedling period

No.	Treatments	2009		2010		2011	
		Mean; St. Dev.	GD	Mean; St. Dev.	GD	Mean; St. Dev.	GD
1.	Control	18.00 ± 0.269 [b]	Base	16.70 ± 1.302 [e]	Base	18.11 ± 0.580 [e]	Base
2.	Boneprot (optimum)	23.40 ± 1.796 [a]	++	20.50 ± 1.224 [bcd]	++	18.49 ± 0.395 [e]	ns
3.	Boneprot (50%) + Seasol	18.26 ± 0.608 [b]	ns	23.25 ± 2.359 [a]	+++	24.30 ± 0.400 [a]	+++
4.	Lumbrical (optimum)	17.30 ± 0.488 [b]	ns	22.10 ± 0.895 [abc]	+++	19.80 ± 0.200 [de]	+
5.	Lumbrical (50%) + Seasol	22.98 ± 2.616 [a]	++	19.57 ± 0.480 [d]	+	21.82 ± 0.315 [bc]	+++
	$GD_{5\%}$		3.22		2.17		0.90
	$GD_{1\%}$		4.58		2.96		1.26
	$GD_{0.1\%}$		6.63		4.00		1.83

*Duncan's Multiply Range Test, P<0.05

The Multifactorial Analysis of variance (MANOVA, StatSoft) applied for the purpose of analyzing the rate of the net photosynthesis (P_N) in the seedling period during the period of the experiment is presented in Figure 1 below.

The interaction of major factors for pepper growth, i.e. type of biofertiliser and the year of vegetation (different agroecological conditions) was investigated.

The interaction of these factors impacted significantly ($P<0.05$) the rate of the net photosynthesis (P_N).

The impact was significant after feeding with liquid Seasol on Boneprot. Combined biofertilization have greater impact than application of a single biofertiliser.

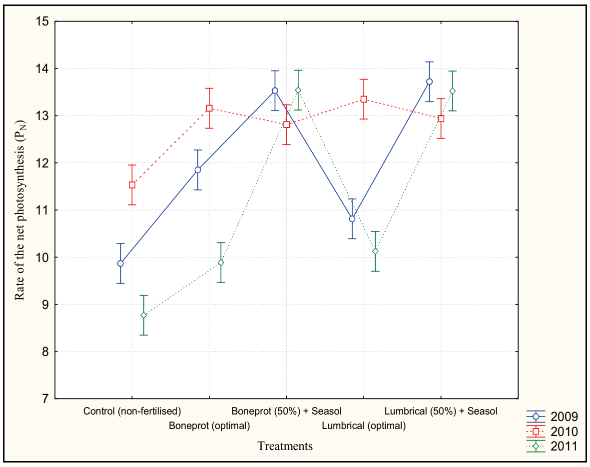

Figure 1. Effect of the interaction of the main factors on rate of the net photosyntesis (2009-2011)

The intensity of transpiration (E) in 2009 at the end of seedling period showed the highest value by the pepper fed with biofertiliser Seasol on the basic fertilization Lumbrical and after optimal concentration of Lumbrical, i.e. 2.00 mmolm^{-2}s^{-1}. The diference by these two variants and the non-fertilized control was significant at $P_{0.1\%}$ (Table 4).

In 2010 the higher values of the dynamics of the net photosynthesis has been reported for plants cultivated on the basic fertilization Lumbrical- 2.42 mmolm^{-2}s^{-1}. The combined application of biofertiliser Seasol on the basic fertilization Lumbrical showed a higher effect on the intensity of the transpiration in comparison with the combined application on the other biofertilisers. In 2011, the data showed the maximum value after single basic fertilization Lumbrical, i.e.

1.88 mmolm^{-2}s^{-1}. Next higher results were shown also after a single application of biofertiliser Boneprot.

The Figure 2 presents the MANOVA (StatSoft) for analyzing the intensity of transpiration (E) in the pepper seedling during the period of the experiment. It was found that there is no uniform tendency, i.e. interaction between the two factors is statistically significant ($P<0.05$) and impacts the intensity of transpiration (E), but it is more profound after application of Lumbrical in optimum concentration as well as after application of the combined treatment of Seasol and basic fertilisation.

The study found a maximum stomatal conductance (g_s) in the pepper seedlings grown on the basic fertilization Lumbrical (2009, 2010) (Table 5).

Table 4. Transpiration intensity E - (mmolm^{-2} s^{-1}) in the end of seedling period

No.	Treatments	2009		2010		2011	
		Mean; St. Dev.	GD	Mean; St. Dev.	GD	Mean; St. Dev.	GD
1.	Control	1.38 ± 0.269 ^{b}	Base	1.82 ± 0.172 ^{d}	Base	1.50 ± 0.029 ^{d}	Base
2.	Boneprot (optimum)	1.26 ± 0.156 ^{bc}	ns	2.32 ± 0.255 ^{bc}	+	1.61 ± 0.110 ^{d}	ns
3.	Boneprot (50%) + Seasol	1.20 ± 0.042 ^{bc}	ns	2.04 ± 0.210 ^{cd}	ns	1.04 ± 0.021 ^{e}	++
4.	Lumbrical (optimum)	2.00 ± 0.035 ^{a}	+++	2.42 ± 0.099 ^{abc}	++	1.88 ± 0.120 ^{c}	++
5.	Lumbrical (50%) + Seasol	2.00 ± 0.042 ^{a}	+++	2.38 ± 0.367 ^{abc}	++	1.09 ± 0.040 ^{e}	++
	$GD_{5\%}$		0.25		0.39		0.26
	$GD_{1\%}$		0.35		0.53		0.38
	$GD_{0.1\%}$		0.51		0.72		0.54

*Duncan's Multiply Range Test, P<0.05

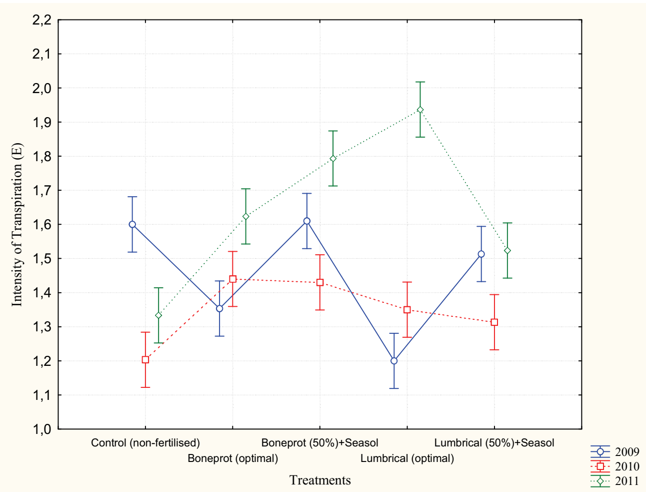

Figure 2. Effect of the interaction of the main factors on (E) (2009-2011)

A good value of stomatal conductance was also detected in the variant treated with the biofertiliser Seasol on the basic fertilization Lumbrical (2009, 2010).

The higher values of stomatal conductance correlate with the observed maximum values of the intensity of transpiration (E). This can be attributed to the influence on the nutrient compounds in accessible form contained in the liquid biofertiliser Seasol and the good combination with the solid biofertiliser applied as a background.

On the other hand, this probably creates conditions for improving the physiological status of the pepper seedlings. Upon comparison of both combined applications of biofertilisers, it was found that there was higher P_N on the basic fertilization Boneprot.

The present study confirmed the findings of Karanatsidis and Berova (2009) that the rate of the net photosynthesis and the intensity of transpiration are significantly increased upon the soil application of the organic nitrogen fertiliser Emosan.

Zlatev and Popov (2013) showed that nutrition with biofertilisers Emosan and Lumbrical increases the parameters of the leaf gas exchange.

Table 5. Stomatal conductance g_s- (mol m^{-2} s^{-1}) in the end of seedling period

No.	Treatments	2009	2010	2011
		Mean; St. Dev.	Mean; St. Dev.	Mean; St. Dev.
1.	Control	0.025 ± 0.007	0.033 ± 0.006	0.030 ± 0.010
2.	Boneprot (optimum)	0.035 ± 0.007	0.033 ± 0.006	0.040 ± 0.010
3.	Boneprot (50%) + Seasol	0.030 ± 0.007	0.040 ± 0.010	0.030 ± 0.005
4.	Lumbrical (optimum)	0.050 ± 0.000	0.045 ± 0.005	0.030 ± 0.010
5.	Lumbrical (50%) + Seasol	0.040 ± 0.007	0.040 ± 0.004	0.030 ± 0.010

CONCLUSIONS

Combined application of biofertilisers of various nature on a basic fertilization had a positive effect on the net photosynthesis (P_N) in comparison with single application of solid biofertilisers in optimum concentrations. This lead to improving the physiological status on the pepper seedlings. A better effect on the P_N was achieves upon the application of the biofertilisers Seasol on the basic fertilization Boneprot (2010, 2011).

The best effect on the intensity of transpiration (E) and stomatal conductance (g_s) of the pepper seedlings was observed after application of the biofertiliser Seasol on the basic fertilization Lumbrical. The effect can be attributed to the nature of the two biofertilisers that provide necessary nutrients for the growth of the seedlings.

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