International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 5, No. 5, p. 148-160, 2014

INNSPUB

RESEARCH PAPER | OPEN ACCESS

Improvement of productivity and quality of pepper (*Capsicum annuum L.*) resulting from biofertilizer applications under organic farming

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Article published on November 23, 2014

Key words: Biofertilizers, *Capsicum annuum* L., Organic agriculture, Productivity, Soil fertility, yield.

Abstract

Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health optimizing biological cycles and soil biological activity. Such optimization often relies on so called biofertilizers which could improve the soil nutrient management. With aim to study the effect of such group of biofertilizers on productivity and quality of economically-importanbt crop such as pepper (*Capsicum annuum* L.) variety Kurtovska Kapiya 1619, an experiment was carried out in 2009-2011 on the experimental fields of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria). Solid biofertilizers, i.e. Boneprot and Lumbrical, and a liquid biofertilizer Baikal EM were tested. The content of the total digestible N, K_2O and humus at the end of the vegetation showed the highest values after application of Boneprot in an optimum concentration. The highest standard yield was measured after Baikal EM on basic fertilization with Lumbrical, i.e. 15980 $kg.ha^{-1}$ in 2009. The result was confirmed in 2011, i.e. 16540 $kg.ha^{-1}$. The increase in comparison with the control variants was by 61.8% and 43.8 % respectively. Upon the combined application of Baikal EM on basic fertilization with Lumbrical, there was an increase of the number of fruits in 2010, i.e. 8.7 pcs/plant that was confirmed in 2011, i.e. 7.9 pcs/plant. The highest mass of the fruits was shown after Baikal EM on basic fertilization with Boneprot (in 2010 and 2011). The maximum value of the pericarp thickness was detected for the fruits of the variant treated with Baikal EM on basic fertilization with Boneprot. The combined application of biofertilizers showed a higher stimulative impact than the single application of biofertilizers. The higher total sugars and Vitamin C content in the pepper fruits after treatment with Baikal EM on basic fertilization with Lumbrical, determined this combination as favourable.

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Introduction

Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem health, including biological cycles, and soil biological activity (Kristiansen *et al.*, 2006). Within this holistic production management, maintaining the desirable soil fertility and soil quality is of utmost importance for producing healthy food and feed (Anderson, 2011; Agamy *et al.*, 2013; Fatima Baby, 2013; Sobieralski *et al.,* 2013) by providing plants with the necessary nutritional elements without having undesirable impact on the environmental resources (Njoroge and Manu, 1999; Suge *et al.*, 2011). Therefore, it is necessary to regularly update the existing technological practices of so called bio- fertilization to achieve optimum yields in organic production without compromising plant quality.

There are many attempts to search for optimal solutions for providing sustainable nutritional supply for economically-important crops such as pepper (Vlahova and Popov, 2013 a,b). Biofertilizers as such are important components of integrated nutrients management (Mohammadi and Sohrabi, 2012). They are cost-effective, eco-friendly and renewable sources of nutrients and as such they play a vital role in maintaining a long-term soil fertility and sustainability (Aggani, 2013). Many biofertilizers contain living microorganisms, which provide direct or indirect beneficial effects on plant growth and crop yield through different mechanisms (Fuentes-Ramirez and Caballero-Mellado, 2005; Ismail *et al.,* 2013). They are suggested as an alternative to mineral fertilization and for mitigating the environmental pollution (Padhi and Swain, 2009). However, the above-mentioned research does not sufficiently investigate typical varieties adapted to specific local conditions and also do not sufficiently cover wide range of mutually-connected parameters of pepper growth.

In order to address the high nutritional demands of pepper in organic farming, it is necessary to achieve a high level of soil organic matter (Naturland, 2001). Therefore, this study is focused on the use of biofertilizers, which are key elements for maintaining and improving soil fertility under the conditions of organic farming. The expected outcomes would highlight the possibility for optimization of the biological potential of the pepper crop. Worldwide, the studies related to the effect of application of biofertilizers on vegetable crops are still limited and the most are focused on vegetable greenhouse production.

Considering the existing data and the research arguments for this study, the following objectives were set up, i.e.:

1. To investigate impact of application of biofertilizers on agroecological factors (i.e. soil agro-chemical parameters) and their effect on production of economically-important crops such as pepper under organic farming conditions.
2. To obtain more complex information on the impact of biofertilizers on productivity and quality of pepper aimed at providing guidance to organic producers on how to organise more economically-efficient organic vegetable production.

Materials

Place of experiment

This experiment was carried out in 2009-2011 of the certified Demonstration organic farm on the experimental fields of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria). Plovdiv is a part of the Transitional continental climatic sub-region of Bulgaria to the Continental European climatic region and climatic region of East-Central Bulgaria (Ahmed, 2004).

Vegetable tested

Pepper (*Capsicum annum* L.) is an annual crop and belongs to the Genus *Capsicum* of Family *Solanaceae,* is one of the most varied and widely used foods in the world (Abu- Zahra, 2012).

Agrotechnology

The pepper variety Kurtovska Kapiya 1619 grown as mid-early field production in conformity with the principles of organic agriculture. The experiment was setup in the framework of a 6-field crop rotation with bean (*Phaseolus vulgaris*) as a preceding crop during all three-year study period. Soil cultivation included deep-ploughing in autumn and fine cultivation and high-levelled seed-bed formation in spring. Agricultural practices during vegetation included maintaining optimal soil moisture, within-rows weeding, monitoring of pests and disease attacks on plants and in-time plant protection.

The seedlings were planted on a permanent place during the third decade of May, on a high-levelled seed-bed using a sowing scheme 120+60x15 cm. The experiment design included the method of long plots, in four replications and a size of a test plot of 9.6 m^2.

Treatments (Variants)

1. Control (non- fertilized); **2.** Basic fertilization with Boneprot (optimum concentration); **3.** Basic fertilization with Boneprot (50 %) + Baikal EM; **4.** Basic fertilization with Lumbrical (optimum concentration); **5.** Basic fertilization with Lumbrical (50 %) + Baikal EM.

Characteristics of tested biofertilizers

This study includes solid biofertilizers Boneprot and Lumbrical, and a liquid biofertilizer Baikal EM. Their active ingredients are among permitted substances list of the Regulation (EC) No. 889/2008 (Enclosure No. 1) concerning organic farming in the European Union.

Boneprot

(Arkobaleno, Italy) is a pellet organic fertilizer consisting solely of cattle manure and has following composition: organic nitrogen (N)- 4.5 %; phosphorus anhydride (P_2O_5) total- 3.5 %; K_2O- 3.5 %; CaO-5-8 %; organic carbon of biological origin- 30 %; degree of humification-40-42 %; humidity-13-15%; pH in water- 6- 8.

Lumbrical

(v. Kostievo, Plovdiv, Bulgaria) is a product obtained from processing animal manure and other organic waste by Californian red worms (*Lumbricus rubellus* and *Eisenia foetida*) and consists of their excrements. The commercial product has humidity of 45-55% and organic substance content of 45-50 %. Ammonium nitrogen ($N-NH_4$)- 33.0 ppm; nitrate nitrogen ($N-NO_3$)- 30.5 ppm; P_2O_5- 1410 ppm and K_2O- 1910 ppm. It contains useful microflora $2x10^{12}$ pce/g; humic and fulvic acids; pH in water- 6.5-7.0.

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 *Saccharomices cerevisiae*. The product has the following chemical composition: Organic carbon- 0.15 %; total nitrogen- 0.01 %; P_2O_2- 0.001 %; K_2O- 0.02 %; pH- 3.2 and secondary microflora, a total titer of 10^6- 10^7.

Fertilization

Two basic fertilizations, using biofertilizers Boneprot and Lumbrical, were applied into the soil through incorporation prior to planting of the seedlings on the field. The biofertilizers were applied in two concentrations, i.e. an optimum (700 $kg.ha^{-1}$ for the basic fertilization Boneprot and 4000 L/ha for the basic fertilization Lumbrical) and a reduced (i.e. 50 % of the optimum concentration). The third biofertilizer i.e. the liquid Baikal EM was introduced twice in soil during vegetation, i.e. at the pepper growing stage 'flower bud' and after a formed 'mass fruitset' stage in concentration 1:1000 (Vlahova, 2013).

Parameters studied

1. The agrochemical soil parameters investigated: assimilated forms of nitrogen ($N-NH_4$ and $N-NO_3$- BDS ISO 14255- mg/kg); mobile forms of P_2O_5 and mobile forms of K_2O (according method of Egner-Reem-mg/100g); aqueous-extract pH 1:2.5; organic C (BDS ISO 14235 (g/kg) and humus- calculated on the basis of organic C (%). Soil samples were taken using a probe from the 0-20 cm layer with replicates from each variant in the beginning and in the end of vegetation.

2. Yield as a standard yield (kg.ha^{-1})

3. Economic productivity of plants as:

a. Number of fruits per plant (pcs/plant) from 10 plants per treatment.

b. Mass of fruits (g) from 10 fruits per treatment.

c. Pericarp thicknesses (mm)- 10 fruits per treatment.

4. Production quality- biochemical analysis was carried out on an average sample of 20 fruits from each treatment. The following parameters were analysed: dry matter content (refractometrically, in %), vitamin C (according to Tilman's reaction, in mg %) and total sugars (according to Schoorl-Regenbogen method, in %) (Genadiev *et al.,* 1969).

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

Impact of biofertilizers on the agrochemical soil parameters

In the *beginning of vegetation,* the *total absorbable nitrogen* content was within the range that determines the soil as weekly- alkaline (Tomov *et al.*, 2009) (2009, 2010 and 2011) (Table 1). Also, the *P_2O_5* content in the soil was within an average level of supply content (2009) and low-level of supply content (according to the Tomov *et al.*, 2009) (2010 and 2011). The *K_2O* content in the soil showed a good level of supply (2009, 2010 and 2011). By determining soil *humus* content, the soil can be classified into a group with a low level of humusness (2009 and 2010) and a very low level of humusness (2011) according Orlov and Grishina classification's (Totev *et al.*, 1991). The active *soil reaction* (pH) was slightly alkaline, according to the classification of the Bulgarian soils (2009, 2010 and 2011).

Table 1. Main agrochemical parameters in the beginning of vegetation.

Parameters	pH 1: 2,5 (H_2O)	EC mS/cm $^{-1}$	N- NO_3 mg/kg	N- NH_4 mg/kg	Total digestible nitrogen Nmg/kg	P_2O_5 mg/100g	K_2O mg/100g	Humus %
2009	7.30	2.83	13.0	3.6	16.6	16.40	22.10	2.00
2010	7.50	2.25	10.8	3.8	14.6	8.20	24.21	2.01
2011	7.30	2.49	11.5	3.9	15.4	8.42	25.10	1.95
Average	*7.37*	*2.52*	*11.8*	*3.8*	*15.5*	*11.01*	*23.80*	*1.99*

At the end of the vegetation period the results on the nitrate *N-NO_3* content showed the highest values for the variant treated with the biofertilizer Boneprot in an optimum concentration (2009 and 2011, Table 2), with the highest average value of 10.17 mg/kg for the study period.

Comparison of the variants regarding the nitrogen *N-NH_4* content shows variability. As an average for the period, the highest value was observed after a combined application of Baikal EM on basic fertilization with Lumbrical, i.e. 2.90 mg/kg. The result may be attributed to the impact of the composition of the microbial biofertilizer Baikal EM and the appropriate combination with the biofertilizer Lumbrical.

The content of *the total digestible nitrogen* in the end of the vegetation was the highest after application of Boneprot in an optimum concentration. This result was reported in 2009 and was confirmed in 2011. As an average for the period of the experiment, the value for this specific variant was the highest, i.e. 13.03 mg/kg. This proved that the biofertilizer Boneprot enriched the soil with digestible nitrogen, which was beneficial for the next crop in the crop rotation.

There was no unidirectional tendency for *P_2O_5* content in the soil for the three-year study period. Considering so called 'border-values' for the level of supply of soil with mobile phosphates, the observed soils had different levels of supply (Table 3). The highest P_2O_5 content was shown in 2009 and confirmed in 2011 after application of Boneprot in an optimum concentration. The level of soil P_2O_5 reserve was at a good level in 2009 and at an average level in 2011, according to the classification of the Bulgarian soils.

Table 2. Agrochemical parameters in the end of the vegetation- pH, N-NO_3, N-NH_4 and total nitrogen.

Parameters	Years	Treatments /variants/				
		Control	Boneprot (opt.)	Boneprot (50 %) + Baikal EM	Lumbrical (opt.)	Lumbrical (50 %) + Baikal EM
pH 1: 2.5 (H_2O)	2009	7.26 ^{c}	7.55 ^{ab}	7.51 ^{b}	7.48 ^{b}	7.59 ^{a}
	2010	7.46 ^{b}	7.48 ^{b}	7.58 ^{a}	7.46 ^{b}	7.47 ^{b}
	2011	7.54 ^{ab}	7.57 ^{a}	7.51 ^{b}	7.51 ^{b}	7.58 ^{a}
	Average	*7.42*	*7.53*	*7.53*	*7.48*	*7.55*
N- NO_3 mg/kg	2009	10.2 ^{c}	16.3 ^{a}	11.1 ^{b}	7.1 ^{d}	10.8 ^{b}
	2010	3.4 ^{e}	5.8 ^{c}	5.2 ^{d}	6.7 ^{b}	8.3 ^{a}
	2011	6.6 ^{b}	8.4 ^{a}	5.4 ^{c}	5.4 ^{c}	5.4 ^{c}
	Average	*6.73*	*10.17*	*7.23*	*6.4*	*8.17*
N- NH_4 mg/kg	2009	2.7 ^{b}	3.6 ^{a}	3.5 ^{a}	2.6 ^{b}	2.5 ^{b}
	2010	1.9 ^{d}	2.4 ^{c}	2.0 ^{d}	3.4 ^{b}	3.7 ^{a}
	2011	2.2 ^{c}	2.6 ^{a}	2.7 ^{a}	2.1 ^{c}	2.5 ^{ab}
	Average	2.27	2.87	2.73	2.70	2.90
Total digestible N mg/kg	2009	12.9	19.9	14.6	9.7	13.3
	2010	5.3	8.2	7.2	10.1	12.0
	2011	8.8	11.0	8.1	7.5	7.9
	Average	*9.0*	*13.03*	*9.97*	*9.10*	*11.07*

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

The *K_2O* content in the soil in the end of the vegetation was the highest after Boneprot in an optimum concentration for the study period. The measured level of soil K_2O reserve, screened towards the border values, was determined at a good level (2009) and at an average level (2010 and 2011). Higher values were also reported for the combined variant of biofertilizer Baikal EM on basic fertilization with Boneprot in 2009 (a good level of supply) and 2010 (an average level of supply). The results might be attributed to the content of K_2O in the composition of the biofertilizer Boneprot (3.5 %).

The soil *humus* content (Table 3) showed slight variation in the three-year period. The soil can be classified into the group of low-humus content soils. All applied biofertilizers had an impact on the enriching the humus content of soil. The highest average value for the period was detected after Boneprot in an optimum concentration, i.e. 2.64 %. This variant also showed a maximum value of total nitrogen, which might be attributed to the impact of the nature and composition of the biofertilizer Boneprot providing a large quantity of nutritional substances to the soil. Aiming at long-term sustainable soil fertility, a range of solid biofertilizers could be used among which is the Boneprot. They release slowly the necessary nutrients in the soil, while the liquid biofertilizers are quickly absorbed by plants or can be washed off by the soil water.

During the three years of the experiment, in the end of the vegetation there were some small changes in *the soil pH*, as compared to the reported in the beginning of the vegetation, but overall, all soil showed a slightly alkaline reaction.

Standard Yield

The highest *standard yield* was measured after Baikal EM on basic fertilization with Lumbrical, i.e. 15980 kg.ha^{-1} in 2009. The result was confirmed in 2011, i.e. 16540 kg.ha^{-1}. The increase in comparison with the control variants was by 618 g.kg^{-1} and 438 g.kg^{-1} respectively (Table 4).

Table 3. Agrochemical parameters at the end of vegetation, i.e. P_2O_5, K_2O and humus.

Parameters	Years	Treatments /variants/				
		Control	Boneprot (opt.)	Boneprot (50 %) + Baikal EM	Lumbrical (opt.)	Lumbrical (50 %) + Baikal EM
P_2O_5 (mg/100g)	2009	23.53^{c}	28.87^{a}	27.08^{b}	22.12^{d}	21.43^{e}
	2010	8.98^{d}	12.35^{b}	10.33^{c}	13.02^{a}	8.98^{d}
	2011	6.29^{e}	17.06^{a}	8.33^{d}	12.78^{b}	11.23^{c}
	Average	*12.93*	*19.43*	*15.25*	*15.97*	*13.88*
K_2O (mg/100g)	2009	27.28^{d}	31.69^{a}	28.61^{b}	27.95^{c}	23.47^{e}
	2010	15.49^{c}	18.17^{a}	17.83^{b}	14.60^{d}	14.37^{e}
	2011	13.47^{d}	19.49^{a}	14.37^{c}	14.35^{c}	17.95^{b}
	Average	*18.75*	*23.12*	*20.27*	*18.97*	*18.60*
Humus (%)	2009	2.90^{e}	3.35^{a}	2.93^{d}	3.25^{b}	2.99^{c}
	2010	2.45^{b}	2.45^{b}	2.57^{a}	2.16^{d}	2.25^{c}
	2011	2.05^{e}	2.11^{d}	2.20^{c}	2.25^{b}	2.49^{a}
	Average	*2.47*	*2.64*	*2.57*	*2.55*	*2.58*

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

Table 4. Standard Yield (kg.ha^{-1}), variety of Kurtovska Kapiya 1619 (from 2009 to 2011).

Years		Treatments /variants/				
		Control	Boneprot (opt.)	Boneprot (50 %) + Baikal EM	Lumbrical (opt.)	Lumbrical (50 %) + Baikal EM
2009	Mean; St. Dev.	9870 ± 230.3^{b}	11510 ± 84.2^{ab}	15460 ± 102.4^{ab}	12690 ± 545.4^{ab}	15980 ± 267.7^{a}
	GD	Base	n.s	+++	n.s	+++
	GD $_{5\%; 1\%; 0.1\%}$	295.92; 403.59; 546.15;				
2010	Mean; St. Dev.	10960 ± 316.2^{a}	13810 ± 349.7^{a}	16930 ± 526.3^{a}	14810 ± 155.2^{a}	16790 ± 118.2^{a}
	GD	Base	++	+++	+++	+++
	GD $_{5\%; 1\%; 0.1\%}$	169.44; 231.09; 312.72;				
2011	Mean; St. Dev.	11500 ± 138.0^{c}	12450 ± 66.5^{bc}	16150 ± 22.1^{a}	13010 ± 14.7^{b}	16540 ± 49.0^{a}
	GD	Base	ns	+++	++	+++
	GD $_{5\%; 1\%; 0.1\%}$	97.73; 133.30; 180.38;				
Average		*11777*	*12590*	*16180*	*13503*	*16437*

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

A significantly higher yield of 16930 kg.ha^{-1} (Table 4) compared to control variants was observed in 2010 after Baikal EM on basic fertilization with Boneprot, as the increase compared to the control was 545 g.kg^{-1}. The differences between the combined variants with the application of Baikal EM in comparison with the control values were well proven for $P_{0.1\%}$ (2009, 2010 and 2011). During the three vegetation years the increase of the standard yield after application of biofertilizers varied from 166 g.kg^{-1} to 618 g.kg^{-1} in 2009, from 260 g.kg^{-1} to 545 g.kg^{-1} in 2010, and from 83 g.kg^{-1} to 438 g.kg^{-1} in 2011, in comparison with the control. For both variants with the single application of biofertilizers in an optimum concentration, the higher yield was reported for Lumbrical, which was confirmed in the three experimental years. The increase in comparison with the non-fertilised plants was by 285 g.kg^{-1} in 2009, by 351 g.kg^{-1} in 2010 and by 131 g.kg^{-1} in 2011. As an average for the period, the highest yield was shown by pepper treated with Baikal EM on basic fertilization with Lumbrical, i.e. 16437 kg.ha^{-1}.

Table 5. Number of fruits per plant, variety of Kurtovska Kapiya 1619 (from 2009 to 2011).

Years		Treatments /variants/				
		Control	Boneprot (opt.)	Boneprot (50 %) + Baikal EM	Lumbrical (opt.)	Lumbrical (50 %) + Baikal EM
2009	Mean; St. Dev.	4.3 ± 0.707 ^{c}	5.4 ± 0.882 ^{b}	6.1 ± 0.782 ^{b}	7.9 ± 0.782 ^{a}	7.7 ± 0.500 ^{a}
	GD	Base	++	+++	+++	+++
	GD $_{5\%; 1\%; 0.1\%}$	0.78; 1.06; 1.43;				
2010	Mean; St. Dev.	4.2 ± 0.833 ^{c}	6.0 ± 0.500 ^{b}	8.3 ± 0.500 ^{a}	6.6 ± 0.726 ^{b}	8.7 ± 0.500 ^{a}
	GD	Base	+++	+++	+++	+++
	GD $_{5\%; 1\%; 0.1\%}$	0.67;0.91;1.24;				
2011	Mean; St. Dev.	4.3 ± 1.225 ^{c}	4.8 ± 0.667 ^{c}	7.3 ± 0.707 ^{a}	5.8 ± 1.093 ^{b}	7.9 ± 1.054 ^{a}
	GD	Base	n.s.	+++	+++	+++
	GD $_{5\%; 1\%; 0.1\%}$	0.69; 0.95; 1.28;				
Average		*4.3*	*5.4*	*7.2*	*6.8*	*8.1*

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

Table 6. Mass of fruits and thickness of pericarp, variety of Kurtovska Kapiya 1619.

Parameters	*Years*	Treatments /variants/				
		Control	Boneprot (opt.)	Boneprot (50 %) + Baikal EM	Lumbrical (opt.)	Lumbrical (50 %) + Baikal EM
Mass of fruits (g)	2009	66.5 ± 1.069 ^{d}	68.3 ± 0.473 ^{c}	70.1 ± 0.902 ^{b}	67.1 ± 0.971 ^{cd}	76.8 ± 0.265 ^{a}
	2010	69.2 ± 0.757 ^{c}	70.5 ± 1.756 ^{bc}	77.3 ± 0.473 ^{a}	71.3 ± 0.850 ^{b}	76.4 ± 0.737 ^{a}
	2011	69.1 ± 2.684 ^{b}	69.6 ± 0.551 ^{b}	76.1 ± 0.520 ^{a}	70.2 ± 0.907 ^{b}	75.2 ± 0.551 ^{a}
	Average	*68.3*	*69.5*	*74.5*	*69.5*	*76.1*
Thickness of pericarp (mm)	2009	4.37 ± 0.699 ^{b}	4.58 ± 0.706 ^{b}	5.80 ± 0.621 ^{a}	5.15 ± 0.562 ^{ab}	5.72 ± 0.764 ^{a}
	2010	4.10 ± 0.307 ^{c}	4.81 ± 0.283 ^{b}	5.72 ± 0.730 ^{a}	5.03 ± 0.494 ^{b}	5.67 ± 0.562 ^{a}
	2011	4.53 ± 0.28 ^{c}	4.61 ± 0.47 ^{c}	5.51 ± 0.34 ^{a}	4.73 ± 0.39 ^{bc}	5.02 ± 0.26 ^{b}
	Average	*4.33*	*4.67*	*5.68*	*4.97*	*5.47*

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

The results from the Multifactorial Analysis of variance (MANOVA) (Statistica, StatSoft) to analyze the standard yield on pepper plants during the the three experimental years is presented on Figure 1. There is no significant difference in standard yield of the pepper between treatments (at $p>0,05$), when consider the interaction between major factors, i.e. experimental years and type of treatment (variants). However, the analysis detected significant differences (at $p<0.05$) between variants (treatments with different combinations of biofertilizers, Figure 2) when a comparison of average values from the three experimental years is made. Both analyses show that the standard yield was impacted positively after treatments with biofertilizer Baikal EM on Lumbrical and Boneprot as compared to the control (non-fertilized) pepper plants.

Table 7. Content of dry matter, total sugars and vitamin C in the fruits.

Parameters	*Years*	Treatments /variants/				
		Control	Boneprot (opt.)	Boneprot (50 %) + Baikal EM	Lumbrical (opt.)	Lumbrical (50 %) + Baikal EM
Dry matter (%)	2009	6.20 ^{d}	8.94 ^{a}	8.30 ^{b}	8.10 ^{bc}	8.00 ^{c}
	2010	8.20 ^{a}	7.80 ^{b}	8.00 ^{ab}	8.00 ^{ab}	8.20 ^{a}
	2011	7.40 ^{d}	8.50 ^{a}	8.30 ^{b}	8.10 ^{c}	8.10 ^{c}
	Average	*7.27*	*8.41*	*8.20*	*8.07*	*8.10*
Total sugars (%)	2009	5.92 ^{e}	6.68 ^{d}	8.32 ^{b}	8.20 ^{c}	8.48 ^{a}
	2010	8.36 ^{b}	7.21 ^{d}	7.87 ^{c}	7.33 ^{d}	8.91 ^{a}
	2011	6.80 ^{d}	7.20 ^{c}	7.80 ^{b}	7.30 ^{c}	8.40 ^{a}
	Average	*7.03*	*7.03*	*8.00*	*7.61*	*8.60*
Vitamin C (mg%)	2009	163.0 ^{d}	187.0 ^{c}	205.9 ^{b}	185.0 ^{c}	220.0 ^{a}
	2010	166.6 ^{b}	191.3 ^{a}	173.7 ^{b}	173.7 ^{b}	175.4 ^{b}
	2011	163.0 ^{d}	180.7 ^{b}	175.6 ^{c}	172.0 ^{c}	221.4 ^{a}
	Average	*164.2*	*186.3*	*185.1*	*176.9*	*205.6*

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

Economic productivity of plants

a. Number of fruits per plant

During the period of the research, there was no unidirectional tendency observed in regards to formation of *number of fruits per plant*. The highest value of 7.9 pcs/plant was observed in 2009 after fertilisation with Lumbrical applied in an optimum concentration (Table 5).

Upon the combined application of Baikal EM on basic fertilization with Lumbrical, there was an increase of the number of fruits in 2010, i.e. 8.7 pcs/plant that was confirmed in 2011, i.e. 7.9 pcs/plant. In this variant, the difference compared to the control was significant for $P_{0.1\%}$ (in 2009, 2010 and 2011). Similar results were shown in 2010 and 2011. The average of 8.1 pcs/plant for this combination of biofertilizers was superior towards all other combinations and the control variant.

b. Mass of fruits

The parameter *average mass of the fruits* has also shown no unidirectional tendency during the three-year period. The highest mass was shown after Baikal EM on basic fertilization with Boneprot (in 2010 and 2011), and after Baikal EM on basic fertilization with Lumbrical (in 2009). The positive impact on mass of the fruits was found upon combination of Baikal EM on both basic fertilizations in optimum concentrations. As an average for the period, the highest value was detected after the combination of Baikal EM on basic fertilization with Lumbrical, i.e. 76.1 g. The results after combinations of biofertilizers show that the stimulating effect on the mass of the fruits can be attributed to the microbial nature of the biofertilizer Baikal EM (Table 6).

The MANOVA (Statistica, StatSoft) to analyze the differences in the mass of pepper fruits after treatment with combinations of biofertilizers (Figure

3) showed a significant impact (at p<0.05) of interaction of major factors, i.e. experimental year and type of applied biofertilizer, on this parameter. Similar to the standard yield (see Fig. 1 and 2), the mass of fruits was positively impacted after treatments with biofertilizers Baikal EM on Lumbrical and Boneprot as compared to the control (non-fertilized) pepper plants.

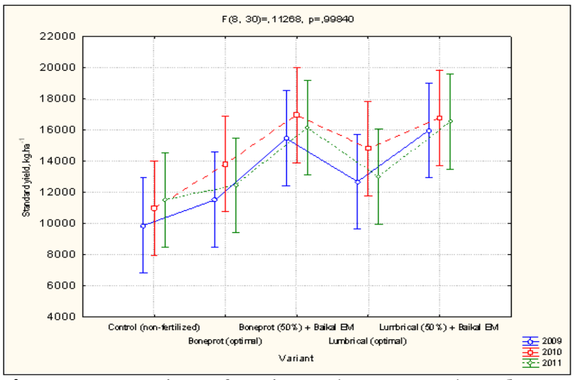

Fig. 1. Interaction of variants (treatments) and year of application on standard yield of pepper (from 2009 to 2011).

c. Pericarp thickness

The maximum value of the *pericarp thickness* was detected for the fruits of the variant treated with the combination of Baikal EM on basic fertilization with Boneprot (Table 6). The higher values for this combination were observed for the three study years, as the average for the period was 5.68 mm. The combined application of biofertilizers showed more stimulative impact than the single application of biofertilizers. All variants with biofertilizers exceeded the non-fertilized control in all three experimental years.

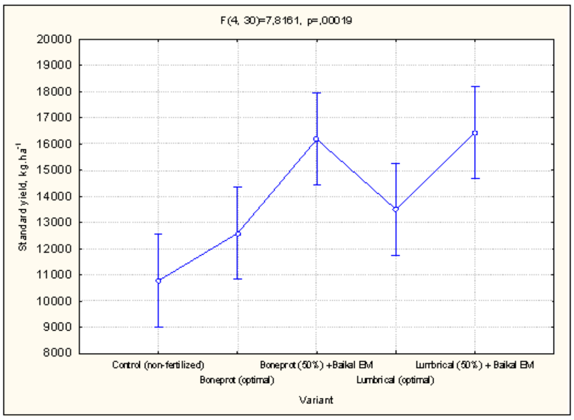

Fig. 2. Differences in standard yield between variants after a combined application of biofertilisers (as an average from the three experimental years).

Quality of the pepper

The data regarding the *dry matter* content of pepper fruits showed a unidirectional tendency in the period of the experiment. The highest value was detected in the fruits from a variant with the biofertilizer Boneprot applied in an optimum concentration (i.e. in 2009 and 2011) (Table 7). High values of the dry matter were reported in the fruits after combined application of the biofertilizer Baikal EM on basic fertilization with Boneprot (i.e. 2009 and 2010). As an average for the period of the experiment, the highest value was observed in the fruits of the variant with the biofertilizer Boneprot applied in an optimum concentration, i.e. 8.41 %.

The *total sugars* content in the fruits showed a slight variation between the individual variants. The highest value was shown after a combined application of Baikal EM on basic fertilization with Lumbrical, which was established in the three vegetation years (Table 7). The combined variants on basic fertilization with Boneprot and Lumbrical exceeded the values of their optimum concentrations, which determined the positive impact of the combined fertilization. As an average for the period, the highest value was reported for the combined variant with the biofertilizer Baikal EM on basic fertilization with Lumbrical- 8.60 %. All variants exceeded the control (2009 and 2011).

The highest *Vitamin C* content in the fruits was reported after application of Baikal EM on basic fertilization with Lumbrical, i.e. 220.0 mg% in 2009, which was confirmed in 2011 (i.e. 221.4 mg%), as the average for the period was 205.6 mg% (Table 7). The single application of Boneprot in an optimum concentration lead to increased Vitamin C content in 2010 and 2011. All tested biofertilizers stimulated the accumulation of Vitamin C in plants as the Vitamin C content was higher in treated compared to non-fertilized (control) plants in the three-year study period. The higher total sugars and Vitamin C content in the pepper fruits after treatment with Baikal EM on basic fertilization with Lumbrical, determined this combination as favourable. The stimulative effect can be attributed to import of efficient microorganisms

(EM) with the treatment with the biofertilizer Baikal EM.

Discussion

Having regards to dynamics and variation of agroecological conditions, selected and suggested by this study combinations of solid (Boneprot and Lumbrical) and liquid (Baikal EM) biofertilizers showed an impact on soil processes. They offer a relatively long-term release of nutrients to vegetable plants thus determining a higher plant productivity compared to liquid biofertilizers that are quickly absorbed by plants or can be washed off by the soil water.

The single applications of the solid biofertilizer Boneprot (in optimal concentrations) impacted the soil agro-chemical features in the end of vegetation, i.e. increased content of *total digestible N,* P_2O_5 and K_2O. It could be also a valuable resource of nutrients for the following crops in the rotation. The soil humus content showed slight variation in the three-year period, but generally there was an increase of the humus content of soil.

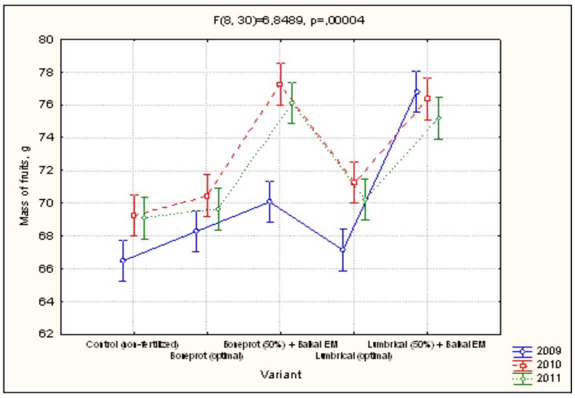

Fig. 3. Interaction of variants (treatments) and year of application on mass of fruits of pepper (from 2009 to 2011).

Pepper responded positively to the improved soil agro-chemical features, i.e. the highest *standard yield* was observed in 2009 (i.e. 15980 kg.ha^{-1}) and then in 2011 (i.e. 16540 kg.ha^{-1}) after application of Baikal EM on basic fertilization with Lumbrical. The increase of yield compared to the control (non-fertilized) variants was by 618 g.kg^{-1} and 438 g.kg^{-1} respectively thus confirming the findings of Karem *et al.* (2000), who also suggested that a large number of microorganisms, usually found in the rhizosphere, contribute to the increase in the soil fertility and the standard yield of the crops.

The increase of the standard yield of pepper variety of "Kurtovska Kapiya 1619" is achieved through increased productivity of treated plants, i.e. the *number of fruits* and their *mass*, in comparison with the control (non- fertilized) plants, thus confirming the conclusions drawn by Atiyeh *et al.* (2000) and Vermany (2007) and Chatterjee and Khalko (2013). Upon combined application of Baikal EM on basic fertilization with Lumbrical, there was an increase of the number of fruits, i.e. 8.7 pcs/plant in 2010, than 7.9 pcs/plant in 2011. The highest mass of fruits was shown after Baikal EM on basic fertilization with Boneprot (in 2010 and 2011), and after Baikal EM on basic fertilization with Lumbrical (in 2009). The positive impact on mass of the fruits was found upon combination of Baikal EM on both basic fertilizations in optimum concentrations. These findings conclusions made by Atiyeh *et al.* (2001) and Cabanillas *et al.* (2006). The maximum value of the pericarp thickness was detected for the fruits of the variant treated with the combination of Baikal EM on basic fertilization with Boneprot showing a significant increase compared to control ($p<0.05$), i.e. 5.80 mm (2009) и 5.72 mm (2010).

Overall, the combined application of biofertilizers showed more stimulation impact on pepper growth than the single application of biofertilizers.

The quality of pepper fruits was also improved, i.e. higher total sugars and Vitamin C content in the pepper fruits after treatment with Baikal EM on basic fertilization with Lumbrical, which confirms the findings of Szafirowska and Elkner (2009) and Sharma *et al.* (2005), Zaki *et al.* (2014) and Kazimierczak *et al.* (2011), and determines this combination as most favourable compared to others. The stimulative effect can be attributed to the import of efficient microorganisms (EM) after application of biofertilizer Baikal EM to the soil. This combination of biofertilizers is promising because it can be also used by organic farmers to achieve optimal pepper productivity.

Conclusions

To achieve an optimal productivity of agroecosystems, organic farmers may use soil application of additional substances of organic nature.

The research shows that the goals of achieving higher biological productivity of pepper and uncompromising environmental quality can be achieved by application of solid biofertilizers. They release the necessary nutrients more gradually in the soil and therefore are absorbed for a longer period by the targeted crop. A similar effect was found after the treatments with the liquid biofertilizer Baikal EM, which contains mixed microbiological cultures existing under a regime of activity and interacting with soil solution upon application. The soil-plant agroecosystem develops based on the input of energy by the biofertilizers, as it remains stable but at the same time enriched with nutritious substances. The improvement of the ecological sustainability of the agroecosystem through improved technological systems of fertilization depends also on the availability local resources and the existing ecological and socioeconomic conditions.

Acknowledgements

This research work was carried out with the support of Project "Vegetative and reproductive manifestations of pepper cultivated under the conditions of organic agriculture and possibilities for the application of some types of biofertilizers", No.03 - 09 of the Scientific Research Centre at Agricultural University of Plovdiv, Bulgaria.

Reference

Abu-Zahra TR. 2012. Vegetative, Flowering and Yield of Sweet Pepper as Influenced by Agricultural Practices. Middle-East Journal of Science Research **11 (9),** 1220- 1225.

Agamy R, Hashem M, Alamri S. 2013. Effect of soil amendment with yeasts as bio-fertilizers on growth and productivity of sugar beet. African Journal of Agricultural Research **8(1),** 46-56.

Aggani SL. 2013. Development of Bio-Fertilizers and its Future Perspective. Scholars Academic Journal of Pharmacy **2(4),** 327-332.

Ahmed T. 2004. Study of Allelopathic Relationship in the Soil-Weed-Tomato System. PhD thesis, Agricultural university- Plovdiv, Bulgaria [in Bulgarian].

Anderson R. 2011. Success Stories of Agricultural Long-term Experiments. Editor: Kerstin Hideborn Alm, KSLA, ISSN 0023-5350, 105.

Atiyeh RM, Arancon N, Edwards CA, Metzger JD. 2000. Influence of earthworm processed pig manure on the growth and yield of greenhouse tomatoes. Bioresource Technology **75,** 175-180.

Atiyeh RM, Edwards CA, Subler S, Metzger JD. 2001. Pig manure vermivompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth. Bioresource Technology **78,** 11-20.

Cabanillas C, Ledesma A, del Longo O. 2006. Biofertilizers (vermicomposting) as sustainable alternative to urea application in the production of basil (*Ocimum basilicum* L.). Molecular Medicinal Chemistry **11,** 28-30.

Chatterjee R, Khalko S. 2013. Influence of Organic Amendments and Inorganic Fertilizers on Late Blight Incidence and Yield of Tomato (*Lycopersicon esculentum* Mill.). International Journal of Geology, Agriculture and Environmental Sciences **1(1),** 36- 38. http//www. woarjournals.org/IJGAES,

Duncan D. 1955. Multiply range and multiple F-test. Biometrics, **11,** 1-42.

Fuentes-Ramirez LE, Caballero-Mellado J.

2005. Bacterial Biofertilizers (Chapter 5). PGPR: Biocontrol and Biofertilization, 143- 172.

Fatima Baby D. 2013. A Study on Organic Tomato Cultivation in Palamedu Panchayat, Madurai District. Global Journal of Science Frontier Research Agriculture and Veterinary Sciences **13(3),** 44- 51.

Genadiev A, Kalchev D, Tavekeliev N, Chavdarov N. 1969. Food Production, Analytical Technology, 696. [in Bulgarian].

Ismail EG, Mohamed WW, Khattab S, El-Sherif F. 2013.Effect of Manure and Bio-fertilizers on Growth, Yield, Silymarin content, and protein expression profile of *Silybum marianum*. International Journal of Medicinal and Aromatic Plants **3(4),** 430- 438.

Karem HA, Radwan SMA, Attia M. 2000. Impacts of Certain Biofertilizers on Cowpea Plants in Sludge Amanded Sandy Soil. Pakistan Journal of Biological Science **3(3),** 423-428.

Kazimierczak R, Hallmann E, Treščinska V, Rembiałkowska E. 2011. Ocena wartości odżywczej dwóch odmian buraków ćwikłowych (Beta vulgaric) z uprawy ekologicznej i konwencjonalnej. Journal of Research and Applied Agricultural Engineering **56 (3),** 206- 210 [in Polish].

Kristiansen P, Taji A, Reganold J. 2006. Organic Agriculture A Global Perspective. http://orgprints.org/14043

Mohammadi K, Sohrabi Y. 2012. Bacterial Biofertilizers for sustainable crop production: A Review **7(5),** 307- 316.

Naturland. 2001. Organic Farming in the Tropics and Subtropics. Exemplary Description of 20 Crops. Pepper.

Njoroge WJ, Manu C. 1999. Organic Farming. A Textbook for Post-Secondary Education. Kenya Institute of Organic Farming. Nairobi. Kenya.

Padhi S, Swain P. 2009. Effective role of microorganisms and seaweeds as biofertilizers in organic ferming for a sustainable environment. http://scholar.google.bg/

Regulation (EC). No. 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No. 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union L 250/1.18.9. 2008, 84.

Sharma S, Pradhan K, Satya S, Vasudevan P. 2005. Potentiality of Earthworms for Waste Management and in Other Uses- A Review. The Journal of American Science **1(1),** 4- 16.

Sobieralski K, Siwulski M, Sas-Golak I. 2013. Nutritive and health-promoting value of organic vegetables. Acta Scientiarum Polonorum Technologia Alimentaria **12(1),** 113-123. http://www.food.actapol.net/

Suge JK, Omunyin ME, Omami EN. 2011. Effect of organic and inorganic sources of fertilizer on growth, yield and fruit quality of eggplant (*Solanum Melongena* L). Journal of Applied Sciences Research **3(6),** 470-479.

Szafirowska A, Elkner K. 2009. The comparison of yielding and nutritive value of organic and conventional pepper fruits. Vegetable Crops Research Bulletin **71,** 111-121.

Tomov T, Rachovski G, Kostadinova S, Manolov I. 2009. Agrochemistry Manual. Academic Publishing House at the Agricultural University-Plovdiv, [in Bulgarian].

Totev T, Gribachev P, Nechev H, Artinova N. 1991. Soil Science Exercise Manual. PH „Zemizdat", Sofia, [in Bulgarian].

Vermany S. 2007. Knowledge and Adoption of Bio-Fertilizers in Horticultural and Other Crops in Rural Communities of Haryana. Journal of Horticultural Sciences **36 (1&2),** 86-88.

Vlahova VN. 2013. Agroecological aspects of the mid-early production of pepper *(Capsicum annuum* L.*),* PhD thesis, Agricultural university-Plovdiv, Bulgaria [in Bulgarian].

Vlahova V, Popov V. 2013 a. Impact of Biofertilisers upon Quality Parameters of Pepper Fruits (*Capsicum annuum* L.) Cultivated under the Conditions of Organic Agriculture, Journal of International Scientific Publications, Agriculture & Food **1(1),** 327- 333.

Vlahova V, Popov V. 2013 b. Quality of Pepper Fruits (*Capsicum annuum* L.) upon the Application of the Biofertilisers Cultivated under the Conditions of Organic Agriculture. Journal of International Scientific Publication, Ecology & Safety **7(3),** 4-10.

Zaki ME, ShafshakNS, Gabal MR, Shams AS. 2014. Effects of N-fertilizer source, biofertilizer and foliar spray with Delfan (amino acids) or garlic extract on growth, yield and fruit quality of sweet pepper plants.
http://www.academia.edu/482860