



Biological efficiency of biofertilizers emosan and seasol on pepper (*Capsicum annuum* L.) cultivated under organic farming conditions

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Abstract

Biofertilizers based on processed organic manure are proven alternative to mineral fertilizers. The experiments were carried out in 2009 to 2011 on an organic farm of the Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria). The study included pepper variety „Sofiiska Kapiya”. The biofertilizers Seasol (Earthcare) and Emosan (Hemozym Bio N5) were applied as vegetative feeding on top of two basic fertilizations with the solid biofertilizers Boneprot and Lumbrical. The objectives were to investigate impact of biofertilizers on agroecological conditions for pepper growth and on biological and productive parameters of pepper under organic farming. The investigated parameters were standard yield and economic productivity of plants (i.e. average number of fruits per plant, mass of fruits and pericarp thicknesses). The present study found that treatments with biofertilizers Emosan and Seasol on the basic fertilizations ensured more nutrients in the soil comparison with the single applications of the basic fertilization. It was shown by the agrochemical status of soil, i.e. at the end of vegetation, a higher total absorbable N after application of the biofertilizer Emosan on basic fertilization with Boneprot was found. Nevertheless, the treatment with biofertilizer Boneprot lead to a higher humus and major nutrient content compared to control (non-fertilized) plants. All treatments showed ‘a good level of supply’ of the soil with K₂O. This determines the positive impact of the biofertilization for enrichment of soil with potassium thus assuring a valuable reserve for the next crops. The optimal agroecological conditions provided by the addition of biofertilizers reflected in higher standard yield of pepper compared to the control plants. The results showed the positive effect of application of biofertilizer Emosan, regardless of the type of basic fertilization. The highest standard yields in all three experimental years were shown by the pepper plants fed with the biofertilizer Emosan on the basic fertilization Lumbrical, i.e. the average increase was 47.2 % compared to the control plants. The biofertilizers applied as single treatments or as combined treatments increased the number of fruits per plant (i.e. 1.5- 4.0 pcs) and the average pericarp thickness of fruits by 0.58 - 1.16 mm. The research findings provide grounds for suggesting the studied biofertilizers as an effective solution for maintaining soil fertility in organic production of pepper.

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Introduction

Organic agriculture is the best known production methods that are supportive of the environment (Nerkhede *et al.*, 2011). The organic certification of agricultural land in Bulgaria has so far achieved modest results (Paull and Hennig, 2013) with 25,022 hectares certified for organic agriculture along with 543, 655 ha certified for organic wild collection (Willer *et al.*, 2013).

As part of the sustainable agriculture movement, organic farming has become the focal point for environmental and socio-economic change to modern industrialized agriculture (Egri, 1997). Biofertilizers have been used as sources of improvement of plant nutrients in sustainable agriculture (Han *et al.*, 2006). Use of biofertilizers, in combination with organic manures, offers a great opportunity to increase the crop production and to achieve sustainability (Vishwanath, 2002). In recent years, biofertilizers also emerged as an important component of the integrated nutrient supply system, as they hold a great promise to improve crop yield through environmentally better nutrient supplies (Marozsán *et al.*, 2009).

Organic materials were found to play critical role in both short and long-term nutrient availability and maintenance of soil organic matter in small holder systems and represent key materials in reversing nutrient depletion (Daudu *et al.*, 2006). They can be used as an alternative to inorganic fertilizers (Makinde *et al.*, 2007). Sharma and Mitra (1991) as quoted by Ayoola and Makinde (2007), state that nutrients contained in organic manures are released more slowly and are stored for a longer time in the soil, thereby ensuring a long residual effect.

The composition and concentration of beneficial components of pepper fruit may vary in dependence on weather and growing conditions such as the way of fertilization, nutrient accessibility, soil complex and humus content, etc. (Szafirowska and Elkner, 2008). The modern organic farming aims at stable yields and produce of a good quality. Still, the yields are lower

than conventional. One of the basic reasons is the insufficient nitrogen availability, provided particularly by fertilizers. Soil organic matter after humification and mineralization can be a source of nitrogen. In organic farming system soil serves as reservoir and transformer of nutrients for crop productivity. An overall objective of organic production is to increase the chemical, physical and biological soil fertility. The more stabilized soil environment the better crop productivity (Szafirowska and Elkner, 2009). According to Reganold *et al.*, (1987) the organically-farmed soil has a significantly higher organic matter content, a thicker topsoil depth and less soil erosion than the conventionally farmed soil.

Having regards to all the above, the arguments for this research were following:

1. The modern agroecosystems that use environmentally-friendly technologies need to investigate the effect of application of a holistic approach, i.e. interactions of agroecological factors, with aim to obtain optimal production at low ecological risk.
2. There is a need for updated and complex information on the anticipated impact of biofertilizers on vegetation and productivity of economically-important crops (e.g. pepper). It is expected that optimization of soil nutrient supply may affect positively the overall agroecological conditions for crop growing.
3. The data from such multi-aspect study may guide organic producers to organize more economically-efficient vegetable production, i.e. to use less energy and nutrient inputs.

Therefore, the presented research study aimed at investigating the impact of selected biofertilizers on certain agroecological conditions (i.e. agroclimatic and agrochemical) as well as on biological and productive parameters of pepper grown under organic farming.

Materials and methods

This experiment was carried out in the period of 2009- 2011 on the organically-certified farm of the

Agroecological Centre at the Agricultural University-Plovdiv (Bulgaria). Plovdiv is situated in the Central part of the Thracian plain in the longitude of 24°45' according to Greenwich, in the latitude of 42° N, and in the altitude of 160 m (Ahmed, 2004).

Materials

Vegetable tested

Pepper is an annual crop and belongs to the Genus *Capsicum* of Family *Solanaceae*. The pepper variety was cultivated in conjunction with the principles of organic agriculture and according to the technology for mid-early field production. Pepper is a very demanding crop regarding the predecessor. Suitable predecessors are the vegetable varieties from the Family *Fabaceae* and the Family *Cucurbitaceae* (Panayotov *et al.*, 2007).

Experimental setup

The seedlings were planted on a permanent place during the third decade of May, on a high-levelled seed-bed using the 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 m².

Treatments (variants):

1. Control (non-fertilized); 2. Basic fertilization with Boneprot (optimum concentration); 3. Basic fertilization with Boneprot (50 %) + Seasol; 4. Basic fertilization with Boneprot (50 %) + Emosan; 5. Basic fertilization with Lumbrical (optimum concentration); 6. Basic fertilization with Lumbrical (50 %) + Seasol; 7. Basic fertilization with Lumbrical (50 %) + Emosan.

Fertilization

Solid biofertilizers Boneprot and Lumbrical were incorporated in the soil prior to planting of the seedlings on the field. They were applied in two concentrations, i.e. an optimal, corresponding to 70 kg/da for the basic fertilization with Boneprot and 400 L/da for the basic fertilization with Lumbrical) and reduced concentrations, i.e. optimal concentration decreased by 50 %. The liquid

biofertilizers Seasol and Emosan were introduced in soil twice during vegetation, i.e. at the pepper growing stages 'flower bud' and in 'mass fruit set' in concentration 1:500, i.e. 0.3 - 0.4 L/da for Seasol and 15 L/da for Emosan (Vlahova, 2013).

Characteristics of tested biofertilizers.

The selected biofertilizers, i.e. Boneprot, Lumbrical, Seasol (Earthcare) and Emosan (Hemozym Bio N5), belong to the list of permitted biofertilizers in the European Union 'Organic' Regulation (EC) No. 889/2008.

Boneprot (Arkobaleno, Italy) is a pellet organic fertilizer consisting mainly of cattle manure and has following composition: organic nitrogen (N)-4.5%; phosphorus anhydride (P₂O₅)-3.5%; potassium (K₂O)-3.5%; organic carbon (C) of biological origin-30%; degree of humification (DH)- 40-42%; humidity-13-15%; pH in water-6-8.

Lumbrical (private producer from Plovdiv region, 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-NH₄)- 33.0 ppm; nitrate nitrogen (N-NO₃)- 30.5 ppm; P₂O₅- 1410 ppm; K₂O- 1910 ppm; pH in water- 6.5-7.0. It contains useful microflora 2x10¹² pce/g, humic and fulvic acids.

Seasol (Earthcare) Seasol International Pty Ltd (Australia) is an extract of brown algae *Durvillaea potatorum*. 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); pH (10.5 ± 0.5% w/w) and has a variety of mineral elements and traces of N (0.10 ± 0.05 % w/w); P (0.05 ± 0.02 % w/w); K (2.0 ± 0.5 % w/w); Cu (0.3 ± 0.2 % w/w) and cytokines.

Emosan, HemoZym NK, Hemozym Bio N5 (Arkobaleno, Italy) contains total nitrogen (N)- 5 %;

organic nitrogen (N)- 5 %; organic carbon (C) of biological origin- 14 %; protein- 34 p/p; humidity- 65 p/p; K- 0.4 p/p; P - 0.06 p/p.; pH- 7.0- 10.0.

Parameters studied

1. The agrochemical soil parameters, i.e. assimilated forms of nitrogen (N-NH₄ and N-NO₃- BDS ISO 14255- mg/kg); mobile forms of P₂O₅ and mobile forms of K₂O (according method of Egner-Reem- mg/100g); aqueous-extract pH 1:2.5; EC saturated paste (mS/cm⁻¹); 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, measured as a standard yield (kg/da)

3. Economic productivity of plants:

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

b. Average mass of fruits (g) from 10 fruits per treatment.

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

Statistical analysis

The MS Office Excel 2007; SPSS (Duncan, 1955) and BIOSTAT packages were used. One-way analysis of variance (ANOVA) was used to analyse the differences between treatments (SPSS treatment 7.5). In the tables below, different letter(s) within a column indicates a significant difference by Duncan's multiple range-test at P<0.05 level. BIOSTAT was used to compare the results with the control (non-fertilized).

Results

Impact of biofertilisers on the agrochemical soil parameters

The main agrochemical parameters in the beginning of the vegetation period showed that the active soil reaction (pH) was slightly alkaline, according to the classification of the Bulgarian soils (Trendafilov and Popova, 2007), throughout the period of three years (Table 1). The content of total absorbable nitrogen was within the range that determines the soil as a weekly-alkaline (Tomov *et al.*, 2009). The content of the mobile soil P₂O₅ (determined according to Tomov *et al.*, 2009) showed variability when analyzed in the beginning of pepper vegetation, i.e. from a low-content in 2010 and 2011 to an average content in 2009. During the study period soil P₂O₅ content showed a narrow range. Regarding the K₂O content, the soil was well-stocked and having a good level of supply according to the threshold-limit values in an extract with calcium lactat mg K₂O/100 g (Tomov *et al.*, 2009) during the entire study period. A significant reserve of K₂O can be attributed to its natural content in the regional soils and to the decomposed plant residues leading to an increased microbiological activity (Vlahova, 2013). Regarding the humus content, the soil can be classified as a low-content one (2009 and 2010) and having a very low level of humus stocks (2011) according to classification of Orlov and Grishina quoted by Totev *et al.* (1991). The results of the analyses showed similar values for three years.

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

| Parameters | pH 1: (H ₂ O) | EC 2,5 mS/cm ⁻¹ | N- NO ₃ mg/kg | N- NH ₄ mg/kg | Total digestible nitrogen N mg/kg | P ₂ O ₅ mg/100g | K ₂ O 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 |

In the end of the vegetation (2009 to 2011) years, there were slight changes in the active soil reaction (pH) under the impact of the biofertilization.

However, the overall pH remained weekly-alkaline that is considered favorable for the vegetative and productive growth of pepper (Table 2). On the

average for the study period, pH of the variants fed with Emosan increased after basic fertilizations with Boneprot, i.e. by 7.51 % and by 7.48 % on the basic fertilization Lumbrical, respectively. On the average for the study period, pH of the variants fed with Seasol decreased after basic fertilization with Lumbrical, i.e. by 7.24 % and by 7.27 % on the basic fertilization with Boneprot. The application of the biofertilizer Seasol lead to slight decrease of pH during the three-year study period. The single application of the biofertilizer Boneprot in optimum concentration increased the pH level and showed the highest values in 2009 and in 2010, as it was 7.55 %

at an average for the period. The humus contents in the end of the vegetation period slightly changed, but in general the soil remained in the same group of humus stocking. On average for the study period, the highest values of the humus were detected upon single application of the biofertilizer Boneprot in optimum concentration, i.e. 2.88 % and upon combined application of Emosan on basic fertilization with Boneprot, i.e. 2.81 %. Regarding soil agrochemical status, biofertilization, both as single application in optimum concentration and as in combined application with basic fertilization, showed a positive impact on the soil humus contents.

Table 2. Agrochemical parameters at the end of the vegetation - pH and Humus.

| Treatments /variants/ | pH | | | | Humus (%) | | | |
|-------------------------------------|---------------------------|--------------------|--------------------|-------------|--------------------|-------------------|-------------------|-------------|
| | 1: 2.5 (H ₂ O) | | | Ave rage | | | | Ave rage |
| | 2009 | 2010 | 2011 | | 2009 | 2010 | 2011 | |
| Control | 7.17 ^{cd} | 7.42 ^{bc} | 7.47 ^d | 7.35 | 2.94 ^e | 2.53 ^c | 2.09 ^c | 2.52 |
| Boneprot (opt.) | 7.48 ^a | 7.63 ^a | 7.54 ^c | 7.55 | 3.49 ^a | 3.10 ^a | 2.06 ^c | 2.88 |
| Boneprot (50%) + Seasol | 7.11 ^d | 7.32 ^d | 7.39 ^e | 7.27 | 3.25 ^b | 2.51 ^c | 2.53 ^a | 2.76 |
| Boneprot (50%) + Emosan | 7.40 ^{ab} | 7.50 ^b | 7.64 ^b | 7.51 | 3.06 ^{cd} | 3.10 ^a | 2.26 ^b | 2.81 |
| Lumbrical (opt.) | 7.21 ^c | 7.41 ^c | 7.71 ^a | 7.44 | 3.23 ^b | 2.74 ^b | 2.29 ^b | 2.75 |
| Lumbrical (50%) + Seasol | 7.10 ^d | 7.27 ^d | 7.36 ^e | 7.24 | 2.99 ^{de} | 2.78 ^b | 2.04 ^c | 2.60 |
| Lumbrical (50%) + Emosan | 7.37 ^b | 7.48 ^{bc} | 7.59 ^{bc} | 7.48 | 3.09 ^c | 2.75 ^b | 2.27 ^b | 2.70 |

Duncan's Multiply Range Test (P<0.05)

The study reports higher values of soil nitrogen N-NO₃ compared to those of N-NH₄. It was observed in

all variants and confirmed during the study period (Table 3).

Table 3. Agrochemical parameters in the end of the vegetation N- NO₃, N- NH₄ and total nitrogen.

| Treatments /variants/ | N- NO ₃ | | | | N- NH ₄ | | | | Total digestible N | | | |
|-------------------------------|--------------------|-------------------|------------------|-------------|--------------------|------------------|------------------|-------------|--------------------|------|------|-------------|
| | mg/kg | | | Ave rage | mg/kg | | | Ave rage | mg/kg | | | Ave rage |
| | 2009 | 2010 | 2011 | | 2009 | 2010 | 2011 | | 2009 | 2010 | 2011 | |
| Control | 12.0 ^c | 4.3 ^e | 6.3 ^c | 7.5 | 3.8 ^b | 1.9 ^b | 1.9 ^c | 2.5 | 15.8 | 6.2 | 8.2 | 10.1 |
| Boneprot (opt.) | 19.2 ^a | 10.4 ^a | 5.9 ^d | 11.8 | 4.8 ^a | 2.0 ^b | 2.2 ^b | 3.0 | 24.0 | 12.4 | 8.1 | 14.8 |
| Boneprot (50%) + Seasol | 9.6 ^e | 4.5 ^e | 5.3 ^f | 6.5 | 2.9 ^e | 2.0 ^b | 1.6 ^d | 2.2 | 12.5 | 6.5 | 6.9 | 8.6 |

| | | | | | | | | | | | | |
|--|-------------------|------------------|------------------|------|-------------------|------------------|------------------|-----|------|------|------|------|
| Boneprot (50 %) + Emosan | 15.0 ^b | 9.9 ^b | 8.9 ^b | 11.3 | 3.4 ^c | 2.0 ^b | 1.6 ^d | 2.3 | 18.4 | 11.9 | 10.5 | 13.6 |
| Lumbrical (opt.) | 11.4 ^d | 4.5 ^e | 5.6 ^e | 7.2 | 3.3 ^{cd} | 2.3 ^a | 1.9 ^c | 2.5 | 14.7 | 6.8 | 7.5 | 9.7 |
| Lumbrical (50 %) + Seasol | 6.3 ^f | 5.6 ^b | 9.9 ^a | 7.3 | 2.3 ^f | 2.0 ^b | 2.4 ^a | 2.2 | 8.6 | 7.6 | 12.3 | 9.5 |
| Lumbrical (50 %) + Emosan | 11.4 ^d | 7.0 ^c | 5.3 ^f | 7.9 | 3.2 ^d | 2.0 ^b | 1.8 ^c | 2.3 | 14.6 | 9.0 | 7.1 | 10.2 |

Duncan's Multiply Range Test (P<0.05)

The highest N-NO₃ content was detected in the variant fed with single application of the biofertilizer Boneprot in optimum concentration (in 2009 and 2010, at p<0.05), i.e. the average for the period was 11.8 mg/kg. The N-NO₃ content was also higher upon application of Emosan on the basic fertilization with Boneprot with an average of 11.3 mg/kg for the study period. All other variants showed insignificant differences. The maximum for the average N-NH₄ content for the study period was 3.0 mg/kg. The content of total digestible nitrogen was highest for the variant fed with single application of the biofertilizer Boneprot in optimum concentration. It was detected in 2009 and confirmed in 2010, as the average value for the period was 14.8 mg/kg that was significantly higher compared to other variants. The effect can be attributed to the higher nitrogen content in the composition of the biofertilizer Boneprot.

Upon the application of the biofertilizer Seasol on the basic fertilization with Boneprot, the content of total nitrogen was detected in its lowest levels in 2010 and

in 2011. This can be attributed to the high level of absorption of the macroelement of pepper plants during the vegetation, which reflected on the vegetative growth of the plants, i.e. on their height (i.e. 62.00 cm in 2010 and 60.10 cm in 2011), and on the formed number of leaves per plant (i.e. 159.0 pcs/plant in 2010 and 151.1 pcs/plant in 2011) (Vlahova, 2013). Depending on the border values of the level of supply of the soil with accessible nitrogen compounds, the soil can be classified as 'a low-level of supply'. It may be attributed to more intensive nitrogen absorption by crops during vegetative stages of growth.

The highest phosphorus P₂O₅ content in the soil was detected upon combined application of biofertilizer Seasol on the basic fertilization with Boneprot, as the average value for the study period was 20.9 mg/100g (Table 4). After single application of the biofertilizers in optimum concentration, it was found that the P₂O₅ content was higher after Boneprot than after Lumbrical (2009 and 2010, at p<0.05).

Table 4. Agrochemical parameters on P₂O₅ and K₂O in the end of the vegetation.

| Treatments /variants/ | P ₂ O ₅ (mg/100g) | | | | Ave rage | K ₂ O (mg/100g) | | | | Ave rage |
|-----------------------------|---|--------------------|--------------------|------|--------------------|----------------------------|--------------------|------|--|-------------|
| | 2009 | 2010 | 2011 | | | 2009 | 2010 | 2011 | | |
| Control | 27.44 ^{bc} | 9.66 ^d | 9.38 ^e | 15.5 | 36.08 ^b | 15.73 ^c | 19.01 ^c | 23.6 | | |
| Boneprot (opt.) | 29.14 ^a | 13.24 ^b | 11.36 ^c | 17.9 | 37.85 ^a | 18.40 ^a | 19.02 ^c | 25.1 | | |
| Boneprot (50 %) + Seasol | 29.27 ^a | 20.66 ^a | 12.84 ^b | 20.9 | 37.65 ^a | 16.62 ^b | 23.37 ^a | 25.9 | | |

| | | | | | | | | |
|----------------------------------|---------------------|--------------------|--------------------|------|--------------------|--------------------|--------------------|------|
| Boneprot (50 %)+ Emosan | 24.69 ^d | 7.18 ^f | 13.33 ^b | 15.1 | 37.64 ^a | 14.38 ^e | 19.74 ^b | 23.9 |
| Lumbrical (opt.) | 28.00 ^b | 10.56 ^c | 15.31 ^a | 18.0 | 34.73 ^c | 15.28 ^d | 17.29 ^d | 22.4 |
| Lumbrical (50 %)+ Seasol | 26.53 ^c | 8.09 ^e | 11.86 ^c | 15.5 | 28.35 ^e | 16.40 ^b | 14.57 ^e | 19.8 |
| Lumbrical (50 %) + Emosan | 27.44 ^{bc} | 8.08 ^e | 10.37 ^d | 15.3 | 31.56 ^d | 14.37 ^e | 14.82 ^e | 20.2 |

Duncan's Multiply Range Test (P<0.05)

The highest potassium K₂O content in the soil was shown after single application of the biofertilizer Boneprot in optimum concentration (2009 and 2010). It determines the positive impact of the application of biofertilizers, e.g. basic fertilization with Boneprot, for improved supply of soil with potassium. The highest average K₂O content for the period was detected after application of the biofertilizer Seasol on the basic fertilization with Boneprot (25.9 mg/100g).

Another investigated soil property was the electrical conductivity (EC). It is used to determine the level of the salts dissolved in the soil (Table 5). The results showed that the EC was highest for the variant with single application of the biofertilizer Boneprot in optimum concentration (in 2009 and 2010). The research found that the applied biofertilizers did not increase the concentration of the salts dissolved in the soil.

Table 5. Agrochemical parameters in the end of the vegetation- EC (mS/cm⁻¹).

| Treatments /variants/ | 2009 | 2010 | 2011 | Average |
|----------------------------------|--------------------|-------------------|---------------------|---------|
| Control | 2.38 ^d | 2.01 ^c | 1.65 ^{bc} | 2.01 |
| Boneprot (opt.) | 3.30 ^a | 2.89 ^a | 1.70 ^{ab} | 2.63 |
| Boneprot (50 %) + Seasol | 2.60 ^{bc} | 1.70 ^e | 1.62 ^c | 1.97 |
| Boneprot (50 %) + Emosan | 2.64 ^b | 2.20 ^b | 1.67 ^{abc} | 2.17 |
| Lumbrical (opt.) | 2.62 ^b | 1.82 ^d | 1.46 ^d | 1.97 |
| Lumbrical (50 %) + Seasol | 2.28 ^d | 2.00 ^c | 1.66 ^{bc} | 1.98 |
| Lumbrical (50 %) + Emosan | 2.50 ^c | 1.97 ^c | 1.73 ^a | 2.07 |

Duncan's Multiply Range Test (P<0.05)

The high electrical conductivity (EC) may have unfavorable impact on the development of pepper, because pepper is sensitive to constant high salt content (Sári and Forró, 2007). The EC-values were within the range of 1.96 mS/cm⁻¹ to 3.97 mS/cm⁻¹ and did not have a negative impact on the growth of the pepper, which belongs to the group of average-susceptible crops with a threshold of the salt index of about 1.5 dS/m (Hanlon *et al.*, 1997). A higher content of soluble salts was found after application of

Emosan on the basic fertilization with Boneprot, as an average for the study period was 2,17 mS/cm⁻¹. The results from the soil analyses showed that the biofertilizers had an impact on the content of absorbable nutrients.

Standard yield

Economic yield is one of the most important parameters for assessing the biological effect of application of the technology for pepper cultivation,

including the application of biofertilizers. The study showed that the standard yield of pepper plants increased in the variants with biofertilizers in comparison with the control (non-fertilized) plants (Table 6). The highest standard yield in all three experimental years was shown by the variant fed with the biofertilizer Emosan on the basic fertilization with Lumbrical, i.e. 1541 kg/da (2009), 2258 kg/da (2010), and 2006 kg/da (2011). The average increase in all three years compared to the control was by 47.2 %. A high yield ($p < 0.05$) was reported upon the additional feeding with the biofertilizer Emosan on the basic

fertilization with Boneprot, i.e. 1512 kg/da (2009), 2184 kg/da (2010) and 1900 kg/da (2011), as the average increase in all three years compared to the control was by 41.9 %. The results obtained showed the positive effect of the application of the biofertilizer Emosan, regardless of the type of basic fertilization. From a statistical point of view, there are significant differences ($P_{0.1\%}$) between the two variants fed with the biofertilizer Emosan during the period of three years compared to the control. The results also showed the impact of the two basic fertilizations.

Table 6. Standard Yield (kg/da) variety of Sofiiska Kapiya (from 2009 to 2011).

| Treatments /variants/ | 2009 | | 2010 | | 2011 | | Average | |
|---------------------------------|----------------------------|--------------|-----------------------------|--------------|----------------------------|--------------|---------|-------|
| | Mean; St.Dev. | GD | Mean; St. Dev. | GD | Mean; St. Dev. | GD | kg/da | % |
| Control | 1119 ± 221.3 ^e | Base | 1405 ± 261.0 ^e | Base | 1420 ± 29.103 ^f | Base | 1314.7 | 100.0 |
| Boneprot (opt.) | 1235 ± 313.5 ^{de} | ns | 1618 ± 19.0 ^{de} | ns | 1550 ± 47.226 ^e | ++ | 1467. | 111.6 |
| Boneprot (50 %) + Seasol | 1283 ± 443.5 ^{cd} | ++ | 1737 ± 33.9 ^{bcde} | + | 1704 ± 41.016 ^d | +++ | 1574.7 | 117.7 |
| Boneprot (50 %) + Emosan | 1512 ± 132.0 ^{ab} | +++ | 2184 ± 26.3 ^{ab} | +++ | 1900 ± 77.117 ^b | +++ | 1865. | 141.9 |
| Lumbrical (opt.) | 1339 ± 402.2 ^{cd} | ++ | 1797 ± 208.4 ^{bcd} | + | 1602 ± 29.501 ^e | +++ | 1579. | 120.1 |
| Lumbrical (50 %) + Seasol | 1140 ± 56.0 ^e | ns | 1952 ± 56.7 ^{abcd} | ++ | 1580 ± 69.788 ^e | +++ | 1557. | 118.4 |
| Lumbrical (50 %) + Emosan | 1541 ± 142.6 ^a | +++ | 2258 ± 120.0 ^a | +++ | 2006 ± 15.885 ^a | +++ | 1935. | 147.2 |
| GD _{5%} | | 120.1 | | 318.9 | | 75.51 | | |
| | | 5 | | 3 | | | | |
| GD _{1%} | | 163.8 | | 434.9 | | 102.9 | | |
| | | 7 | | 8 | | 9 | | |
| GD_{0.1%} | | <u>221.7</u> | | <u>588.6</u> | | <u>139.3</u> | | |
| | | 6 | | 4 | | 7 | | |

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

The composition of the applied biofertilizer as well as its combination with the basic fertilization could have had an impact on the quantity of yield. The feeding

with the biofertilizer Seasol did not show unidirectional results after application on the two basic fertilizations. Higher yield was reported upon

the application of the biofertilizer Seasol on the basic fertilization Boneprot, i.e. 1283 kg/da (2009) and 1704 kg/da (2011).

Economic productivity of plants

a. Average number of fruits per plant

The yield parameters are directly interconnected and impact plant quantity. The maximum number of fruits per plant was observed on the variant fed with the biofertilizer Emosan on the basic fertilization with Lumbrical, i.e. 9.9 pcs/plant in 2009, 9.3 pcs/plant in 2010 and 8.4 pcs/plant in 2011 (Table 7). A higher number of fruits were detected upon the application

of the biofertilizer Emosan on the basic fertilization Lumbrical, as this was confirmed in 2009 and in 2010. A similar tendency was observed for the biofertilizer Seasol. On the average for the study period, the increase of the number of fruits was by 2.7 pcs/plant for the plants fed with the basic fertilization Lumbrical and on by 2.1 pcs/plant for the plants fed with the basic fertilization Boneprot. The study also confirmed the findings of Alves *et al.* (2009) that the use of liquid biofertilizers is one of the organic agriculture practices that aims to achieve balanced plant nutrition.

Table 7. Number of fruits per plant, variety of Sofiiska Kapiya

| <i>Treatments /variants/</i> | <i>2009</i> | | <i>2010</i> | | <i>2011</i> | | <i>Average</i> |
|----------------------------------|---------------------------|-------------|---------------------------|-------------|--------------------------|-------------|----------------|
| | Mean; St. Dev. | GD | Mean; St. Dev. | GD | Mean; St. Dev. | GD | |
| Control | 5.4 ± 0.726 ^e | Base | 5.0 ± 0.707 ^f | Base | 5.2 ± 0.441 ^d | Base | 5.2 |
| Boneprot (opt.) | 7.7 ± 0.866 ^{cd} | +++ | 6.2 ± 0.667 ^e | ++ | 6.1 ± 0.782 ^c | ++ | 6.7 |
| Boneprot (50 %) + Seasol | 7.7 ± 0.866 ^{cd} | +++ | 6.6 ± 0.527 ^{de} | +++ | 7.6 ± 0.726 ^b | +++ | 7.3 |
| Boneprot (50 %) + Emosan | 7.1 ± 0.601 ^{cd} | +++ | 8.1 ± 0.333 ^b | +++ | 8.4 ± 0.726 ^a | +++ | 7.9 |
| Lumbrical (opt.) | 7.6 ± 0.527 ^{cd} | +++ | 7.3 ± 0.707 ^c | +++ | 6.2 ± 0.667 ^c | ++ | 7.0 |
| Lumbrical (50 %) + Seasol | 8.9 ± 0.928 ^b | +++ | 7.3 ± 0.866 ^c | +++ | 6.4 ± 0.882 ^c | +++ | 7.5 |
| Lumbrical (50 %) + Emosan | 9.9 ± 1.364 ^a | +++ | 9.3 ± 0.500 ^a | +++ | 8.4 ± 0.527 ^a | +++ | 9.2 |
| GD _{5%} | | 7.48 | | 0.67 | | 0.54 | |
| GD _{1%} | | 1.02 | | 0.91 | | 0.74 | |
| GD_{0.1%} | | 1.38 | | 1.24 | | 1.00 | |

Duncan's Multiply Range Test (P<0.05)

Throughout the study period the variants of combined application of biofertilizers as vegetative feeding on the basic fertilization Lumbrical provided nutritional compounds for the formation of a larger number of fruits than the variant on single

application of the biofertilizer Lumbrical in optimum concentration. This was also found for the basic fertilization with Boneprot in 2010 and in 2011. Regarding the the variants of combined application of biofertilizers as vegetative feeding on the basic

fertilizations, the difference the control variants was proven for $P_{0.1\%}$ (2009, 2010 and 2011).

b. Average mass of fruits

The pepper yield depends not only on the number of the fruits formed per plant, but also on the average mass of fruits. The study showed highest values of the average mass of fruits during the three experimental years after application of biofertilizers Emosan on the basic fertilization with Lumbrical i.e. 79.7g (2009), 78.5g (2010) and 76.1g (2011) (Table 8). Positive effect on the average mass of the fruits was also found

for the variant fed with the biofertilizer Emosan, but on the Boneprot basic fertilization, i.e. the average for the period was 77.2 g. The application of biofertilizers provided necessary nutrients, so that an increase of average mass of fruit in comparison with the non-fertilised control during the three experimental years was observed.

c. Pericarp thickness

Although insignificant, differences in fruit pericarp thickness between variants were detected (Table 9) depending on the applied fertilization.

Table 8. Mass of fruits, variety of Sofiiska Kapiya, g.

| <i>Treatments</i> <i>/variants/</i> | <i>2009</i> | <i>2010</i> | <i>2011</i> | Average |
|--|----------------------------|----------------------------|----------------------------|----------------|
| Control | Mean; 64.9 ^b | Mean; 65.3 ^f | Mean; 63.1 ^e | 65.4 |
| Boneprot (opt.) | 66.2 ^{ab} | 66.2 ^{cd} | 66.3 ^{bc} | 66.2 |
| Boneprot (50 %) + Seasol | 65.8 ^a | 68.1 ^{de} | 72.0 ^{ab} | 68.6 |
| Boneprot (50 %) + Emosan | 78.2 ^{ab} | 77.7 ^a | 75.8 ^a | 77.2 |
| Lumbrical (opt.) | 66.1 ^a | 68.2 ^{cde} | 66.8 ^{bc} | 67.0 |
| Lumbrical (50 %) + Seasol | 57.1 ^a | 70.0 ^e | 67.8 ^{bc} | 65.0 |
| Lumbrical (50 %) + Emosan | 79.7 ^a | 78.5 ^{bc} | 76.1 ^a | 78.1 |

Duncan's Multiply Range Test

Table 9. Thickness of pericarp of pepper fruits, variety of Sofiiska Kapiya, mm.

| <i>Treatments</i> <i>/variants/</i> | <i>2009</i> | <i>2010</i> | <i>2011</i> | Ave rage |
|--|--|--|--|---------------------|
| Control | Mean; St.Dev. 4.14 ± 0.578 ^b | Mean; St.Dev. 4.01 ± 0.145 ^f | Mean; St.Dev. 4.44 ± 0.405 ^e | 4.20 |
| Boneprot (opt.) | 4.74 ± 0.606 ^{ab} | 4.72 ± 0.364 ^e | 4.87 ± 0.426 ^{cde} | 4.78 |
| Boneprot (50 %) + Seasol | 4.89 ± 0.290 ^a | 4.83 ± 0.442 ^{de} | 4.73 ± 0.350 ^{de} | 4.82 |
| Boneprot (50 %) + Emosan | 5.07 ± 0.361 ^a | 5.71 ± 0.457 ^a | 5.29 ± 0.500 ^{abcd} | 5.36 |
| Lumbrical (opt.) | 4.85 ± 0.348 ^a | 4.79 ± 0.316 ^{de} | 4.98 ± 0.402 ^{bede} | 4.87 |
| Lumbrical (50 %) + Seasol | 5.01 ± 0.641 ^a | 5.13 ± 0.366 ^{cd} | 5.46 ± 0.346 ^{abc} | 5.20 |
| Lumbrical (50 %) + Emosan | 4.99 ± 0.311 ^a | 5.33 ± 0.355 ^{bc} | 5.48 ± 0.472 ^{ab} | 5.27 |

Duncan's Multiply Range Test (P<0.05)

During the three experimental years the highest value of pericarp thickness was detected for the variant fed with the biofertilizer Emosan on the basic fertilization Boneprot, i.e. 5.36 mm on the average for the period. Variant fed with the biofertilizer Emosan on the basic fertilization Lumbrical showed higher values, i.e. an average of 5.27 mm which can be attributed to the high level of proteins in the composition of Emosan. Higher values of pericarp thickness were observed by the pepper fed with biofertilizer Seasol applied on the basic fertilization Lumbrical 5.20 mm. This may be attributed to the alginates in the biofertilizer Seasol known for their direct impact on plants through activation of beneficial soil microflora.

Upon comparing the two basic fertilizations, the study found that the effect of the application of the biofertilizer Emosan was higher on the basic fertilization Boneprot, the increase on the pericarp thickness in comparison with the control was by 22.5 % (2009) and by 42.4 % (2010) respectively. All variants exceeded the control throughout the period of three years, thus proving the positive effect of the application of the biofertilizers on the pericarp thickness.

Discussion

The low stocks of organic compounds and macroelements detected by the agro-chemical analysis argued the necessity for additional feeding with organic fertilizers through basic fertilization in soil and during vegetation. Such fertilization could provide opportunity for stabilization of soil agrochemical content. The results from the agrochemical analysis provided grounds to claim that the soil conditions were at their optimum for establishing a biofertilization experiment and were appropriate for development of the pepper crop.

According to existing agrotechnology, in the beginning of the vegetation the plants should be provided with the necessary nutrients on the basis of their requirements. In the system of organic agriculture the 'green' fertilisation is a significant element of the natural soil enrichment (Proishen and Bernat, 1987). To form an optimum vegetative and

reproductive system plant extract part of the available nutrients in the soil. Therefore, a reduction of soil nutrients is usually observed at the end of the vegetation period. The quantity of nutrients necessary for the formation of one ton of pepper is approximately 4.6 kg of N, 1.7 kg of P₂O₅, and 4.5 kg of K₂O (Rankov *et al.*, 1983).

Effect of biofertilization on agrochemical parameters

The highest pH values and the N content shown after fertilization of Emosan on Boneprot may be attributed to the composition of fertilizers, i.e. a high content of organic nitrogen in the biofertilizer Boneprot and significant protein content in the biofertilizer Emosan. It is in conjunction with Lin *et al.* (2002) who conclude that the increase of the active soil acidity in the variants fed with biofertilizers is due to the weak organic acids formed as a result of microbial decomposition of the organic substances or due to the activity of the soil microorganisms. The higher phosphorus content (P₂O₅) after application of biofertilizer Boneprot and its efficiency may be attributed to the enrichment of the soil medium with P₂O₅ compounds. Depending on the border values of the level of supply of the soil with mobile phosphates, the soil in all variants can be classified as having 'a good level of supply' (i.e. 2009) and 'an average level of supply' (i.e. 2011). All treatments showed 'a good level of supply' of the soil with potassium K₂O according to the border values. This determines the positive impact of the biofertilization for enrichment of soil with potassium thus assuring a valuable reserve for the next crops in the crop rotation. Here, the present study confirms the findings of Koynov *et al.* (1998) that the soils of the region of Plovdiv have a 'good level of supply' with available K₂O forms in fluvial soils. The increased K₂O content detected at the end of the vegetation indicates the positive impact of biofertilizer Seasol and its favourable combination with the basic fertilization with Boneprot. This can be attributed to the composition of the biofertilizers Seasol (i.e. 2.0 ± 0.5 w/w K₂O) and Boneprot (3.5 % K₂O), the application of which stimulates and enriches the soil with major nutrients.

The soil organic matter is crucial in organic agriculture requires as it determines the slow but regular supply of nutrients to plants. But due to the relatively shorter vegetation period of the pepper (i.e. 140-150 days), the positive impact of biofertilizers on the humus content is less profound as also concluded by Tuev (1989). The results of the agrochemical analysis done by the present study provide grounds to conclude that the soil nutrient pool may be maintained by adding nitrogen-rich fertilizers applied in the form of basic fertilisation that slowly release nutrients and improve soil conditions.

The changes in the total concentration of soluble salts among the variants with biofertilizers were in conjunction with the processes of biological immobilization and the following mobilization of the nutrients. The total concentration of soluble salts in the end of the vegetation was higher upon application of Boneprot in an optimum concentration in comparison with the combined variants on Boneprot during the period (2009 and 2010).

Effect of applied biofertilizers on standard yield of pepper

The highest standard yield was detected after application of Emosan on the basic fertilization with Lumbrical, i.e. 2258 kg/da (2010) and 2006 kg/da (2011), followed by application of Emosan on the basic fertilization with Boneprot, i.e. 2184 kg/da (2010) and 1900 kg/da (2011). The increase of the yield of treated pepper compared to the non-fertilised (control) pepper was by 60.8% and 41.3 %, (2010, 2011 respectively) and by 55.5 % and 33.8 % (2010, 2011 respectively). The positive impact by the biofertilizer Emosan on the standard yield was distinctive on both basic fertilizations (i.e. Boneprot and Lumbrical). The highest standard yield was shown after Emosan on the basic fertilization Lumbrical (2009, 2010 and 2011). The stimulating effect of Lumbrical on the yield can be attributed to its physical and chemical composition, which makes it more easily assimilated by plants (Vlahova and Popov, 2013). Overall, the results confirmed the expectation that the combined application of biofertilizers stimulate the pepper growth in organic

farming. The results are in conjunction with the findings of El- Zawily *et al.* (2002) quoted by El-Sayed and Elzaawely (2010) as well as Szafirowska and Elkner (2008) who mention the positive influence of organic fertilizers on pepper yield. The latter, quoted Buczkowska (2005) that organic fertilization may increase the yield of sweet pepper by 30-40 %. Also Berova and Karanatsidis (2008) reported that the biofertilizer Lumbrical influences the yield by 18 % of the Buketen 50 cultivar and by 22 % of the Gorogled 6 cultivar.

The pepper in the variants treated with combined biofertilisers, showed higher yields compared to the yield of variants with single application of the biofertilizer in optimum concentration. The only exception was the variants fed with biofertilizer Seasol on the basic fertilization Lumbrical (i.e. in 2009 and 2011), which showed lower yields compared to the optimum concentration of the basic fertilization Lumbrical. It can be attributed to the provision of more nutritional substances released after the combined application of the biofertilizers in comparison with the single application of biofertilizers in optimum concentration.

Influence of biofertilization upon economic productivity of pepper plants

The highest values of the average mass of fruits during the three experimental years after application of Emosan on the basic fertilization Lumbrical reflected in highest standards yields and highest productivity (total number of fruits), compared to other variants (other biofertilizers combinations). It also confirms the effectiveness of biofertilizers in vegetable farming. It is in conjunction with the findings of Atiyeh *et al.* (2000, 2001), Berova and Karanatsidis (2008), Premuzic *et al.* (1998) quoted by Cabanillas *et al.* (2006) and Subramanian *et al.* (2006) quoted by Direkvandi *et al.* (2008). The biofertilisers, applied either individually or in combination, increased the average pericarp thickness by 0.58- 1.16 mm average for the period, compared to untreated pepper plants. This confirms the findings of Dincheva *et al.* (2008) who report an

increase of the pericarp thickness upon application of biofertilizer Biohumus compared to untreated plants.

Conclusions

Agroecosystems are open systems that exhibit certain stability in functioning of biological cycles. One of the aims of agroecosystems is to obtain higher biomass (i.e. biological productivity). This may be achieved on the basis of provision of additional nutrients in soil through application of biofertilizers. The biofertilizers, however, should function within certain agroecological conditions, thus changing the media in which pepper plants grow.

As an intensive vegetable crop that is demanding soil nutrients, the pepper plants uptake the necessary nutrients while the soil remains relatively well-stocked for the next crop in the rotation. The present study found that the soil agrochemical conditions after combined treatment with liquid biofertilizer Emosan improved at the end of vegetation, i.e. soils showed higher total absorbable N after Emosan on basic fertilization with Boneprot (2009, 2010, and 2011) and after single treatment with basic fertilization with Boneprot. Therefore, a positive impact on soil nutrient stock and on growth of the next crop in the rotation can be expected. The single application of both basic fertilization (i.e. Boneprot and Lumbrical) leads to higher P_2O_5 content in the soils treated with Boneprot in optimal concentration (2009 and 2010). Similar results are shown by the K_2O content in the soil. The positive effect of the studied biofertilizer on soil P_2O_5 content reflects on improved growth characters of the pepper, which can be attributed to the physiological role of P on the meristemic activity of plant tissues and the consequent increase of the plant growth.

The study showed that optimal agroecological conditions for pepper growth were assured. This involved addition of biofertilizers, which it reflected in higher standard yield of the treated pepper plants compared to the control (non - fertilized) ones.

The present study found that application of biofertilizers resulted in higher number of fruits per plant and average mass of fruits, i.e. after treatments with Emosan on both basic fertilizations (i.e. Boneprot and Lumbrical) compared to non - fertilized plants (i.e. by 1.5- 4.0 pcs per plant on average). Later, it reflected in higher standard yield that is a function of the two productivity parameters.

The results of the study show that fertilization with liquid biofertilizer Emosan on basic application of solid Boneprot or Lumbrical improve the soil nutrient conditions which reflects in improved feeding regime of pepper plants. This reflects in improved (higher) yields and productivity of plants. The findings provide grounds for suggesting the studied biofertilizers as an effective agro-technical solution in organic production of pepper.

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