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RESEARCH ARTICLE

IMPACT OF THE BIOLOGICAL FERTILIZERS ON THE MICROORGANISMS AND THE NUTRIENT ELEMENTS IN THE SOIL

[1]Plamena Yankova, [1]Pavlina Naskova, [2]Bojka Malcheva and [1,]*Dragomir Plamenov

[1]Department of Plant Production, Technical University – Varna, Varna, Bulgaria
[2]Department of Soil Science, University of Forestry – Sofia, Sofia, Bulgaria

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ABSTRACT

The present experimental work is set as a vessel experiment in a non-heated greenhouse of department „Plant Production", Technical University – Varna. There are used three biological products – Extrasol, Herbagreen, Life Bat Guano and one mineral fertilizer – NPK. It is followed the impact of the fertilizers on the microbiological activity, as there is analyzed and the content of macroelements in the soil. From the carried out analyses is established that the biological fertilizers leave the soil well stocked with nitrogen, movable phosphates and absorbable potassium, as highest results are reported at usage of Extrasol. In the composition of the microbocenosis prevailing are the non-spore forming bacteria, and least are the micromycetes. The activity of the microorganisms is highest at usage of a combination of mineral (NPK) and biological fertilizer (Herbagreen). At combination of Extrasol and Herbagreen is reported the weakest development of microorganisms, but at the expense of this the mineralization coefficient is highest.

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INTRODUCTION

The biological agriculture is developed in more than 164 countries in the world, as their number constantly increases. The areas with biological production in the world amount to 37,5 million hectares. As per continents this share is highest in Oceania (12.2 mln ha, 32%), followed by Europe (11.2 mln ha, 30 %), Latin America (6.8 mln ha, 18 %), Asia (13.2 mln ha, 9%), Northern America (3 mln ha, 8%) and Africa (1.1 mln ha, 3%) (Willer and Lernoud, 2014). The fast development of the biological agriculture on a world scale imposes to be developed ecologically appropriate methods, which to conform to the requirements of the European Union for obtaining of a biological production. The microorganisms have significant importance in all agricultural practices. Particularly important is their role for the structure and the fertility of the soil, including the interrelations plants – microorganisms, for the preservation and the processing of the plant production and for the recycling of wastes from the crop. The participation of the microorganisms in the soil fertility, biocontrol and the bioremediation includes the including of natural and transgenic microbial inoculants. During the last years the basic scientific researches in this field concern the production and the composition of microorganism inoculants. The microbial communities are a more reactive component of the earth ecosystem to external stress than the plants and the animals (Panikov, 1999). The changes in them can be used in order to be foreseen stress impacts of the ecosystems of organic and conventional practices for management (Bending *et al*., 2000; Bruggen-Van and Semenov, 2000; Poudel *et al*., 2002). The fertilization is in condition directly to stimulate the growth of the microbial populations, as fully through giving of nutrient substances, but also in particular to affect the composition of the separate microbial communities in the soil (Khonje *et al*., 1989). The usage of chemical fertilizers independently is not an effective approach for improvement of the nutrient status of the soil (Kang *et al*., 2005). In comparison to the conventional agriculture, the biological agriculture has potential advantages – increasing of the biodiversity of the soil (Doles *et al*., 2001; Maeder *et al*., 2002; Oehl *et al*., 2004), decreasing of the pollution of the environment (Horrigan *et al*., 2002;. Macilwain, 2004). On a world scale there is observed intense development and applying of microbial fertilizers because of the realizing of the harmful impact, which it has on the environment the excessive and/or improper usage of chemical fertilizers, as well as in connection with the accumulation of more and more information for the interactions between the plants and all risospheric microorganisms. This potential stimulates the scientists towards experiments for isolation and selecting of strains microorganisms, which are capable of stimulating the growth through direct and indirect

Corresponding author: Dragomir Plamenov,
Department of Plant Production, Technical University – Varna, Varna, Bulgaria.

improvement of the suction of the nutrient substances by the plants. The biofertilizers are defined as product formulae, which contain one or more microorganism, which improve the nutrient status (and the growth) of the plants, as they restore the nutrient substances in the soil or make them easily absorbable and assist the access of the plants to them. The applying of the biofertilizers satisfies the basic requirements for sustainable production: stability and quality of the production, preservation of the ecological equilibrium, and all this contributes for increase of the level of the nutrient safety and the positive financial effect from the production. Numerous researches show the favaourable impact of the biofertilizers on physical, chemical and microbiological properties of the soil (Higa and Parr, 1994; Reddy, 2005; Tognetti *et al*., 2005). In this way is increased the dynamics and the direction of the microbiological processes in the soil, which have indirect positive impact on the maintaining and the increasing of the levels of the soil fertility. Besides, the biofertilizers stimulate the delivery of mineral nutrient elements to the plants, which gives opportunity to the plants to absorb phosphorus easier and to assist the growth, as they produce phytohormones, antibiotics and biologically active substances (Lucy *et al*. 2004; Carvajal-Muñoz and Carmona-Garcia, 2012; Saharan and Nehra, 2011). The purpose of the present development is to be followed the impact of biological fertilizers during vegetation of greenhouse cucumbers sort Kiara F_1, as well as to be established the impact of the bioproducts on the soil fertility.

MATERIALS AND METHODS

The experimental work was carried out in year 2015 in a non-heated greenhouse of Technical University – Varna with greenhouse cucumbers sort Kiara F_1.

Production of seedlings

The seed were put on 01 April 2015 in Petri dishes, as after germination in a thermostat are planted in plant pots with volume 0.5 l, full with turf-perlitic mixture. In period 3-4 real leaf they are transferred in plant pots with volume 12 l. The experiment is set as vessel in 3 repetitions. Before planting to the plants is carried out an agrochemical analysis of the soil. There is followed the nutrient reserve of the soil with nitrogen (ammonium and nitrate), movable forms of phosphorus, absorbable potassium and pH of the soil solution.

Scheme of the experiment

There are used three biological fertilizers – Extrasol, Herbagreen, Life Bat Guano. The experiment is set in seven variants with three repetitions as per the following scheme:

1. NPK;
2. NPK + Herbagreen;
3. Extrasol;
4. Extrasol + Herbagreen;
5. Life Bat Guano;
6. Life Bat Guano + Herbagreen;.
7. Control – not fertilized

The fertilizer norms are recalculated for the separate variants. At the variant with NPK are brought in as per 2 g, before the plants to be set out. Extrasol is applied in period beginning of the flowering, as there are brought in as per 2 ml. Herbagreen is brought in period 6-7 leaf, as there is prepared a working solution 40 g in 10 l water. Each one of the plants was sprayed, as the treating is carried out from bellow upwards. Life Bat Guano is applied 3 times at an interval of 15 days, as the plants are also sprayed from bellow upwards, in dose of 5 ml. The hybrid Kiara F_1 is characterized with lengthened, cylindrical, fruits with length 30-33 cm, with average weight 340-350 g, with intensive dark-green colour of the peel and strong coating, corrugated. The pulp is tender, crunchy, without cavities, without bitterness. The crop is 150-200 t/ha.

Extrasol is a microbial liquid fertilizer, which contains risosphere bacteria from genus *Bacillus subtilis*, content of dry substance not less than 19 %, organic substances 58-64% from the dry substance, humic acids 50-85% from the organic substance, fulvic and low-molecular organic acids 15-50% from the organic substance, potassium not less than 9 % from the dry substance. Salts of the humic acids 80-90 % from the dry substance, microelements.

Herbagreen is 100% natural product, obtained from calcitic microparticles $CaCO_3$). It acts reinforcing to the plants and to the activating of the primary and secondary metabolism. This advantages for the better steadiness of the plant against the biotic and abiotic factors. It contains CaO 44.10 %, MgO 2.20 %, Fe_2O_3 1.20 %, Al_2O_3 – 0.70 %, SiO_2 9.10 %, SO_4 0.11 %.

Life Bat Guano is a natural fertilizer from bats, with rich composition of macro and microelements, as well as enzymes. The organic substance is 20 %, nitrogen 2 %, pH 4.2-6.3. The enzymes and the containing calcium and magnesium stimulate the soil microorganism and under their impact the nutrient substances from the guano are released gradually. It contributes for the better and easier absorption of the nutrient substances from the plants. The guano improves the quality and the production of the obtained crop.

The content of nitrate and ammonium nitrogen is specified photometrically with Nitrospectral as a result of extraction with solution of calcium dichloride ($CaCl_2$) (ISO 14255:2002). The content of movable phosphates and absorbable potassium is specified as per standard ISO 11263:2002, through double lactate method of Egner-Riehm. The method is based on extracting of the movable compounds of the phosphorus and potassium with solution of calcium lactate $(CH_3CH.OH.COO)_2Ca$. The active reaction of the soil (pH) is specified in water extract, in compliance with Bulgarian State Standard ISO 10390:2011.The microbiological analyses include determining of ammonification bacteria (non-spore forming bacteria and bacilli), actynomycetes, micromycetes, bacteria, which absorb mineral nitrogen. They are specified as per the method of dilution and a culture of solid nutrient media (mesopeptonic agar – for specifying of non-spore forming bacteria and bacilli; starch-ammonia agar – for specifying of actynomycetes and bacteria, which absorb mineral nitrogen; Czapek-Dox medium – for specifying of micromycetes), cultivation in a thermostat and next reporting of colony-forming units, recalculated to 1 g absolutely dry soil. There are calculated average values from three repetitions and a coefficient of variation. The data are included in a dispersion and correlation analysis, as there is used statistical product SPSS for Windows version 16.

RESULTS AND DISCUSSION

Before setting of the experiment, there is carried out an agrochemical analysis of the soil for content of nitrogen, absorbable potassium and movable phosphates. The results are presented in table 1. From the carried out analysis was established that it is well stocked with absorbable potassium (63.38 mg/100 g). Regarding the phosphate ions, the soil is with average nutrient reserve (8.17 mg/ 100 g). There are reported low values of nitrate (6.24 mg/kg) and ammonium nitrogen (0.41 mg/kg). The soil reaction is slightly alkaline (pH 7.3). In the carried out experiment is used mineral fertilizer NPK as a variant for comparison of the biological products (table 2). Namely after fertilizing with it are reported statistically proven highest values of nitrate (63.80 mg/kg) and ammonium nitrogen (3.33 mg/kg) in the soil. From the biological fertilizers, highest results of nitrate nitrogen are established in the variants with brought in Extrasol (52.20 mg/kg) and Extrasol + Herbagreen (52.02 mg/kg), which the dispersion analysis does not distinguish and because of this it classifies them in one group. High results of nitrate nitrogen are reported and in the variants with brought in Life Bat Guano + Herbagreen (47.20 mg/kg) and Life Bat Guano (45.73 mg/kg). Regarding the ammonium nitrogen, highest value in the experiment is established at fertilizing with Extrasol (3.48 mg/kg), as the dispersion analysis does not report statistically proven difference in comparison with the above-indicated value after usage of NPK. From the biological fertilizers, the variant with Life Bat Guano shows the next as per value results for content of nitrogen in the soil (2.06 mg/kg). Lowest result is registered in the variant Life Bat Guano + Herbagreen (1.36 mg/kg). For comparison, the not-fertilized variant is with reported content of the nitrate nitrogen 4.08 mg/kg and 1.94 mg/kg of ammonium nitrogen. The soil reaction is from neutral to slightly alkaline, as it varies within the interval pH 6.8 – 8.2. In Table 3 are presented the results for content of potassium and phosphorus in the soil. Regarding absorbable potassium, statistically highest are the values at the variants, fertilized with Extrasol (126.63 mg/100 g soil) and Extrasol + Herbagreen (125.58 mg/100 g). In a separate group is classified the variant with used product Life Bat Guano, as it is registered slightly lower content of absorbable potassium (123.70 mg/100 g). At the variants with brought in NPK and NPK + Herbagreen are established respectively 121.60 mg/100 g and 121.14 mg/100 g absorbable potassium. Lowest values are reported at Life Bat Guano + Herbagreen (111.59 mg/100 g). From the carried out analysis for content of movable phosphates, highest values from the biological products are reported at Extrasol (54.07 mg/100 g). Comparing the biological variant towards the conventional with used NPK (54.11 mg/100 g), the difference between them is very small and it is not proven. The high content of phosphorus in the soil is registered at fertilizing with Life Bat Guano (33.35 mg/100 g). Lowest values of the investigated index are established in the variants with brought in Herbagreen. At the control are reported lowest values of absorbable potassium (45.31 mg/100 g) and movable phosphates (2.98 mg/100 g).

The qualitative and quantitative content of the investigated groups of microorganisms is presented in table 4. The biogenity (the total microflora) of the soil samples is calculated totally of the quantity of non-spore forming bacteria, bacilli, actynomycetes and micromycetes. The investigated soil sample before setting of the experiment as per total number of microorganisms occupies a medium position in comparison with the fertilized samples. However, not always the higher quantity of microorganisms supposes a higher activity. The mineralization coefficient is lowest at the soil sample taken before setting of the experiment and the soil fertilized only with the preparation Extrasol. Higher is the biogenity at the fertilized samples (with exception of the fertilizing with Extrasol + Herbagreen), in comparison with the not fertilized control sample. There is established highest total microflora at the sample fertilized with a combination of mineral fertilizer and biological product NPK + Herbagreen, followed by the sample fertilized with Life Bat Guano + Herbagreen – about 2 times above the not fertilized control sample. The organic fertilizers increase the quantity of nutrient substances, the microbe activity and the production potential of the soil, while the usage only of chemical fertilizers leads to decrease of the microbe activity and the production potential of the soil (Kang *et al*., 2005). The variants, at which is applied only one preparation – Life Bat Guano and Extrasol show respectively total quantity of microorganisms 1.6 and 1.3 times above the control. Weakest is the development of the microorganisms at the development of the microorganisms at the sample fertilized with Extrasol + Herbagreen, but the coefficient of mineralization at this soil is highest, which once again shows that the activity of the microorganisms does not depend only on their quantity. The low microbe biomass in the soils from the conventional agroecosystems is frequently caused by decreased content of organic carbon in the soil (Fliebach and Mader, 2000). Organically treated sections show maximum number of microorganisms (micromycetes and bacteria) and microbe carbon from biomass, followed by non-organically processed plots and the control soil (Nakhro and Dkhar, 2010).

Table 1. Content of nitrogen, phosphorus and potassium in the soil before setting of the experiment

Sample	pH	K_2O, mg/ 100g	P_2O_5, mg/ 100g	NO_3 –N, mg/kg	NH_4-N, mg/kg
Soil	7.3	63.38	8.17	6.24	0.41

Table 2. Content of nitrate and ammonium nitrogen after harvesting

№	Variant	pH	NO_3 –N, mg/kg		NH_4-N, mg/kg	
1	NPK	6.8	63.80	a	3.33	a
2	NPK + Herbagreen	7.1	61.98	b	2.50	b
3	Extrasol	7.3	52.20	c	3.48	a
4	Extrasol + Herbagreen	7.9	52.02	c	1.70	cd
5	Life Bat Guano	8.2	45.73	e	2.06	bc
6	Life Bat Guano + Herbagreen	8.1	47.20	d	1.36	d
7	Control	7.9	4.08	f	1.94	bcd

a,b,c,d,e,f – Duncan′s multiple range test ($p<0.05$)

Table 3. Content of potassium and phosphorus after harvesting

№	Variant	K_2O, mg/ 100g		P_2O_5, mg/ 100g	
1	NPK	121.60	c	54.11	a
2	NPK + Herbagreen	121.14	c	21.75	e
3	Extrasol	126.63	a	54.07	a
4	Extrasol+ Herbagreen	125.58	a	23.68	d
5	Life Bat Guano	123.70	b	33.37	b
6	Life Bat Guano + Herbagreen	111.59	d	24.91	c
7	Control	45.31	e	2.98	f

a,b,c,d,e,f – Duncan′s multiple range test ($p<0.05$)

The present results show that the development of the microorganisms is activated most strongly when there is used a combination from non-organic and organic fertilizer, but the activity of the microorganisms is highest at the combined fertilizing with two bio products – Extrasol and Herbagreen.

Table 4. Qualitative and quantitative content of the microorganisms (cfu x 10^3/g abs. dry soil) ± C.V.; (%)

Variant	Total microflora	Non-spore-forming bacteria	Bacilli	Micromycetes	Actinomycetes	Bacteria, assimilating mineral nitrogen	Coefficient of mineralization
Soil (before betting experience)	5587,2	4915,2 ± 0,061 (88,0)	384,0 ± 0,208 (6,9)	96,0 ± 1,042 (1,7)	192,0 ± 0,470 (3,4)	3379,2 ± 0,083	0,64
NPK	4313,4	3286,4 ± 0,074 (76,2)	284,4 ± 0,598 (6,6)	347,6 ± 0,345 (8,1)	395,0 ± 0,152 (9,2)	3539,2 ± 0,034	0,99
NPK + Herbagreen	7894,8	6880,0 ± 0,113 (87,1)	326,8 ± 0,184 (4,1)	258,0 ± 0,775 (3,3)	430,0 ± 0,186 (5,4)	7017,6 ± 0,037	0,97
Extrasol	5342,4	4704,0 ± 0,085 (88,1)	235,2 ± 0,473 (4,4)	100,8 ± 0,992 (1,9)	302,4 ± 0,298 (5,7)	3091,2 ± 0,023	0,63
Extrasol+ Herbagreen	3744,0	1872,0 ± 0,388 (50,0)	414,0 ± 0,105 (11,1)	144,0 ± 0,694 (3,8)	1314,0 ± 0,076 (35,1)	6624,0 ± 0,060	2,90
Life Bat Guano	6426,0	4216,0 ± 0,085 (65,6)	782,0 ± 0,102 (12,2)	340,0 ± 0,270 (5,3)	1088,0 ± 0,084 (16,9)	5576,0 ± 0,036	1,12
Life Bat Guano + Herbagreen	7361,2	5297,6 ± 0,064 (72,0)	554,4 ± 0,108 (7,5)	200,2 ± 0,300 (2,7)	1309,0 ± 0,084 (17,8)	4681,6 ± 0,038	0,80
Control (not fertilized sample)	4108,0	2780,8 ± 0,076 (67,7)	489,8 ± 0,490 (11,9)	189,6 ± 0,475 (4,6)	647,8 ± 0,334 (15,8)	3160,0 ± 0,316	0,97

Table 5. Correlation dependency between the total microflora and the chemical elements in the soil

№	Variant	K_2O	P_2O_5	NO_3-N	NH_4-N
1	NPK	-0.71	0.68	0.68	0.67
2	NPK + Herbagreen	-0.57	-0.58	0.57	-0.57
3	Extrasol	0.65	0.67	0.64	0.64
4	Extrasol + Herbagreen	0.49	0.50	0.33	0.50
5	Life Bat Guano	-1	-0.99	-0.99	-1
6	Life Bat Guano+ Herbagreen	-0.64	0.64	-0.64	-0.64
7	Control (not fertilized sample)	-0.50	-0.50	-0.53	-0.50

On the one hand Extrasol increases the quantity of the organic substances and the bacilli in the soil, since it contains around 60% organic matter and the species *Bacillus subtilis*. The bacilli participate mainly in the decomposition of the organic matter. On the other hand, the preparation Herbagreen increases the activation of the initial and secondary metabolism, it advantages for the better steadiness as of the plant, as well as of the microorganisms against the biotic and abiotic factors. Main percentage share in the composition of the microflora occupies the non-spore forming bacteria, followed by the actynomycetes and the bacilli. They participate most actively in the initial stages of degradation of the organic matter. Most weakly presented are the micromycetes, with exception of the sample, fertilized with NPK, where their quantity is higher than the one of the bacilli. The bacteria, which absorb mineral nitrogen are best presented at the soil with the preparations NPK + Herbagreen, and their quantity is lowest at the soil, which is fertilized with Extrasol. The higher quantity of this bacteria and the lower of the non-spore forming bacteria and bacilli specify higher values of the mineralization coefficient and respectively higher speed of decomposition of the organic matter. Higher quantity of this group, as well as of the total microflora correlates to higher quantity of nitrogen at the variant NPK + Herbagreen. The better nutrient reserve of the fertilized samples with the macroelements nitrogen, phosphorus and potassium determines as a whole higher quantity and higher activity of these samples in comparison with the control. According to researches of Bogdanov *et al*. (2015) the micromycetes improve its development at most of the variants of fertilizing, especially at complete fertilizing with average norm of nitrogen and high for phosphorus and potassium, but they are slightest presented in the composition of the total microflora. The actynomycetes have decreased presence in almost all fertilized sections in comparison with the control, but they are with higher percentage participation than the micromycetes. The increased participation of ammonifying bacteria (non-spore forming bacteria and bacilli) in the soil microbocenosis shows that under impact of the brought in mineral fertilizers is activated the passing of the microbic processes, connected with the transformation of organic nitrogen compounds from the organo-mineral soil complex. This on its behalf leads to enriching of the soil with absorbable for the plants nutrient substances. The increased biogenity of the fertilizing soil samples can be explained with the actual aftereffect of the fertilizing, including and through more active root exo-osmosis, which helps for the development of the soil microorganisms. The deep and fast growing root system stimulates the microbiological activity, it improves the structure of the soil, it increases the water permeability and the aeration. The root system is the factory of the plant, there are processed and synthesized the nutrient compounds for the growth (Koedzhikov, 1975). The root system of the cucumbers, however, is shallow, with weak sucking ability, which determines the big requirements of the cucumber towards the water and nutrient regime. Therefore, the proper fertilizing of the soils for growing of this culture is exceptionally important for its development.

There is established a correlation dependency between the total microflora and the macroelements in the soil (Table 5). In the variant with brought in NPK is established negative and positive correlation between the total microflora and the chemical indexes of the soil. A negative correlative coefficient (-0.71) is calculated at the total microflora and K_2O, and a positive coefficient is established at the rest of the indexes, which are comparatively close to each other (P_2O_5 0.68; NO_3-N 0.68; NH_4-N 0.67). The established correlation coefficients show that the total microflora and the chemical indexes have positive, medium as per strength correlation. At the combination of fertilizers NPK + Herbagreen there is also observed a correlation, which can be analyzed as moderate up to significant (from -0.58 up to +0.57). At the variant with Extrasol the calculated correlation coefficients prove that there exists a correlation between the investigated dependencies. The correlation is positive and as per strength of impact it is medium up to high (from 0.64 up to 0.67). At the combination of fertilizers Extrasol + Herbagreen the correlation is also positive, but it is with lower coefficients, which allows us to determine it as weak (NO_3-N 0.33) up to moderate (0.49 at K_2O; 0.50 at P_2O_5 and NH_4-N). The strongest correlation is calculated in the variant with Life Bat Guano. In this case the correlation is very high, complete negative (-0.99 up to -1). At the combination of fertilizers Life Bat Guano + Herbagreen the established correlation coefficients prove the presence of a correlation between the microorganisms and the chemical indexes of the soil. The established correlation is negative and significant as per strength. At the control variant there is established negative, moderate correlation dependency between the total microflora and the investigated chemical elements.

Conclusion

The biological product Extrasol leaves behind itself in the soil most macroelements – nitrate nitrogen (52.20 mg/kg), ammonium nitrogen (3.48 mg/kg), phosphorus (54.07 mg/100 g) and potassium (126.63 mg/100 g). The fertilized soils show higher quantity total microflora and higher activity of the microorganisms in comparison with the not fertilized control sample. Main percentage share in the composition of microbocenosis occupies the non-spore forming bacteria, followed by the actynomycetes and bacilli. As a whole, most poorly presented are the micromycetes, but their quantity increases at fertilizing. The biogenity of the soils increases most strongly at the usage of a combination of mineral fertilizer and biological product – NPK and Herbagreen, followed by the sample fertilized with Life Bat Guano + Herbagreen. The variants, at which is applied only one biological product – Life Bat Guano and Extrasol show respectively total quantity of microorganisms 1.6 and 1.3 times above the control, while the total microflora at the sample fertilized only with mineral fertilizer NPK is close as per value with the control not fertilized sample. The weakest is the development of the microorganisms at the sample, which is fertilized with a combination of two bio products Extrasol + Herbagreen, but the coefficient of mineralization at this soil is highest, which shows that the activity of the microorganisms depends only on its quantity.

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