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The effect of the same microbial products on basic biological activities of soil under cereal crops

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ABSTRACT

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The aim of this research was a preliminary evaluation of the effectiveness of using three preparations which improve soil fertility and yield of plants. Field tests with microbial products: EM (effective microorganisms), EmFarma Plus and UG_{max} were carried out in the Agricultural Research Centre in Grabowo, Poland. The experimental plants were: spring triticale, spring barley and winter wheat. Bioproducts were applied directly into stubble or straw left in the field after harvesting grain and straw with an addition of nitrogen and were compared against control treatments, without the use of the above preparations. The implied treatments are determined for soil biological activity and the basic chemical properties of the soil. The microbiological tests showed a statistically significant difference between the control treatments and treatments with EM and UG_{max} preparations (a statistically significant increase in microbial biomass and activity of dehydrogenases). Analysis of principal component analysis (PCA) explained 52.54% of the variation and separated the three groups: I (UG_{max}), II (EM) and III (control and EmFarma Plus). It was found that the average yield of triticale grains was approximately only by 4% higher in treatments where EM and EmFarma Plus were applied, while in treatments with UG_{max}, triticale yielded at control level.

Keywords: microorganisms; soil enzymes; total organic carbon and nitrogen; cereal plants; soil quality

The soil environment was inhabited by various species of microorganisms (bacteria, actinomycetes, fungi). One gram fresh weight of the soil may contain even billions bacteria (colony forming units). The composition of soil microorganisms and their activity is determined by many biotic and abiotic factors, but the main limiting factor is the availability of the rate of organic matter decomposition, nutrient cycling and their availability. The term soil fertility is defined as the natural ability of soils to meet the plant nutrients and is affected by soil-forming processes and minerals, soil colloids, organic matter and microorganisms abundance taking part in them. The fertility can be increased by using appropriate crop rotation, cultivation and fertilization (organic and mineral) and the proper relationship between water and air (Bowles et al. 2014).

The negative effects of intensification of crop production carried out by conventional methods have led to the reduction of biological activity and acidification and degradation of soils. Soil biological activity, including soil microbial biomass and enzymatic activity, is influenced by a range of physicochemical and environmental parameters. The new ecological methods are being searched for reducing the use of chemicals and restoring soil fertility. They would aim at increasing the fertility of the soil by, for example, increasing soil microbial activity which would increase the organic matter content and improve the performance of biological, chemical and physical properties of the

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soil (Khan et al. 2007). It seems that those methods include the attempts of the formation of soil fertility using preparations with useful microorganisms. Microbial inoculants of plant growth-promoting microorganisms (PGPM) used as bioproducts are attracting growing interest in such management strategies (Khaliq et al. 2006, Javaid et al. 2008).

The industrially produced microbial preparations represent a mixture of naturally occurring microorganisms. There are positive opinions concerning the beneficial influence of the microbial preparations on the vegetative growth of plants and improvement of plant general condition, which contributes to plants' greater resistance to diseases (Muthaura et al. 2010). However, also critical opinions are known concerning possibilities of using this type of preparations in agricultural production (Mayer et al. 2010). Currently, in agricultural practice many microbial preparations may be found which, according to the manufacturers, may modify plant metabolism and increase the use of its yield-producing potential.

The aim of this study was a preliminary assessment of the effectiveness of the applied bioproducts used for improving the performance of selected parameters of biological activities.

MATERIAL AND METHODS

Field experiment. Field studies were carried out at the Agricultural Experimental Station (AES) of Institute of Soil Science and Plant Cultivation – State Research Institute (IUNG-PIB) in Grabow, the Mazowieckie province, Poland (52°13'N, 19°37'E) in 2012–2014. The research at the AES in Grabow was conducted on a grey brown podsolic soil formed from light loam. The basic properties of soil are presented in Table 1. The experiment was established with the equivalent method of sub-blocks: split-block-split-plot, in three replicates. The study was conducted for a total of 108 plots, each with a gross area of 48 m^2 and net area (for harvest) 25.5 m^2.

Plants. The cereals were used as experimental plants: in 2012, it was spring triticale of cv. Nagano, in 2013, winter wheat cv. Figura, in 2014 spring barley of cv. Kucyk.

Microorganisms preparations. The two selected microbial preparations EM (Greenland Technologia

Table 1. The basic physicochemical properties of soil (0–30 cm, average for treatments)

Treatment	Stubble					Stubble + straw					Stubble + straw + N				
	pH_{KCl}	g C_{org} per 1 kg of soil	P_{Egner}	K_{Egner}	Mg	pH_{KCl}	g C_{org} per 1 kg of soil	P_{Egner}	K_{Egner}	Mg	pH_{KCl}	g C_{org} per 1 kg of soil	P_{Egner}	K_{Egner}	Mg
			(mg per 1 kg of soil)					(mg per 1 kg of soil)					(mg per 1 kg of soil)		
Spring triticale (2012)															
Control	6.5	8.2	100.4	102.0	35	6.7	8.2	110.4	90.4	31	6.3	8.4	103.0	93.7	36
EM	6.7	8.2	117.4	101.2	31	6.6	8.4	121.8	105.3	32	6.5	8.1	111.3	111.1	33
EmFarma Plus	6.6	8.3	112.6	90.4	27	6.6	8.3	117.0	100.4	30	6.6	8.1	118.7	94.5	31
UG_{max}	6.4	7.9	105.6	90.4	24	6.3	8.3	103.9	92.1	28	6.4	8.0	115.2	93.7	26
Winter wheat (2013)															
Control	6.6	8.3	97.3	94.5	36	6.6	8.1	92.9	94.5	38	6.4	8.3	94.3	96.2	34
EM	6.5	8.3	110.9	102.0	34	6.3	8.3	121.3	105.3	35	6.3	8.3	105.6	112.8	38
EmFarma Plus	6.5	8.4	108.2	92.1	32	6.4	8.5	103.0	104.5	34	6.5	8.1	101.7	93.7	34
UG_{max}	6.5	7.9	111.8	93.7	29	6.5	8.4	1030	102.0	33	6.3	8.3	105.6	112.8	38
Spring barley (2014)															
Control	6.3	7.8	108.3	103.7	29	6.6	8.1	120.5	115.3	30	6.3	8.0	107.8	125.3*	33
EM	6.4	7.9	127.0*	120.3*	32	6.5	8.0	117.4	110.3	30	6.4	7.9	120.9	126.9*	31
EmFarma Plus	6.3	7.7	120.3	98.7	23	6.7	7.7	128.7*	119.4*	29	6.2	8.0	123.1*	128.6*	31
UG_{max}	6.3	7.5	118.3	118.6*	24	6.6	7.7	113.5	129.1*	29	6.3	8.0	130.9*	113.9	24

*$P \leq 0.05$

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EM Sp. z o.o.) and EmFarma Plus (ProBiotics Poland) and also one soil fertilizer – Ug_{max} (P.P.H.U. BOGDAN, Poland) were used in field experiment.

Microbiological preparations were used every year in three combinations: directly onto the stubble or straw left behind in the field after harvesting of grain and straw, as well as onto the straw with an addition of nitrogen and were compared to the control treatment without the use of the above preparations. Microbiological preparations were used in doses recommended by the manufacturer. In case of EM and EmFarma Plus – 30 L/ha and in case of UG_{max} – 0.9 L/ha. All preparations were used every year in September as a dilution in 300 L of water and then sprayed on the stubble, stubble with straw cut and left behind in the field after harvest grain and stubble with straw with the nitrogen (30 kg N/ha) addition. Right after (the same day) preparations were dosed, the surfaces of the experimental fields were covered with 10 cm layer of soil with the use of compact disc harrow. The effect of the tested products was compared to the control objects, without the use of microbiological preparations.

Factors of the experiment. In this study, three factors were applied:

– microbial preparations + control without treatments;
– manner of use of the microbial products: stubble, stubble with straw, and stubble with straw with the nitrogen (30 kg N/ha) addition; nitrogen (N) as NH_4NO_3 applied in spring;
– two levels of N fertilization: the first year: (NI) 60 kg N/ha and 180 kg N/ha, and in the next two years: 0 kg N/ha and 140 kg N/ha (NII).

Soil samples. Soil samples were collected in June of 2012–2014 from the plough layer (0–30 cm) in three replicates. The moist soil samples were sieved through a 2 mm sieve and stored in a refrigerator (4°C) until analysis.

Bacterial communities analysis. Microbiological counts were expressed as a number of colony forming units (CFUs) per g of dry soil. The number of microorganisms was determined by the dilution method on agarized selective medium. Total number of microorganisms was determined by the dilution method on agarized soil extract (Wallace and Lochhead 1950). The total number of fungi was determined on Martin's medium (Martin 2003). The number of oligotrophic and copiotrophic bacteria were determined, too (Hattori and Hattori 1980). The yeast content was determined on LB medium (lysogeny broth) and ammonifying bacteria (AM) according of Rodina (1968). The total content of phosphate solubilizing bacteria (PSB) was determined on Pikowska medium in modification of Rodina (1968).

The enzymatic activities analysis. Dehydrogenase (DHA) activity in soils was determined according to the method described by Casida et al. (1964). The phosphatases activity was determined spectrophotometrically by the *p*-NPP method (Tabatabai and Bremner 1982).

Microbial biomass C and N. Microbial biomass was determined by the chloroform-fumigation-extraction method by extracting fumigated and unfumigated soils. The results of microbial biomass C and N were calculated according to the following formula:

$$C_{mic} = E_C/k_{EC}$$

Where: E_C = soluble C in fumigated samples – soluble C in control (unfumigated) samples; k_{EC} = 0.45.

$$N_{mic} = E_N/k_{EN}$$

Where: E_N = soluble N in fumigated samples – soluble N in control (unfumigated) samples and k_{EN} = 0.54 (Jenkinson and Ladd 1981, Ghani et al. 2003).

Statistical analysis. Analysis of variance ANOVA, principal component analysis (PCA) and all significance analyses were carried out at $P \leq 0.05$ (Statistica 10.0, StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

The bioproducts claim to enhance plant growth and yields and also to improve soil fertility, but often the composition of microbial is not specified in detail, making it difficult for the users to evaluate the product and for scientists to prove its effectiveness (Bajwa 2005). The grey brown podsolic soil formed from light loam was characterized by a regulated pH, preferred quantity basic macro and micronutrients (only low Mg contents) (Table 1). The biological activities of the soil are closely related to weather conditions. The meteorological conditions during the growing season (2011–2014) are presented in Table 2. Both air temperature and sum of precipitation differed significantly between the years. The physico-chemical properties of the soil were evaluated as the one of the parameters

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Table 2. Meteorological conditions during the growing season (2012–2014)

Year	Month												Σ
	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	
Sum of precipitation (mm)													
2011–2012	3.6	21.5	0.3	40.6	42.1	21.8	20.9	37.8	36.5	54.3	81.6	64.2	425
2012–2013	21.8	83.5	29.4	27.4	45.4	37.5	41.1	29.9	112.0	116.3	20.8	11.6	577
2013–2014	63.9	3.0	48.0	15.7	39	19	31	58	172	93	68	117	728
Average air temperature (°C)													
2011–2012	14.7	7.9	2.2	2.2	–1.2	–6.8	5.1	9.6	15.3	17.7	20.9	18.8	9.2
2012–2013	14.5	8.1	5.3	–3.4	–3.6	–1.1	–2.1	8.3	15.3	18.6	19.7	19.2	8.2
2013–2014	11.8	9.5	5.2	1.6	–2.2	2.0	6.7	10.7	14.3	16.5	20.9	18.3	9.6

used for the assessment of effectiveness of microbial preparations (Table 1). The use of the above microbiological preparations did not significantly affect the pH, organic C content, concentrations of available potassium and magnesium in the soil and the content of trace elements necessary for the plant. The only positive tendency was an increase of the amount of available phosphorus in the soil due to the use of EM's or EmFarma Plus's in all three combinations (Table 1).

The dynamics of soil microorganisms development expressed by changes in the number of the particular groups of soil is a measurable indicator of the biological life in soil environment (Khan et al. 2007, Bowles et al. 2014).

Analysis of PCA explained 52.54% of the variation in the variables examined (Figure 1) variation and separated the three groups: I. UG_{max}; II. EM and III. control and EmFarma Plus. The results of PCA (PCA1 and PCA2 factors) analysis as the correlation coefficient values are presented in Table 3.

Soil microbial biomass is the main driving force in the decomposition of organic materials and is frequently used as an early indicator of changes

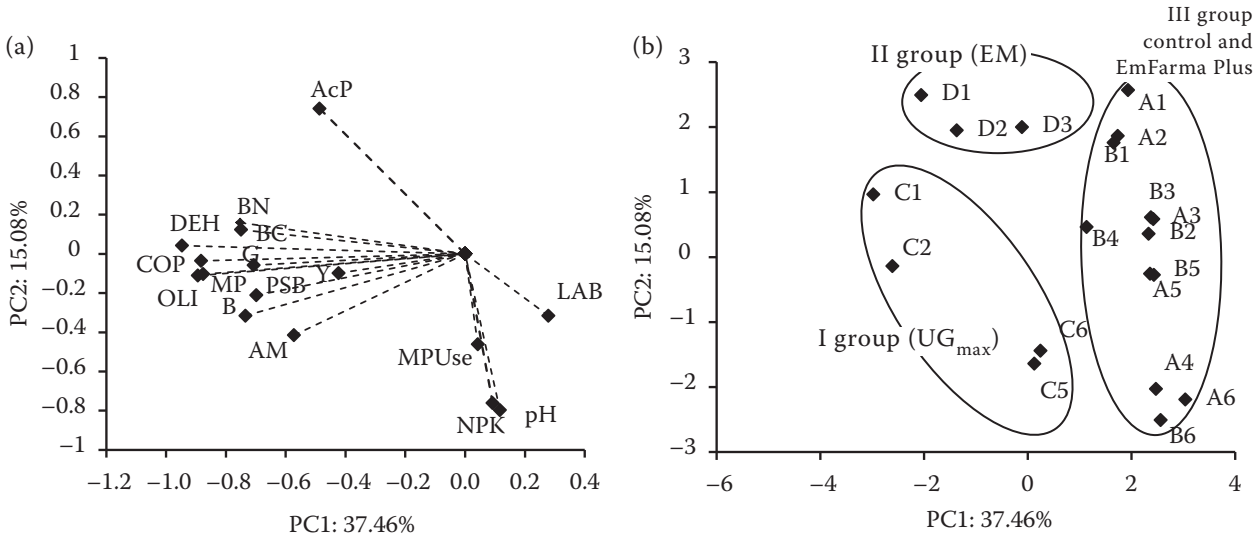

Figure 1. The results of PCA analysis (principal component analysis) – variable for 2014. A1 – control, stubble + straw with nitrogen (NI) + (NII); A2 – control, stubble + straw with nitrogen (NI); A3 – control, stubble + straw + (NII); A4 – control, stubble + straw; A5 – control, stubble + NPK+ (NII); A6 – control, stubble; B1 – EmFarma Plus, stubble + straw with nitrogen (NI) + (NII); B2 – EmFarma Plus, stubble + straw with nitrogen (NI); B3 – EmFarma Plus, stubble + straw + (NII); B4 – EmFarma Plus, stubble + straw; B5 – EmFarma Plus, stubble + (NII); B6 – EmFarma Plus, stubble; C1 – UG_{max}, stubble + straw with nitrogen (NI) + (NII); C2 – UG_{max}, stubble + straw with nitrogen (NI); C3 – UG_{max}, stubble + straw + (NII); C4 – UG_{max}, stubble + straw; C5 – UG_{max}, stubble + (NII); C6 – UG_{max}, stubble; D1 – EM, stubble + straw with nitrogen (NI) + (NII); D2 – EM, stubble + straw with nitrogen; D3 – EM, stubble + straw + (NII); D4 – EM, stubble + straw; D5 – EM, stubble + (NII); D6 – EM, stubble

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Table 3. The results of PCA analysis (principal component analysis) – correlation coefficient values with PCA1 and PCA2 factors

Variable	PCA1	PCA2
Microbial product	–0.882*	–0035
Manner of use microbial products	0.092	–0.761*
Fertilization (N)	0.042	–0.460*
Bacteria (10^8 CFU/g DM of soil)	–0.706*	–0.060
Fungi (10^4 CFU/g DM of soil)	0.020	–0.279
Yeast (10^4 CFU/g DM of soil)	–0.423	–0.098
Ammonifying bacteria (10^7 CFU/g DM of soil)	–0.697*	–0.210
Copiotrophics bacteria (10^6 CFU/g DM of soil)	–0.893*	–0.110
Oligotrophics bacteria (10^7 CFU/g DM of soil)	–0.876*	–0.103
Phosphate solubilizing bacteria (10^4 CFU/g DM of soil)	–0.734*	–0.315
LAB bacteria (10^6 CFU/g DM of soil DM/24)	0.279	–0.315
Dehydrogenases (ug formazan/g DM of soil)	–0.947*	0.042
Alkaline phosphatase (ug *p*-nitrofenol/g DM of soil/1 h)	–0.572*	–0.415
Acid phosphatase (ug *p*-nitrofenol/g DM of soil/1 h)	–0.486*	0.741*
Biomass carbon (ug/g DM of soil)	–0.749*	0.123
Biomass nitrogen (ug/g DM of soil)	–0.752*	0.159
pH	0.116	–0.797*

CFU – colony forming units; DM – dry matter; *$P \leq 0.05$

in soil properties resulting from environmental stresses in agricultural ecosystems (Ghani et al. 2003). Statistically significant increase of the organic biomass carbon content was found in the soil after the application of two microbial preparations: EM and UG_{max} into the soil. There was not found any statistically significant increase in the biomass carbon and nitrogen contents depending on the ways of application of these preparations into the soil.

Enzymes activities are good and widely used indicators of changes of soil microbial parameters. Dehydrogenase activity (DHA) was proposed as a sensitive indicator for evaluation of microbial oxidative activity in soils (Table 2, Figure 1a). Statistically significant higher activities of dehydrogenases, acid and alkaline phosphatase were observed after application of EM and UG_{max} preparations. Dehydrogenases rapidly decompose under normal soil conditions and do not accumulate (Gałązka et al. 2017).

Phosphatase activity (PA) is sensitive to abiotic and biotic factors of the environment, thus may be a suitable selection for inclusion in a soil quality index (Frąszczak et al. 2012). The statistically significant effects of preparation used, way of application and N fertilization on changes in alkaline phosphatase activity in soil, were not found. In contrast, statistically significant effect was observed in the case of acid phosphatase activity assays (Table 3). The highest acid phosphatase

Table 4. Effects of microbial products on grain yields of cereals (average for groups)

Combination	Stubble	Stubble + straw	Stubble + straw + nitrogen
	grain yield (t/ha)		
Spring triticale (2012)			
Control	3.74	3.65	3.86
EM	4.15	3.85	3.98
EmFarma Plus	4.0	4.12	3.92
UG_{max}	3.82	3.79	3.89
Winter wheat (2013)			
Control	5.75	5.72	6.11
EM	6.0*	5.94	6.23
EmFarma Plus	5.84	5.75	6.24
UG_{max}	5.82	6.12*	6.21
Spring barley (2014)			
Control	4.96	5.05	4.94
EM	4.92	5.12	5.23*
EmFarma Plus	5.52*	5.22*	5.51*
UG_{max}	4.90	5.09	5.13

*$P \leq 0.05$

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activity was observed in the first and the second year of study after application of EM and UG_{max}. Statistically significant changes of acid phosphatase activity were observed depending on the ways of application of these preparations in the soil and N fertilization.

The grain yield of spring triticale and wheat increased on average about 3–4% (no statistical difference after application of preparations) in the first two years of study while in the third year spring barley grain yield increase was significant not only because of the preparations but also due to N fertilization (Table 4).

The obtained results confirm an increase in some biological activities of soils (mainly enzymatic activity, microbial biomass) after the application of bio preparations.

Many authors suggest that under field conditions it is difficult to get the positive effects of microbiological preparations, mainly because of the complex interactions between soil organisms and the impact of a changing weather conditions and abiotic soil properties on the development of soil microorganisms (Górski and Kleiber 2010, Bowles et al. 2014).

In most cases, the number of microbes introduced into the soil is usually reduced to the level naturally occurring in the particular soil (Bajwa 2005). In agricultural soils, well fermented manure is of great importance in improving soil fertility and plant health. Due to the ambiguous effect of the tested preparations on some parameters of soil fertility and plant yields, the study of these preparations requires further investigation. The 3-year research period seems too short, under varying weather conditions, to state definite conclusions in this regard.

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