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Efficacy of Biofertilizers and Organic Additive Application in Sweet Basil (*Ocimum Basilicum* L.) Cultivation Depending on the Type of Soil

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Abstract

Basil (*Ocimum basilicum* L.) is one of the most important medicinal and aromatic plants of a considerable economic role; therefore research on this plant is required, especially because of the continuous and increasing demand for such products. Literature sources lack information on the improvement of growth conditions for sweet basil with no requirement for preparations containing chemicals, which is of particular importance in organic farming, in which the application of synthetic pesticides and artificial fertilizers are banned. Biofertilizers (UG Max, EM1, PRP SOL) were tested in two-year pot experiments with different types of soil. The aim of the study was to evaluate the efficacy of the application of biofertilizers and organic additives in basil grown on different types of soil. The addition of straw to mineral soil provided an effect comparable to cultivation in organic soil, in which greater dry mass of leaved shoots of basil was obtained than in mineral soil. The application of all tested biofertilizers, particularly UG Max, in growing basil on mineral soil provides a similar yield of dry mass of leaves as that grown on organic or mineral soil with an addition of straw.

Keywords:

Biofertilizers; EM1; *Ocimum basilicum* L.; PRP SOL; UG Max; Herb

Introduction

In contemporary agriculture the tendency is to search for preparations stimulating growth and development of plants, which have an advantageous effect also on quality of produced raw materials, thanks to which they may improve economic effects in a manner safe for humans and the environment. Such actions to a considerable degree are consistent with the principles of sustainable agriculture, which primary objective is to limit the use of artificial fertilizers and synthetic pesticides. At present we have a broad spectrum of microbial preparations referred to as biofertilizers, which according to the declarations of their manufacturers have an advantageous effect on growth and yielding of plants thanks to improved soil conditions as a result of inoculation with beneficial microorganisms or stimulation of soil microorganisms. In many cases the microbial composition of such preparations is not specified in detail by the manufacturer, which makes it more difficult for users to evaluate the product and for researchers to assess their efficacy [1]. After positive effects of the application of such preparations have been verified, they could be recommended for use in organic farming, in which in accordance with the binding regulations synthetic pesticides and artificial fertilizers are banned [2]. In recent years demand for products of herbal plants has been increasing, particularly in urban areas [3]. Eastern and Southeast European countries play a significant role within the European and global herb trade. Poland as well as Bulgaria, Hungary and Turkey are important source countries for this commodity [4]. Poland is an important exporter of herb raw materials and products to many countries worldwide, although 31.5% of exports [5] are sent to Germany. Moreover, increasing the area cropped to herb plantations may be a chance for the development of organic farming. In view of the above it is advisable to search for new solutions to improve plant growth conditions in methods other than the application of chemicals. Nowadays medicinal and aromatic plants occupy a special economic position because of the continuous and increasing demand for their products from the local and foreign markets. Basil is one of the most important plants in this respect [6]. Both fresh and dried basil herb is used as an aromatic spice and a source of essential oil [7], which is extensively used in several European countries and the USA for flavoring and food stuffs, confectionery goods, condiments and toiletry products such as mouth washes and dental creams [6]. Fresh basil leaves, which retain their taste and aroma also after being frozen or preserved in vinegar and oil, are the most valuable spice [8], which is confirmed by its Latin name, derived from the Greek word *okimoin* – "*it smells*" and the Latin word *basilicum* – "*royal*" [9].

Many publications concern agronomical aspects of basil cultivation and as a result of both conducted research and practical efforts; there is a great variability in the cultivation methods of basil around the world [10] and to date several studies have been carried out concerning its cultivation [11]. Still there is a shortage of reports describing the use of microbiological preparation and organic additives especially in temperate climates such as in Central Europe [12], with only few reports describing these issues in this species [11,13,14].

The focus of this study was to evaluate the effectiveness of the application of biofertilizers and organic additives in basil grown on different types of soil.

Materials and Methods

Two-year pot experiments were conducted in a greenhouse at the Department of Agronomy, the Poznań University of Life Sciences. Soil for the experiments was collected from the topsoil of a field located in Złotniki (N 52° 29.193'; E 016° 20.569') near Poznań and organic soil ('*Athena*') purchased at a Garden Centre (Table 1). Soil was placed in pots of 2 dm^3. Except for the control samples the soil was supplemented with the following biofertilizers: UG Max, EM1 and PRP SOL (Table 2), and mineral soil was supplemented with straw. After 14-day incubation seeds of sweet basil were sown and after germination 3 well-developed seedlings were left per pot. In the course of the experiment standard cultivation measures were applied, consisting in regular watering with distilled water, weeding

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and loosening of soil. At full anthesis plants were hand-harvested by cutting at a height of 5 cm above the soil and vegetative growth parameters, i.e. herb dry weight (g/m^2), shoot dry weight (g/m^2) and root dry weight (g/m^2), were recorded in both seasons. The chlorophyll index of plants was examined using a pocket Hydro N-Tester (Minolta, Japan). Height and root volume were measured at the end of vegetation season. The roots were separated from the soil by gently washing off the soil core with water. The root volume was determined by immersing the roots into a partially filled graduated cylinder and recording the resulting water displacement. Descriptive statistics were applied for the values of height, the chlorophyll index and root volume data. Analysis of variance (ANOVA) was used to evaluate vegetative growth parameters for different soil types and biofertilizers. In the case of statistically significant differences as the result of variance analysis, related categorization was performed applying Tukey's test. Data were transferred to the computer MS Office system and analyzed statistically using Statistica (ver.10) software package for Windows.

Results and Discussion

The results of descriptive statistics and plant height, the chlorophyll index and root volume of basil plants depending on treatment type are given in Table 3.

Basil prefers moderately fertile or humus-rich, well-drained loamy or sandy-loam soils [10]. Analyses showed that vegetative development of basil plants depended on the type of used soil (Table 3). In the conducted experiments plants grown in mineral soil were the tallest. In an experiment conducted by Nazim et al. [14] basil plants growing in sandy soil with manure reached a similar height (46 cm) as in this study. The height of plants growing in organic soil was by 3.1 cm lower than that of plants grown in mineral soil. The organic admixture (rye straw) added to mineral soil caused a slight decrease in plant height, amounting on average to 0.9 cm. These differences were confirmed statistically.

Soil type also had a significant effect on values of the leaf chlorophyll index. The organic admixture to mineral soil added in the form of rye straw resulted in the significantly greatest value of this index in this experiment. In comparison to the other combinations, i.e. mineral and organic soil, the value was by 36.5 and 57 SPAD units lower. In turn, organic soil promoted the development of the root system, as significantly the greatest root volume of basil plants was recorded for this soil. Root volume in basil plants growing in mineral soil was by 4 cm^3 lower in comparison to that recorded for organic soil, while the organic admixture to mineral soil caused an increase in root volume by 1.2 cm^3.

The application of UG Max stimulated growth in basil plants, which were by 2.2 cm taller than the control. In turn, the use of PRP SOL limited plant growth by 1.8 cm in relation to the control. Similarly, the application of effective microorganisms during the cultivation of sweet basil inhibited plant growth in a study by Frąszczak et al. [13], who obtained plants characterized by a significantly lower height in the combinations, in which the plants were growing in the substrate with an addition of EMs. This study did not confirm growth inhibition in plants growing in soil with an addition of EM1, as their height did not differ significantly from that of plants in the control.

In comparison to the control the application of the EM1 and PRP SOL preparations reduced values of the chlorophyll index by 4 and 6 SPAD units. Similarly, chlorophyll relative content (SPAD) was found to be higher in sweet basil plants growing without Ems in the experiments conducted by Frąszczak et al. [13]. In field trials on rape, winter wheat, spring barley and potatoes the application of PRP SOL resulted in an increased number of SPAD units in comparison to the control [18,19,20]. In turn, in this study following the application of UG Max the chlorophyll index was by 21.2 SPAD units higher than in the control, although the differences were not confirmed statistically. Among the tested soil admixtures only UG Max slightly, statistically non-significantly stimulated root growth in basil plants. In contrast, the other preparations in comparison to the control significantly reduced root volume.

An interaction of the tested factors was found when investigating their effect on plant height, dry mass of whole plants, leaves and stems and on the proportion of leaf mass in the mass of whole plants (Figure 1a). Plants of sweet basil were tallest when grown in mineral

Type of soil	N mg·kg^{-1}	P mg·kg^{-1}	K mg·kg^{-1}	pH in 1 M KCl	Sand (%)	Clay (%)	Dust (%)
Mineral*	18	270	189	6,5	17	17	12
Organic	180	69.8	182.6	7.2	-	-	-

**According to soil quality classification it belongs to the good rye complex with luvisol soils, sand texture (UG2) formed from glacial moraine clay [15].*

Table 1: Composition of used soil types.

Components	UG Max[1] (mg·kg^{-1})	EM1[2] (mg·kg^{-1})	PRP SOL[3] (mg·kg^{-1})
N	1200	-	-
P	500	-	-
K	3500	-	-
Mg	100	-	48000
Ca	-	-	228600
Mn	3	-	-
Other (mg·kg^{-1})	Na - 200	-	Na - 35000; 3 - 5% admixtures (48 trace elements)
Microorganisms	Lactic acid bacteria, Photosynthetic bacteria, Azotobacter, Pseudomonas, yeast, Actinomycetes	Photosynthetic bacteria, Lactic acid bacteria, yeast, Actinomycetes	-
Dose	0.026 ml/pot	2.8 ml/pot	12 g/pot

UG Max[1] [16]; EM1[2] [17]; PRP SOL[3] [18]

Table 2: Composition of used biofertilizers.

Compared feature	Treatment type	Min	Max	Mean±SD	CV(%)	p-value
Plant height (cm)	M+S	35.0	56.0	45.1±6.5	14.5	0.041**
	M	38.0	61.0	46.0±5.0	10.9	
	O	27.0	57.0	42.9±9.3	21.7	
	Control	36.0	57.0	44.7±6.1	13.7	0.047*
	UG Max	37.0	61.0	46.9±6.3	13.5	
	EM 1	27.0	53.8	44.1±7.9	18.0	
	PRP SOL	30.0	56.0	42.9±8.2	19.2	
chlorophyll index (SPAD)	M+S	165.0	516.0	257.3 ±67.2	26.1	0.000**
	M	135.0	298.0	220.8±33.5	15.2	
	O	112.0	375.0	200.3±76.6	38.3	
	Control	112.0	334.0	223.3±65.5	29.3	0.331ns
	UG Max	135.0	516.0	244.5±92.1	37.7	
	EM 1	115.0	274.0	219.3±43.6	19.9	
	PRP SOL	124.0	322.0	217.3±52.4	24.1	
Root mass volume (cm^3)	M+S	2.6	8.8	6.2±1.3	21.0	0.000**
	M	1.3	9.1	5.0±2.1	43.4	
	O	5.0	15.6	9.0±2.1	23.2	
	Control	3.9	10.4	7.0±2.3	33.4	0.001**
	UG Max	3.9	15.6	7.7±2.7	34.6	
	EM 1	1.3	9.1	5.9±2.6	44.6	
	PRP SOL	2.6	11.3	6.2±2.2	35.2	

*SD – standard deviation; * – statistically significant differences (p < 0.05), ** – statistically highly significant differences (p <0.01), ns – no statistically significant effect on tested trait (p>0.05), cv – coefficient of variation; M+S – mineral soil with straw, M- mineral soil, O-organic soil*

Table 3: Descriptive statistics and measurement of height, chlorophyll index and root volume of basil plants.

soil following the application of UG Max. The use of this preparation in mineral soil with an addition of straw resulted in a non-significant reduction of plant height on average by 6.75 cm. The tendency to produce lower plants than in the other tested combinations was observed in the case of organic soil with an addition of EM1 (Figure 1a).

The application of tested biofertilizers in mineral soil with an addition of straw and in organic soil led to a reduction of dry mass of whole plants. A significant decrease in the values of this parameter was recorded following the application of PRP SOL and amounted to 14.75 and 44.15 g/m^2, respectively, for mineral soil with straw and for organic soil. In this study only in mineral soil dry mass of whole plants increased after the application of all the tested preparations and in comparison to the control this increase amounted to 23.93 (UG Max), 11.91 (EM1) and 8.13 (PRP SOL) g/m^2 (Figure 1b).

We need to stress here that dry mass of leaves and stems, similarly as the mass of whole plants, decreased following the application of tested biofertilizers in mineral soil with an addition of straw and in organic soil (Figure 1c, 1d). It may be assumed that microorganisms supplied with the preparations competed with plants for nutrients coming from mineralization of carbon from straw and from organic soil. As a result the availability of nitrogen decreased, since microorganisms transformed it into protein. Similarly, Mayer et al. [12] found that the observed checking of plant growth following EM application might also have been caused by an increased competition for nitrogen between bacteria and crop plants. In turn, Schenck et al. [1] found no effects of EM on N mineralization and microbial biomass C and N in soil without amendments and with application of wheat straw and coarse meal of yellow lupins. In this study only the application of UG Max in mineral soil led to a significant increase in the yield of leaf and stem dry mass by 8.48 and 15.49 g/m^2 in comparison to the control.

The percentage share of leaf mass in herb mass is one of the elements determining the value of the raw material [8]. In a study by Jadczak et al. [21] conducted on basil this share was 49.8–56.9%, depending on the sowing date applied in the experiment, whereas in this study the range of values was by 3.11–11.79 percentage points lower. In these experiments an interaction was shown for the EM1 and PRP SOL preparations with soil. Following the use of these biofertilizers in organic soil the share of leaf mass in the mass of whole plants increased in the comparison to the control by 15.78 and 14.14 percentage points, which was confirmed statistically (Figure 1e).

Roots of basil developed best in mineral soil with an addition of straw (Figure 2a). Their dry mass was by 9.6 g/m^2 higher in comparison to root mass produced in organic soil. Moreover, the addition of straw to mineral soil caused a significant increase in dry matter of leaved shoots of basil on average by 5.4 g/m^2. In turn, the greatest dry mass of stems with leaves was obtained from plants grown in organic soil, amounting to 55.4 g/m^2. Organic matter content is a major property modifying soil structure [22], promoting constant, although slow release of essential nutrients [23]. Many authors showed in their studies a positive effect on soil structure for organic admixtures such as manure [24,25], post-harvest residue in the form of straw [26], or an interaction of both these additives [27]. In a study by Nazim et al. [14] conducted on sandy soil with manure the produced dry mass of basil shoots was comparable to that recorded in this study for mineral soil with an addition of straw, while dry mass of roots per plant was lower than in this study on average by 2.42g. The findings reported by Putwattana et al. [28] indicated that the addition of 20% cow manure greatly enhanced the dry biomass production of *O. basilicum*. Khalid et al. [6] also found that the highest values of vegetative growth characters resulted from the treatments of static compost or tea of static compost. In Egypt reclaimed soil may be used for basil production when soil quality is improved with 30–40 t/ha organic matter [10]. In an experiment by Zheljazkov et al. [29] three basil cultivars yielded differently when grown at four different locations. Yields of cv. Mesten were the highest on very fine sandy loam, lower on silt loam, and the lowest on sandy loam. Air-dry matter yields of

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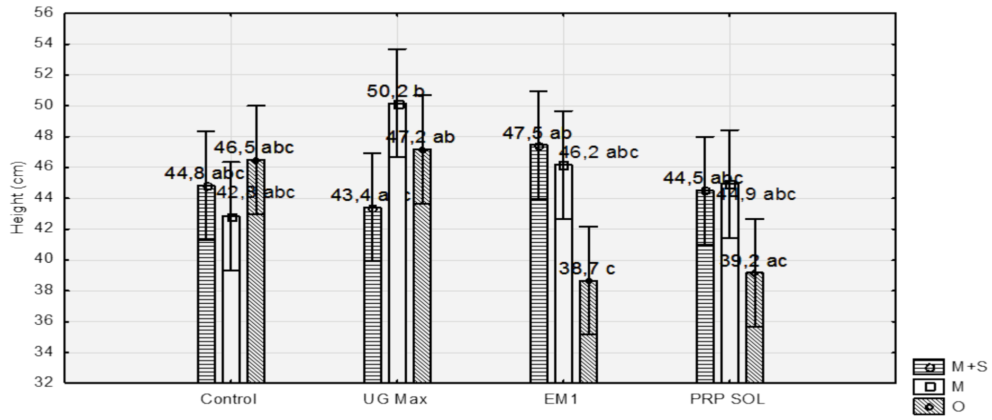
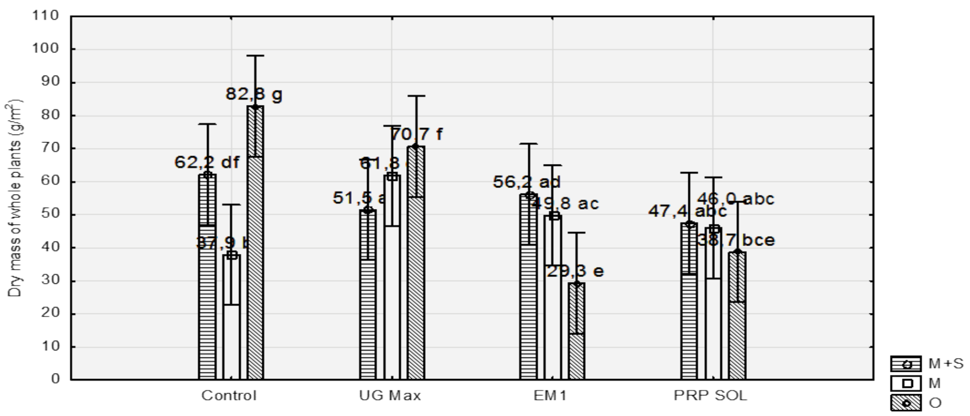


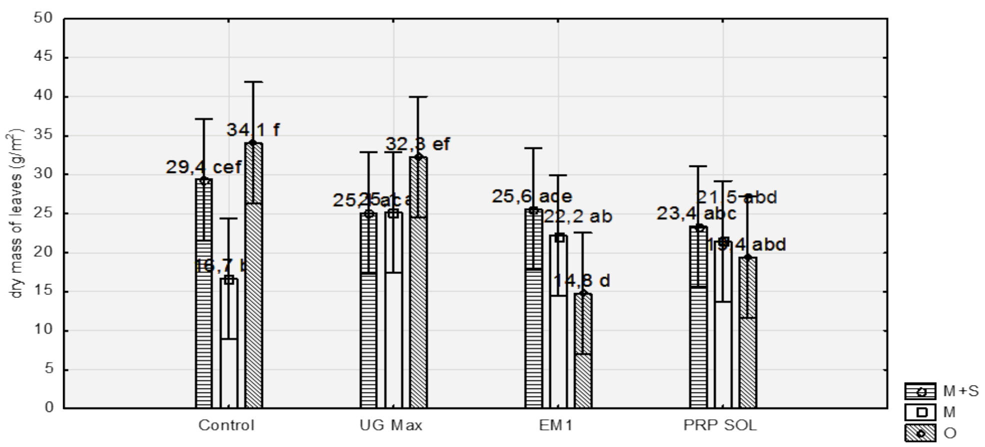
M+S – mineral soil with straw, M - mineral soil, O - organic soil; Mean values that do not differ significantly are have the same letter; a, b, c – homogeneous groups (Tukey's test)

Figure 1a: Effect of soil type and biofertilizers in the years of study on height (cm).



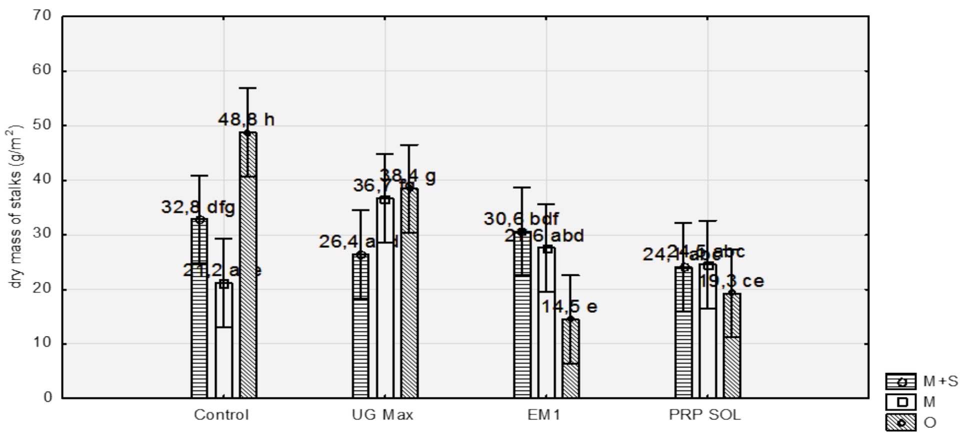
M+S – mineral soil with straw, M - mineral soil, O - organic soil; Mean values that do not differ significantly are have the same letter; a, b, c – homogeneous groups (Tukey's test)

Figure 1b: Effect of soil type and biofertilizers in the years of study on dry mass of whole plants (g/m²).



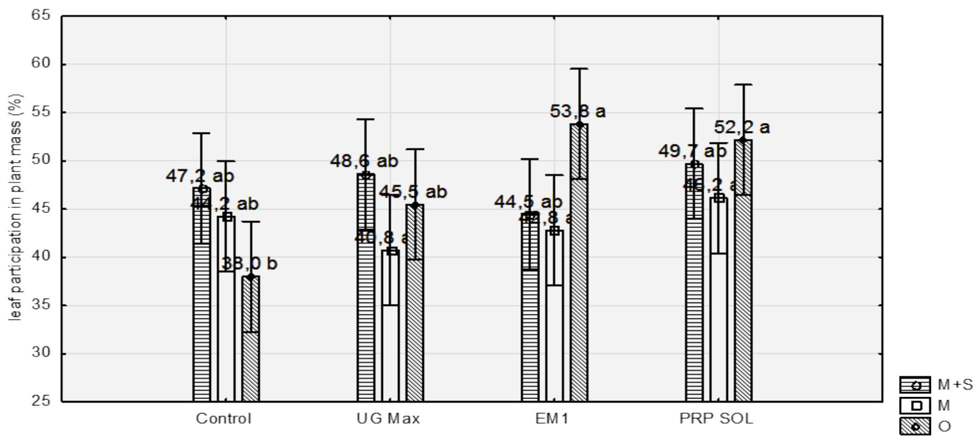
M+S – mineral soil with straw, M - mineral soil, O - organic soil; Mean values that do not differ significantly are have the same letter; a, b, c – homogeneous groups (Tukey's test)

Figure 1c: Effect of soil type and biofertilizers in the years of study on dry mass of leaves (g/m²).



M+S – mineral soil with straw, M - mineral soil, O - organic soil; Mean values that do not differ significantly are have the same letter; a, b, c – homogeneous groups (Tukey's test)

Figure 1d: Effect of soil type and biofertilizers in the years of study on dry mass of stalks (g/m²).



M+S – mineral soil with straw, M - mineral soil, O - organic soil; Mean values that do not differ significantly are have the same letter; a, b, c – homogeneous groups (Tukey's test)

Figure 1e: Effect of soil type and biofertilizers in the years of study on leaf proportion in plant mass (%).

sweet basil cv. Germanof were the highest on silt loam, lower on very fine sandy loam and silt loam, and the lowest on sandy loam. Thus these results showed that the type of soil must be selected not only for the species, but also for variety.

Following the application of UG Max a trend was observed towards the accumulation of root mass greater by 4.6 g d.m./m², while that of leaved shoots was by 0.4 g d.m./m² higher than in the control. The other tested preparations had an adverse effect on the development of roots and leaved shoots. Following the application of EM1 and PRP SOL dry mass of roots decreased by 5.1 g/m² and 3.0 g/m², respectively, although the differences were not confirmed statistically. Moreover, following the application of the above mentioned preparations a marked decrease was observed in dry mass of leaved shoots by 15.9 and 17.0 g/m², while the differences in relation to the control were statistically significant (Figure 2b). Based on the results reported by other authors it may be stated that the application of microbial preparations remains disputable. However, experiments conducted by Frąszczak et al. [13] on sweet basil confirm the results recorded in this study and definitely show a strong growth inhibition in plants growing in the substrate supplemented with EMs. They were characterized by a smaller height, fresh mass, as well as smaller leaf area in comparison with plants growing in the substrate without any addition of effective microorganisms. Also Javaid et al. [30] observed a reduced yield of pea growing in soil with an addition of EM. Similarly, Kaczmarek et al. [31] showed that the application of Em-A in different fertilization variants is of limited efficacy or even unjustified, particularly from the point of view of cultivation practice due to the deterioration of certain soil properties. Similar results were recorded by Mayer et al. [12], who confirmed that '*Effective microorganisms*' did not improve yields or soil quality during 4 years of application in the field experiment under the temperate climatic conditions of Central Europe. Analogously, studies by Bajwa et al. [32], Khaliq et al. [33] and Okorski et al. [34] showed that the effect of EM was slight and frequently negatively influenced the development and yields of chickpea, cotton, wheat or pea. According to Priyadi

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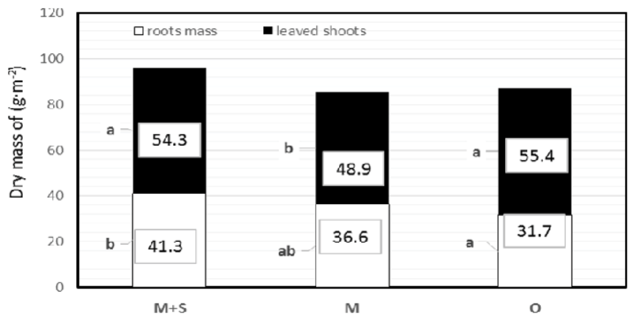
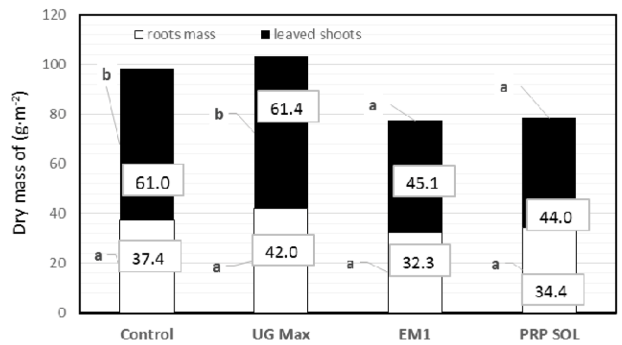


M+S – mineral soil with straw, M - mineral soil, O - organic soil; Mean values that do not differ significantly are have the same letter; a, b, c – homogeneous groups (Tukey's test)

Figure 2a: The effect of soil type treatment on dry mass of roots and leaved shoots.



M+S – mineral soil with straw, M - mineral soil, O - organic soil; Mean values that do not differ significantly are have the same letter; a, b, c – homogeneous groups (Tukey's test)

Figure 2b: The effect of biofertilizer treatment on dry mass of roots and leaved shoots.

et al. [35] and van Vliet et al. [36] the application of EM had no effect on yields of maize or grasses. Cóndor et al. [37] warned against the use of EM and claimed that there is a great amount of unreliable information concerning such preparations. Their beneficial effect is supported by experiments conducted in tropical countries, although no reliable and testable data have been published, thus more research is needed.

Similarly, the results of studies published on the application of PRP SOL so far with respect to the effects of this soil additive on soil properties and crop yields are controversial [38]. Certain results confirm the assumptions of the technology developed by the manufacturer, aiming at increasing the enzymatic activity of soils and improving their physical properties [39]. In contrast, other reports do not confirm the advantageous effect of this preparation. Niewiadomska et al. [40] showed a stimulating action of PRP SOL on the microbial activity of soil cropped to rape, while under barley they observed an opposite effect. Experiments by Martyniuk et al. [41] showed that phosphatase activity, total counts of bacteria and fungi, the counts of Azotobacter spp., MPN of rhizobia, counts of spores of AM fungi, glomalin content in soil under winter wheat, corn and spring barley generally did not differ significantly from those found in soil fertilized with NPK compared with PRP SOL. In turn, Bielińska et al. [42] showed a beneficial effect of PRP SOL, EM-A, UG Max on the activity of dehydrogenases, urease and protease and a negative effect on phosphatase activities, although no statistical assessments of these results were performed. The application of UG Max in an arable field in the temperate climate of northern Poland over a three-year period of experiments conducted by Piotrowska et al. [43] showed no clear effects on the biological and physico-chemical properties of soil. Martyniuk et al. [41] stressed that both in their study and in most other investigations conducted by Polish researchers UG Max showed no advantageous effect on plant yields or soil quality, thus according to those authors its application in farming practice is unreasonable.

Conclusion

Introduction of straw to mineral soil made it possible to obtain a similar yield of dry mass of basil leaved shoots as that produced in organic soil and higher than in mineral soil. The application of all tested biofertilizers in organic soil and mineral soil with an addition of straw led to inhibition of plant growth and accumulation of lower dry mass of leaves, stems and whole plants than in the case of mineral soil. This could have been caused by an increased competition of microorganisms and plants for nitrogen.

The addition of all tested biofertilizers, particularly UG Max, in the culture of basil in mineral soil makes it possible to produce a similar yield of dry mass of leaves as in organic soil or mineral soil with an addition of straw.

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