

Full Length Research Paper

Impact of effective microorganisms on yields and nutrition of sweet basil (*Ocimum basilicum* L.) and microbiological properties of the substrate

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The objective of the performed investigations was to assess the effect of application of effective microorganisms (EM), employed in the form of substrate inoculant (I), seed inoculation (II) and foliar application in the form of a spraying solution (III), on growth, development and macroelement uptake as well as microbiological properties of the root zone in sweet basil (*Ocimum basilicum* L.) cultivated in a peat substrate. The application of effective microorganisms for sweet basil cultivation resulted in the inhibition of plant growth dynamics, among other things, reduction of plant height and fresh mass. A significantly higher macro-element content was observed for the application of EMs in form I – on the improvement of plant nutrition with nitrogen (N) and potassium (K) and in the form III – on the improvement of plant nutrition with nitrogen (N). The application of the EM inoculum was found to reduce the total number of bacteria, numbers of fungi, copiotrophs and oligotrophs. Recapitulating, it can be concluded that the application of EMs for a short cultivation period of spice plants in pots fails to yield any positive effects in the form of improved yield.

Key words: Chlorophyll content, macroelements, nutrient content, soil microbiology, spice plants.

INTRODUCTION

One of the preparations used to improve plant yield and soil fertility is effective microorganisms (EM), which can be defined as a commercial mixture of photosynthesizing bacteria, actinomycetes, lactic acid bacteria, yeasts and fermenting fungi (*Aspergillus* and *Penicillium*) (Muthaura et al., 2010; Wielgosz et al., 2010). The microbiological composition of the EM concentrate (population size in 1 ml given in brackets) is as follows: *Streptomyces albus* (10^5), *Propionibacterium freudenreichii* (10^5), *Streptococcus lactis* (10^5), *Aspergillus oryzae* (10^5), *Mucor hiemalis* (10^5), *Saccharomyces cerevisiae* (10^5) and

Candida utilis (10^5) (Formowitz et al., 2007). Moreover, EMs also contains an unspecified amount of *Lactobacillus* sp., *Rhodopseudomonas* sp. and *Streptomyces griseus*. Until recently, a number of experiments were carried out, in which effective microorganisms (EMs) were used in cultivation of various species of agricultural crops (cotton, maize, sweet potatoes, rice, triticale, wheat) as well as horticultural plants (rose, gerbera, apple, apricot) as reported in numerous studies (Kengo and Hui-lian, 2000; Klama and Kleiber, 2010; Shah et al., 2001; Eissa, 2002; Khaliq et al., 2006; Sahain et al., 2007; Boligłowa and Gleń, 2008; Górski and Kleiber 2010), confirming their positive influence on most plants, while it failed to demonstrate a positive effect Mayer et al. (2010) on the yield of few.

According to Higa (2003) and Wielgosz et al. (2010),

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Table 1. Experimental design.

Substrate							
EM +				EM -			
Seeds							
EM+		EM-		EM+		EM-	
Spraying							
1. EM+	2. EM-	3. EM+	4. EM-	5. EM+	6. EM-	7. EM+	8. EM-

EM+, With effective microorganism; EM-, without effective microorganism.

effective microorganisms can exert influence on the conditions for other microorganisms, causing growth of autochthonous groups of microorganisms, thanks to which the microflora of a given environment becomes richer, in turn, affecting growth and development of plants. The population size of microorganisms depends on soil fertility, cultivated plant species, climatic conditions and various ecological factors. Advantages of the EM preparation include its wide spectrum of action, associated with the multifaceted activity of various groups of antagonistic microorganisms contained in it (Janas, 2009).

Higa (2003) maintains that EMs are capable of producing antioxidants, counteracting the development of free radicals by oxygen. Free radicals contribute to the development of certain diseases, while antioxidants produced by EMs eliminate, counteract, or reverse the effects of oxygen activity. A significant number of microorganisms making up EMs have been used to produce food products, such as beer, wine, bread, various dairy products and sauerkraut. Effective microorganisms, once they are provided with appropriate substrates in the medium, activate their metabolic mechanisms leading to the development of useful substances, such as vitamins, antioxidants or organic acids, enhancing the resistance of organisms living in this specific environment (Mau, 2007).

Cultivation of spice plants in containers is one of the youngest branches of the greenhouse vegetable growing. Currently, cultivation technology of herbs in containers is advanced and has changed from a marginal cultivation area in a separate glasshouse crops (Frąszczak et al., 2011). Basil is one of the most popular spices, both in Europe and over the world. It is also an often grown spice herb in containers (Koch et al., 2007).

This study evaluates the effect of application of effective microorganisms (EM) on growth, development and macroelement uptake as well as root zone microbiological properties in sweet basil (*Ocimum basilicum* L.) cultivated in containers on peat substrate.

MATERIALS AND METHODS

Experiments were carried out in vegetation chambers at the

Experimental Station of the Departments of the Faculty of Horticulture and Landscape Architecture, the Poznań University of Life Sciences, Poland. Their objective was to assess the effect of effective microorganisms (EM) applied as an inoculum for the substrate (I), seed inoculation (II) and foliar solution to spray plants (III), on growth and development of sweet basil, uptake of macroelements (nitrogen, phosphorus, potassium, calcium and magnesium) by plants as well as root zone microbiological properties. A three-factorial, random block design experiment in eight combinations was carried out and each combination was made up of 6 replications, that is, single pots. The experiment was conducted in 2009 in two cultivation cycles.

Climatic conditions

Experiments were carried out in vegetation chambers under controlled and constant climatic conditions. The following conditions were maintained: day and night temperatures of 25 and 20°C, respectively, and daylight and dark periods of 16 and 8 h, respectively. The source of light were fluorescent TL'D 36/W/840 (Philips, Poland) tubes with 350 to 700 nm wavelength and white light PPFD of 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Vegetation experiment

Eight experimental combinations were applied (Table 1) and each combination was replicated in 6 treatments. The control had no form of EM.

Experimental plants were grown in a mixture of peat and sand substrate mixed at a 5:1 volume ratio in pots measuring 7 x 7 x 6 cm (volume = 294 cm^3). The described experiments were conducted on cv. *Kasia*, obtained from the Institute of Natural Fibres and Herb Plants in Poznań, Poland. This cultivar is recommended for pot cultivation (Seidler-Łożykowska, 2004). Fifty seeds were sown in one pot and were then covered with a thin layer of sand. The number of plants grown in pots was identical and amounted to 42 to 45. The pots with the substrate and sown seeds were then placed on cultivation tables lined with infiltration mats and perforated black foil. The moisture content of the substrate was maintained at 70% throughout the vegetation period. Plants were cultivated for 5 weeks (35 days) counting from the day of emergence. Emergence was assumed as the time at which 80% of plants in each pot emerged and developed seed leaves. The following discussed procedures were used in individual combinations

Preparation of the substrate for sweet basil cultivation

EM-A solution was prepared at a 1:50 proportion (50 ml EM-A per 450 ml H_2O). The inoculum prepared in this way was mixed with the

part of the substrate intended for inoculation in the amount of 14 L solution per 252 L of substrate. The EM-A solution was applied 3 days prior to seed sowing into the substrate in order to allow penetration through peat structure. The substrate was put into pots three days after inoculation.

Inoculation of sweet basil seeds and their sowing

An appropriate batch of seeds was soaked for 20 min in a diluted (1:5) EM-A solution.

Spraying of plants

Plants were sprayed 15 days after their emergence. The EM-A solution used in spraying was diluted at a 1:10 ratio and 4 ml of the EM-A solution was used per each pot. Plants in the control combination were sprayed with clean water.

Plant morphological assessment

Morphological and green mass measurements were performed on the 35th day of the experiment, that is, on the day of its termination. Yields of the fresh mass were estimated for each pot and these measurements comprised plant height, leaf area and relative chlorophyll content (with the assistance of a SPAD apparatus, Minolta Co.). Fresh mass was determined for all plants in the pot, whereas plant height and area were determined for 40 plants per pots, while relative chlorophyll content was determined for 40 plants in each replication. The obtained results were subjected to statistical analysis followed by Duncan's test ($\alpha = 0.05$).

Plant chemical analysis

Aboveground plant parts for chemical analyses were collected individually from each experimental combination on the day of the trial termination, and they were subsequently dried at a temperature of 45 to 50°C and ground. In order to determine total N, P, K, Ca and Mg forms, the plant material was mineralised in concentrated sulphuric acid as described in Kleiber and Komosa (2010). After mineralisation of the plant material, the following methods were used to conduct appropriate chemical analyses: total N, according to Kjeldahl based on Parnas-Wagner distillation; P, colorimetrically with ammonium molybdate, K, Ca and Mg, by atomic absorption spectrometry (AAS). The results of plant material chemical analyses were processed statistically by Duncan's test ($\alpha = 0.05$).

Microbiological methods

A representative pooled sample was prepared by collecting 5 g of the substrate from each of the 6 pots, making up a given experimental combination and which was subsequently used to prepare a series of consecutive soil dilutions. Samples were collected on four consecutive dates:

- I - prior to seed sowing,
- II - 20 days after seed sowing, prior to plant spraying with the EM preparation,
- III - 10 days after plant spraying with the EM preparation,
- IV - before harvesting of mature plants.

Analyses of the substrate microbiological condition were conducted using the method of deep plate inoculation by determining the total numbers of bacteria, actinomycetes, fungi, copiotrophs and oligotrophs. Cultures were performed on selective substrates: total

count of bacteria on the ready-to-use standard count agar by MERCK (28°C, for 7 days), actinomycetes - on the Pochon medium (Kańska et al., 2001) (28°C, for 7 days), fungi - on the medium according to Martin (1950) (24°C, for 7 days), copiotrophs - on nutrient broth (28°C, for 7 days) and oligotrophs - in a diluted nutrient broth (Ohta and Hattori, 1980) (28°C, for 21 days). The obtained results of microbiological analyses were subjected to statistical analyses with the Tukey test ($\alpha = 0.05$).

RESULTS

Plant growth

The application of effective microorganisms during the cultivation of sweet basil inhibited plant growth (Table 2). Plants were characterised by a significantly lower height in the combinations in which plants were growing on the substrate with an addition of EMs, in comparison with the cultivation without EMs. The lowest height (6.08 cm) of plants was observed in the case of the substrate with EM supplementation, while the greatest height (13.84 cm) on the EM-substrate, that is, in the combination (for both values) where seeds were not inoculated, but plant spraying was applied. Plant spraying and seed inoculation did not exert a significant effect on plant height.

Substrate treatment with EMs had a significant effect on the fresh mass of sweet basil plants. Plants growing in the substrate without EM supplementation were characterised by a significantly higher green mass than plants growing in the substrate to which EMs were added. Seed inoculation and plant spraying with EMs failed to affect the fresh mass of sweet basil plants (Table 2).

Also, synergy between the substrate, seed inoculation and plant spraying, exerted a significant effect on the examined parameter. The greatest fresh mass was found in the case of plants without EM applied into the substrate and on seeds, but when EMs was sprayed on plants. The worst growth dynamics was recorded in the case of plants growing in the EM+ substrate, irrespective of the other applications, as well as in plants growing in the EM- substrate with EM+ seeds and EM spraying.

The greatest leaf area in the pot (140.65 cm²) was recorded for plants growing in the EM- substrate from EM- seeds with EM+ spraying, while the smallest leaf area were observed in the case of basil plants growing in EM+ substrate and with EM+ spraying, with no difference regarding seed inoculation (Table 2). The addition of EMs exerted a significant effect on leaf area in sweet basil. Plants growing in EM- substrates were characterised by significantly greater leaf areas in comparison with those growing in EM+ substrates. Plant spraying and seed inoculation failed to have a significant influence on this factor.

Chlorophyll relative content

Chlorophyll relative content was found to be higher in

Table 2. Effect of EM application on the biometrics parameters of sweet basil plants.

EM-spraying	Substrate				Mean for EM-spraying
	EM+		EM-		
	Seeds				
	EM+	EM-	EM+	EM-	
	Height (cm)				
EM+	8.17 ^{c*}	6.08 ^d	11.75 ^b	13.84a	9.96a*
EM -	8.89 ^c	7.13 ^{cd}	12.00 ^b	13.28ab	10.32a
Mean for substrate	7.57 ^{b*}		12.71 ^a		
Mean for seeds	10.20 ^{a*}		10.08 ^a		
	Fresh mass (g)				
EM+	7.50 ^{b*}	6.43 ^b	9.00 ^b	18.38 a	10.33a*
EM -	7.77 ^b	5.58 ^b	16.82 ^a	16.25 a	11.60a
Mean for substrate	6.82 ^{b*}		15.11 ^a		
Mean for seeds	10.27 ^{a*}		11.66 ^a		
	Leaf area (cm² pot⁻¹)				
EM+	56.07 ^{c*}	56.71 ^c	95.22 ^b	140.65 a	87.16a*
EM -	66.58 ^{bc}	80.96 ^{bc}	96.11 ^b	67.35bc	77.75a
Mean for substrate	65.08 ^{b*}		99.83 ^a		
Mean for seeds	78.49 ^{a*}		86.41 ^a		
	Chlorophyll content (SPAD)				
EM+	23.41 ^{c*}	19.93 ^d	25.70 ^b	26.30 b	23.84b*
EM -	26.87 ^b	21.72 ^{cd}	27.15 ^b	29.30 a	26.27a
Mean for substrate	22.98 ^{b*}		27.12 ^a		
Mean for seeds	25.78 ^{a*}		24.32 ^b		

*Values marked with the same letter do not differ statistically at p=0.05, separately for each of means.

sweet basil plants growing in EM- substrate from EM+ seeds and at EM- spraying (Table 2). The highest relative chlorophyll content (29.30) was recorded in the combination in which no EM form was applied, while the smallest content of this pigment was found in plants growing in the EM+ substrate from EM- seeds and at EM+ spraying.

The growth dynamics of sweet basil plants was affected most strongly when EMs was added to the substrate. Plants growing in the substrate supplemented with EMs were characterised by strong growth inhibition. They were characterised by smaller height, fresh mass, as well as smaller leaf area in comparison with plants growing in the substrate without any addition of effective microorganisms.

Nutrient content in aboveground plant parts

Nitrogen

A significant and stimulating effect was determined in case of the uptake of nitrogen by the aboveground plant

parts (Table 3) as a result of the application of effective micro-organisms in the form of spraying and as an inoculant added to the substrate. The best nitrogen nutrition (2.70% N) was found in plants growing in the EM+ substrate from EM- sown seeds and without EM spraying, while the worst (1.33% N) – in plants without EM application by any of the examined treatment methods. Mean nitrogen contents in the case of plants sprayed with the EM solution were determined at 2.48% N in comparison with 2.13% N (EM -) and when the inoculant was applied into the substrate at 2.45 and 2.15% N, respectively. Seed inoculation with effective microorganisms did not modify significantly plant nutrition with nitrogen.

Phosphorus

The application of effective microorganisms, both in the form of a spray or as a seed inoculant, significantly reduced phosphorus uptake by plants (the decrease in relation to EM- plants amounted to 27.1 and 28.6%, respectively). The highest phosphorus content from

Table 3. Effect of EM application on macroelements content in sweet basil herbage.

EM-spraying	Substrate				Mean for EM-spraying
	EM+		EM-		
	Seeds				
	EM+	EM-	EM+	EM-	
Nitrogen (% N in DM)					
EM+	2.28 ^{a*}	2.59 ^a	2.52 ^a	2.52 ^a	2.48 ^{a*}
EM -	2.24 ^a	2.70 ^a	2.24 ^a	1.33 ^b	2.13 ^b
Mean for substrate	2.45 ^{a*}		2.15 ^a		
Mean for seeds	2.32 ^{a*}		2.28 ^a		
Phosphorus (% P in DM)					
EM+	0.30 ^{c*}	0.40 ^b	0.38 ^b	0.26 ^c	0.35 ^{b*}
EM -	0.31 ^c	0.66 ^a	0.41 ^b	0.54 ^a	0.48 ^a
Mean for substrate	0.44 ^{a*}		0.40 ^a		
Mean for seeds	0.35 ^{b*}		0.49 ^a		
Potassium (% K in DM)					
EM+	3.28 ^{c*}	6.71 ^a	4.79 ^b	3.30 ^c	4.52 ^{a*}
EM -	3.23 ^c	6.73 ^a	3.96 ^c	4.44 ^b	4.59 ^a
Mean for substrate	4.99 ^{a*}		4.12 ^b		
Mean for seeds	3.81 ^{b*}		5.29 ^a		
Calcium (% Ca in DM)					
EM+	1.21 ^{c*}	2.08 ^b	2.09 ^b	1.19 ^c	1.64 ^{b*}
EM -	1.37 ^c	2.26 ^b	2.00 ^b	3.97 ^a	2.40 ^a
Mean for substrate	1.73 ^{b*}		2.31 ^a		
Mean for seeds	1.67 ^{b*}		2.38 ^a		
Magnesium (% Mg in DM)					
EM+	2.49 ^{c*}	3.60 ^b	4.32 ^a	2.13 ^c	3.13 ^{b*}
EM -	2.90 ^c	3.52 ^b	3.91 ^a	3.77 ^a	3.52 ^a
Mean for substrate	3.13 ^{b*}		3.53 ^a		
Mean for seeds	3.40 ^{a*}		3.26 ^a		

*Values marked with the same letter do not differ statistically at p=0.05, separately for each of means.

among the examined combinations (0.66% P) was determined in plants growing on the EM+ substrate (without any other additional application), while the lowest P content (0.26%) was found in sweet basil plants, in which EM spraying was applied (without EM application by other methods) (Table 3).

Potassium

Effective microorganisms were found to affect the status of plant potassium nutrition depending on the method of their application (Table 3). The application of the microbiological inoculum to inoculate seeds significantly reduced potassium uptake by 28%, whereas when it was used to inoculate the substrate, it improved plant nutrition

with this element by 21.1%. Spraying plants with EMs did not affect significantly the content of potassium in plants. The highest content of this element (6.73% K) from among all the experimental combinations was determined in plants grown in the EM+ substrate (without any other EM application), while the lowest was in plants cultivated in the EM+ substrate from EM+ seeds (but without EM spraying).

Calcium

Irrespective of the application method, a general trend was found, indicating a significant deterioration in the calcium nutrition status of sweet basil following treatment with effective microorganisms: in the case of combination

I by 25.1%, II by 29.8% and III by 31.7%, respectively. The highest calcium content from among all the examined combinations was determined in plants growing in EM- substrate and in the absence of seed inoculation and plant spraying with EMs (3.97% Ca), while the lowest (1.19% Ca) – when EM- seeds were sown into EM- substrate and at plant spraying with EMs (Table 3).

Magnesium

The application of EMs in the form of plant spraying and substrate inoculation was found to exert a significantly deteriorating effect on the state of plant nutrition with magnesium by 11.1 and 11.3%, respectively (Table 3). Seed treatment with EMs did not affect plant nutrition with this element. The highest magnesium content from among the examined combinations (4.32% Mg) was determined in plants growing from EM+ seeds in EM-substrate and sprayed with EMs, while the lowest (2.13% Mg) was in plants sown without seed inoculation with EMs into EM- substrate and a simultaneous spraying with an aqueous EM solution.

Microbiological analyses

Total bacterial counts

When analysing variations in total bacterial counts in successive experimental combinations, no marked changes were observed in their numbers potentially attributable to the application of EM inoculum, although a tendency towards a decline in total bacterial counts occurred in the combinations in which sweet basil seeds were inoculated with the experimental biopreparation (Table 4). The difference between combinations in which seeds were inoculated and those in which no such treatment was applied amounted to 36.8%.

The highest reductions in the total counts of the examined groups of microorganisms were recorded in the case of the combined application of all the three forms of plant inoculation with the EM preparation. This difference turned out to be highly significant and constituted nearly 67% of the value determined in the control.

Actinomycetes

The performed analyses of actinomycete counts in the course of experiments revealed their considerable variability (Table 4). On the last date of analyses, their numbers increased following the application of EMs both into the substrate and in the form of plant spray. The determined number of actinomycetes in this experimental combination was characterised by a highly significant

difference in comparison with the control.

Fungi

The performed analyses of fungi numbers in the majority of the applied variants of EM inoculum applications showed that they reduced fungi numbers in comparison with the control (Table 4). A trend was noticed that together with an intensified application of the EM biopreparation, numbers of fungi in the substrate declined. Counts of the determined fungi within a given combination were characterised by considerable variability on consecutive dates of sample collection.

Copiotrophs

The obtained results regarding numbers of copiotrophs subjected to statistical analysis revealed highly significant differences in the counts of these microorganisms in the combinations with experimental EM applications in comparison with the control (Table 4). The difference between the control and the combination, in which all EM application variants were used, amounted to 72.8%.

Oligotrophs

When comparing changes in oligotroph counts in consecutive dates of analyses, a distinct reduction in their numbers was observed when the EM preparation was applied (Table 4). The mean differences in their numbers from all the dates of analyses between the control and the combinations, in which EMs were applied, amounted to 36.55%. The most significant difference between the control and the combination with EMs was observed on date IV. The inhibiting effect of the EM biopreparation on oligotroph development was confirmed by the significant drop (up to 135.4%) in their numbers in the combination, in which all methods of EM application were used (substrate and seed inoculation, plant spray).

DISCUSSION

Plant growth

A majority of articles concerning the effect of EMs on plant yields describe their action as very small and frequently, as in our experiments, as negative (Bajwa 2005; Javaid 2006; Okorski et al., 2008). Some investigations failed to determine any influence of EMs on crop plant yields (Priyadi et al., 2005; van Vliet et al., 2006). According to Mayer et al. (2010), the observed checking of plant growth following EM application might also have been caused by an increased competition for

Table 4. Effect of EM application on in the rhizosphere of sweet basil (cfu 10⁵ 1 g⁻¹ DM of substrate).

Parameter	Substrate		EM+				EM-			
	Seeds		EM+		EM-		EM+		EM-	
	EM-spraying		EM+	EM-	EM+	EM-	EM+	EM-	EM+	EM-
Total bacterial counts	Date	I				54.1				48.0
		II		42.8		38.5		41.0		50.0
		III	26.7**	41.3**	72.5	52.0**	48.6	63.7	89.3	80.2
		IV	84.0	27.7**	74.8*	53.4**	37.8**	49.9**	78.3	103.2
Numbers of actinomycetes	Date	I				56.2				22.5
		II		23.1**		74.5*		64.9		73.7
		III	51.9**	77.4*	56.6**	67.2**	52.5**	81.3**	119.6	110.7
		IV	155.0**	99.2	210.9**	51.0	44.4	52.7	39.3*	70.7
Numbers of fungi	Date	I				12.5				10.2
		II		4.2**		12.0		10.5		17.3
		III	5.3	6.8	7.6	18.1	20.7**	9.8	10.0	8.6
		IV	15.7**	17.1**	14.8**	10.9**	6.6**	5.3**	9.8**	35.3
Numbers of copiotrophs	Date	I				63.9				41.7
		II		22.5**		34.5**		20.3**		68.4
		III	43.9**	42.6**	70.0**	91.2**	151.0	142.2	81.6**	130.8
		IV	53.5**	41.4**	45.0**	41.8**	35.9**	19.8**	70.6**	154.0
Numbers of oligotrophs	Date	I				131.3				107.7
		II		90.0**		273.4		199.5*		222.0
		III	182.2**	197.6**	235.7**	205.0**	284.3	239.4**	374.6	389.6
		IV	135.0**	200.0**	132.0**	260.0**	153.0**	167.5**	160.0**	368.7

*Values in the same line are significantly different at $p < 0.05$; **values in the same line are highly significantly different at $p < 0.01$. I, prior to seed sowing; II, 20 days after seed sowing, prior to plant spraying with the EM preparation, III, 10 days after plant spraying with the EM preparation, IV, before harvesting mature plants.

nitrogen between bacteria and crop plants. An absence of the EM effect on crop plants was also demonstrated by Javaid et al. (2008) in his investigations, in which rye was cultivated in containers with a substrate treated with EMs. In the experiments reported by Javaid (2006), similar to our study, the addition of EMs to the substrate and EM spray application during plant vegetation resulted in a decline of pea yields. On the other hand, field experiments carried out in Asia or in a tropical climate, demonstrated a positive effect of EMs on plant yields, especially when nitrogen fertilisation was also decreased (Iwaishi, 2000; Khaliq et al., 2006). However, according to Córdor et al. (2007), scientific investigations carried out so far failed to demonstrate a significant influence of EMs on crop plant yields, with the exception of cultivations in tropical regions.

Nutrient content in aboveground plant parts

Concentrations of nitrogen determined in our experiments

Remained within the range given by Markiewicz (2000) (0.78 to 2.8% N) as well as by Jadcak et al. (2006) (2.44 to 3.07% N). Seidler-Łożykowska et al. (2006) reported that sweet basil plants in ecological cultivations contained 2.44 to 3.77% N, while in the case of conventional cultivation, it was 2.96% N. Distinctly higher nitrogen contents (5.23 to 5.42% N) were determined in experiments conducted by Dzida (2010). In the discussion, papers dealing with the effect of mycorrhiza are also presented due to the scarcity of studies concerning the effect of EMs on plant nutrition status. Our own research results are corroborated by Khaliq et al. (2006), who found a positive effect of EMs on plant nitrogen nutrition. Sahain et al. (2007) reported a positive and stimulating influence of effective microorganisms on the status of apple tree nutrition with nitrogen, magnesium and zinc. On the other hand, Golcz and Bosiacki (2008) demonstrated a significant effect of the application of a mycorrhizal inoculum on nitrogen content in leaves and stems of thyme herbs.

The application of effective microorganisms was found

to significantly decrease phosphorus uptake by plants. Khaliq et al. (2006) as well as Sahain et al. (2007) demonstrated a significant improvement of plant nutrition status with phosphorus following an EM application. Experiments earlier carried out yielded similar ranges of phosphorus content in sweet basil (Markiewicz, 2000; Seidler-Łożykowska et al., 2006, 2009). Also, Jadczak et al. (2006) in their studies on the effect of different types of covers in sweet basil cultivation, determined contents of this component fluctuating within a similar range from 0.42 to 0.43% P. The applied covers failed to significantly affect plant nutrition with phosphorus. However, phosphorus concentrations are significantly influenced by plant watering (Biesiada and Kuś, 2010). As in the case of nitrogen, phosphorus content determined by Dzida (2010) was distinctly higher in comparison with our investigations. Phosphorus contents in sweet basil ranged from 0.49 to 0.83% P (Ózcan, 2004; Ózcan et al., 2005; Ózcan and Akbulut, 2007).

We have found that effective microorganisms affect the condition of plant potassium nutrition status depending on the method of their application. Sahain et al. (2007) reported increased contents of potassium in leaves of apple trees following their treatment with EMs, while Khaliq et al. (2006) demonstrated a significant increase of potassium content in cotton leaves after the application of the microbiological inoculum. The recorded contents of potassium in sweet basil were similar to those reported by Dzida (2010). Distinctly lower contents of potassium in sweet basil (from 2.46 to 3.28% K) were quoted by Markiewicz (2000), while Seidler-Łożykowska et al. (2006, 2009) reported similar contents of this component. When studying the growth of plants under the influence of varying salinity, Zuccarini and Okurowska (2008) determined contents of this component ranging from 2.32 to 5.74% K, while Jadczak et al. (2006) gave values ranging from 3.01 to 3.74% K. Ózcan (2004) as well as Ózcan and Akbulut (2007) maintain that potassium contents range from 2.48 to 2.76% K. In turn, Biesiada and Kuś (2010) claim that potassium content in sweet basil is modified by watering and plant nutrition with nitrogen.

Golcz and Bosiacki (2008) reported a positive effect of mycorrhizal inoculum on calcium content in leaves and stems of thyme. Calcium contents similar to those determined in our experiments were reported by Dzida (2010). As in the case of potassium, the determined calcium contents in sweet basil were higher in comparison with the earlier studies by Markiewicz (2000). Calcium content in sweet basil can be affected by the type of the applied covers as well as water regime (Jadczak et al., 2006; Biesiada and Kuś, 2010). Contents of this component similar to those determined in our studies were earlier reported by Seidler-Łożykowska et al. (2006) within the range of 1.80 to 3.27% Ca in ecological cultivations and 3.41% Ca in a traditional cultivation, while in a study by Seidler-Łożykowska et al.

(2009), it was 2.26 to 3.45 and 3.09% Ca, respectively. Similar ranges of calcium content (2.74 to 3.36% Ca) were reported by Jadczak et al. (2006).

Similarly, as in case of calcium, Golcz and Bosiacki (2008) reported a positive effect of mycorrhiza application on magnesium content in plants. As in the case of potassium and calcium, Markiewicz (2000) determined distinctly lower magnesium content in sweet basil. Other researchers also reported lower contents of this component in sweet basil plants; Seidler-Łożykowska et al. (2006) in ecological cultivations found 0.54 to 0.97% Mg and in conventional cultivation, 0.46%, Seidler-Łożykowska et al. (2009) in ecological cultivations found 0.59 to 0.85% Mg, while in conventional cultivation, 0.49% Mg, Jadczak et al. (2006) found 0.25 to 0.28% Mg and Dzida (2010) found 0.28 to 0.32% Mg. Ózcan and Akbulut (2007) and Ózcan (2004) also gave markedly lower magnesium contents.

Microbial counts

The recorded decline in the total bacterial counts was caused by the utilisation of nutrients found in the soil by microorganisms from the inoculants. In many environments colonised by different groups of microorganisms, the feed competition phenomenon is a factor which eliminates less specialised groups of microorganisms (Kunicki-Goldfinger, 2001). The recorded reduction in total bacterial counts following seed inoculation with the EM solution could have been caused by substances produced by sprouting seeds. Initiation of sprouting liberates certain amounts of sugars and other organic compounds thanks to the association with microorganisms synthesising vitamins, auxins or substances similar to gibberellins (Myszka and Czaczyk, 2006). However, substances produced by them can exert a negative effect on microorganisms. According to Jeger (2001), growth and development of some microorganisms can be inhibited as a result of higher concentrations of auxins and gibberellins.

In general, numbers of actinomycetes in soil are usually lower than the number of bacteria. However, peat substrates used in the experiment showed a different relationship. The large size of the analyzed group of microorganisms could result from the high content of humus in the peat substrate. Humus is the main source of food for the actinomycetes. Moreover, good oxygenation and substrate moistening of buds have a stimulating effect on their development. The increase in the number of actinomycetes in combination with EM applications may provide stimulating effects of secretions produced by microorganisms contained in the biopreparation on the analyzed microbial group.

The observed antagonistic effect of the employed EM inoculum against fungi from the substrate appears to be an advantageous phenomenon for sanitary reasons.

According to Wielgosz and Szember (2006), the situation in which we observe a strong development of fungi is highly disadvantageous. These microorganisms are capable of producing many toxin-forming and phytopathogenic compounds, which could exert a negative influence not only on the population sizes of other microbial groups, but also on plant development.

The recorded increase in numbers of copiotrophs following the application of the EM inoculum to the substrate on the first date of analyses could probably be attributed to a simultaneous supply of nutrients in the form of molasses, which was contained in the EM solution. During the consecutive dates of the experiment, numbers of microorganisms in different combinations of EM inoculation decreased in comparison with the untreated control. Copiotrophic bacteria develop very intensively after a direct introduction of C sources into the soil. In such situations they are characterised by high physiological activity, especially when there are also favourable conditions for development (pH, temperature). However, when the easily available nutrients are used up, they pass into dormancy and their numbers decline as a result of autolysis (Szostak-Kotowa, 2000).

Oligotrophs comprise a group of microorganisms characterized by low nutritional requirements. They utilize organic matter, which is permanently located in the substrate and their activity remains low (Badura, 2004; Szostak-Kotowa, 2000). The initial stimulation of oligotroph development may have been caused by the content of available organic matter.

Comparing changes in oligotroph numbers in the course of the entire experiment, it may be presumed that the introduction of additional microbial groups, contained in the EM inoculum, into the soil, led to competition for nutrients and reduced numbers of the microorganisms (Kaczmarek et al., 2008).

Conclusion

Intensive proliferation of microorganisms in the substrate following the application of EM at the initial phase of growth resulted in an increased competition for N with plants and a related inhibited plant growth. At a later vegetation period, proliferation of microorganisms was strongly inhibited as a consequence of a rapid depletion of nutrients in comparison to the combination without EMs. This was connected with the fact that the cultivation period in spice plants is short and top dressing is not used. It results from other studies conducted by the authors on tomato, that is, a plant with a longer vegetation period (unpublished data), that after an initial plant growth inhibition, a later soil fertilization treatment caused an intensive growth of plants in the substrate with an addition of EMs. It may be stated that it is not justified to use EMs in the cultivation of crops with a short vegetation period, where substrates rich in humus and

macroelements are used.

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