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The Effects of Various Doses and Types of Effective Microorganism Applications on Microbial and Enzyme Activity of Medium and the Photosynthetic Activity of Scarlet Sage

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Abstract: The aim of this study was to examine the influence of various types and levels of effective microorganism (EM) applications on scarlet sage. For this purpose, EMs were applied at the following three concentrations: 1:10, 1:50, and 1:100. Moreover, two types of treatments (spraying and watering) and a combination of the two were also examined. Photosynthetic intensity was analyzed, including the net photosynthesis rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). Additionally, chlorophyll *a*, *b*, and *a + b*, and the chlorophyll *b/a* ratio were analyzed. The microbial content in the medium and soil enzyme activity were also evaluated to examine the effect of EMs on soil biological properties. The investigations revealed a high positive effect of EMs on the photosynthetic activity of most EM combinations compared with the control. The greatest positive effect was noted for the highest EM concentration application for both types of treatments. There was no such influence on soil activity. An increase was noted only in the number of fungi and dehydrogenase activity, while the rest of the soil biological status parameters revealed significant variability, and mostly small or no effects were recorded.

Keywords: EM; photosynthesis; stomatal conductance; chlorophyll; enzyme activity

1. Introduction

Plant growth and crop yield are dependent on the availability of fertilizer, and thousands of mineral and organic fertilizers are applied to horticultural plant species [1]. However, the availability and uptake of fertilizers by plants are dependent on several factors, both abiotic and biotic. In addition to the economic aspects of proper plant nutrition and growth, there is another aspect, i.e., the potential negative effect on the environment of excess levels of fertilization [2,3]. Hence, recently, biofertilizers have been developed as a supplement or replacement for mineral and natural plant nutrition [4,5] and positive effects of biofertilizers on plant growth have also been reported [6]. Moreover, it has been reported that biofertilizers can enable the conversion of important elements from an unavailable form to an available form through biological processes. Organic compounds are an important source of nutrients, while microorganisms are important to promote the circulation of plant nutrients and to decrease chemical fertilization or improve their

uptake. Plant growth-promoting bacteria (PGPB) were found to significantly improve the impact of nutrients on plant growth (of both above- and belowground plant parts), the physiology and development of plants, and the uptake of nutrients [7,8]. Many species of microorganisms (such as *Bacillus* sp. and *Pseudomonas* sp.) have been found to have direct effects on plant growth [9,10]. These bacteria enable plants to withstand stressful conditions to promote plant growth by improving plant nutrition (N, P, and Fe) and to release various stress-related metabolites, i.e., phytohormone production, phosphate solubilization, and siderophore production [11,12].

The concept of effective microorganisms (EMs) was introduced and developed by Professor Teruo Higa from Japan [13]. A mixture of naturally occurring microorganisms applied as an inoculant was found to improve soil quality, soil health, as well as the growth, yield, and quality of crops [14].

Microorganisms in the soil play important roles as they break down insoluble nutrients [15] and produce enzymes [16]. These enzymes are produced inside and transported outside the cell and catalyze reactions to break down the structure of the nutrient source, making it more accessible. The amount of extracellular enzymes in soil depends on the metabolic abilities of the soil organisms, the number of organisms present, the presence of substrate, and the soil environment [17,18]. The production of enzymes is very energy-consuming, hence, microorganisms produce only as much as is necessary. One of the common extracellular enzymes is phosphatase (EC 3.1.3.2), which is produced to remove the phosphate molecule from organic compounds, such as phospholipids and nucleic acids [19]. Phosphorus is one of the most important limiting factors for plant growth, and phosphatase activity is a good indicator of organic phosphorus mineralization potential and biological activity of soils [20]. Phosphatase activity is related to soil and vegetation conditions [21]. Richardson and Simpson [22] noted that increased activity of phosphatases in soil results from a deficit of phosphorus available to plants and is part of a plant's response to phosphorus starvation.

Other important enzymes in the soil environment are dehydrogenases (EC 1.1.1), which are also treated as an indicator of overall soil microbial activity [23–25] because they occur intracellularly in all living microbial cells [26–28]. Moreover, they are tightly linked with microbial oxidoreduction processes [26]. Importantly, dehydrogenases do not accumulate extracellularly in the soil. Docosahexaenoic acid (DHA) serves as an indicator of the microbiological redox systems and can be considered to be a good and adequate measure of microbial oxidative activities in soil [29]. Urease enzyme (urea amidohydrolase, EC 3.5.1.5) is closely associated with the transformation, biological turnover, and bioavailability of nitrogen [30,31] and is also an important enzyme that can indicate the soil biological status.

Plant nutrition strongly affects photosynthesis. Increased availability increases the uptake of nutrients, and therefore, positively influences photosynthesis. It has also been found that foliar application of EMs significantly increases plant photosynthetic activity [32]. Soil microorganisms promote nutrient uptake and transport in plants [33]. The effectiveness of microbes can be improved by combining cultures of various specific antagonists to pathogens occurring in the soil [34]. Effective microorganisms consist of a combination of several microorganism species that belong to the following five groups: Lactic acid bacteria, photosynthetic bacteria, actinobacteria, yeast fungi, and filamentous fungi [35]. It has also been proven that a properly composed biofertilizer and chemical agent against pathogens can improve the effectiveness of nutrient uptake without a negative effect of chemicals on soil activity, and consequently, can increase yield [36,37]. EMs have also been shown to maintain optimal leaf photosynthesis efficiency of bean plants in sandy soils, as compared with a control [38]. Although EMs have been investigated in many studies [39], there is still a lack of detailed investigations on their effect on plant response, in particular, the relationship between soil microbial activity and plant physiological status. This is especially valid for ornamental plants, which usually are omitted in such investigations due to their poor role in the agricultural economy. However, the investigations in this

study potentially offer a new light from an ecological point of view, as EMs are treated as a partial replacement of mineral fertilization.

The aim of the present study was to determine the influence of the type and amount of EM application on soil microbial activity and whether it had a positive effect on plant physiological response. The detailed aims were as follows: (i) To examine the effect of EM application type and amount on photosynthesis activity and chlorophyll content, (ii) to determine the effect on soil microorganisms and soil enzyme activity, and (iii) to identify relationships among the abovementioned parameters.

2. Materials and Methods

2.1. Experimental Design

Seedlings (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry BBCH scale 16–17) of scarlet sage (*Salvia splendens*) cv. "Saluti Red" were purchased from Vitroflora nurseries (Dobrcz, Poland). This cultivar is one of the best known scarlet sage and is resistant to unfavorable environmental conditions, and widely used in urban areas in flower beds, containers, and green areas. The experiment was conducted under the natural light of glasshouse conditions. The average values of air temperature and relative humidity during the growth period were 20.3 °C and 67.4%, respectively. The plants were cultivated in pots. The material used in the experiments was a peat substrate of 5.5–6.0 pH, which was supplemented with a multicomponent slow-release fertilizer (Osmocote 5–6 M, Heerlen, The Netherlands) in the amount of 3 g·dm⁻³. One seedling of scarlet sage cv. "Saluti Red" was planted in each 12 cm pot containing the abovementioned substrate.

The plants were inoculated with different doses of the EM biopreparation which was applied either onto the leaves or into the peat. The microbiological inoculum used in the study came from Greenland Technologia EM (Trzcianki, Poland). The preparation was diluted in tap water to obtain the following concentrations: 1:10, 1:50, and 1:100. Investigations included the effects of several types of applications together with various EM solutions (Table 1). The abovementioned experimental inoculum was applied in two ways, i.e., onto leaves and into the soil, but always in the amount of 10 mL. Twenty replications of each combination were carried out.

Table 1. Types of effective microorganism (EM) applications (combinations).

Name of Combination	Types of Application
0	Control–peat substrate + plant
1	Peat substrate + plants + watering with EM at concentration 1:10
2	Peat substrate + plants + watering with EM at concentration 1:50
3	Peat substrate + plants + watering with EM at concentration 1:100
4	Peat substrate + plants + spraying with EM at concentration 1:10
5	Peat substrate + plants + spraying with EM at concentration 1:50
6	Peat substrate + plants + spraying with EM at concentration 1:100
7	Peat substrate + plants + watering and spraying with EM at concentration 1:10
8	Peat substrate + plants + watering and spraying with EM at concentration 1:50
9	Peat substrate + plants + watering and spraying with EM at concentration 1:100

The adopted research methodological assumptions used the current developmental phase of scarlet sage as the main factor for determining the moment of collection of substrate samples: Date I, seedling phase (beginning of the experiment, phase of development leaves BBCH scale 17–18); date II, phase of vegetative growth (after 33 days, BBCH scale 33–35); and date III, phase of plant flowering (after 60 days, BBCH scale 63–65).

2.2. Gas Exchange Parameters, Morphological Parameters, and Chlorophyll Contents of Plants

The handheld photosynthesis system CI-340aa (CID BIOSCIENCE Inc., Camas, WA, USA) was used to evaluate the net photosynthesis rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO₂ concentration (C_i). The following constant conditions of measurements in the leaf chamber were maintained: CO₂ inflow concentration (410 μmol (CO₂) mol⁻¹), photosynthetic photon flux density (PPFD) 1000 μmol

(photon) $\text{m}^{-2} \text{s}^{-1}$, chamber temperature $25\text{ }^{\circ}\text{C}$, and relative humidity $40 \pm 3\%$. Mature leaves without visible symptoms of mechanic injuries were selected.

Chlorophyll content was also analyzed. Investigations of the content of chlorophyll $a + b$, as well as a and b in fresh matter, were carried out using the method developed by Shoaf and Lium [40] as well as Hiscox and Israelstam [41]. Extracts of 5 mL of DMSO per 200 mg of leaf tissue, after 20 min of incubation, were measured by colorimetric method at wavelengths 645, 652, and 663 nm (spectrophotometer Hach-Lange DR 2800, Hach, Colorado, USA). The following equations were used to determine chlorophyll contents (in mg per g of fresh matter): $(12.7 D_{663} - D_{645}) \cdot (V \cdot 1000^{-1} \cdot W^{-1})$ for chlorophyll a or $(22.9 D_{645} - D_{663}) \cdot (V \cdot 1000^{-1} \cdot W^{-1})$ for chlorophyll b , and $27.8 D_{652} \cdot (V \cdot 1000^{-1} \cdot W^{-1})$ for chlorophyll $a + b$, where D is absorbance for wavelength; V , total extract volume (cm^3), and W , weight of sample (g). The chlorophyll b/a ratio was also calculated and presented. Three replicates for photosynthesis activity and chlorophyll content were undertaken.

2.3. Microorganisms Number and Enzyme Activity

The samples of peat for each pot (15 pots within a given combination) were collected from four locations using a soil micro sampler.

The count of selected groups of soil microorganisms (total bacterial count, molds, actinobacteria) was measured with the serial dilution method developed by Koch [42]. The measurements were repeated five times. The results were calculated per 1 g of dry mass of soil and expressed as colony-forming units (cfu). The groups of microorganisms were measured on selective mediums, using adequately diluted soil suspensions. The count of individual groups of microorganisms was measured in the following way: Total bacterial count on a ready Merck standard agar after 3 days of incubation at $25\text{ }^{\circ}\text{C}$; molds on a medium developed by Martin [43] after 5 days of incubation at $24\text{ }^{\circ}\text{C}$; actinobacteria on a medium developed by Pochon after 5 days of culturing at $25\text{ }^{\circ}\text{C}$ [44].

In addition, using the spectrophotometric method, dehydrogenase activity was determined in the collected samples of composted material using 1% TTC (triphenyltetrazolium chloride), and then incubation for 24 h at $30\text{ }^{\circ}\text{C}$, with a 485 nm wavelength. The enzyme activity was expressed in $\mu\text{mol TPF} \cdot \text{g}^{-1} \text{ substrate DM} \cdot 24 \text{ h}^{-1}$ [45]. The activity of acid phosphatase was determined using p-nitrophenylphosphate sodium as substrate, after 1 h incubation at $37\text{ }^{\circ}\text{C}$, with wavelength 400 nm. Enzyme activity was expressed in $\mu\text{mol PNP} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ [46]. Urease activity was determined using urea as the substrate, after 1 h incubation at $37\text{ }^{\circ}\text{C}$ and 410 nm wavelength. Enzyme activity was expressed in $\mu\text{g N-NH}_4 \cdot \text{g}^{-1} \cdot 18 \text{ h}^{-1}$ [47].

Enzymatic activity investigations reflected substrate microbiological activity set against the background of the traditional method of determination of total counts of microorganisms using Koch's plate method.

2.4. Statistical Analysis

The results were analyzed by factorial ANOVA with "type of application" and "term of measurement" as fixed factors. Tukey's test was employed to analyze differences between measured parameters. A graphical presentation of Tukey's test results was provided. To determine the structure and relationship among variables, principal component analysis (PCA) was used. In this analysis, the orthogonal transformation of analyzed variables and combinations to a new set of non-correlated variables (components) was performed. The above data analyses were performed with the statistical software STATISTICA 13.1 (Statsoft Polska, Kraków, Poland).

A cluster analysis was performed in order to arrange analyzed parameters in groups, with the highest degree of association within each group and the lowest degree of association between different groups. Euclidean distance measures and Ward hierarchical clustering were used to determine the dendrogram. The Euclidean distance measure can designate a similar structure in interactions between analyzed parameters. These analy-

ses were performed using in accordance with the procedure implemented in the R 3.6.1 environment (R Development Core Team 2019).

3. Results

One-way analysis of variance revealed that the type of EM application had a highly significant effect ($\alpha \leq 0.001$) on all photosynthetic activity parameters (Table 2). An application of EM caused an increase in net photosynthesis rate in almost all examined combinations, excluding combinations 1 and 2, where a similar level or decrease was noted. The highest level of P_N was found in plants treated with the highest solution of EM and both types of application (combination No. 9)(Figure 1a). Higher levels were also recorded in plants in combinations No. 4, 6, and 8. Stomatal conductance (g_s) revealed trends similar to P_N (Figure 1a,b). Intercellular CO_2 concentration was the lowest in plants under combination No. 9, while it was the highest in the control and the first two treatments (Figure 1c). The highest transpiration rate (E) was recorded in plants under the last application. Combinations No. 2 and 3 revealed lower levels as compared with the control, while the other combinations had statistically significantly higher values (Figure 1d).

Table 2. One-way ANOVA of photosynthetic parameters and chlorophyll contents.

Parameter	P_N	g_s	C_i	E	Chl. <i>a</i>	Chl. <i>b</i>	Chl. <i>a + b</i>	Chl. <i>b/a</i> Ratio
F statistics	139.3	176.7	776.1	12.7	12.7	22.8	22.2	17.15
Significance	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001				

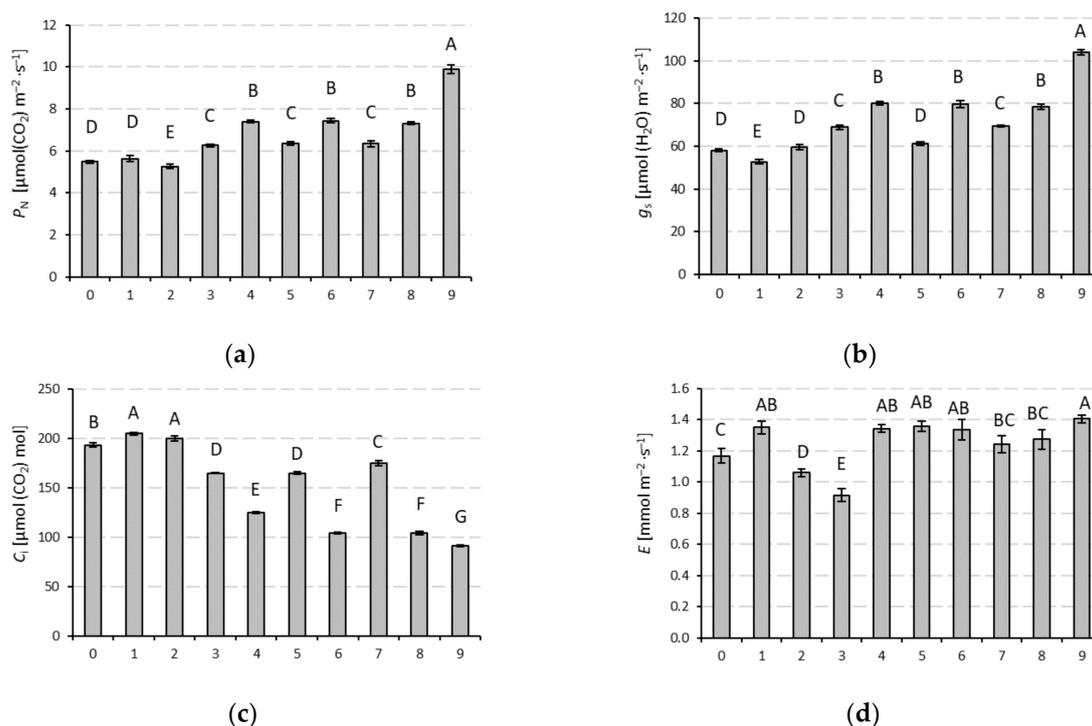


Figure 1. Means \pm SE of scarlet sage under various EM treatments. (a) Net photosynthesis rate, P_N ; (b) Stomatal conductance, g_s ; (c) Intercellular CO_2 concentration, C_i ; (d) Transpiration rate electron E . Letters denote significant differences between means at $p = 0.05$.

Chlorophyll contents also varied between types of applications (Table 2). Notably, the highest level of all chlorophyll forms was observed for the last type of application. This was especially true for chlorophyll *b* and *a + b*, while chlorophyll *a* did not reveal such large differences (Figure 2a). The highest value for the chlorophyll *b/a* ratio was also observed

for the last type of application, which confirmed the high sensitivity of chlorophyll *b* to EM application. A higher level of this parameter was also observed for combination No. 8 (Figure 2b).

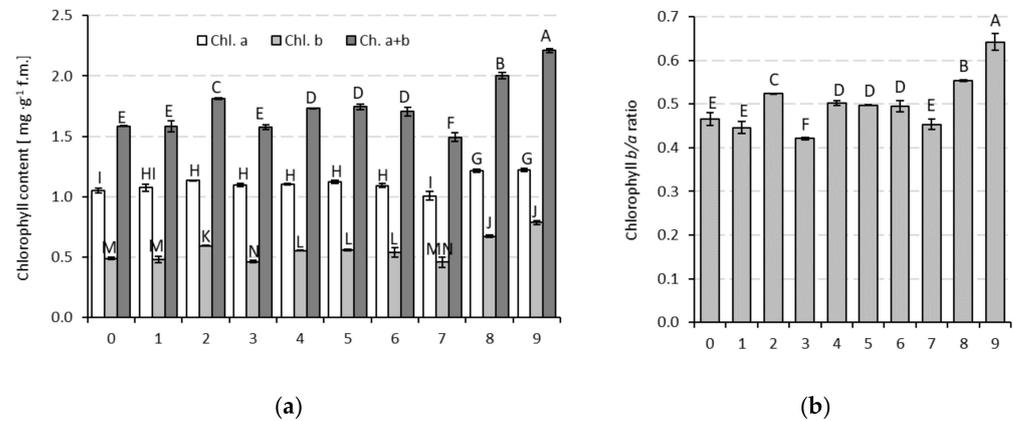


Figure 2. (a) Means \pm SE chlorophyll *a*, *b*, and *a* + *b* contents in fresh weight, (b) Chlorophyll *b/a* ratio. Letters denote significant differences between means at $p = 0.05$.

Two-way analysis of variance, with the influencing factors, i.e., term of measurement and type of EM application, revealed a significant effect on the term of measurement for all microorganisms and enzyme activity, while type of application was not a significant factor for molds (Table 3). It was possible to note variability between terms and combinations, although trends were different as compared with photosynthetic activity parameters and chlorophyll contents. The highest number of molds was recorded in the first term of measurement for all the combinations, while in the second and third terms of measurements, the number of fungi was lower and did not differ between types of application. Moreover, in the first series, a significantly higher level than the control was noted only for combination No. 4. Combination No. 9 revealed an increase, which was not significant (Figure 3a). The number of bacteria revealed significant variability between terms and combinations. The highest levels were recorded in combinations No. 1 and 2 for the first term, as well as for combinations No. 7 and 8. An increase in the number of bacteria as compared with the control in the second and third terms was noted for combination No. 2, while the other combinations revealed similar or lower levels (Figure 3b). The number of actinobacteria also varied between terms and combinations. However, higher levels in most of the combinations were found for the third series. Moreover, the highest value was recorded for combination No 9. In the first and second series, all combinations revealed a lower number of actinobacteria as compared with the control (Figure 3c).

Table 3. Two-way ANOVA of microorganism number and enzyme activities with term measurement and type of EM application fixed factors.

Parameter	Bacteria	Fungi	Actinobacteria	Dehydrogenases	Phosphatases	Ureases
Term	5.3 ^B	377.3 ^A	40.6 ^A	191.3 ^A	646.6 ^A	15.0 ^A
Application	3.6 ^B	1.2 ^{ns}	9.2 ^A	1.7 ^{ns}	24.6 ^A	3.0 ^B
Interaction	2.2 ^C	1.4 ^{ns}	5.5 ^A	2.9 ^C	6.1 ^A	3.4 ^A

^A $p < 0.001$; ^B $p < 0.01$; ^C $p < 0.05$; ^{ns} not significant.

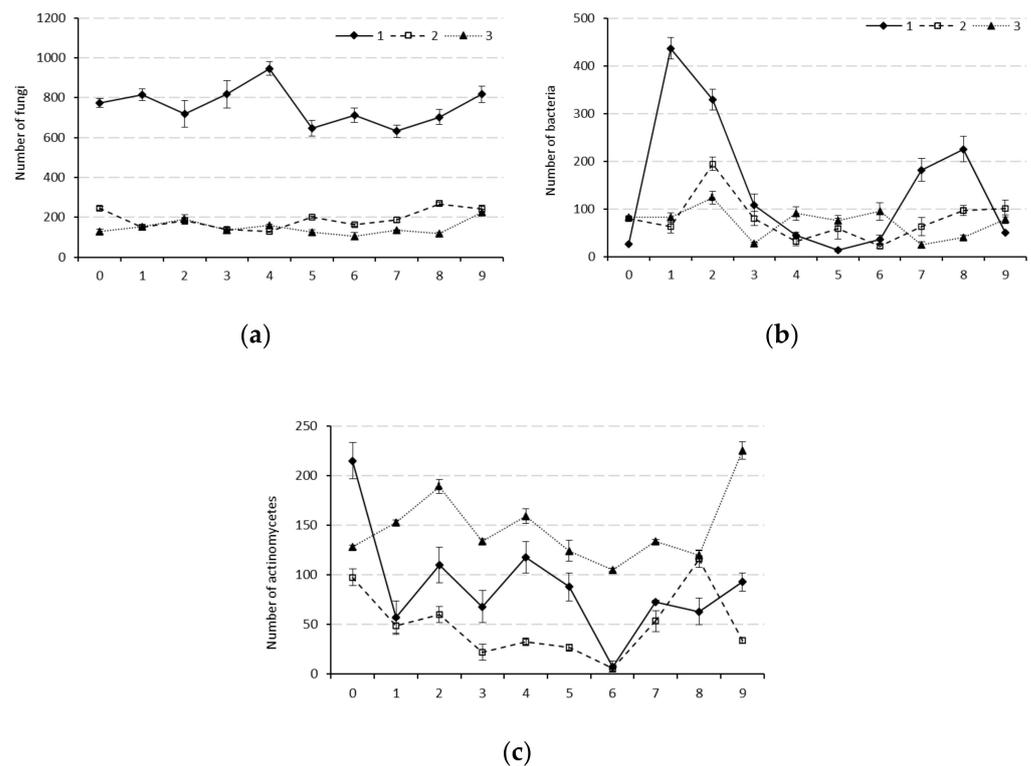


Figure 3. Changes in medium during the growing season. (a) Number of fungi, (b) number of bacteria, (c) number of actinobacteria.

Enzyme activity was found for the last term of measurements in all combinations. Moreover, the highest level was recorded for combination No. 9. In the first series, all combinations revealed lower levels of dehydrogenase activity as compared with the control. Generally, the lowest levels of activity of this enzyme were found in the second series in all combinations, including the control (Figure 4a). Phosphatase activity was the highest in the first series. However, all the combinations revealed lower or similar levels when compared with the control. This was valid for the first and second terms of measurement. Moreover, a decrease in phosphatase activity during the experimental period was found for all combinations (Figure 4b). Urease activity revealed different patterns of changes among terms and combinations. Generally, in most of the combinations, a lower or similar level to the control was observed. In most cases, a higher level of urease activity was recorded in the first term, as compared with the first and second terms (Figure 4c).

The graphic data from principal component analysis for photosynthetic parameters and microbiological soil activity explain a significant part of the data variability, i.e., over 70% of the variability. A strong positive correlation can be observed for parameters, such as P_N , g_s , Chl., fungi, and DHA, and another positively related group is PAC, UR, C_i , bacteria, and actinobacteria. By contrast, negative relationships were noted for W to UR and C_i , as well as P_N and g_s to C_i . Considering combinations, a strong positive relationship was observed for combination No. 9 to P_N , g_s , Chl., E , fungi, and DHA, as well as combination No. 0 to PAC, actinobacteria, bacteria, UR, and CI (Figure 5).

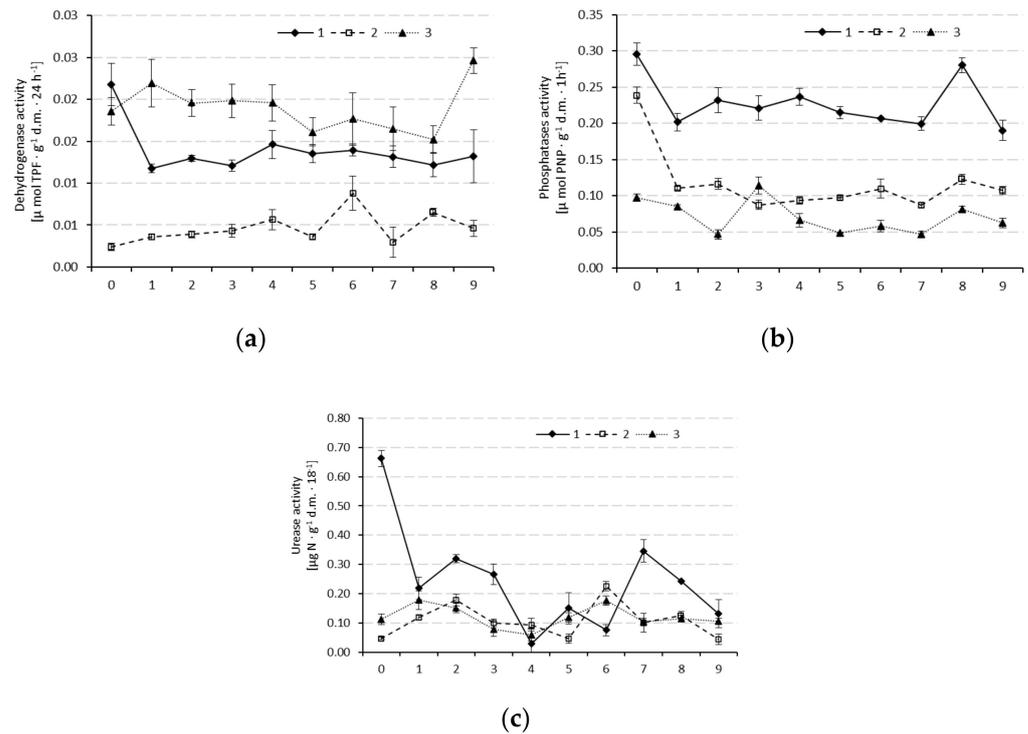


Figure 4. Changes in activities in the medium during the growing season. (a) Dehydrogenases, (b) phosphatases, (c) ureases.

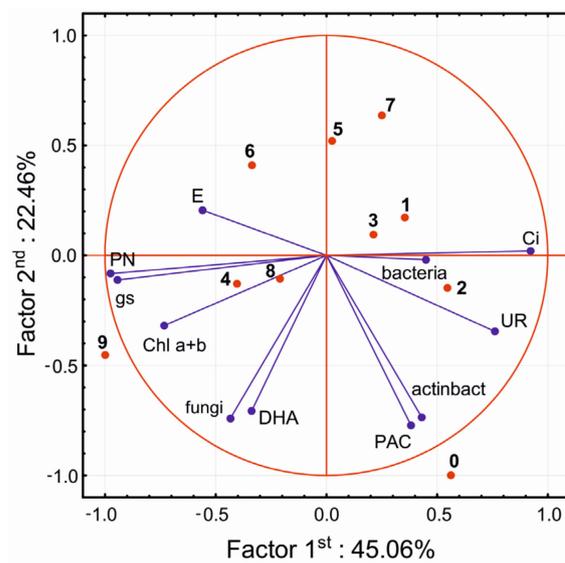


Figure 5. Principal component analysis and cluster analysis of obtained results. Numbers indicate certain combinations of EM application. UR, urease; DHA, dehydrogenase; PAC, phosphatase; PN, net photosynthesis rate; Ci, internal CO₂ concentration; GS, stomatal conductance; E, transpiration rate; actinobact., actinobacteria.

The cluster analysis of photosynthesis and microbiological parameters revealed a division into three groups with a high level of similarities. The first group includes C_i and bacteria. The second group includes the following: P_N, DHA, UR, PAC, E, Chl., g_s, and actinobacteria. Fungi were the most different of these two groups (Figure 6).

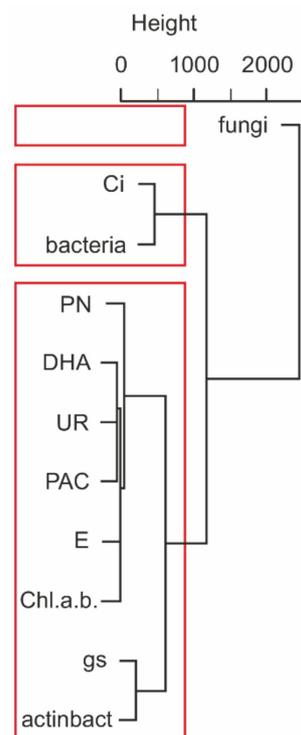


Figure 6. Cluster analysis of photosynthetic parameters and microbial activity. UR, urease; DHA, dehydrogenase; PAC, phosphatase; PN, net photosynthesis rate; CI, internal CO₂ concentration; GS, stomatal conductance; E, transpiration rate; actinobact., actinobacteria.

4. Discussion

Several types of EM applications are found in the literature, such as spraying, irrigation, the EM inoculation of soil, and seed soaking. Most of these investigations were conducted on vegetable and agricultural crops and most of them revealed positive effects on soil microbial activity and plant response [39]. Nevertheless, there is still a low number of investigations on ornamental plants, especially their responses to different types of applications in relation to photosynthetic activity responses. According to our microbiological and biochemical analyses, it can be stated that one of the most important factors significantly influencing changes of quantity and activity of microorganisms in peat substrate under scarlet sage cultivation was the development stage of plants. This was probably related to changes in the quantity and quality composition of root exudates. According to Bais et al. [48] and Biedrzycki et al. [49], the release of exudates by scarlet sage roots includes organic compounds with higher levels of carbon, which is crucial for stimulating the activity of soil microorganisms. Moreover, EM application also caused significant changes in microorganism quantity and analyzed enzymes' activity, which was especially valid for the first term of analyses.

The low number of actinobacteria and molds and low enzyme activity of EM inoculated medium was probably related to possible antagonistic relationships among autochthonous microflora of the medium and microorganisms included in the EM preparation. It has been determined that the EM consortium, especially lactic acid bacteria (LAB), indicates a high capacity for acidification of the environment to unfavorable levels for several microorganisms [50]. Wolna-Maruwka et al. [51] revealed that in-soil application of EM inoculum can decrease soil bacteria development.

Kucharski and Jastrzębska [52], on the one hand, confirmed the lack of effect of an EM preparation on the number and activity of soil microorganisms. Kaczmarek et al. [53] and Kowalska et al. [54], on the other hand, reported a stimulatory effect of the abovementioned preparation on the microbial activity of the soil. EM has been shown to solubilize phosphate [55,56], likely through the production of organic acids. The pool of active soil

enzymes is affected by secretions from the root system and the demand of the crop for specific nutrients at a particular stage of its development (BBCH). The substances contained in the root secretions and in the dying cells of the root tissues are a rich source of nutrients and energy for various physiological groups of microorganisms.

Hupe et al. [57] showed that the development phase of a plant significantly influenced the dynamics of nutrients in its root zone and, thus, the soil enzymatic activity. Increased deposition of carbon and nitrogen in the rhizosphere has also been observed during the period from the emergence to the flowering of plants. It has been pointed out that the amount of nitrogen deposited in the rhizosphere was significantly inhibited after the flowering period, perhaps because of nitrogen displacement in the plants in order to give yield. The decrease in the amount of organic nitrogen substances in the rhizosphere in relation to carbon after the flowering of plants explains the reduced metabolism of some soil enzymes in relation to the count of selected physiological groups of microorganisms.

Our investigations revealed a positive effect of EM on chlorophyll content, which was especially valid for chlorophyll *b*. A positive effect of EM on chlorophyll content has previously been found in many other plants, such as pigweed [10], almond in drought stress conditions [58], potatoes [59], date palm [60], and *Arabidopsis* in salinity stress [61]. An increase in photosynthetic pigments can be caused by increased uptake of mineral fertilizer, including such elements as iron and magnesium, which are responsible for chlorophyll biosynthesis [62]. Moreover, the addition of effective microorganisms can affect the N₂ fixation process and the creation of growth-promoting substances and other compounds of auxin type, which can also have a positive effect on the synthesis of photosynthetic pigments [63]. It is well known that EM influences some growth substances, such as cytokinins [64], which have a positive effect on chlorophyll biosynthesis and on delay of senescence and the destruction process [65].

The positive effect on chlorophyll was also related to an increase in gas exchange parameters. The highest levels of P_N were noted for sprayed plants as well as sprayed and watered plants with a mixture solution ratio of 1:100. A positive effect of spraying plants with EM was also noted by Okorski et al. [66] in their experiment with *Pisum sativum*. The positive effect of EM was related to similar effects on chlorophyll contents, which were mainly related to improved soil properties and rhizosphere microorganisms. The latter have an influence on better availability of minerals and their uptake by plants [33]. It was found that EM has a positive effect on plant photosynthesis due to an enhancement of natural fertilizers, such as chicken and bokashi manure on tomato [67]. The increase in net photosynthesis rate can be related to better nutrient uptake, similarly as in the case of a positive effect on chlorophyll, as these parameters are strongly correlated.

While there have been promising results in many trials with EM, the efficacy of EM is yet to be determined. Many field trials where EM improved crop yield were conducted in tropical or subtropical regions, while most trials in temperate regions have not shown any benefit from EM applications [68]. Hence, there is a further need to test the possibilities of using these EM to improve plant metabolism parameters.

5. Conclusions

Faced with the problems of the unsustainable exploitation of natural resources and climate change, the use of beneficial microorganisms is an opportunity to overcome the negative impacts of agricultural production on the environment, as well as on natural resources. The results of the present study indicate that EMs could have a positive effect on soil microbial and enzyme activity, as well as on photosynthetic intensity of plants, which, in turn, could result in lower mineral fertilizer application and the promotion of sustainable agricultural production.

A positive effect of EM application on photosynthetic activity was noted in almost all EM application types. The greatest positive effect was observed for plants treated with the highest solution of EMs in both types of applications. There were no such unequivocal relationships for soil biological activity parameters. The analyzed correlations

between examined photosynthetic parameters of plants and biological parameters of soil showed that dehydrogenase enzymes and the number of fungi were positively related to photosynthetic parameters (P_N and g_s), while microbial and enzyme parameters revealed small or no associations with photosynthetic activity.

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