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Original Research

Impact of Microbiological Inoculum on Numbers and Activity of Microorganisms in Peat Substrate and on Growth and Flowering of Scarlet Sage

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Abstract

The aim of our study was to determine the dynamics of development of select groups of microorganisms and the activity of dehydrogenases in a substrate containing a microbiological inoculum (BAF_1) intended to improve scarlet sage growth and flowering. The material used in the investigations was peat substrate of 5.5-6.0 pH into which plants were planted and then inoculated with different doses of the BAF_1 biopreparation (1:10, 1:50, 1:100). Samples of the substrate were collected during the following three phases: seedling planting, vegetative growth, and flowering. The following parameters were determined: developmental dynamics of total bacteria number, actinomycetes, molds (Koch plate method), and dehydrogenases activity (spectrophotometric method). Moreover, plant morphological parameters such as plant height, shoot number and length, number of buds and inflorescences, as well as content of chlorophyll *a*+*b, a,* and *b,* and leaf greenness index (SPAD) were also measured. The application of the BAF_1 inoculant into the peat substrate contributed to increased number of heterotrophic bacteria, actinomycetes, molds, and dehydrogenases activity. The number of the studied microorganisms were stimulated most significantly by the applied foliar application of the biopreparation at 1:10 concentration, while their metabolic activity also was stimulated by the foliar application of the experimental inoculum applied at a concentration of 1:50.

The applied BAF inoculum failed to exert a significant effect on the number and greenness index of leaves or on leaf blade width and length. However, irrespective of the dose and method of application of the inoculum, it improved the degree of coloring of inflorescence buds and affected the length of inflorescences (in particular, the foliar and soil application of 1:50 concentration) and increased the chlorophyll content in plants (especially the soil application with the biopreparation at 1:50 concentration, as well as foliar application at 1:100 concentration).

Keywords: microorganisms, dehydrogenases, inoculum, peat, scarlet sage

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Introduction

Discoveries in the field of chemistry made at the end of the 19th and beginning of the 20th centuries caused chemical compounds used in plant protection as well as mineral fertilizers to be commonly applied in agriculture and horticulture. The desire to increase profits and multiply yields led to huge abuses in the application of chemical preparations. Many of these compounds do not undergo biodegradation easily and thus accumulate in the substrate [1]. Obviously, this cannot remain without influence on the environment, primarily on the health of both contemporary and future generations. Bearing in mind the consequences associated with the utilization of chemical preparations in plant protection and cultivation and, hence, out of concern for the natural environment, attempts are being made to use microbiological inocula in the cultivation of ornamental plants [2]. It appears that the application of microbiological inocula may have a wide environmental aspect. By metabolizing organic matter contained in the substrate, microorganisms recycle chemical elements essential for agricultural production [3].

Microorganisms in the natural environment frequently enter into various interrelationships not only with other microorganisms but also with plants. The main role of microorganisms found in the substrate is continuous transformation of organic and mineral compounds and providing nutrients to plants [4]. Microorganisms are closely associated with plants and their root systems as well as with root secretions. Moreover, they also participate in the degradation of toxic substances and are responsible for the synthesis of secondary metabolites, exerting a stimulatory effect on plant growth (plant growth hormones, phytochelatins, organic acids, vitamins from B group). Microorganisms can also secrete substances that are toxic in relation to plant pathogens or animal organisms (antibiotics, H_2S) and, in this way, improve the condition and health of plants [5].

It is evident that the application of microbiological inocula consisting of selected strains of bacteria, actinomycetes, and fungi can influence plant protection, chlorophyll content, time of plant flowering, etc. [6].

Higher nutrient absorption by plants is associated with higher activity of selected assimilation enzymes [7]. This, in turn, can have an impact on increased levels of photosynthesis intensity with the aim of maintaining physiological equilibrium, leading to increased biomass production of both above- and underground parts of plants [8, 9]. Investigations carried out earlier revealed improved photosynthesis intensity in many species of inoculated plants, like strawberry [10, 11], *Bromus inermis* [12, 13], and soybean [13].

According to Barea et al. [14], plant inoculation with inocula selected from microorganisms contributes to biological root control against pathogen infestation, participates in making nutrients available for plants, facilitates better rooting of seedlings and affects the quality of the substrate in which plants are cultivated. The above-mentioned inocula can be made up of microorganisms isolated from the plant rhizosphere zone [15] or can be derived from composts prepared from plant residues and other substances [16].

Little is known about the application of effective microorganisms in the cultivation of ornamental plants. Investigations have been carried out only on their impact on the cultivation of geranium, saffron, rose, and gerbera [2, 17, 18]. Therefore, the objective of the present experiments was to assess the microbiological condition of peat substrate as well as the growth and flowering of scarlet sage '*Saluti Red*' following soil and foliar application of the BAF_1 inoculum at different concentrations. The results can contribute to the development of technologies of microbiological preparations and, consequently, to a more rational and environmentally friendly cultivation of bed flowers.

Material and Methods

Experimental Setup

The experiment was established in 2011 in a greenhouse in Marcelin that belongs to the Department of Ornamental Plants of Poznań University of Life Sciences.

Peat substrate of 5.5-6.0 pH (Table 1) supplemented with a slowly released multicomponent fertilizer Osmocote 5-6 M (3 g·dm^{-3}) was used in this experiment. Seedlings of scarlet sage cv. *Saluti Red* were planted in 12 cm diameter pots. The plants were inoculated with different doses of the BAF_1 biopreparation, which was applied either onto leaves or into the soil.

Inoculum

The microbiological inoculum (BAF_1 – bacteria-actinomycetes-fungi) used in the study was designed by the Department of General and Environmental Microbiology. The biopreparation consisted of 15 strains of bacteria, 5 of actinomycetes isolated from mature compost prepared from plant residues and sewage sludge, and 4 strains of *Trichoderma harzianum* fungus derived from the collection of the Institute of Plant Genetics in Poznań.

The above-mentioned strains were examined from the point of view of their proteolytic and cellulolytic activities.

Table 1. Chemical properties of peat.

Type of substrate	Volumetric (g·dm^3)	mg·dm^3 peat						gNaCl·dm^3
		N-NO_3	P	K	Ca	Mg	Cl	
peat	300	644	227	611	1644	197	77	6.12

One milliliter of the employed biopreparation contained $1.55 \cdot 10^6$ cfu of bacteria, $1.99 \cdot 10^3$ cfu of actinomycetes, and $0.98 \cdot 10^2$ of fungi.

The preparation was diluted in tap water with the aim of obtaining the following concentrations: 1:10, 1:50, and 1:100.

The above-mentioned experimental preparation was applied in two ways: onto leaves and into the substrate (always 10 ml). The experiments consisted of the following combinations (each in ten replications):

K – control (peat substrate)+plant
K1 – peat substrate+plant+watering with the preparation – 1:10
K2 – peat substrate+plant+watering with the preparation – 1:50
K3 – peat substrate+plant+watering with the preparation – 1:100
K4 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:10
K5 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:50
K6 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:100
K7 – peat substrate+plant+watering and spraying of plants with the preparation – 1:10
K8 – peat substrate+plant+watering and spraying of plants with the preparation – 1:50
K9 – peat substrate+plant+watering and spraying of plants with the preparation – 1:100

The adopted research methodological assumptions used the current developmental phase of scarlet sage as the main factor determining the moment of collection of substrate samples:

date I – seedling phase (beginning of experiment)
date II – phase of vegetative growth (after 33 days)
date III – phase of plant flowering (after 50 days)

Microbial Analyses

Microbiological analyses were performed on the basis of Koch's plate method and consisted in the determination with the assistance of selective media of colony forming units (cfu) of heterotrophic bacteria, molds, and actinomycetes. The assessment of cfu numbers of the above-mentioned microorganisms using culturing methods is a measure of intensification of microorganisms characterized by current high metabolic activity.

Counts of heterotrophic bacteria were determined on the Merck standard agar medium following 5- to 6-day incubation at 28ºC [19]. Molds were determined on the Martin medium over 5 days at 24ºC [20]. Numbers of actinomycetes were determined on the Pochon selective medium [21] incubating plates for 7 days at 26ºC.

In addition, using the spectrophotometric method, dehydrogenases activity was determined in the collected samples of the composted material using 1% TTC (triphenyltetrazolium chloride) following 24-hour incubation at 30ºC at 485 nm wavelength. The activity of the enzyme was expressed in μmol TPF$\cdot g^{-1}$ substrate DM$\cdot 24h^{-1}$ [22]. Enzymatic activity investigations are believed to reflect substrate microbiological activity set against the background of the traditional method of determination of total counts of microorganisms using Koch's plate method.

Plant Analysis Parameters

Measurements of the following traits were taken during the plant flowering period: leaf floor height, number of leaves, width and length of the leaf blade, and leaf greenness index (SPAD) with the assistance of the N-Tester apparatus. In addition, the percentage of dyed inflorescence buds, indicating the earliness of flowering, and the length of the inflorescence were assessed. Leaf samples for the determination of chlorophyll content also were taken. 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 [23] and Hiscox and Israelstam [24].

Statistical Analyses

Changes in numbers of microorganisms and levels of their metabolic activity in consecutive developmental phases of scarlet sage were elaborated upon statistically by employing two-factorial analysis of variance. In the case of plant morphological parameters as well as $a+b$, a, and b chlorophyll content, a single factor analysis of variance was employed. Moreover, Tukey's test was also used and its results are presented graphically in order to facilitate the interpretation of the obtained differences in the level of the examined parameters [25].

Results and Discussion

Microbial Analyses

The numbers of the analyzed groups of microorganisms (bacteria, actinomycetes, and molds) depended on the applied dose of the BAF_1 inoculum, the method of its application (foliar or into the substrate), and the developmental phase of scarlet sage (Figs. 1, 3, 4).

It was further concluded that on the day of establishment of the trial, the highest total number of bacteria occurred in treatment K1, in which the microbiological BAF_1 inoculum at a concentration of 1:10 was added only to the substrate (Fig. 1). In comparison with the control treatment, the difference was statistically significant at $\alpha_{0.05}$. On the other hand, the smallest number of bacteria was recorded for treatment K8, in which plants were both watered and sprayed with the biopreparation at a concentration of 1:50.

Moreover, in the case of the first date of investigations, the greatest numbers of bacteria were observed, primarily, in combinations in which the BAF_1 preparation was applied at the highest concentration, i.e. 1:10 (Fig. 1).

During vegetative growth phase (date II), bacteria proliferation declined rapidly in all combinations (opposite to the

Table 2. Pearson correlation coefficients between the number of chosen groups of microorganisms and enzymatic activity in composts.

Combination	Pearson Correlation Coefficient		
	bacteria × dehydrogenases	molds × dehydrogenases	actinomycetes × dehydrogenases
K (control) peat substrate+plant	-0.98	-1	0.88
K1 – peat substrate +plant+watering with the preparation – 1:10	-1	-0.99	-0.98
K2 – peat substrate+plant+watering with the preparation – 1:50	-0.97	-0.97	0.98
K3 – peat substrate+plant+watering with the preparation – 1:100	-0.89	0.99	0.96
K4 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:10	-0.93	-0.89	-0.89
K5 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:50	0.99	-0.97	0.99
K6 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:100	-0.96	0.94	-0.95
K7 – peat substrate+plant+watering and spraying of plants with the preparation – 1:10	-0.99	0.98	-0.92
K8 – peat substrate+plant+watering and spraying of plants with the preparation – 1:50	-0.98	0.92	-0.93
K9 – peat substrate+plant+watering and spraying of plants with the preparation – 1:100	-0.99	0.88	-0.97

flowering plant phase, date III). The recorded increase in bacterial number during the flowering phase was associated mostly with the presence of root secretions whose quantities and composition during the flowering phase can change. According to Różycki and Strzelczyk [26], root secretions stimulate the activity of microorganisms and, hence, may affect their growth and development. Also, Wielgosz and Szember [27] reported that the greatest total number of bacteria occurred during the period of plant generative growth.

The greatest mean bacterial number during the performed investigations were recorded in combination K1 (watering of plants with the biopreparation at the concentration of 1:10), while the lowest were observed for K8 (watering+spraying of plants with the inoculum at the concentration of 1:50) (Fig. 1).

Lower substrate pH could have been among the causes of lower bacteria proliferation in the case of treatment K8. The mean pH value from the three dates for K8 was 6.5, while for combination K1 it was in the order of 7.18, i.e. optimal for bacterial development (Fig. 2).

It was further found that irrespective of the date of analyses, the application of the BAF_1 inoculum, in the

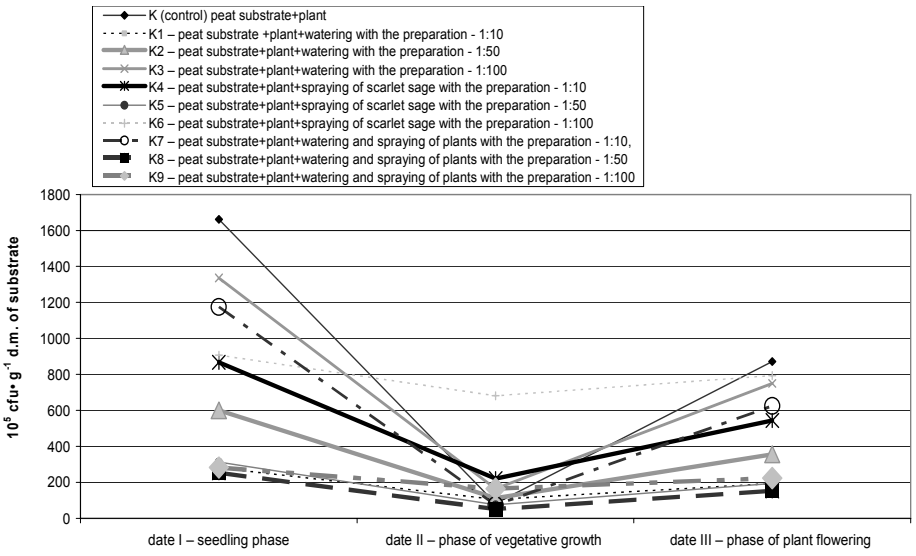

Fig. 1. Changes in the total bacteria number.
LSD – least significant difference

majority of experimental combinations, contributed to the increase in total bacterial number in relation to the control treatment. Different research results were obtained by Wolna-Maruwka et al. [2], who reported that the application of a microbiological preparation, in general, exerted an inhibitory impact on the development of bacteria in the peat substrate under geranium cultivation. Also, Kucharski and Jastrzębska [28] found that soil inoculation with an effective microorganisms microbiological inoculum inhibited growth of most groups of heterotrophic bacteria as well as of actinomycetes and molds.

Changes in the dynamics in numbers of actinomycetes in the analyzed peat substrates were identical to those observed in the case of true bacteria. Due to their ability to decompose many complex compounds, including proteins, pectins, cellulose, hemicellulose, lignins, and chitin, as well as their ability to produce bioactive metabolites, mainly antibiotic compounds [29-31], an attempt was made in this study to determine their numbers. In addition, the performed two-factorial analysis of variance revealed that the application of the BAF_1 inoculum also differentiated significantly numbers of actinomycetes (Fig. 3). Identically to the case of bacteria, the highest, statistically significant (at $\alpha_{0.01}$) differences in numbers of these microorganisms, in comparison with the control, were recorded for treatment K1 (peat substrate+plant+watering with the preparation – 1:10), while their proliferation in this case was the poorest in the K3 substrate (peat substrate+plant+watering with the preparation – 1:100). The numbers of actinomycetes declined on date II (phase of vegetative growth), whereas during the generative stage of scarlet sage, their numbers increased again. This was valid for the majority of combi-

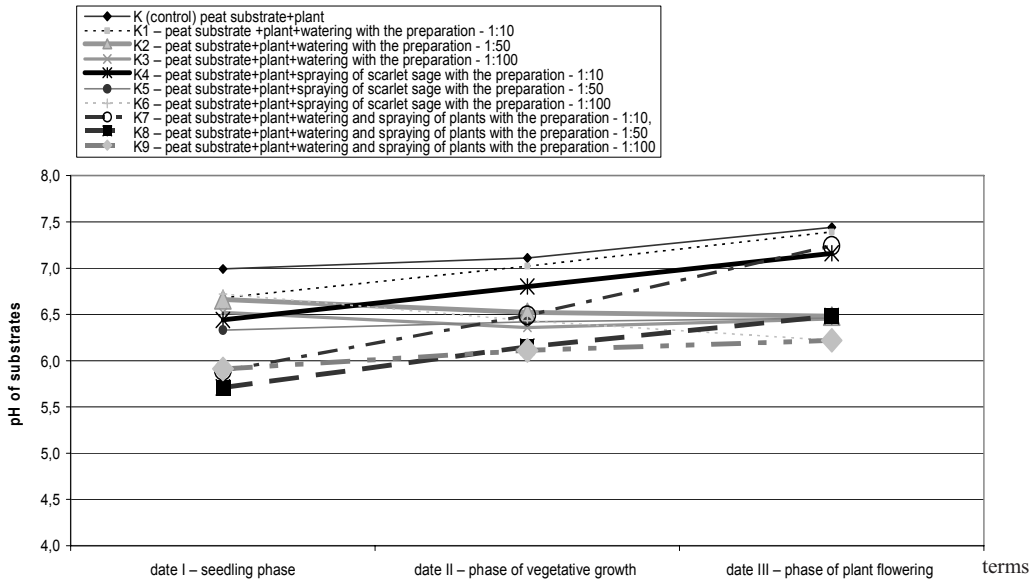

Fig. 2. Changes in substrate pH levels.

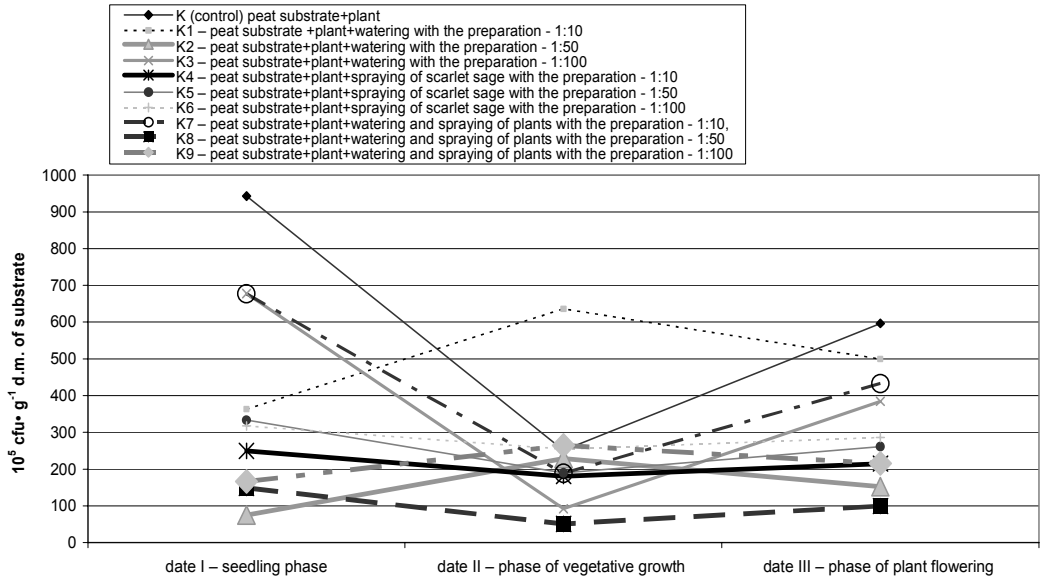

Fig. 3. Changes in total actinomycetes number.
LSD – least significant difference

nations. Probably, root secretions were again the main factor responsible for changes in numbers of actinomycetes in soil.

The composition and number of soil microflora and the production of enzymes connected with it depends not only on the plant species, but also on their developmental phase [32]. Moreover, on the basis of the performed statistical analysis (Table. 2), it was concluded that the growth of pH values in substrates only in the case of some combinations correlated positively with changes in numbers of actinomycetes.

It was further found that statistically significant differences (at $\alpha_{0.01}$ or $\alpha_{0.05}$) in numbers of actinomycetes in relation to control combinations occurred in object samples watered or sprayed with the BAF_1 inoculum at 1:10 and 1:50 concentrations (K1, K2, K4, K8) (Fig. 3).

Besides bacteria and actinomycetes, molds also play a key role in the process of mineralization of organic matter in the substrate [33]. It was further determined that some species of molds produce several secondary metabolites with a broad spectrum of antimicrobial activity: siderophores, chitinases, glucanases, and antibiotics [34, 35].

The BAF_1 preparation, irrespective of the method of its inoculation and dosage, in most experimental combinations caused a stimulatory effect on the development of mold fungi in peat substrate. The highest, statistically significant differences (at $\alpha_{0.01}$ or $\alpha_{0.05}$) in numbers of the discussed microorganisms in relation to the control were recorded in combinations K1 (peat substrate+plant+watering with the

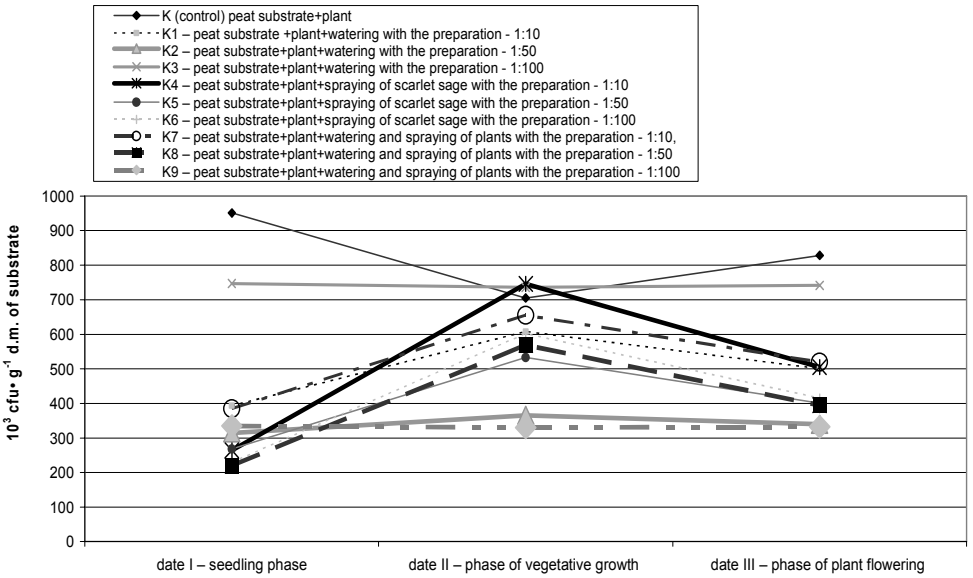

Fig. 4. Changes in total mold numbers.
LSD – least significant difference

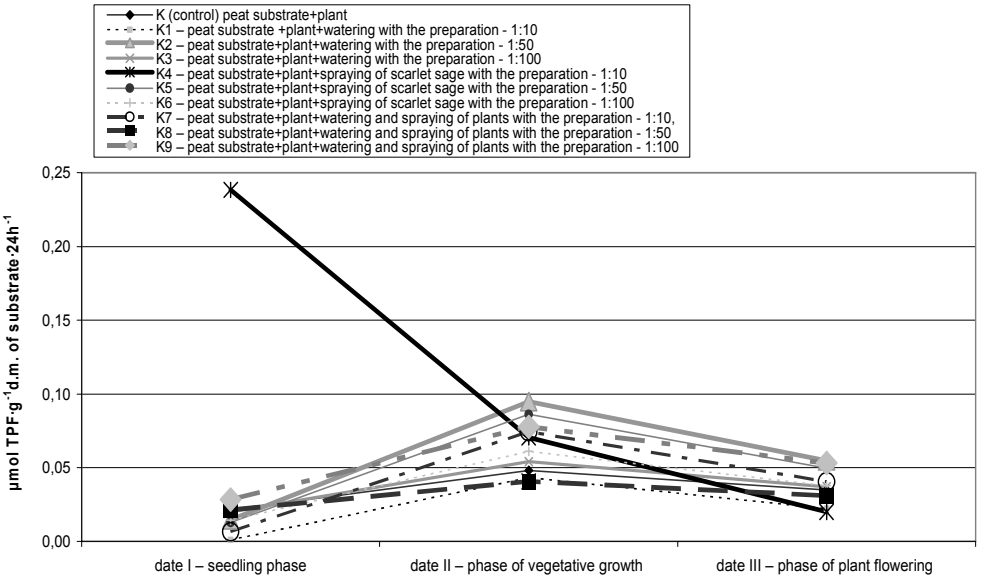

Fig. 5. Changes in dehydrogenase activity.
LSD – least significant difference

preparation – 1:10), K4 (peat substrate+plant+spraying of scarlet sage with the preparation – 1:10), and K5 (peat substrate+plant+spraying of scarlet sage with the preparation – 1:50) (Fig. 4).

The highest number of molds occurred during the plant vegetative growth phase, and the lowest – during the flowering phase (date III) in most treatment combinations (except K1). These results are in agreement with those obtained by Wolna-Maruwka et al. [2]. They reported a reduction in numbers of these microorganisms during the flowering phase of geranium cultivated on peat substrate following inoculation by a microbiological preparation. Changes in the number of molds in successive plant developmental phases in our experiment could have been associated with changes in the qualitative and quantitative composition of plant root secretions, also reported by Garcia et al. [36]. Wielgosz et al. [37], for example, also observed the greatest number of fungi during the vegetative phase of growth of *Sida hermaphrodita*, *Salix wiminalis*, and *Lathyrus*. Moreover, it is evident from experiments carried out by Wolna-Maruwka et al. [38, 39] that the plant developmental phase is one of the main factors affecting the population size of microorganisms in the substrate.

The observed reduction in the proliferation of molds in the majority of analyzed peat samples during the phase of plant flowering could also have been caused by pH, which in the last analyses date reached the highest values (Fig. 2).

Enzymatic Analyses

The activity of dehydrogenases in the substrate is widely accepted as an indicator of the intensity of the respiratory metabolism of microorganisms. According to Brzezińska and Włodarczyk [40], it is possible to estimate the physiological condition of microorganisms on the basis of the activity of these enzymes.

The highest dehydrogenase activity occurred in combination K5, where the BAF_1 inoculum was sprayed onto leaves and into soil at 1:50 concentration (Fig. 5). The above-mentioned activity was statistically significantly higher (at $\alpha_{0.01}$) in comparison with the control treatment. The lowest activity of the discussed enzymes was observed in combination K2 (peat substrate+watering with the preparation at the concentration of 1:50).

Analyzing the mean activity value of dehydrogenases in the course of the performed experiments, it was found that it was highest in combination K5 and lowest in combination K2. Analyzing changes in the level of dehydrogenase activity in consecutive developmental phases of scarlet sage (Fig. 5), it was observed that, irrespective of the kind of treatment, enzyme activity reached maximum values in the course of vegetative plant growth (phase II).

According to Wielgosz and Szember [27], as well as Wolna-Maruwka et al. [41], plant root secretions can cause stimulation of dehydrogenase activity. On the other hand, Kucharski [42] maintains that, apart from microorganisms, underground plant parts as well as soil fauna can also be sources of enzymes.

Moreover, the metabolic activity of microorganisms, similarly to their numbers, depends on the substrate pH value as well as on the organic matter content in the substrate [43, 44].

The number of molds was positively correlated with levels of dehydrogenase activity (Table 2) because, according to the assumptions of Wysocki and Lira [45], a strong Pearson linear correlation between given factors takes place when $0.75 \leq |r| < 0.95$.

In the case of true bacteria and actinomycetes (Table 2), in the majority of peat samples, a negative correlation was noted between changes of their numbers and levels of dehydrogenase activity. Previous investigations revealed that the number of microorganisms does not always correlate positively with the level of metabolic activity of microorganisms [46]. According to them, a large population of microorganisms can exhibit either low or high enzymatic activity.

Plant Analyses

The single-factorial analysis of variance revealed a significant impact of the method of application of BAF_1 on the level of chlorophyll $a+b$ ($p<0.05$) as well as b ($p<0.01$), but it failed to show a significant influence on the level of chlorophyll a.

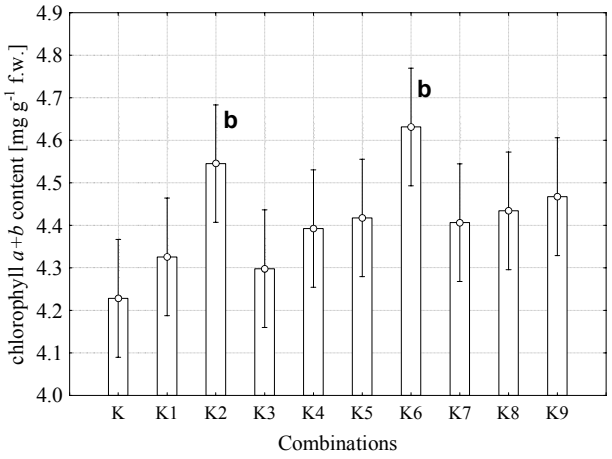

Fig. 6. Chlorophyll $a+b$ content in fresh weight of Scarlet Sage leaves. Significant differences to control (at level $\alpha=0.05$) are shown as *b*.
K (control) peat substrate+plant
K1 – peat substrate +plant+watering with the preparation – 1:10
K2 – peat substrate+plant+watering with the preparation – 1:50
K3 – peat substrate+plant+watering with the preparation – 1:100
K4 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:10
K5 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:50
K6 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:100
K7 – peat substrate+plant+watering and spraying of plants with the preparation – 1:10
K8 – peat substrate+plant+watering and spraying of plants with the preparation – 1:50
K9 – peat substrate+plant+watering and spraying of plants with the preparation – 1:100

Table 3. The influence of BAF1 on morphological parameters of Scarlet Sage.

Combination	Trait					
	Height of leaves layer	Length of inflorescences	Number of leaves	Width of leaf blade	Length of leaf blade	Greening index of leaves SPAD
K (control) peat substrate+plant	13.4 a	9.5 a	28.6 a	9.4 bc	8.9 ab	62.7 ab
K1 – peat substrate +plant+watering with the preparation – 1:10	15.0 c	10.3 ab	28.5 a	9.5 c	8.9 ab	64.6 ab
K2 – peat substrate+plant+watering with the preparation – 1:50	16.2 d	10.6 ab	29.9 a	9.2 abc	9.2 ab	62.9 ab
K3 – peat substrate+plant+watering with the preparation – 1:100	15.3 c	10.7 ab	30.2 a	9.4 bc	9.3 ab	63.5 ab
K4 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:10	14.4 ab	12.2 c	29.0 a	9.6 c	9.4 b	61.9 a
K5 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:50	14.4 ab	12.1 c	28.4 a	9.3 bc	8.7 ab	64.2 ab
K6 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:100	13.8 ab	12.3 c	28.4 a	9.7 c	8.9 ab	65.0 ab
K7 – peat substrate+plant+watering and spraying of plants with the preparation – 1:10,	14.2 ab	12.4 c	28.6 a	9.6 c	9.1 ab	65.8 ab
K8 – peat substrate+plant+watering and spraying of plants with the preparation – 1:50	13.2 a	15.1 e	30.2 a	8.7 a	8.7 ab	64.4 ab
K9 – peat substrate+plant+watering and spraying of plants with the preparation – 1:100	13.7 ab	13.7 d	28.6 a	8.9 ab	8.6 a	66.5 b

Means followed by the same letters do not differ significantly at α=0.05

The level of chlorophyll *a*+*b* in plants inoculated with BAF_1 was higher than in the control plants, although only in the case of K2 (peat substrate+plant+watering with the preparation – 1:50) and K6 (peat substrate+plant+spraying of scarlet sage with the preparation – 1:100) combinations were significant differences found in the level of the examined parameter in comparison with the control plants (Fig. 6). For the content of chlorophyll *a* (Fig. 7), again higher levels of the examined parameter (mostly statistically non-significant) were observed in plants treated with the BAF_1 preparation than in control plants. Significantly higher levels of chlorophyll *a* were observed only in the case of combination K6. The content of chlorophyll *b* is the most varied (Fig. 8) although, as in the case of chlorophyll *a*+*b*, statistically significant differences in comparison with the control plants were recorded only in the case of leaves in K2 and K6 combinations. Higher levels of chlorophyll *b* were observed in plants of K7-K9 treatments in comparison with the control plants, although the differences were not statistically significant.

Chlorophyll concentration is associated with the level of photosynthesis intensity. Despite the absence of statistical significance in all methods of BAF_1 application in comparison with the control plants, the content of all three forms of chlorophyll was found higher in plants inoculated with BAF_1, and this corroborates the results of earlier investigations that indicate a positive impact of inoculation on the level of photosynthetic activity [7], chlorophyll content [6] and, associated with it, growth and development of the aboveground and underground plant parts [8, 9].

Furthermore, it was also possible to conclude on the basis of the performed experiments that the application of the BAF_1 biopreparation affected the height of the leaf floor, length of inflorescences, and earliness of flowering, etc. (Fig. 9, Table 3).

The height of the leaf floor depended significantly on the method of application as well as concentration of the BAF_1 inoculum. The best results were obtained when the experimental inoculum was applied into the peat at 1:50 concentration (K2). On the other hand, in the case of the length of the inflorescence, good results were recorded when the inoculum was applied jointly in the form of watering and spraying at 1:50 and 1:100 concentrations (K8 and K9).

The application of the experimental inoculum primarily influenced the flowering of plants, i.e. length of inflores-

cences as well as earliness of flowering. Earlier flowering of plants was achieved following foliar and soil application of the BAF_1 inoculum, especially at 1:50 concentration. It is evident from that that the percentage of dyed buds in these combinations was higher in relation to the control and the r other combinations (Fig. 9).

Earlier flowering of plants following the application of microbiological inocula was also reported by Wolna-Maruwka et al. [2] in investigations on bed geranium.

The longest and also the most impressive inflorescences were formed by plants that were both watered and sprayed with BAF_1 at 1:50 concentration. Longer inflorescences in relation to the control were also observed in the case of plants sprayed with different inoculum concentrations. Significant differences in the length of inflorescences in comparison with the control were not recorded only in the case of plants watered with inoculum solutions, irrespective of their concentrations.

A favorable impact of a microbiological inoculum (EM) applied in the form of foliar or soil application on the diameter of rose flowers and number of gerbera inflorescences was reported by Górski and Kleiber [18]. Analyzing the obtained results regarding the height of the leaf floor, it was found that the longest segments of shoots covered with leaves were obtained following watering of plants with the BAF_1 solution, especially when its concentration was 1:50. In the other cases, following foliar or joint foliar and soil application of the experimental inoculum, the statistical analyses did not revealed any significant differences in relation to the control plants.

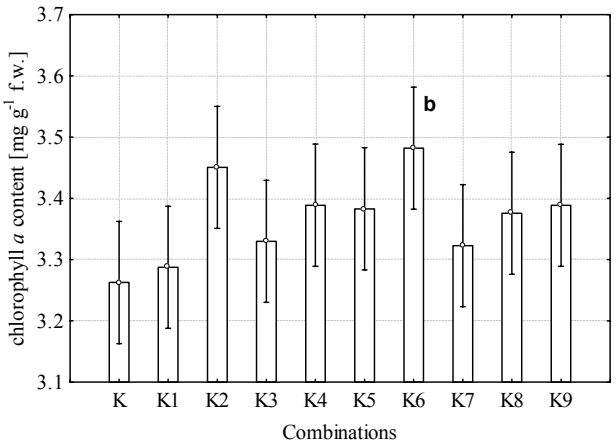

Fig. 7. Chlorophyll *a* content in fresh weight of Scarlet Sage leaves. Significant differences to control (at level α=0.05) are shown as *b*.
K (control) peat substrate+plant
K1 – peat substrate +plant+watering with the preparation – 1:10
K2 – peat substrate+plant+watering with the preparation – 1:50
K3 – peat substrate+plant+watering with the preparation – 1:100
K4 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:10
K5 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:50
K6 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:100
K7 – peat substrate+plant+watering and spraying of plants with the preparation – 1:10
K8 – peat substrate+plant+watering and spraying of plants with the preparation – 1:50
K9 – peat substrate+plant+watering and spraying of plants with the preparation – 1:100

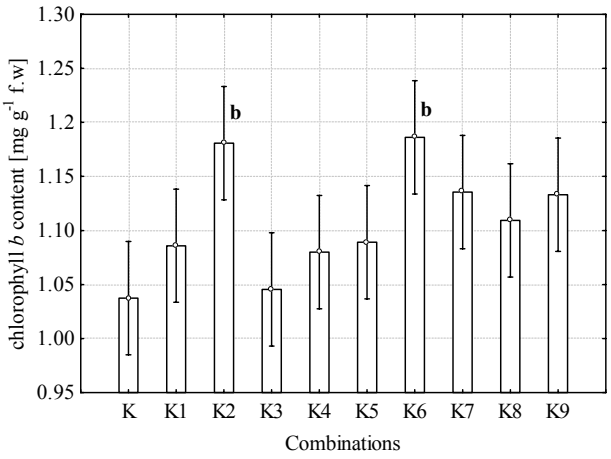

Fig. 8. Chlorophyll *b* content in fresh weight of Scarlet Sage leaves. Significant differences to control (at level α=0.05) are shown as *b*.
K (control) peat substrate+plant
K1 – peat substrate +plant+watering with the preparation – 1:10
K2 – peat substrate+plant+watering with the preparation – 1:50
K3 – peat substrate+plant+watering with the preparation – 1:100
K4 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:10
K5 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:50
K6 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:100
K7 – peat substrate+plant+watering and spraying of plants with the preparation – 1:10
K8 – peat substrate+plant+watering and spraying of plants with the preparation – 1:50
K9 – peat substrate+plant+watering and spraying of plants with the preparation – 1:100

The employed experimental BAF_1 inoculum did not exhibit any significant influence on such plant vegetative traits as number of leaves, length and width of leaf blades, or leaf greenness index. Our results are in agreement with those obtained by Wolna-Maruwka et al. [2] and Chowdhury et al. [47], who also reported lack of significant influence of EM inoculum on leaf numbers of bed geranium and spinach.

Conclusions

1. Independent of the dosage of the applied inoculum as well as the method of its application, numbers of the discussed microorganisms and the activity of the analyzed dehydrogenases in the examined peat substrates were found to increase.

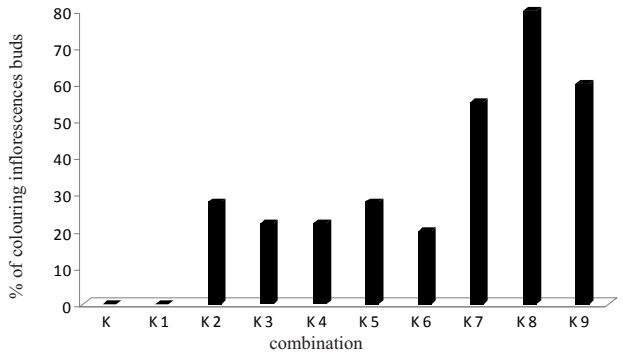

Fig. 9. The influence of BAF on percentage of colouring inflorescences buds of Scarlet Sage.
K (control) peat substrate+plant
K1 – peat substrate +plant+watering with the preparation – 1:10
K2 – peat substrate+plant+watering with the preparation – 1:50
K3 – peat substrate+plant+watering with the preparation – 1:100
K4 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:10
K5 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:50
K6 – peat substrate+plant+spraying of scarlet sage with the preparation – 1:100
K7 – peat substrate+plant+watering and spraying of plants with the preparation – 1:10
K8 – peat substrate+plant+watering and spraying of plants with the preparation – 1:50
K9 – peat substrate+plant+watering and spraying of plants with the preparation – 1:100

2. In the performed experiment, the highest, statistically significant development of microorganisms was recorded following foliar application of the experimental BAF_1 inoculum at the concentration of 1:10 (K1); the activity of dehydrogenases was the highest in the treatment with foliar application of the inoculum at 1:50 concentration (K5).
3. The developmental phase of scarlet sage was one of the major factors affecting the dynamics of quantitative changes of the analyzed microorganisms as well as levels of their metabolic activities.
4. The greatest proliferation of bacteria and actinomycetes was observed during the flowering phase, and molds during the phase plant vegetative growth.
5. The highest level of dehydrogenase activity took place during the flowering phase of scarlet sage.
6. Application of the BAF_1 inoculum increased levels of chlorophyll content; this was particularly evident in treatments K2 (watering with the preparation at 1:50 concentration) and K6 (spraying with the experimental preparation at the concentration of 1:100).
7. The examined BAF_1 inoculum did not significantly affect leaf number and greenness index or the width and length of the leaf blade. Irrespective of the dose and method of inoculum application, it did not increase the coloring of inflorescence buds.
8. The longest inflorescences were developed by plants watered and sprayed by BAF_1 inoculum at 1:50 concentration (K8).

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References

1. APLADA-SARLIS P., MILIADIS G.E. Monitoring of agricultural products in Greece for residues of pesticides. Fresen. Environ. Bull. **10**, (5), 423, **2001**.
2. WOLNA-MARUWKA A., SCHROETER – ZAKRZEWSKA A., BOROWIAK K. Effect of EM inoculum on the microbiological state of substrate designed for pelargonium (Pelargonium × hortorum) Science Nature and Technology. **4**, 6 #98, **2010**.
3. PIDRE J., TOJA R J., ALONSO E. Biological phosphorus removal from waste water: influence of sludge retention time and carbon concentration. Fresen. Environ. Bull. **10**, (12), 245, **2001**.
4. BLUM U. Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interaction. J. Chem. Ecol. **24**, 685, **1998**.
5. MARSCHNER P. Plant-microbe interactions in the rhizosphere and nutrient cycling. In: Marsner P., Rengel Z. (Eds.) Nutrient Cycling in Terrestrial Ecosystems, Soil Biology 10. Springer-Verlag Berlin Heidelberg. pp. 159, **2007**.
6. BOELENS J., VANDE WOESTYNE M., VERSTRAETE W. Ecological importance of motility for plant growth – promoting rhizopseudomonas strain ANP15. Soil. Biol. Biochem. **26**, 269, **1994**.
7. TRAPPE J.M. Mycorrhiazae and productivity of arid and semiarid rangelands. In: Manassah J.T., Briskey E.J. (Eds.) Advances in food producing systems for arid and semiarid lands. Academic Press, New York. pp. 581, **1981**.
8. MARTIN F., BOTTON B. Nitrogen metabolism of ectomycorrhizal fungi and ectomycorrhiza. Advanced Plant Phatology. **9**, 83, **1993**.
9. SMITH S.E., READ D. Mycorrhizal symbiosis. Third edition. Academic Press, London, pp. 800, **2008**.
10. FAN Y., LUAN Y., AN L., YU K. Arbuscular mycorrhizae formed by *Penicillium pinophilum* improve the growth, nutrient uptake and photosynthesis of strawberry with two inoculum-types. Biotechnological Letters. **30**, 1489, **2008**.
11. KASCHUK G., KUYPER T.W., LEFFELAAR P.A., HUNGRIA M., GILLER K.E. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? Soil. Biol. Biochem. **41**, 1233, **2009**.
12. BILDUSAS I.J., DIXON R.K., PFLEGER F.L., STEWART L. Growth, nutrition and gas exchange of *Bromus inermis* inoculated with *Glomus fasciculatum*. New Pyhtologist. **102**, 303, **1986**.
13. ZHOU H.-J., LIANG Y., CHEN H., SHEN S.-H., JING Y.-X. Effects of rhizobia inoculation and nitrogen fertilization on photosynthetic physiology of soybean. Photosynthetica. **44**, (4), 530, **2006**.
14. BAREA J.M., AZCÓN R., AZCÓN-AGUILAR C. Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van Leeuwenhoek. **81**, 343, **2002**.
15. VESTBERG M., KUKKONEN S., SAARI K., PARIKKA P., HUTTUNEN J., TAINIO L., DEVOS N., WEEKERS F., KEVERS C., THONART P., LEMOINE M.C., CORDIER C., ALABOUVETTE C., GIANINAZZI S. Microbial inoculation for improving the growth and health of micropropagated strawberry. Appl. Soil. Ecol. **27**, 243, **2004**.

16. PHAE C. G., SASAKI M., SHODA M., KUBOTA H. Characteristic of *Bacillus subtilis* isolated from composts suppressing phytopthogenic microorganisms. Soil. Sci Plant. Nutr. **36**, (4), 575, **1990**.
17. AYTEKIN A., ACIKGOZ A.O. Hormone and microorganism treatments In the cultivation of saffron (*Crocus sativus* L.) plants. Molecules, **13**, 1135, **2008**.
18. GÓRSKI R., KLEIBER T. Effect of Effective Microoorganisms (EM) on nutrient contents in substrate and development and yelding of rose (*Rosa* x *hybrida*) and gerbera (*Gerbera jamesonii*). Ecological Chemistry and Engineering. **17**, (4), 505, **2010**.
19. Merck-Polska.101621 Standard count agar for microbiology, 1, **2004**.
20. MARTIN J. P. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**, 215, **1950**.
21. KAŃSKA Z., GRABIŃSKA-ŁONIEWSKA A., ŁEBKOWSKA M., ŻECHOWSKA E. Laboratory exercises in sanitary biology. Warsaw Polytechnic, Warsaw, **193**, **2001**.
22. THALMANN A. Methodology for the determination of the activity dehydrogenase in soil from triphenyltetrazolium chloride (TTC). Landwirtsch. Forsch. **21**, 249, **1968**.
23. SHOAF W.T., LIUM RW. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. Limnol. Oceanogr. **21**, 926, **1976**.
24. HISCOX J.D., ISRAELSTAM G.F. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. **57**, 1332, **1979**.
25. OTT L. An introduction to statistical methods and data analysis. PWS Publishers, Boston, pp. 750, **1984**.
26. RÓŻYCKI H., STRZELCZYK E. Organic bonds secreted by soil microorganisms and plant roots. Adv. Appl. Microbiol. **24**, (4), 285, **1985**.
27. WIELGOSZ E., SZEMBER A. Occurrence of natural complexes of soil microorganisms in near-root zone of plants used designing house garden. Annales UMCS, Sec. E. **61**, 75, **2006**.
28. KUCHARSKI J., JASTRZĘBSKA E. Role of Effective Microorganisms (EM) and soil microorganisms in the development of soil microbiological properties. Advances of Agricultural Science Problem Issues. **506**, 315, **2005**.
29. DAHM H., RÓŻYCKI H., STRZELCZYK E. Bacteria and actinomycetes of soils and root zone of forest trees. Advances in Microbiology. **XXV**, (1/2), 103, **1986**.
30. MORDARSKA H., PAŚCIAK M. Actinomycetes in nature and biotechnology. Biotechnology. **4**, 142, **2002**.
31. OSKAY M., TAMER A., AZERI C. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology. **3**, 441, **2004**.
32. WIELGOSZ E. Microbial and enzymatic activity in brown soil under the cultivation of *Sida hermaphrodita* and *Helianthus tubersus*. Annales UMCS. **35**, (36), 173, **1999**.
33. BIS H. Occurrence of toxicogenic fungi in soil environment. Activity of microorganisms in different environments. Kraków, pp. 35, **2002**.
34. NENWANI V., DOSHI P., SAHA T., RAJKUMAR S. Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth. Journal of Yeast and Fungal Research. **1**, (1), 009, **2010**.
35. RENWICK A, CAMPBELL R, COE S. Assessment of *in vivo* screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. Plant Pathol. **40**, 524, **1991**.
36. GARCIA C., RODAN A., HERNANDEZ T. Changes in microbial activity after abandonment of cultivation in semiarid Mediterraneam environment. J. Environ. Qual. **26**, 285, **1997**.
37. WIELGOSZ E., SZEMBER A., PRYCIAK I. Influence of selected plants on occurrence of complexes of soil microorganisms. Annales UMCS. Sec. E. **59**, (4), 1679, **2004**.
38. WOLNA-MARUWKA A., KLAMA J., NIEWIADOMSKA A. Effect of fertilization using communal sewage sludge on respiration activity and counts of selected microorganisms in the grey brown podzolic soil. Pol. J. Environ. Stud. **16**, (7), 899, **2007**.
39. WOLNA-MARUWKA A., SAWICKA A., KAYZER D. Size selected groups of microorganisms and soil respiration activity fertilized by municipal sewage sludge. Pol. J. Environ. Stud. **16**, (1), 129, **2007**.
40. BRZEZIŃSKA M., WŁODARCZYK T. Enzymes of intracellular redox (oxidoreductase) changes. Acta Agrophysica. **3**, 11, **2005**.
41. WOLNA-MARUWKA A., NIEWIADOMSKA A., KLAMA J. Biological activity of grey-brown podzolic soil organically fertilized for mazie cultivation in monoculture. Pol. J. Environ. Stud. **18**, (5), 931, **2009**.
42. KUCHARSKI J. Relations between enzyme activity and soil fertility. In: Microorganisms in environment. Occurrence, activity and significance. Ed. Financed by KBN and Faculty of Agronomy of AR Wrocław, pp. 327, **1997**.
43. FUCHS J.G. Interactions between beneficial and harmful microorganisms: from the composting process to compost application. Springer-Verlag Berlin Heidelberg, 213, **2010**.
44. SUNDBERG C., SMÅRS S., JÖNSSON H. Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. Bioresource Technol. **95**, (2), 140, **2004**.
45. WYSOCKI F., LIRA J. Descriptive statistics. Agricultural University Publisher in Poznań, Poznań, pp. 203, **2003**.
46. LYNCH J.M., PANTING L.M. Cultivation and the soil biomass. Soil Biol. Biochem., **12**, 29, **1980**.
47. CHOWDHURY A.R., HOSSAIN M.M, MIA M.S., KARIM A.J.S., HAIDER J., BHUIYAN N.I., SAIFUDDIN K. Effect of organic amendments and EM on crop production in Bangladesh. Proceedings of the Second International Conference on Kyusei Nature Farming. October 7-11, Sao Paulo, Brazil, **8**, **1991**.