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INFLUENCE OF THE LIGHT COLOR AND MICROBIOLOGICAL INOCULUMS ON THE ZONAL PELARGONIUM QUALITY AND MICROBIOLOGICAL AND ENZYMATIC STATE OF THE SUBSTRATE

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ABSTRACT

Two inoculums: Effective Microorganisms (EM) specimen available on the market and microbiological BAF_1 inoculum, were applied in the experiment. The plants were cultivated in the growth chamber equipped with shelves with fluorescent or LED lamps. The highest number of inflorescences was under the influence of white color of light emitted by fluorescent lamps and blue color of light emitted by LED lamps, especially after application of BAF_1 inoculum. Irrespective of microbiological inoculum, no significant effect of the color of light and type of lamps on such traits as height of leaves layer, number of leaves, greening index of leaves (SPAD) and length of inflorescences, was found. The white color light emitted by fluorescent lamps stimulated actinobacteria multiplication, especially after EM application. Regardless of the inoculum application, it was the blue color light emitted by LED lamps that stimulated the multiplication of moldy fungi. After the use of fluorescent lamps, the increase in dehydrogenase activity was observed, especially after the application of BAF_1 inoculum. The activity of acid phosphatase was stimulated by blue and white+blue light emitted by LED lamps. The increase in the activity of urease was observed under fluorescent lamps emitting the green, blue and white color of light, after the application of EM.

Key words: light quality, LEDs, microorganisms, enzymatic activity

INTRODUCTION

Zonal pelargonium (*Pelargonium zonale*) has been highly rated among balcony plants for years. Recently, the distribution centers of these plants have changed. They are purchased not only at flower shops and gardening centers, but also, more and more often, in supermarkets and discount stores. Light and temperature conditions in the latter are much worse and they differ significantly from those optimal for the species. Improper warehousing results in quality and plant health decline, which, in turn, leads to smaller consumer interest and, as a consequence, very low sales. Dark storage induced leaf yellowing decreasing the visual quality.

Cultivation in closed chambers with no access to natural light – so called "closed system" was started in Japan in the 80s of the twentieth century. At that time, it was leafy vegetables that were planted in such chambers. Afterwards, fruit and ornamental plants were also grown in this way [Chun and Kozai 2002, Kozai et al. 2006]. At the end of 2000, "closed system" became popular all over the world, especially in Asian countries. As Goto [2012] states, genetically

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modified plants and medicinal plants can be cultivated in this way. This production technology involves cultivation in an air-conditioned room, on shelves under the lamps used for plant illumination. Unused rooms can be adapted for this purpose, which highly lowers the costs of production, connected for example with building a greenhouse. The closed system is an environmentally friendly system for plant production. Heating load is small, therefore oil or natural gas is not required for heating in the closed system. The floor area of the closed system is only 10% of the greenhouse floor area for the same plant production, thus material resources and labor for constructing and managing the production system can be saved significantly. Electricity cost for cooling and lighting is small. Disinfection of the closed system is relatively easy [Kozai et al. 2006]. Initially, high pressure sodium lamps were used, then fluorescent lamps were introduced. Recently, diode lamps have become more and more popular among producers as they are much more energy-efficient and this is why they are often seen as the solution for the future [Watanabe 2011]. Due to the specific construction, heat is carried through radiators, which is why the lamps do not overheat and can be placed directly above the plants. Recently, LEDs have been developed as alternative light sources for plants because of their wavelength specificity and narrow bandwidth.

At present, microbiological preparations are gaining in popularity. The combined action of different groups of microorganisms contained in a given inoculum used in farming or horticultural practice has an advantageous effect on fertility in a given substrate, improving the plant growth conditions e.g. by facilitating the nutrient uptake, enhancing plant growth, preventing from the development of plant pathogens and facilitating gradual reconstruction of the substrate [Wielgosz et al. 2010]. Application of microbiological specimens in cultivation can bring many benefits. As stated by Stielow [2003], application of EM inoculum into the substrate can limit rotting processes, increase resistance to drought, retard the development of plant pathogens and also contribute to more abundant flowering. The above mentioned factors are crucial while storing plants with no access to natural light. According to Marschner [2007], microorganisms can release substances that are toxic towards plant pathogens or animal organisms, for example antibiotics, H_2S, improving in this way plant condition and health.

The purpose of the conducted experiment was the assessment of the light and microbiological inoculum influence on the zonal pelargonium quality and also on the microbiological and enzymatic parameters of the substrate, in which the plants were grown.

MATERIAL AND METHODS

Zonal pelargonium (*Pelargonium zonale*) 'Tamara' cuttings were planted into pots of 12 cm in diameter and of volume of 659 cm^3. Experiments were conducted in 2013–2014 in a controlled environment growth chamber located at the Experimental Station of the Departments of the Faculty of Horticulture and Landscape Architecture, the Poznań University of Life Sciences (Poland). High-peat-based substrate with pH 5.5-6.0 was enriched with slow-release compound fertilizer Osmocote 5-6 M in the amount of 3 $g \cdot dm^{-3}$. Two weeks after planting, the plants were treated with leaf- and soil-applied microbiological inoculums (EM or BAF_1) diluted with water at the ratio of 1 : 100. The preparation was applied in the amount of 10 ml per plants. The plants were inoculated with the EM bio-preparations. Specimen available on the market. The microbiological composition of the Effective Microorganisms (EM) concentrate was as follows (population size in 1 cm^3 given in brackets): *Streptomyces albus* (10^5), *Propionibacterium freudenreichil* (10^5), *Streptococcus lactis* (10^5), *Aspergilius oryzae* (10^5), *Mucor hiemalis* (10^5), *Saccharomyces cerevisiae* (10^5) and *Candida utilis* (10^5). Morever, EM contain an unspecified amount of *Lactobacillus* sp., *Rhodopseudomonas* sp. and *Streptomyces griseus* [Formowitz et al. 2007].

The microbiological inoculum BAF_1 (Bacteria-Actinobacteria-Fungi) was formulated in the Department of Agricultural Microbiology. The bio-preparation BAF_1 consisted of 15 strains of bacteria, 5 of actinomycetes isolated from mature compost prepared from plant residues and sewage sludge as well as 4 strains of *Trichoderma harzianum* derived from the collection of the Institute of Plant Genetics in Poznań. The above-mentioned strains were examined, among others, from the point of view of their proteolytic, cellulolytic and phytosanitary activities. One milliliter of the employed bio-preparation con-

tained $1.55 \cdot 10^6$ cfu of bacteria, $1.99 \cdot 10^3$ cfu of actinomycetes and $0.98 \cdot 10^2$ of fungi.

After two weeks of growing plants in the greenhouse, pots with plants were placed in the growth chamber equipped with 120 × 50 cm shelves. The experiment was performed using two types of lamps: fluorescent (Philips TLD) and LED Tube (Leuchtek). The lamps emitted the following light colors: white, green, blue, blue+red (75 : 25) and white+blue (50 : 50). Each shelve was equipped with a different lamp and covered with a shutter, so that the colors of light didn't mix with one another. Conditions in the growth room were stable: the temperature was 20°C and the length of the day was 12 hours. The quantum irradiance measured by means of phyto-photometer was 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Spectral characteristic of lamps was performed by means of spectro-radiometer (USB 4000) and it is presented in Figures 1 and 2. Measurement of the following traits and microbiological analyses were taken during the plant flowering period: level of leaves height, number of leaves, leaf greening index (SPAD) with the assistance of the N-Tester apparatus. In addition, the number of inflorescences and length of inflorescences were assessed. The experiment was established in two culture cycles. Results of measurement were given as mean from two years of research. Measurement results were statistically elaborated by means of the analysis of variance with the use of Duncan's test at $P < 0.05$ level.

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 the temperature of 28°C. Molds were determined on the Martin medium in the period of 5 days at the temperature of 24°C [Martin 1950]. Numbers of actinobacteria were determined on the Pochon selective medium [Kańska et al. 2001], incubating plates for 7 days at the temperature of 26°C. 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 the temperature of 30°C at 485 nm wavelength. The activity of the enzyme was expressed in $\mu mol\ TPF \cdot g^{-1}$ substrate $DM \cdot 24\ h^{-1}$ [Thalmann 1968]. The activity of acid phosphatase was determined using the substrate p-nitrophenylophosphate sodium, after one-hour incubation at 37°C at the wavelength 400 nm. Enzyme activity was expressed in $\mu mol\ PNP \cdot g^{-1} \cdot h^{-1}$ [Tabatabei and Bremner 1969]. Urease activity was determined using as substrate urea, after one-hour incubation at 37°C at wavelength 410 nm. Enzyme activity was expressed in $\mu g\ N \cdot g^{-1} \cdot 18\ h^{-1}$ [Hoffman and Teicher 1961]. 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.

Changes in numbers of microorganisms and levels of their metabolic activity were processed statistically employing two-factorial analysis of variance. 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.

RESULTS AND DISCUSSION

Scarce information has been reported on the application of natural origin preparations in growing of ornamental plants. Studies conducted by Wolna-Maruwka et al. [2010, 2012, 2015] show that the use of a microbiological preparation in growing of pelargonium, Scarlet sage and French marigold lead to earlier plant flowering and an increased number of buds and flowers. Experiments performed by Górski and Kleiber [2010] on roses (*Rosa* × *hybrida*) and gerberas (*Gerbera jamesonii*) showed an increased number and diameter of flowers in both these plant species.

On the basis of the conducted analyses, irrespective of microbiological inoculum on the color of light and type of lamps, no significant effect was found on such traits as height of level leaves, number of leaves, leaves greening index (SPAD) (except for the white light emitted by LED lamps, in the case of which the darker leaves formed after the application of BAF1 inoculum) and length of inflorescences.

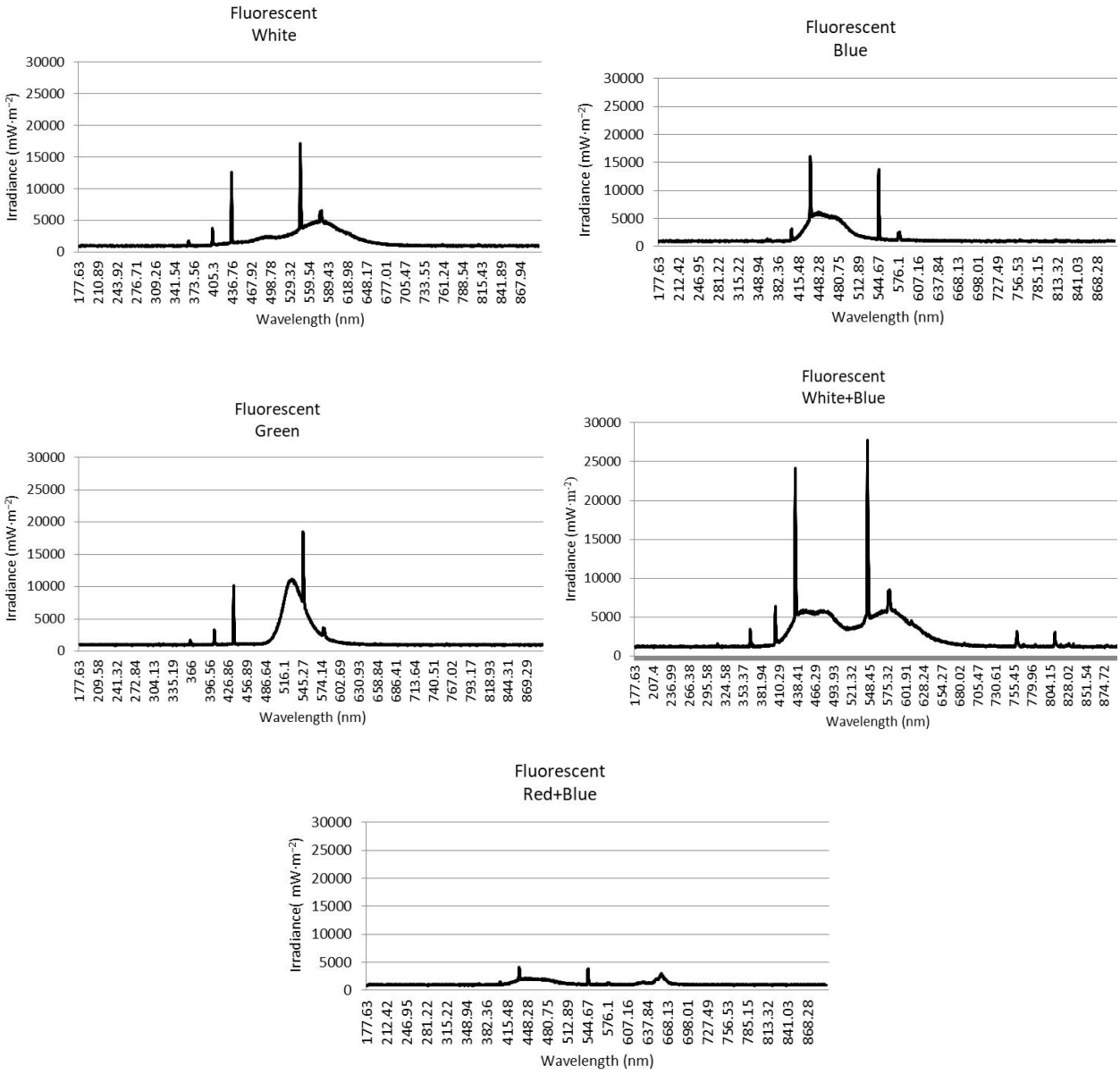

Fig. 1. Spectral characteristics of fluorescent lamps

In the conducted experiment, the number of inflorescences depended on the light color, lamp type, and also on the type of microbiological inoculum applied (Tab. 1). The highest number of inflorescences after leaf and soil application of BAF_1 inoculum, and also under the influence of the white color light emitted by fluorescent lamps and blue light emitted by LED lamps, was observed. Number of inflorescence was significantly reduced under fluorescent and LED lamps, especially those emitting the red+blue color light, irrespective of microbiological inoculum. Heo et al. [2002] showed that development of flower buds in marigold was much greater in fluorescent lamps plus red LED lamps, being about five times greater than blue and red LED lamps. As Jerzy et al. [2011] states, the number of overblown flower heads in chry-

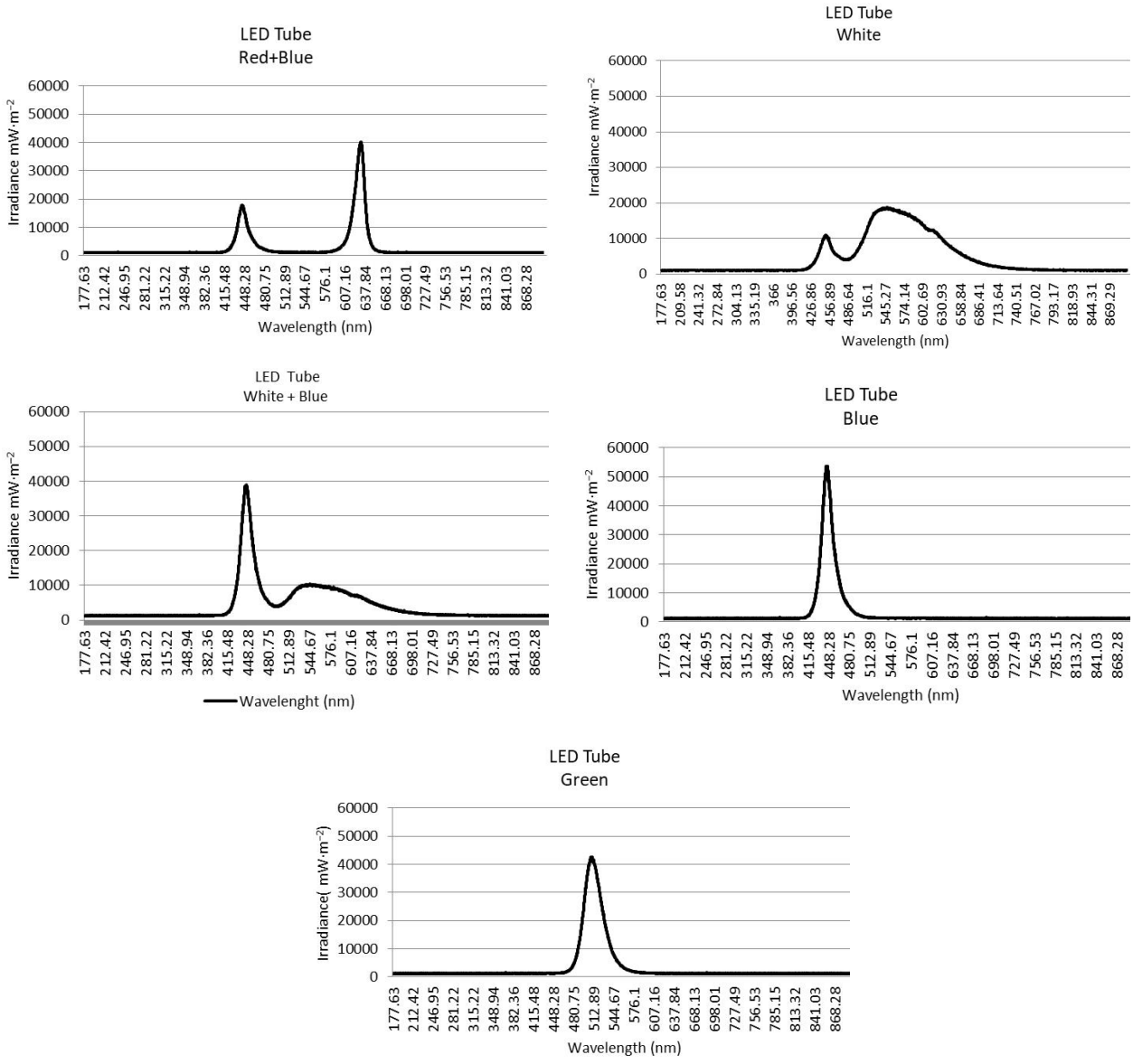

Fig. 2. Spectral characteristics of the LED lamps

santhemum is not dependent on the light color. In the case of hyacinths forced in pots, red light accelerated withering, while white and blue light had a favorable effect onto the length of the blooming period [Śmigielska and Jerzy 2009].

Due to the pioneering character of the conducted research, there is little information on the influence of the light color on the microbiological and enzymatic parameters of the substrate used in the available literature.

In higher plants, light is indispensable for the photosynthesis and morphogenesis processes, however, as stated by Ambra et al. [2004], it also stimulates morphogenesis in non-photosynthetic organisms. According to Arjona et al. [2009] and Corrochano [2007], light and especially the appropriate electromagnetic

Table 1. Influence of color of light, type lamp and microbiological inoculum on morphological traits of zonal pelargonium

Color of light	Type of lamps					
	Fluorescent			LED		
	control	BAF_1	EM	control	BAF_1	EM
	Height of level leaves (cm)					
White	16.2 a	17.3 a	16.2 a	17.4a	17.6 a	17.4 a
Blue	17.0 a	16.9 a	17.0 a	16.8 a	17.7 a	17.2 a
Green	16.9 a	16.0 a	16.9 a	16.7 a	16.7 a	16.8 a
White+blue	17.3 a	17.4 a	17.3 a	16.9 a	17.8 a	17.0 a
Red+blue	16.7 a	17.1 a	17.6 a	16.8 a	16.7 a	16.9 a
	Number of leaves					
White	31.1 a	30.4 a	31.0 a	30.7 a	30.2 a	30.3 a
Blue	31.2 a	29.8 a	29.9 a	28.9 a	29.9 a	30.2 a
Green	28.9 a	29.1 a	28.7 a	29.8 a	29.9 a	30.0 a
White+blue	29.2 a	29.7 a	30.3 a	30.0 a	30.2 a	29.5 a
Red+blue	30.1 a	30.3 a	29.9 a	30.1 a	30.0 a	29.8 a
	Length of inflorescences (cm)					
White	17.2 a	16.7 a	16.4 a	17.1 a	17.3 a	17.4 ab
Blue	17.0 a	16.4 a	16.5 a	16.7 a	17.1 a	17.3 a
Green	16.5 a	16.1 a	17.0 a	16.7 a	17.0 a	16.8 a
White+blue	17.4 ab	16.7 a	16.8 a	17.8 ab	17.2 a	16.7 a
Red+blue	16.8 a	16.6 a	16.7a	16.9 a	17.3 a	17.1 a
	Number of inflorescences					
White	3.6 b	5.1 c	3.4 b	3.5	2.7 ab	3.6 b
Blue	2.0 ab	3.1 b	3.1 b	2.3 ab	5.4 c	3.5 b
Green	2.2 ab	2.3 ab	2.2 ab	3.0 b	2.0 ab	3.2 b
White+blue	3.4 b	2.1 ab	2.1 ab	2.1 ab	3.0 b	3.1 b
Red+blue	1.0 a	1.3 a	1.1 a	1.3 a	1.2 a	1.3 a
	Leaves greening index (SPAD)					
White	51.6ab	55.2 ab	54.5 ab	49.7 a	62.3 b	49.8 a
Blue	48.1 a	52.3 ab	52.7 ab	56.7 ab	50.7 ab	55.1 ab
Green	48.6 a	49.1 a	52.4 ab	59.0 b	55.4 ab	55.7 ab
White+blue	51.4 ab	52.7 ab	49.0 a	56.8 ab	60.2 b	55.1 ab
Red+blue	60.4 b	58.0 ab	59.7 b	53.1 ab	57.2 ab	58.5 ab

Means followed by the same letters do not differ significantly at $\alpha = 0.05$

wavelength, influences the growth, metabolism and metabolite production by moldy fungi. Indurum and Heitman [2005] claim that different parts of the light spectrum have different influence onto the development and metabolic activity of fungi as they react not only to the light color, but also to its intensity [Shu et al. 2010]. While analyzing the influence of light onto microbiological and enzymatic parameters of the substrate used, it was observed that this influence was dependent on the light color, lamp type and the inoculum applied. As far as the total bacteria count in the substrate with no inoculum applied is concerned, the highest counts were observed under the red+blue light color emitted by fluorescent lamps, and also under the blue color light emitted by LED lamps. After BAF_1 application, the highest bacteria counts were observed in the substrate, in which pelargoniums were cultivated under the fluorescent lamps emitting the green color light and under LED lamps emitting the blue color light. The total bacteria count differed under the application of EM inoculum. In this case, bacteria developed significantly under LED lamps emitting the green color light (Fig. 3). Actinobacteria, apart from bacteria, are the second group of microorganisms that are responsible for significant changes of the complex compounds of both carbon and nitrogen in the substrate. Actinomycetes in peat substrate constitute a numerous and varied group of organisms. In the opinion of Hawrot-Paw et al. [2012], these microorganisms serve a significant role in the substrate, metabolizing a wide range of sparingly degradable compounds. As demonstrated by the own research, the white color light emitted by fluorescent lamps stimulated multiplication of actinobacteria, especially after EM application. The red+blue color light emitted by LED lamps, on the other hand, retarded this process, regardless of the plant treatment (Fig. 4). Apart from bacteria and actinomycetes, it is also molds that play essential role in the process of organic matter mineralization. According to Senger [1987], fungi exhibit the

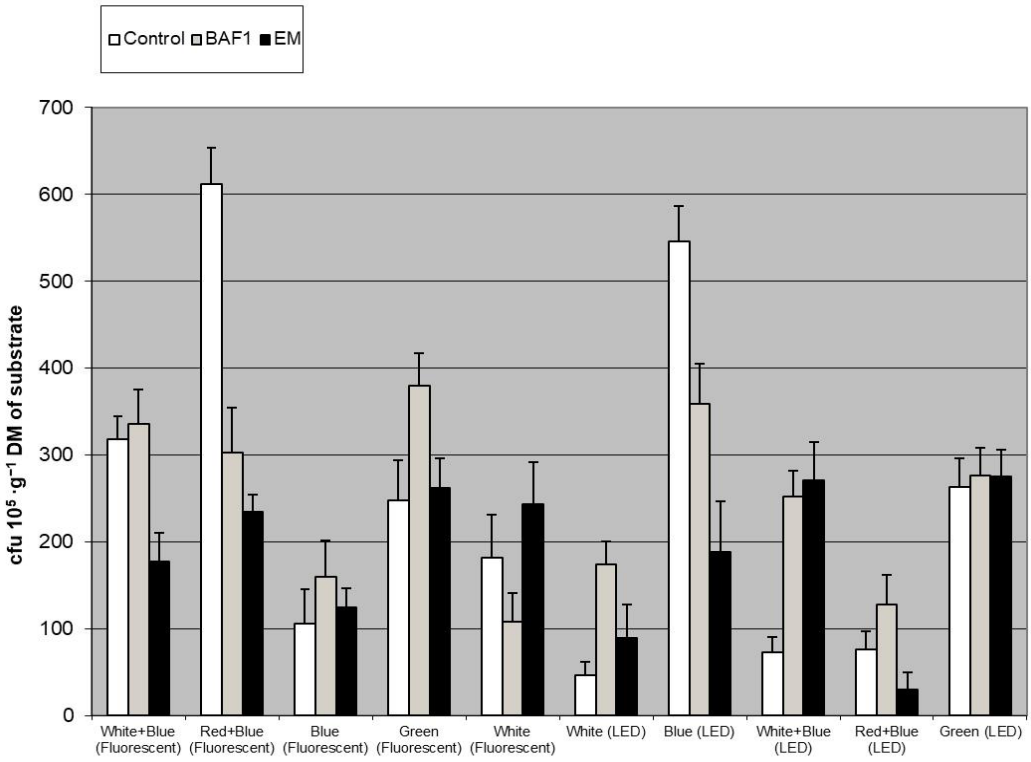

Fig. 3. Changes of the total bacteria number

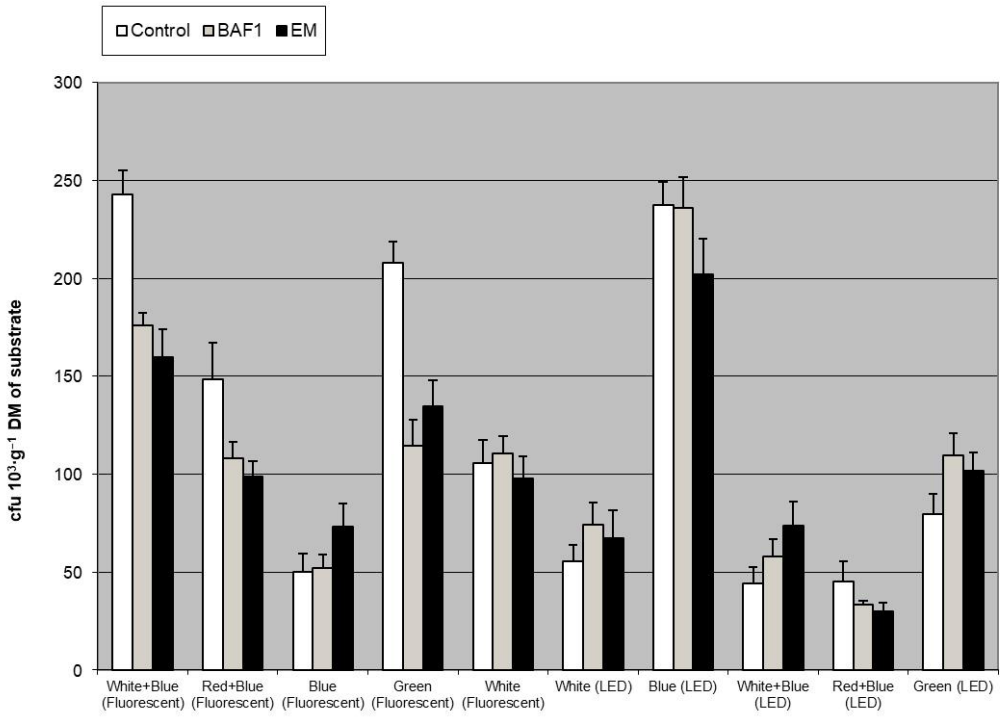

Fig. 4. Changes of the total molds number

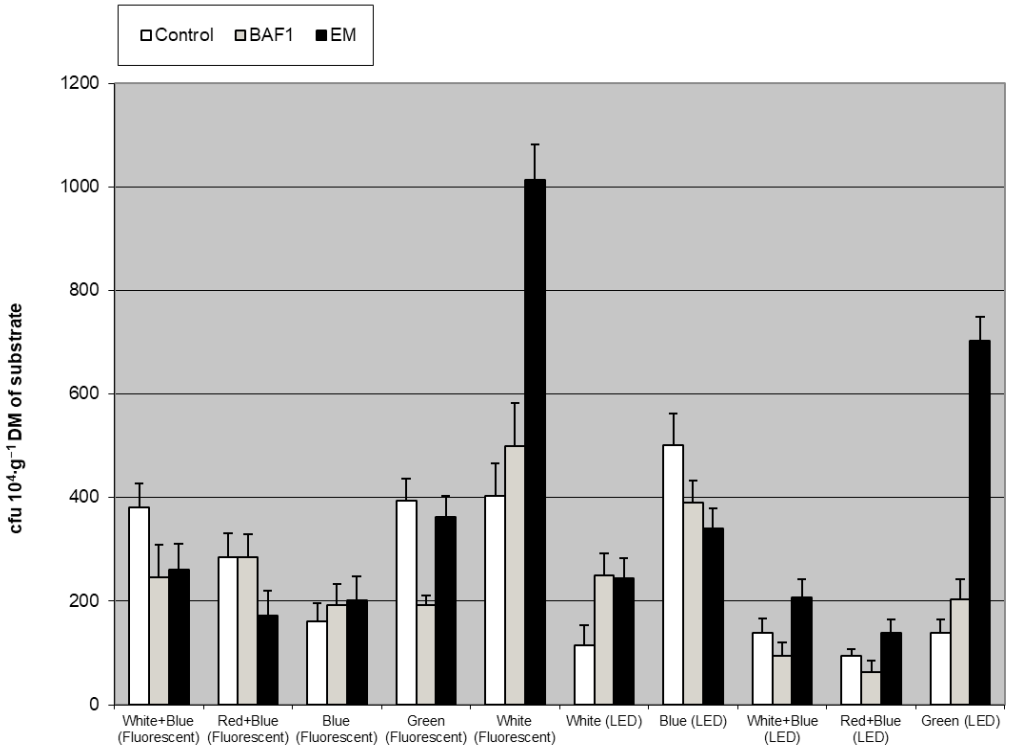

Fig. 5. Changes of the total actinobacteria number

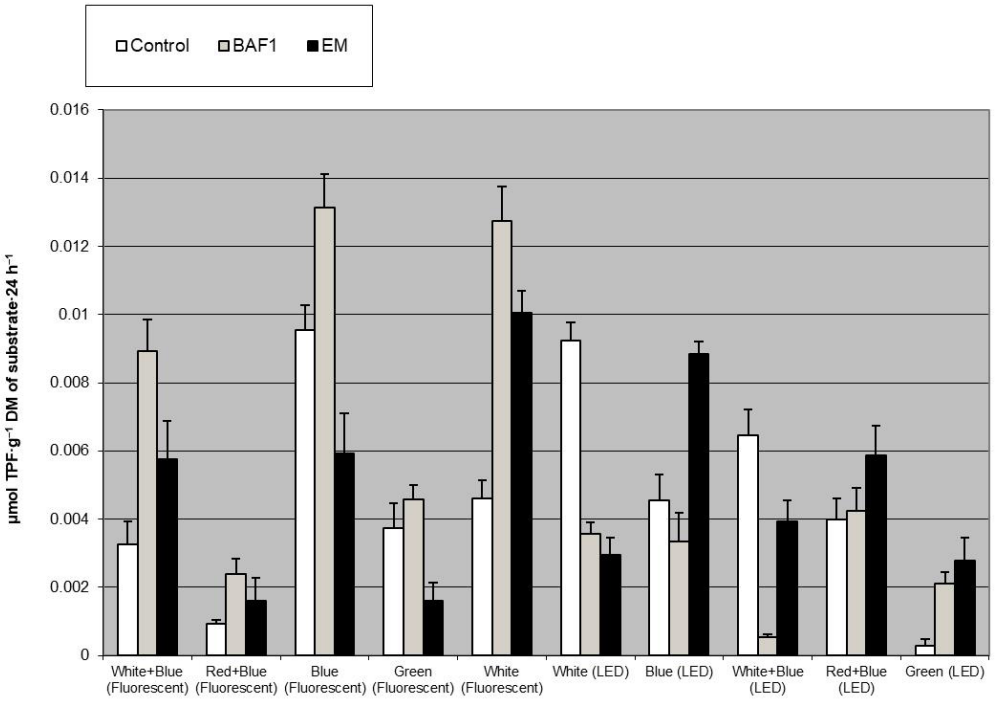

Fig. 6. Changes of the dehydrogenases activity level

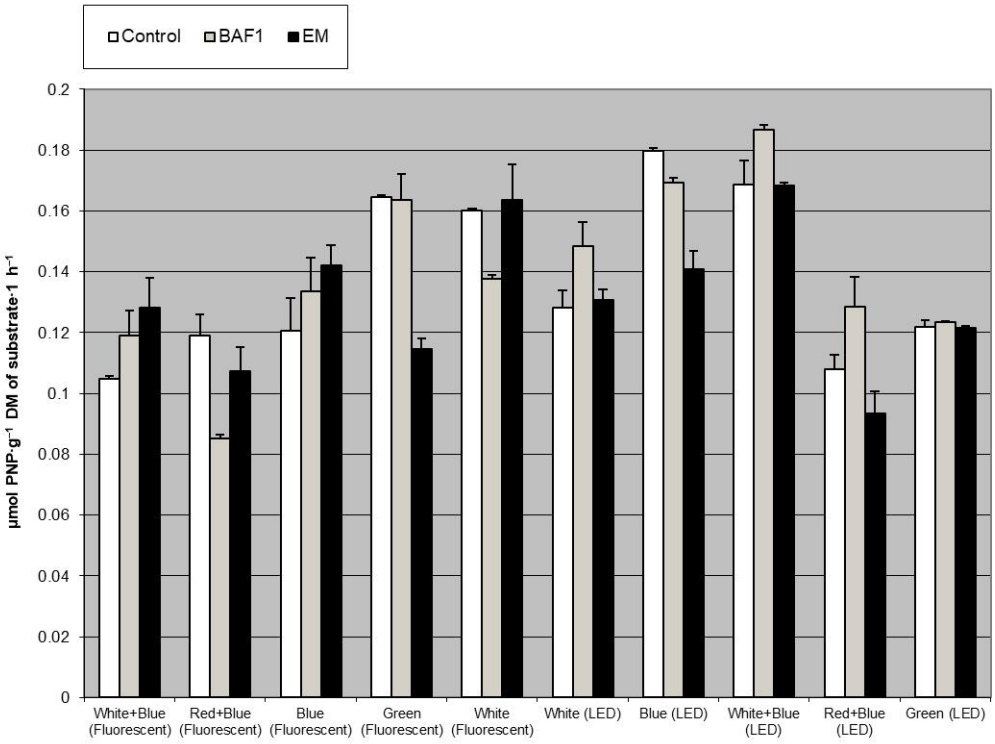

Fig. 7. Changes of the acid phosphatase activity level

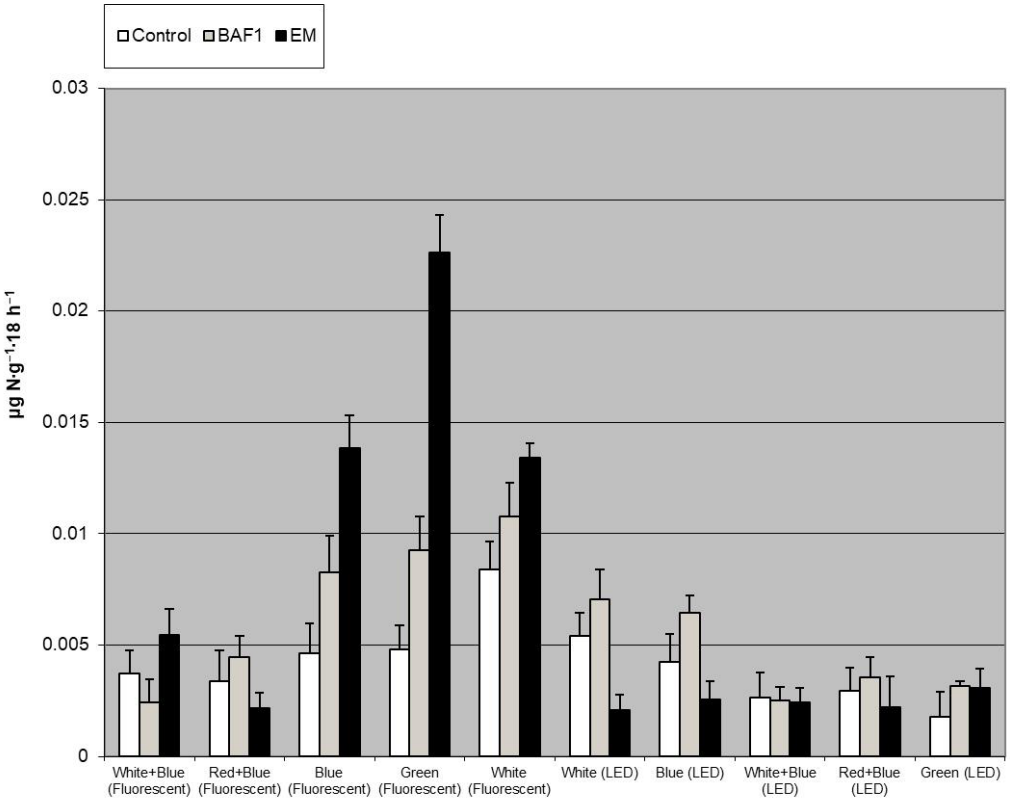

Fig. 8. Changes of the urease activity level

highest sensitivity to the influence of the blue color light. In these organisms, the light of lower intensity is sufficient for the purpose of photo morphogenesis induction, when compared to higher plants. In the opinion of Niewiadomska et al. [2010], it is this group of microorganisms that contributes to the natural cycle of biogenic elements through mineralization and mobilization of dead organic matter. According to Lioussanne et al. [2010], molds together with other microorganisms create a specific environment in the area of plant roots, i.e. mycorhizosphere. Due to that fact, plants obtain nutrients, thus their growth and productivity are stimulated. In the conducted experiment, it was the lamp type and the light color that had a significant influence onto the number of molds. Significantly low number of molds was observed in the substrate, in which plants were grown under fluorescent lamps emitting the blue+white, red+blue and green light. Regardless of the fact whether the plants were treated with inoculums or not, the blue color light emitted by LED lamps influenced the number of these organisms (Fig. 5). As stated by Shu et al. [2010], the blue color light emitted by fluorescent lamps has a retarding effect onto fungi multiplication in the substrate.

The physiological state of microorganisms in the given substrate can be determined on the basis of the enzymatic activity level. Dehydrogenases are enzymes classified as oxidoreductases. Their soil activity is strictly connected with respiratory metabolism of soil microorganisms. Dehydrogenase activity is considered to be a general indicator of the number and the activity of microorganisms in soil. The level of dehydrogenase activity determines the rate of oxidoreductase changes in a given substrate [Król et al. 2006]. In the research conducted by Ramirez et al. [2010], five light colors: white, yellow, red, blue and ultraviolet, were applied and their influence onto the peroxidase activity level was examined. Peroxidases, similarly to dehydrogenases, are also classified as oxidoreductases. On the basis of the obtained results, the authors stated that the green light color emitted by LED lamps had a

favorable (by over 20%) effect onto the peroxidase activity. Moreover, it also significantly increased the biomass production in the fungus under examination. The blue color light and the ultraviolet light significantly retarded the activity of this enzyme. As far as other light colors are concerned, the effect was diverse. In the own research as a result of using fluorescent lamps, the increase in dehydrogenase activity in the substrate inoculated with microbiological inoculums (especially after the application of BAF_1 inoculum) in comparison with the control group, was observed (Fig. 6). The substrate that was not treated with inoculums and was irradiated with LED lamps emitting the green light color, was characterized by the lowest activity of the enzymes in question. In the opinion of Tarafadar and Jungk [1987], the level of acid phosphatase activity depends on the contents of organic matter in the substrate. Moreover, plants may release those enzymes to the substrate in response to phosphorus requirement or stimulate microorganisms to their production. When analyzing changes in the activity of the discussed enzymes in the tested peat objects, the lowest activity of acid phosphatase was recorded at green light color emitted by fluorescent lamps. Acid phosphatase activity was stimulated by the use of the blue light color and the simultaneous treatment with blue and white light color emitted by LED lamps (Fig. 7). As far as urease is concerned, the tendency towards the activity growth was observed under the fluorescent lamps emitting the green, blue and white color light, especially after the application of EM specimen. Lower activity was observed after the treatment with blue+white and red+blue fluorescent light and also after the treatment with LED lamps (Fig. 8). In the opinion of Bais et al. [2006] and Walker et al. [2003], the composition of root exudates may provoke microorganisms to produce urease due to the increased nitrogen requirement of plants at the flowering phase.

CONCLUSIONS

The highest number of inflorescences was found after application of BAF_1 inoculum, especially under white color of light emitted by fluorescent lamps and blue color of light emitted by LED lamps. The total bacteria count was dependent on the light color, lamp type and the inoculum applied. The white color light emitted by fluorescent lamps stimulated the actinobacteria multiplication, especially after EM application. Regardless of the inoculum application, the increase in the number of moldy fungi was stimulated by the blue light emitted by LED lamps. After the use of fluorescent lamps, the increase in the dehydrogenase activity, especially after the application of BAF_1 inoculum, was observed. The activity of the acid phosphatase was stimulated by the blue light and white+blue light emitted by LED lamps. The increase in the urease activity was observed under the fluorescent lamps emitting the green, blue and white light after the EM application.

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