The use of biostimulant microalgae to influence the growth and development of ornamental plants

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Abstract. The article discusses the use of biostimulant microalgae, known for their bioactive compounds. Understanding the positive impacts of biostimulants is essential for future applications. Research conducted in the Department of Plant Sciences at the Széchenyi István University has revealed that algae produce plant hormones and possess beneficial properties that influence the water, soil and plant systems. The effects of microalgae on various ornamental plants are being studied with a focus on improving root and general plant development. The methodology involves testing different algae extracts in ornamental plants in controlled environments. Data collection includes measuring plant height, leaf and bud numbers, chlorophyll content and other plant parameters through laboratory and destructive tests. The results indicate positive changes in plant parameters after treatments with biostimulant microalgae. In conclusion, biostimulant microalgae offer a promising and eco-friendly alternative to synthetic chemicals in the cultivation of ornamental plants. Continued research and innovation in this field is crucial to realise the full potential of biostimulants in sustainable agriculture.

1 Introduction

Ornamental plants extensively utilized for landscaping and for both interior and exterior decoration. They are particularly effective in shading technology, especially during warmer seasons. The demand for such plants is rapidly increasing, prompting the exploration of various methods to enhance their growth and development. Biostimulants represent one such method capable of naturally boosting plant growth and development. Understanding the positive impacts of biostimulants is crucial for exploring future possibilities.

Beyond their ecological role in oxygen production and as fundamental components of aquatic food chains, algae carry significant economic importance as sources of crude oil, food, and numerous pharmaceutical and industrial products for human consumption. The cultivation of ornamental plants is an exceptionally important technological element involving the replenishment of necessary quantities and qualities of natural materials, nutrient supplements, soil improvers, and various plant conditioning agents into the soil, as well as into the plant-soil system. The number of microalgae products available in

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commercial markets worldwide is increasing. This is due to the fact that their favourable effects and useful properties are becoming increasingly well-known. Microalgae are most commonly used in plant cultivation as biofertilizers, soil conditioners, and growth regulators, but recently there has been a growing interest in their positive effects on plant growth as well. The visual appearance of flowering and foliage potted plants plays a crucial role in defining their quality.

The hormone production of algal strains from the Mosonmagyaróvár Algae Culture Collection (MACC) has been studied for almost two decades in the Department of Plant Sciences. Through bioassays and analytical methods, it has become apparent that both microalgae and macroscopic marine algae produce plant hormones [1], which makes them suitable for special plant treatments. Among other effects, they reduce transpiration, improve fruit formation, increase chlorophyll content in leaves, increase protein content in fruits, promote root and shoot development [2]. In addition to their use of solar energy, they have numerous beneficial properties that influence water, soil and plant systems: their extracellular polysaccharides (EPS), such as alginates, agars, carrageenans and fucoidans, are widely used in food, pharmaceutical, and other industries [3]. Furthermore, they produce plant growth regulators (PGR) and secrete antimicrobial agents [4]. The application of hormone-producing algae in suspension form to plants can lead to improvements in crop quality and yield and can address current plant protection issues [5]. Biostimulants are one of the methods that can naturally enhance plant growth and development [6]. Studies have been conducted in rapeseed and potatoes using growth-promoting algal preparations containing marine algal extracts to increase crop security [7]. Targeted experiments with specific hormone-producing strains have also been carried out to prevent harmful leaf abscission in sugar beets [8]. In recent years, the effects of some microalgae on paprika have been investigated [9]. Certain algae inhibit the proliferation of pathogenic plant fungi, prevent infection or indirectly reduce plant susceptibility and sensitivity to certain plant diseases [10].

In the Department of Plant Sciences, the effects of microalgae-derived abscisic acid (ABA), auxins, cytokinins, ethylene and gibberellins have been studied in plants [11, 12,13,14], as well as the capacity of certain strains to produce extracellular polysaccharides [15] and genus-level identification has been performed [16].

Based on the results acquired previously, our chosen task is to use MACC strains to positively influence the development of roots and shoots in the propagation of ornamental plants. It is crucial that during the process, the genetic makeup of ornamental plants remains unchanged, without mutation or degradation. In addition, it is essential to establish a resilient and virus-free stock. Throughout the execution of this task, proper data integrity and a statistically thorough evaluation of the results obtained are indispensable.

This research is based on the remarkable diversity of the Mosonmagyaróvár Algae Culture Collection, which offers numerous potential applications for industry and agriculture. Our aim is to use biostimulant microalgae to influence the growth and development of ornamental plants.

2 Methodology

The Department of Plant Sciences at the Faculty of Agriculture and Food Sciences of the Széchenyi István University, Mosonmagyaróvár, has the fifth largest collection of soil microalgae in Europe. The department provides all necessary conditions for the laboratory cultivation of the selected microalgae. The test plants will be subjected to two different types of algae extracts to see if there are positive (or negative) effects and if there is any difference that can be detected in the impact mechanisms of the treatments. Based on previous research results provided by the university, we concluded that ornamental plants will have the best

chance of a positive vital plant reaction using the two microalgae listed in Table 1, where 'T' stands for 'test' and 'K' for control.

Table 1. Suggested algae types from MAAC.

ID.	MAAC	Algae Name	Algae Type
T1	612	*Nostoc piscinale*	Blue algae
T2	922	*Chlorella vulgaris*	Green algae
K	Control	N/A	N/A

For the effective testing of the biostimulant microalgae on various plants, we are going to use a 300 m^2 foil tent located at 9167, Bősárkány, Győr-Moson-Sopron County, Hungary. (GPS coordinates: 47.690455 | 17.251585). When selecting the test plants, we need to ensure that all the selected plant types are commercially available on the market in the following years. This is important because if the relevant test results are proven to be positive, the same tests will have to be repeated at the same location, with the same algae types, with the same plant cultivar in the following 2 years. Furthermore, we would like to examine the impact of algae treatments on 3 different types of commercially available Pelargonium genotypes: (*Pelargonium* × *Zonale, Pelargonium* × *Peltatum, Pelargonium* × *Lateripes*) [17]. We have chosen the following ornamental plants for the tests listed in Table 2.

Table 2. Selected pelargonium genotype.

ID.	Type	Commercial name
A	*Zonale*	Andria ™
B	*Zonale*	Savanna Realy Red ™
C	*Peltatum*	Great Balls Of Fire ™
D	*Peltatum*	Atlantic Dark Red ™
E	*Lateripes*	Classic Single Villa Paris Lilac ™
F	*Lateripes*	Classic Single Villa Dresden ™

From each type of ornamental plant, we selected 10 test subjects for each treatment, which resulted in 180 test plants.

$$10 \text{ (test subject)} * 3 \text{ (treatment)} * 6 \text{ (plant genotype)} = 180 \text{ plants} \quad (1)$$

For statistical data analysis and proper, transparent and easily understandable graphics during evaluation, we need to create a matrix from the test subjects (See Table 3):

Table 3. Test subject matrix.

MAAC 612	MAAC 922	Control
AT1	AT2	AK
BT1	BT2	BK
CT1	CT2	CK
DT1	DT2	DK
ET1	ET2	EK
FT1	FT2	FK

Now that data integrity is planned, we have to select the following vital properties, features, and parameters so that we can indicate the impact of biostimulant microalgae treatment. Variables examined over time / developing plant parameters are plant height, number of leaves, number of buds, visual ranking (green mass and colour intensity).

2.1 Chlorophyl content

The most important source of energy for plant growth is photosynthesis [18], and one of the most important pigments of the plant is chlorophyll. This pigment largely determines the photosynthetic capacity, hence the growth of plants [19].

To determine and compare the chlorophyll content, chlorophylls can be extracted from the protein using organic solvents that allow the estimation of chlorophyll concentration within a leaf [20]. With these methods, we can determine the chlorophyll a, chlorophyll b, and carotenoid content [21, 22]. We have measured the chlorophyll content with a spectrophotometer (Varian™ Cary 50) in the Plant Sciences Laboratory of Széchenyi István University [23].

2.2 Application of biostimulant microalgae and execution of data collection

To The microalgae strains (MACC-922 and MACC-612) are derived from the MACC, Hungary. The strains were incubated at 25 ±2 °C, in a 12:12 light/dark cycle. The microalgae biomass was produced in laboratory culture units. It was illuminated from below with a light intensity of 130 µmol m-2 s-1 and grown in Tamiya nutrient solution [24], with a starting concentration of 10 mg L-1 algal dry weight. 20 L h-1 of filtered compressed air enriched with 1.5% CO2 during the light period was used for aerating the culture strains [25]. The cultures grown in these conditions for 10 days were then centrifuged for 15 mins at 3000 rpm and freeze-dried using Gamma 1–20 and stored at -18 °C. Biomass samples were re-suspended in distilled water and sonicated (VirSonic™ 600 Ultrasonic Cell Disruptor) before plant treatments. To collect adequate data, the first measurements of the previously determined critical properties were made directly after planting. The application of the microalgae extract (Concentration: 1g/l) directly to the root collar of the seedlings, happened right after the measurements. Later on there was no further microalgae application. The plants were potted in plastic (Teku VCG™ ⌀120 mm) pots with the volume of 0,69 l. The composition of the growing medium is the following:

- Peat (pre-moistened with tap water)
- Hawita™ Uni 20-II
- Slow-release fertiliser - Osmoco™ Exact High K. (Concentration: 3g/l)
- Biostimulant microalgae
- MAAC 922 / 612

2.3 Destructive testing

Parameters examined through destructive testing are plant height, root length, root collar diameter, (fresh) plant weight, (fresh) root weight, (dry) plant weight. The acquired data will be analysed in Windows™ 10 Home operating system, with Microsoft Excel™ and Mini-TAB™ statistical software. In addition to these parameters, we will look for any type of abnormalities, the development of phytotoxicity, or any undesired effect in the plants as a result of the treatments. To remove the plants from the growing medium, we first removed the plastic pot from each subject examined, then with a tap water stream we carefully washed the plants, so the parameters became visible and available. No detergent or washing agent was used for this process to avoid interference with the test results.

After cutting the plants, we were able to measure the plant height, the diameter of the root collar, and the length of the root. After measuring the chosen parameters of the dissected plants, we had to immediately bind the associated plant parts back together (for example, with zip-lock), with the related code name-tag, not to mix up the components and ensure the traceability during the whole time of the research. It was very important because the same

plants would be re-measured again after a month of drying to measure the dry weight of the plants as well (Figure 1).

Fig. 1. Dissection and wet/dry measurements of ornamental plants.

3 DATA EVALUATION

3.1 Leaf number

See the results of the counted leaf numbers evaluated with the help of the Minitab™ software in Figure 2. (Where DAP means days after planting).

The effect of algae treatments on the Peltatum and Zonale variety resulted in significantly higher leaf numbers compared to the control group. In the case of the Lateripes geranium no improvement in the number of leaves was observed in the treated populations.

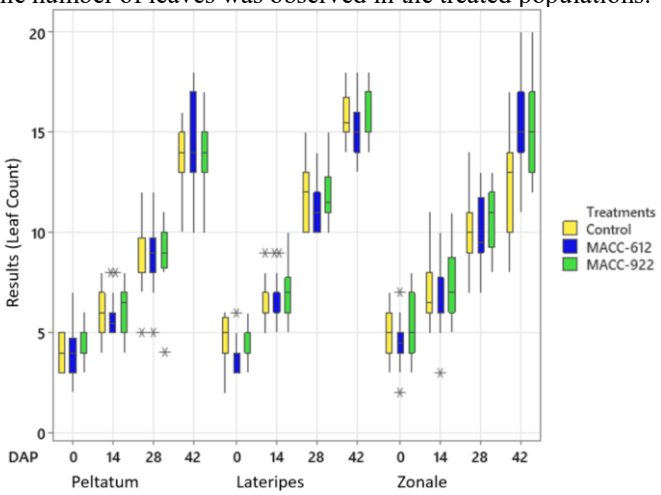

Fig. 2. Leaf number results

3.2 Plant height

See the measured height results evaluated with the help of Minitab ™ Software in Figure 3.

In the case of Peltatum (Hybrid, C and D genotype), both algae treatments positively influenced the growth of the plants. In the case of *Pelargonium Lateripes* (Trailing geranium, E, and F genotype), the green algae clearly had a beneficial effect on plant development.

However, in the case of *Pelargonium* × *Zonale* (Standing Geranium, A, and B genotype) there is no observable improvement in growth due to algae biomass treatment.

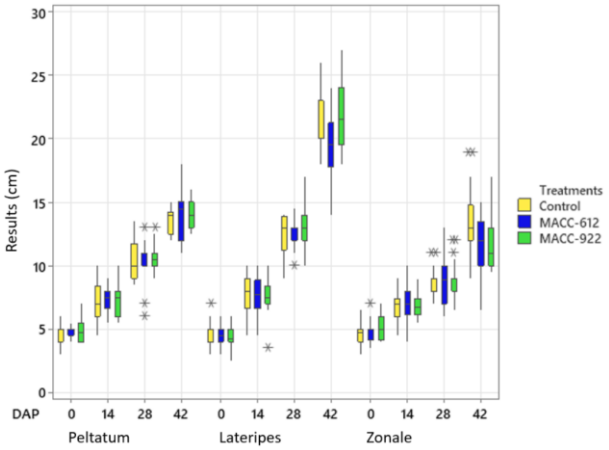

Fig. 3. Plant height results.

3.3 Bud number

See the results of the counted bud number evaluated with the help of the Minitab ™ software in Figure 4.

Bud formation started much earlier due to the treatment in *Pelargonium* × *Zonale*, and as time progressed, the treated plants exhibited a greater number of buds. However, in the case of the other plants, there was no observable difference in the aspect of the bud numbers between the control group and the threat subjects.

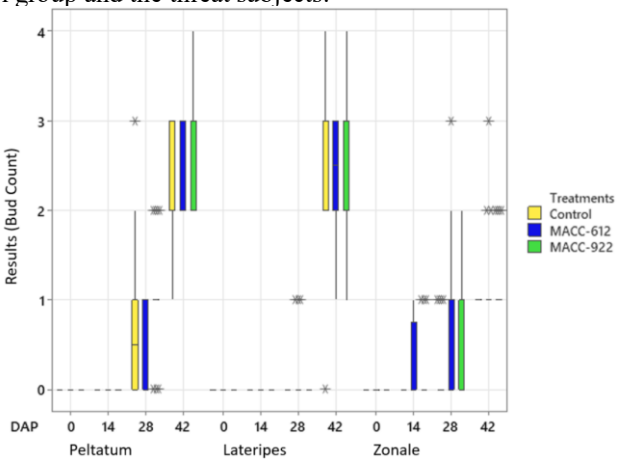

Fig. 4. Results of the number of bud numbers.

3.4 Visual ranking

See evaluation of the ranking of the subjective assessment of the examined plants with the help of Minitab ™ Software in Figure 5.

Subjective assessment indicates that treated populations received higher scores more often. The more colourful the plants were (had higher bud count) and the more volume they

had (with higher leaf count), they appeared to be more viable and had a higher chance of resistance to biotic and abiotic stresses during the life cycle.

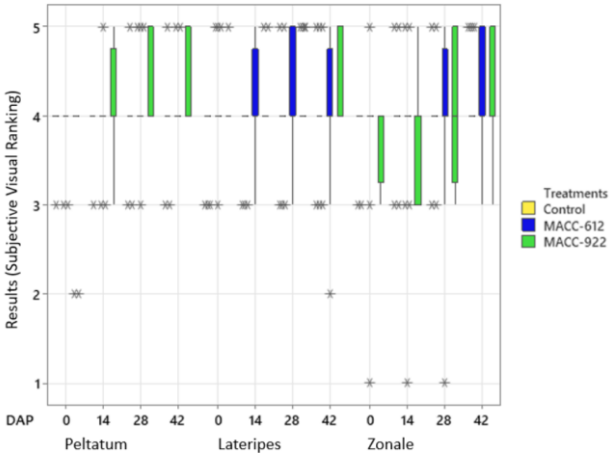

Fig. 5. Visual ranking

3.5 Chlorophyll content

See the results of the measurement of the chlorophyll content in Figure 6. (See the y-axis codes in Table 3.)

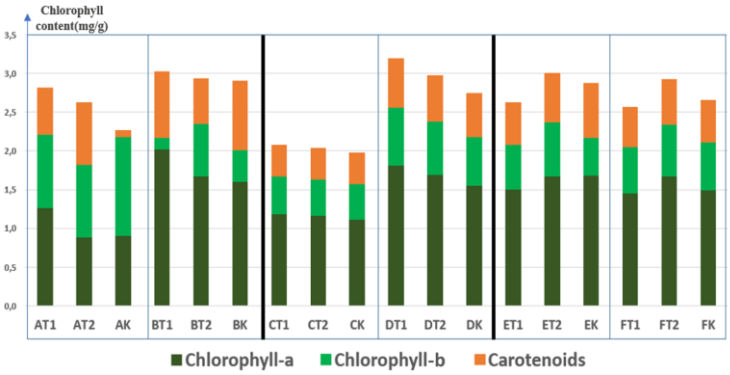

Fig. 6. Chlorophyll-a, chlorophyll-b and carotenoid content

In the case of *Pelargonium* × *Zonale*, the chlorophyll content changes positively due to algae treatments. For *Pelargonium* × *Peltatum*, algae treatments have a slightly lesser but detectably positive effect on the chlorophyll content of plants. For *Pelargonium* × *Lateripes* the green algae treatment shows a higher chlorophyll content.

3.6 Plant height

Figure 7 presents the average values of the measured data calculated in Excel software.

Pelargonium × *Peltatum* and *Pelargonium* × *Zonale* show a definitive positive reaction microalgae treatment. *Pelargonium* × *Lateripes* does not show significant differences between the treated and control groups in the matter of plant height.

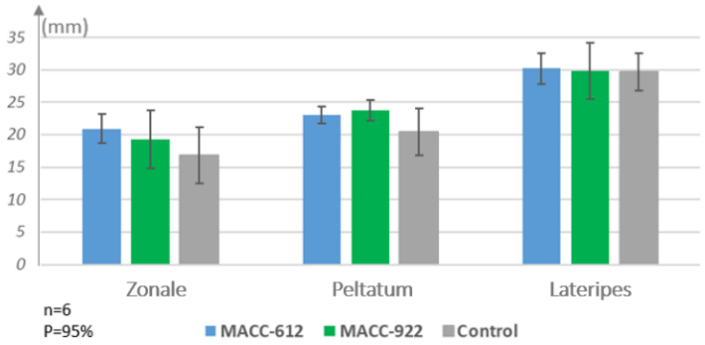

Fig. 7. Measured plant height

3.7 Root length

Figure 8 presents the average values of the measured data calculated in Excel software.

Pelargonium × *Lateripes* shows significantly better results in the case of green algae treatment, and both other geranium genotype respond well to biostimulant microalgae treatment.

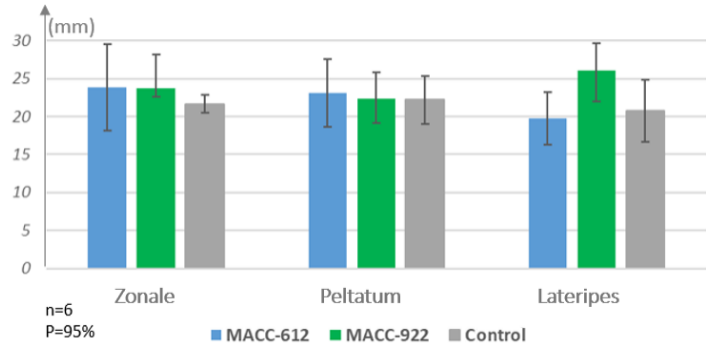

Fig. 8. Measured root length

3.8 Root collar diameter

Figure 9. presents the average values of the measured data calculated in Excel software.

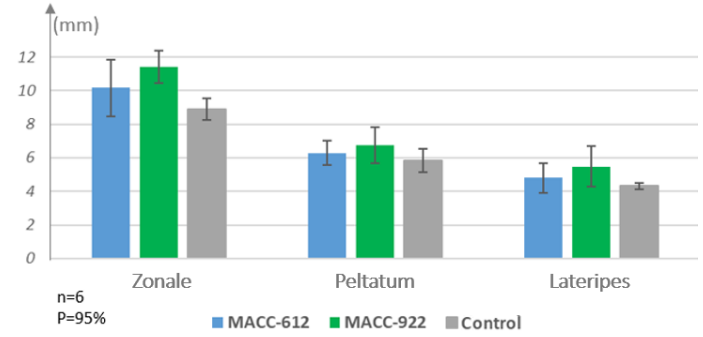

Fig. 9. Measured root collar diameter.

Pelargonium × *Zonale* shows an exceptionally positive reaction to green and blue algae treatments. This characteristic is extremely important because this is the bottleneck of

nutrient flow. The larger the diameter of the root collar, the better the plant's nutrient uptake, making the plant more resistant and viable.

4 CONCLUSIONS

Microalgae, renowned for their diverse bioactive compounds, are gaining attention as potential biostimulants because of their beneficial effects on plant growth and development. These biostimulants help improve nutrient mobilisation, stress tolerance, and overall plant quality. The method of application of biostimulants through root immersion positively influences plant metabolism. Based on the acquired results, we can observe that treatment with biostimulant microalgae resulted in a positive change in the selected parameters of the test plants. From this we can also infer that treated plants not only have a better appearance, but also have better chances of vitality during their annual life cycle compared to their untreated counterparts. Based on the statistical analysis of the measurement results obtained, it can generally be stated that ornamental plants treated with MACC 922 green algae yielded better results in the aspects examined compared to untreated plants and plants treated with blue algae. Biostimulant microalgae can be a viable and eco-friendly alternative to substitute hazardous synthetic chemicals and offers promising pathways for sustainable ornamental plant growth. Continued research efforts and innovation in this domain are imperative to fully exploit the potential of biostimulants in shaping the agricultural landscape of the future.

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