

USE OF MICROALGAE *SCENEDESMUS QUADRICAUDA* AND *CHLORELLA VULGARIS* LIVING-CELLS SUSPENSIONS FOR PLANT BIOSTIMULATION

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Abstract: Up to date research on microalgae as plant biostimulants remains fragmented and use of living microalgal cell suspensions is understudied. This research focuses on use of *Scenedesmus quadricauda* and *Chlorella vulgaris* living-cells suspensions as growth stimulants for garden cress (*Lepidium sativum*). Different concentrations of microalgal biomass suspensions were tested. It was found that plant treatment with *S. quadricauda* biomass 0.8 g/L (dry weight) suspension resulted in a significant improvement in *L. sativum* seeds germination and growth rate.

Key words: microalgae, plant biostimulants, plant growth, microalgal biomass, *Lepidium sativum*

Introduction

Currently chemical plant stimulants and synthetic fertilisers remain widespread and cause environmental damage. Microalgae is a valuable but at the same still understudied group of microorganisms, which is attracting an attention as a prospective mean for use as sustainable plant biostimulants (Colla and Rouphael, 2020). This is associated with microalgae ability to produce complex compounds, e.g., lipids, proteins, antioxidants, antimicrobial compounds, polysaccharides, phytohormones. However, up to date the use of microalgae as a sustainable plant biostimulant remains limited (Chiaiese et al., 2018).

Current research shows that microalgal biostimulants trigger natural processes in crops improving nutrient uptake, as well as promoting tolerance to abiotic stress (Colla and Rouphael, 2020). These microalgal properties can promote the replacement of chemical biostimulants or biofertilizers with nature-friendly and sustainable alternatives. Mainly use of microalgal biomass in a form of extracts has been reported up to date, i.e., cell wall is initially disrupted or extraction of bioactive compounds from the cell has been performed by mean of varied solvents and techniques (Colla and Rouphael, 2020; Bella et al., 2021). For instance, in a study conducted by Bella et al. (2021) *C. vulgaris* extract positively influenced the growth of lettuce seedlings, by increasing shoots growth and plant biomass production, chlorophyll and carotenoids concentration in leaves, as well as protein content. However, it can be assumed that use of living cells as foliar sprays

can decrease the costs associated with biomass post-processing and potentially stimulate the synthesis of phytohormones and anti-microbial compounds.

This study focuses on evaluation of effects of living-cells biomass of *Scenedesmus quadricauda* and *Chlorella vulgaris* for treatment of garden cress (*Lepidium sativum*) seeds during germination and further growth in nutrient depleted soil substrate.

Methods

Microalgal strains

Two freshwater microalgae *Scenedesmus quadricauda* CCAP 276/16 and *Chlorella vulgaris* CCAP 211/111 were obtained from Culture Collection of Algae and Protozoa (CCAP, United Kingdom). Both strains were maintained and cultivated in Bold's basal medium with triple nitrogen and vitamins (3N-BBM-V) (Yee et al., 2019).

Preparation of microalgal biomass suspensions

S. quadricauda and *C. vulgaris* were cultivated statically for 21 days in order to achieve high biomass density. Photoautotrophic cultivation has been performed in presence of LED light source (3000 lux), day : night cycle 16 : 8 h, at 25 °C. After the cultivation microalgal was harvested by centrifugation at 6000 rpm for 5 minutes. Supernatant was decanted and the biomass resuspended in distilled water which followed by centrifugation. This procedure was repeated twice to remove medium residues. Subsequently, obtained microalgal biomass was resuspended in distilled water at three concentrations (0.4, 0.8 and 1.2 g/L) and used as foliar spray.

Assessment of plant germination

In order to assess the germination rate, *L. sativum* seeds were placed into containers with 50 g of dry nutrient depleted soil substrate. The soil substrate was watered every two days with tap water. Additionally, 5 mL of microalgae biomass spray (cell suspension) at 0.4 g/L concentration (water as control) was added to respective containers at 1st and 3rd day. After 5 days seed germination rate was calculated.

Plant growth experiment

For assessment of changes in plant shoot system length and plant biomass production, 10 *L. sativum* seeds were placed in pods with 50 g of dry nutrient depleted soil substrate. Each experimental group was grown in triplicates. Approximately 5 mL of microalgal biomass suspension at 0.4, 0.8 and 1.2 g/L concentration was sprayed at plants once a day every two days. For the control group 5 mL of tap water was used instead. Additionally, each container was watered with 10 mL of tap water once a day every two days. After 8 days the length and dry weight of plant shoot system was measured and compared to control. Experiments were performed in controlled environment chambers with 16 : 8 day : light cycle and 22 °C temperature.

Data analysis

SPSS (BM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, USA) was used in order to compare means (analysis of variance) at significance level $p = 0.05$.

Results and discussion

Our research shows, that use of live microalgal cells suspension can promote the germination of *L. sativum* seeds in nutrient depleted soil compared to control (Table 1). After five days of treatment with *S. quadricauda* CCAP 276/16 suspension 60% seed germination rate has been found. Additionally, *C. vulgaris* CCAP 211/11 also promoted the germination of 20% of seeds. Noteworthy that there was no seed germination observed in the control group with no treatment with microalgal suspensions applied. This can be associated with increase of bioavailability of minerals, and presence of growth promoting factors, i.e., phytohormones, provided by microalgae. Additionally, a significant increase in water holding capacity was found in microalgae supplemented soil. It seems, that living microalgal cells can promote the soil moisture for longer time compared to control group which required additional watering.

Analysis of shoot system's length showed, that use of live microalgae cells suspension can promote the length of shoot system (Figure 1). It was demonstrated, that *S. quadricauda* promoted a significantly greater increase in *L. sativum* length compared to *C. vulgaris*. Therefore *S. quadricauda* suspension was used in further studies in order to assess the optimal concentration of microalgal cell suspension. Additionally, the untreated control group showed a significantly lower growth rate compared to microalgal supplemented cell groups.

Table 1. Germination of *L. sativum* seeds after 5 days in the nutrient depleted substrate with negative control (water) and *S. quadricauda* or *C. vulgaris* suspensions (0.4 g/L) moisturised containers

Germination rate			
	Control	<i>S. quadricauda</i>	<i>C. vulgaris</i>
Germinated seeds, %	0	60	20

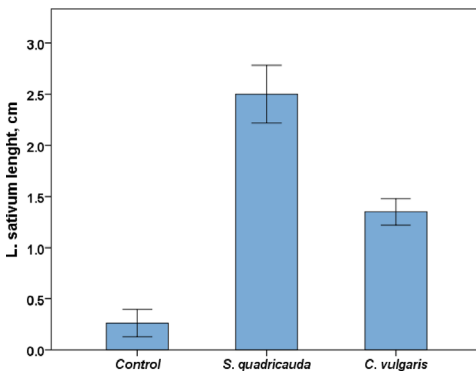


Figure 1. The length of shoot system of *L. sativum* after 5 days of growth in the nutrient depleted substrate with negative control (water) and *S. quadricauda* or *Chlorella vulgaris* suspension at 0.4 g/L treated containers

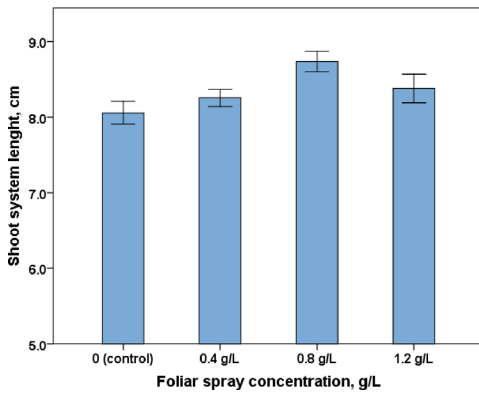


Figure 2. The length of shoot system of *L. sativum* after 8 days of growth in the nutrient depleted substrate with negative control (water) and *S. quadricauda* suspension (0.4, 0.8, 1.2 g/L) treated containers

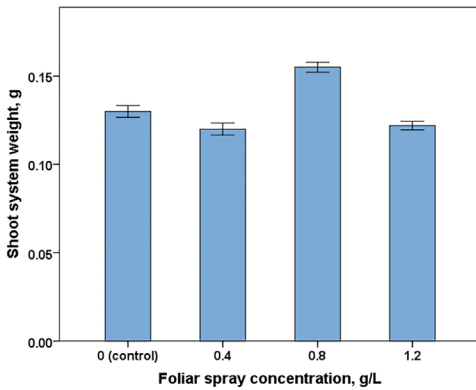


Figure 3. The dry weight of shoot system of *L. sativum* after 8 days of growth in nutrient depleted substrate with negative control (water) and *S. quadricauda* suspension (0.4, 0.8, 1.2 g/L) treated containers

Subsequently, different concentrations of selected microalgae (*S. quadricauda*) cell suspension sprays were tested (Figure 2). A statistically significant ($p < 0.05$) difference between experimental groups was found for microalgal suspension with 0.8 g/L of biomass. As shown in Figure 2, use of cell suspension with higher biomass concentration (1.2 g/L) showed a slight inhibition of plant shoot system growth. This can be associated with excess presence of compounds responsible for growth rate, for example phytohormones (abscisic acid) or relevant compounds (Wang et al., 2022). Further studies should focus on detection of specific stimulants and growth effecting factors present in *S. quadricauda* biomass.

Additionally, plant group treated with 0.8 g/L suspension showed highest increase in dry weight of its shoot system. Such improvement can be associated with increase in uptake of nutrients that microalgal biostimulants can promote (Colla and Roupael, 2020).

Further research should be carried out to verify whether the improvement was connected to the phytohormonal activity of microalgae or with the release of additional nutrients from the microalgal biomass. Overall, higher germination rate and faster growth of *L. sativum* when microalgal biomass applied on the seeds or plant shoot system were observed.

Similar improvements in plant growth were observed after use of *Desmodesmus subspicatus* microalgal extracts at 0.5, 1.0, 1.5, and 2.0 g/L biomass concentrations. Additionally, a 0.4 g/L extract significantly improved leaf areas and development compared to lower as well as higher extract concentrations (Mazepa et al., 2021).

Conclusion

Results indicate that use of *S. quadricauda* and *C. vulgaris* biomass resulted in a significant improvement of seed germination and plant growth compared to negative control groups. Among tested microalgae the treatment with *S. quadricauda* biomass had a higher positive effect on seed germination and shoot system length compared to control with no microalgal biomass applied or *C. vulgaris* suspensions. Assessment of different concentrations of *S. quadricauda* biomass suspension showed, that at 0.8 g/L the most pronounced impact on *L. sativum* growth can be achieved. Further studies are required in order to select suitable microalgal strains for plant biostimulation, assess optimal biomass concentrations in suspensions and to evaluate the effect of microalgae on specific plant developmental stages.

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