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THE CAPACITY OF SOIL MICROALGAE TO IMPROVE GERMINATION AND INITIAL GROWTH OF WHITE RADISH AND KOHLRABI

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SUMMARY

*Biofertilizers and biostimulators have become alternative sources of plant nutrients. Biofertilizers derived from microalgae represent a new approach in plant production. The aim of the study was to examine the capacity of the microalgal cell suspension to improve germination and initial growth (root and shoot length) of white Icicle Radish (*Raphanus sativus*) and Kohlrabi (*Brassica oleracea* var. *gongylodes*). By applying soil microalgal biofertilizer, initial growth was improved. Compared to the control, the root length of white radish was increased by 64.24% and 41.32%, using *Chlorella* sp. and *Dictyosphaerium* sp. cell suspension, respectively. Kohlrabi root growth was stimulated by application of *Chlorella* sp. and *Dictyosphaerium* sp. suspension by 60.97% and 55.02%, respectively. A significant difference in the shoot length was recorded, as well.*

Key words: *Chlorella* sp., *Dictyosphaerium* sp., white radish, kohlrabi, germination

Abbreviations: C45 – *Chlorella* sp.; D37 – *Dictyosphaerium* sp.; dH₂O – distilled water

INTRODUCTION

The human population is growing globally. It is estimated that the population is going to be doubled (9 billion people) by 2050, which means that crop yield has to increase to ensure enough food (Raja, 2013). On the other hand, new strategies in crop production have to provide food safety and agricultural production sustainability. Biostimulants and biofertilisers are considered as environmentally friendly and cost-effective alternatives to synthetic fertilisers (Kawalekar, 2013). Biofertilisers are products containing living microorganisms or natural substances that can improve soil chemical and biological properties, stimulating plant growth, and restoring soil fertility (Rongachner, 2012). Microalgae have the ability to grow and reproduce themselves in freshwater and marine environments (Nabti et al., 2017), but also in soil. There are more than 800.000 microalgae species, while 50.000 species are described (Ronga et al., 2019), but only a few species are in use for widespread application. The microalgal species examined as potential components of biofertilizers primarily belong to the branch of blue-green (*Cyanophyta*) and green (*Chlorophyta*) algae (Hernandez et al., 2009; Bumandalai & Tserennadmid, 2019). Microalgae have proven to be suitable for use in plant production because they contain micronutrients as well as macronutrients. They are usually used as soil amendment or plant growth stimulator applied by foliar spraying. Seed growth with a significant enhancement of soluble carbohydrate, soluble protein and total free amino acids content can be improved compared to the control. *Chlorella vulgaris* based biofertilisers can increase growth and physiological response of the treated seeds. By its application, it is possible to increase seedlings dry weight, yield and pigment content (Hajnal-Jafari et al., 2020a). The effects of green alga *C. vulgaris* cell extract as a foliar fertiliser on the nutrient status, growth and yield of wheat plants were studied and compared to a commercial

synthetic micronutrient foliar fertiliser (Shaaban, 2001). The best results were obtained with 50% and 100% v/v of *C. vulgaris* microalgal extract. *C. vulgaris* can improve germination, fresh and dry weight of lettuce seedlings, and its pigment content when applied to soil (Faheed & Abd-El Fattah, 2008). Results of Vig et al. (2012) indicated no difference in achieving higher yields in corn production between two biofertilisers containing *Chlorella sp.* extract, living cells, and powdered *Chlorella sp.* cells. *Dictyosphaerium sp.* is a single-celled microalgae, highly valued for its exopolysaccharides production (Kumar et al., 2017). The genus *Dictyosphaerium* consists of 16 species and these algae have specific growth of oval cells in groups ranging from 4 to 32 (Bock et al., 2011). *Dictyosphaerium sp.* is also highly efficient in nutrients removal and ecological inactivation of pathogens in wastewater (Xinjie et al., 2019). However, to the best of our knowledge and based on the available literature, there are no data about the effects of *Dictyosphaerium sp.* on seed germination and plant growth so far.

The goal of this study was to evaluate the capacity of two green microalgae to promote germination and initial growth of white radish (*Raphanus sativus*) and kohlrabi (*Brassica oleracea* var. *gongylodes*) in *in vitro* conditions. This paper will provide information for possible application of microalgae suspension, and its biofertilizer potential in agriculture.

MATERIAL AND METHODS

Two soil microalgae *Chlorella sp.* (C45) and *Dictyosphaerium sp.* (D37) were used in the experiment (Algae Collection, Faculty of Agriculture, University of Novi Sad, Serbia). *Chlorella sp.* is single-celled green algae. It has an ellipsoid cell shape, size 4-10µm (young and adult) (Fig. 1a), usually smaller than cells of the genus *Dictyosphaerium*. *Dictyosphaerium sp.* cells are coccoid, mostly in colonies from 4 to 32 cells (Fig. 1b), adult cell size 6-8µm. Before application, morphological observations were made using light microscope (Motic, BA210).

All investigations were carried out in Laboratory of Microbiology at Faculty of Agriculture, University of Novi Sad. Algal strains were multiplied in BG11 medium with the following composition (for 1 l of distilled water): 1.5 g NaNO₃, 0.04 g K₂HPO₄, 0.075 g MgSO₄×7H₂O, 0.036 g CaCl₂×2H₂O, 0.006 g Citric Acid, 0.006 g Feri amonium citrate, 0.001 g EDTA, 0.02 g Na₂CO₃ and 1 ml microelements A5 solution. A mixture of A5 microelements (11 dH₂O) consisted of: 2.86 g H₃BO₃, 1.86 g MnCl₂×4H₂O, 0.22 g ZnSO₄×7H₂O, 0.39 g Na₂MoO₄×2H₂O, 0.08 g CuSO₄×5H₂O, 0.05 g Co(NO₃)₂×6H₂O. Algal growth was carried out as batch cultures with a volume of 250 ml in 500 ml Erlenmeyer glass flasks at room temperature (24±2°C), controlled light: dark photoperiod 12:12 and a light intensity 100µmol photons m² s⁻¹. Aeration was enabled using an open environmental shaker. Two weeks later, the obtained algal biomass was centrifuged and 1 g of fresh biomass was used to prepare an algal cell suspension mixture with sterile distilled water (dH₂O).

Figure 1. Microscopic view of a) *Chlorella sp.* (C45) and b) *Dictyosphaerium sp.* (D37) (magnification x 400)

Seeds were treated with 1% microalgal cell suspension while sterile dH₂O was used for the control. Microalgal cell suspension were applied to white icicle radish (Ledena sveća) and kohlrabi (Bečka bela) seeds (NSSeme, Institute of Field and Vegetable Crops, Novi Sad, Serbia) after sowing on filter paper (substrate) in Petri dishes.

In order to determine the capacity of soil microalgae to improve germination and initial growth of two vegetable cultures, the following parameters were examined: germination percentage (%), roots and shoots lengths (cm) and fresh and dry weight (g) of seedlings. According to the ISTA (International Rules for Seed Testing, 2020), 100 seeds were used per replicate. Algal cell suspensions and dH₂O were added to Petri dishes (Fig. 2) containing Whatman

filter paper after sowing 100 seeds. Dishes were placed in the thermostat (UT-800, Sutjeska) at 28°C. Germination (%) was counted when the radicals protruded through the seed coat, for 5 days (White Radish), and 9 days (Kohlrabi) after sowing. After this, 25 seeds per replicate (100 seeds in total) were chosen and placed on cellulose wadding (15×45cm) previously moistened in the same way (1% C45, 1% D37, and dH₂O). Lanes were rolled up, put in closed plastic bags and incubated in the dark to assess the impact on initial growth. Firstly filter paper and later cellulose wadding were moistened daily with 1-5 ml of sterile dH₂O for the next seven days. The root and shoot length of 100 seedlings were measured with a ruler, from the seed to the end portions. Fresh and dry weight of seedlings was evaluated at the end of the experiments, on a laboratory scale. The constant weight of dry seedlings was achieved in an oven at 70°C after 4 hours.

Figure 2. White radish (above) and kohlrabi (below) treated with dH₂O (a) and *Chlorella sp.* (b) and *Dictyosphaerium sp.* (c)

The experiment was carried out in four replicates. The obtained data were processed by applying the analysis of variance (ANOVA) and least significant differences (LSD), by R program ver. 3.6.3. The results are reported as mean values and the associated standard deviation. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS AND DISCUSSION

White radish initial growth parameters affected by *Chlorella sp.* and *Dictyosphaerium sp.*

The obtained results suggested that the germination percentage of white radish was not improved by the microalgal treatments (Tab. 1). Nevertheless, the morphological parameters such as shoot and root length were significantly different in comparison to the control treatment.

The length of root (9.46 ± 1.1 cm) and shoot (4.48 ± 0.23 cm) were the highest at seedlings treated by C45 and D37, respectively. As compared to the control, the growth of roots increased by 41.32% (D37) and 64.24% (C45), while the shoot length increased by 24.10% (C45) and 34.94% (D37) compared to the control.

The highest fresh and dry weight biomass was obtained on seedlings treated by 1% *Chlorella sp.* cell suspension.

The weight of the fresh biomass was increased by 31.19% (D37) and 43.84% (C45), while the dry biomass yield increased by 21.64% (D37) and 33.67% (C45) in comparison to the seedlings of white radish treated with dH₂O. Both *Chlorella sp.* and *Dictyosphaerium sp.* influenced the dry weight, root and shoot length in similar way, in relation to the untreated control.

Table 1. Germination and morphological changes in white radish seedlings treated with *Chlorella sp.* and *Dictyosphaerium sp.*

Treatments / Parameters	Germination (%)	Length (cm)		Total biomass (g)	
		Root	Shoot	Fresh	Dry
<i>Dictyosphaerium sp.</i>	96.00 ± 2.64 a	8.14 ± 0.99 a	4.48 ± 0.23 a	1.4454 ± 0.05 b	0.1203 ± 0.004 a
<i>Chlorella sp.</i>	97.22 ± 2.31 a	9.46 ± 1.10 a	4.12 ± 0.48 a	1.5848 ± 0.03 a	0.1322 ± 0.002 a
Control	95.66 ± 3.51 a	5.76 ± 0.54 b	3.32 ± 0.22 b	1.1018 ± 0.01 c	0.0989 ± 0.007 b
p-value	ns	**	**	**	**

Values within columns followed by different letters are significantly different ($P < 0.05$ *; $P < 0.01$ ** ; ns-non significant, LSD test)

Kohlrabi initial growth parameters affected by *Chlorella sp.* and *Dictyosphaerium sp.*

The results of the growth parameters obtained for the germination and seedling initial growth of kohlrabi are given in Table 2. None of the treatments had a significant effect on the kohlrabi germination.

Chlorella sp. treated seedlings achieved the highest length of root (4.33 ± 0.50 cm) and shoot (4.93 ± 0.15 cm) of kohlrabi seedlings. The root length of kohlrabi was significantly increased by 55.02% (D37) and 60.97% (C45) in comparison to the control. The shoot length was also significantly increased by 15% for both treatments compared to the control (Tab. 2).

The fresh weight of kohlrabi increased by 12.39% (D37) and 21.71% (C45). Dry weight of kohlrabi seedlings was higher by 16.93% (D37) and 74.28% (C45), as compared to the control.

Table 2. Germination and morphological changes in kohlrabi seedlings treated with *Chlorella sp.* and *Dictyosphaerium sp.*

Treatments / Parameters	Germination (%)	Length (cm)		Total biomass (g)	
		Root	Shoot	Fresh	Dry
<i>Dictyosphaerium sp.</i>	68.33 ± 4.51 a	4.17 ± 0.88 a	4.89 ± 0.25 a	1.1314 ± 0.02 b	0.1091 ± 0.001 b
<i>Chlorella sp.</i>	70.66 ± 5.51 a	4.33 ± 0.50 a	4.93 ± 0.15 a	1.2253 ± 0.03 a	0.1626 ± 0.005 a
Control	68.00 ± 3.60 a	2.69 ± 0.52 b	4.28 ± 0.37 b	1.0067 ± 0.03 c	0.0933 ± 0.003 c
p-value	ns	**	*	**	**

Values within columns followed by different letters are significantly different ($P < 0.05^*$; $P < 0.01^{**}$; ns-non significant, LSD test)

Biofertilizers containing living algal cells can accelerate certain microbial processes in soil which augment the extent of nutrient availability in a form which is easily assimilated by plants. *C. vulgaris* microalgae is an efficient, economical and safe biofertilizer to substitute for chemical fertilizers in enhancing plant growth (Agwa et al., 2017). When it comes to *C. vulgaris*, the results obtained by Hajnal-Jafari et al. (2020b) related to the growth and development of Swiss chard were in accordance with our data. In order to assess the effects of *C. vulgaris* on agronomic and physiological responses, the authors studied the growth parameters in Swiss chard plants using *C. vulgaris* as a biofertilizer. By its application, yield and pigment content were increased proportionately by higher concentration of algal cell in suspension. The best increase in plant development was achieved in treatments where 10% *C. vulgaris* suspension was applied foliarly. Leaf number (80%), stem length (31.81%), root length (12.59%), fresh weight of stem (54.14%) and root (117.65%), total chlorophyll (4.17%) were increased.

Our results showed that application of *Chlorella sp.* and *Dictyosphaerium sp.* had highly significant effect on the initial growth of white radish and kohlrabi seedlings. However, there was no significant effect on germination, which is contrary to the results of Grzesik & Romanowska-Duda (2014). Their research showed that application of *Chlorella sp.* monocultures to the conditioned corn seeds and roots significantly increased the number of the germinated seeds. It also accelerated the growth of seedlings, exhibited faster elongation of the roots (+27.27%) and leaves (+26.24%) and enlarged their fresh (+10.67%, +9.09%) and dry (+20%, +14.29%) biomass.

The presented results of fresh and dry weight are in line with data obtained by Faheed and El Fattah (2008). According to the authors, fresh alga *C. vulgaris* was a suitable substrate for germinating the seeds of lettuce (*Lactuca sativa*), as it significantly enhanced the process of germination (fresh and dry weight of seedlings) compared to the untreated control. The amount of pigments (chlorophyll a, chlorophyll b and carotenoids) in the lettuce seedlings also increased significantly. The best treatment was obtained using 1g of fresh microalgae per kg of soil. Plant responses to the nutrients supplied foliarly are more rapid than applications as a soil amendment (Marschner, 1995; Ronga et al., 2019). Shaaban (2001) investigated the effect of *C. vulgaris* extract on wheat (*Triticum sp.*) growth and yield. His results indicated that application of 50% v/v of microalgae extract was the best treatment in terms of fresh weight (+60.7%) and yield enhancement (+140%) compared to the control. Dry weight (+95%) and 100 seeds weight (+160%) also increased as a result of the application of 100% microalgae extract. Similar results were obtained by Hajnal-Jafari et al. (2016). The authors showed that fresh weight of lettuce plants increased by 56.34% after foliar application of *Chlorella vulgaris*. On the other hand, there are no literature data on the capacity or effectiveness of *Dictyosphaerium sp.* application in plant production. Apart from using *Dictyosphaerium sp.* mainly for removal of wastewater nutrients, our experiment results suggested that it could be very promising when applied for vegetable seed treatment. The measured parameters in both investigated vegetables were similar to the ones obtained in *Chlorella sp.* treated seeds/seedlings, which could be due to the fact that *Dictyosphaerium sp.* produced growth promoting substances. *Dictyosphaerium sp.* might be considered as an alternative biofertilizer due to its fast growth rate and high biomass accumulation, high content of water soluble carbohydrate, as well as strong antioxidant capacity (Wang et al., 2018).

CONCLUSION

White radish and kohlrabi responded in similar manner to the microalgal treatments. No significant influence on the seed germination was achieved in comparison to the untreated control. The best results were obtained with *Chlorella* sp. suspension. *Chlorella* sp. led to statistically significant stimulation of the fresh and dry biomass as well as the root and shoot length of white radish and kohlrabi seedlings. The results indicated that *Chlorella* sp. and *Dictyosphaerium* sp. could be good potential growth promoters in white radish and kohlrabi production.

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