STIMULATING EFFECT OF MICROALGAE ON GERMINATION AND INITIAL GROWTH OF RED RADISH (*Raphanus sativus* L. *var. Radicula* Pers.)

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Abstract
Microalgal application in plant producton are becoming promising alternative practice aiming to enhance seed germination performance. Red radish is one of the most commonly eaten vegetables in early spring in Serbia. The effect of two microalgae on red radish germination and growth promotion was tested. Two concentrations (1 and 2%) of *Chlorella sp.* (strains 71 and 72) suspension were prepared. The root and shoot length, weight of fresh biomass and germination percentage were assessed. The highest germination, root and shoot length were determined after *Chlorella sp.* strain 71 treatment with 2% concentrated suspension. Lower concentration of the same microalga led to the highest fresh shoot biomass. Red radish root (+106 %) and shoot length (+27%) were increased by 1 % and 2% *Chlorella sp.* (72) water suspension, respectively.

Introduction
Healthy and good quality product can be obtained by microalgae application while respecting the environment and maintaining soil fertility through the optimal use of resources. Algal biofertilizers contain live or dormant (metabolically inactive) cells. They can be applied to the soil, used for seed priming [1], or foliarly [2]. Plant growth stimulation is achieved throughout microbes mechanisms such as symbiotic associations, biological control (by production of antibiotics, siderophores, volatile substances or parasitism) and by direct delivery of growth hormones to plants [3].
Regarding the use of biofertilisers, recent published works indicate the positive effect of microalgae on seed germination and plant development. Results of [4], showed enhancement of wheat, maize, bean and lettuce growth parameters for 7.9 to 34.2%. The usage of microalgal based biofertilizers enhanced the growth parameters, fruit yield and vitamin C content in tomato plants [5].
Considering that *Chlorella vulgaris* have been successfully applied as a biostimulant to several crops ([6]; [1]; [7]), this microalga was used in our experiment. This study has been undertaken to estimate the ability of two *Chlorella sp.* strains to stimulate the initial plant growth of red radish. Their effect on red radish germination, fresh biomass and length of root and shoot was evaluated.

Experimental
Two strains of single celled green algae *Chlorella sp.* (strain 71 and 72) form Algae Collection, Faculty of Agriculture, University of Novi Sad, Serbia were used for experiment. *Chlorella sp.* has an ellipsoid cell shape, cell size ranging between 4 to 10μm.
Red radish (*Raphanus sativus* L. *var. Radicula* Pers.) seeds (variety Verica, NSSeme, Institute of field and vegetable crops, Novi Sad, Serbia) were sterilized and then germinated on Whatman filter paper in Petri dishes. Sterilized distilled water (dH_2O) was used as control treatment, while *Chlorella sp.* treatments were prepared as 1 and 2 % suspensions, respectively. Algal strains were grown in BG11 medium for three weeks with periodically added fresh medium. Semi-controlled conditions were obtained during algal growth. Day:night photoperiod 14:10, room

temperature (25°C) and aeration with an aquarium air pump (Champion CX-0088) were maintained. Stock cultures were prepared after centrifugation, using fresh algal biomass. Final algal treatments were prepared by dilution of biomass in dH_2O to the concentration of 1% and 2%.

The experiment was carried out in three replications and completed after 12 days. Germination percentage (%), length (cm) and fresh weight (g) of roots and shoots were measured. According to the ISTA [8], 100 grains were used per replicate for germination assay. Four days after sowing, germination (%) was determined. 100 seedlings were placed in thermostat at 28 °C on wet wadding for initial growth measurements. Eighth days after, fresh weight, roots and shoots lengths were measured.

The data were statistically processed using R software (ver. 4.0.2). The significance of the difference between the applied treatments was determined using Fisher's LSD test ($p<0.01$).

Results and discussion

This research showed that microalgal suspensions stimulated red radish germination and initial growth. Measured parameters average values with standard deviations were determined and presented in Table 1.

The results showed that algal water suspension enabled better germination of treated radish seeds. Lower concentration of algal suspensions led to an increase of 4.67-10 % while higher concentration led to 14.33-21.33 % higher germination than the control. Germination increased as the algal suspension concentrations of both strains increased. Microalgae produce a wide array of biologically active compounds responsible for germination related processes improvement [1]. Our results are supported by the results of other researchers. [14] reported that *Chlorella sp.* strain 56, could enhance germination of barley and wheat seeds, respectively. [7] confirmed that 1 and 2 mg/L of *Chlorella sp.* extract significantly increased the germination percentage of beet. Higher dosages of the same extract did not significantly affect the germination. Our data suggested that the algal suspensions positively affected the germination process at both investigated concentrations. [9] also stated that *Chlorella sp.* extract stimulated the germination of cress.

Table 1. Average values of germination (%), root and shoot length (cm) and fresh biomass (g) of red radish seedlings

Treatments	Germination (%)	ROOT				SHOOT			
		Length (cm)	Min. Length (cm)	Max. Length (cm)	Fresh biomass (g)	Length (cm)	Min. Length (cm)	Max. Length (cm)	Fresh biomass (g)
H_2O	69.33%±2.88 ***c***	3.65±0.61 ***c***	0.8	9.3	1.12±0.01 ***e***	3.41±0.28 ***d***	1	6.3	4.68±0.11 ***d***
Chlorella sp. **71 (1%)**	79.33%±7.02 ***ab***	7.52±0.61 ***a***	2.4	11	2.02±0.06 ***bc***	4.48±0.07 ***b***	2.5	6	8.09±0.14 ***a***
Chlorella sp. **71 (2%)**	90.66%±4.16 ***a***	8.12±0.20 ***a***	3.5	15	2.30±0.03 ***a***	5.40±0.11 ***a***	3	9	6.83±0.09 ***c***
Chlorella sp. **72 (1%)**	74.00%±5.29 ***bc***	7.84±0.19 ***a***	3.2	14	1.95±0.04 ***c***	4.81±0.02 ***ab***	3.1	6.4	8.03±0.13 ***a***
Chlorella sp. **72 (2%)**	83.66%±2.52 ***ab***	7.23±0.23 ***a***	2.8	13.4	2.04±0.02 ***b***	4.26±0.02 ***bc***	2.5	5.6	7.52±0.08 ***b***
Fisher's LSD test *	***p***<0.01**; lsd=12.28	***p***<0.01**; lsd=1.13			***p***<0.01**; lsd=0.084	***p***<0.01**; lsd=0.613			***p***<0.01**; lsd=0.356

* Means with Fisher's lsd test results for which statistically significant difference (p<0.01**) occurred are marked in italics (means with the same letter are not significantly different)

Foliar spraying with the 2% *Chlorella sp.* strain 71 suspension led to significantly (p≤0.01) highest values of root and shoot length and fresh root biomass of red radish seedlings. The lowest values were measured in control treatment. Root (+106.08 and +122.40 %) and shoot length (+31.38 and +58.36 %) of red radish seedlings increased by application of 1% and 2% suspensions concentration, respectively.

Increase in growth of shoot was more even than root within each treated group since disparity between the lowest and the highest values of growth parameters was minor for shoot length. However, increase in growth of roots was more intense than of shoot at each treatment.

According to [10], 10% suspension of live *Acutodesmus dimorphus* triggered faster seed germination (2 days earlier) and greater lateral root development (>5) in comparison to 10% and 100% extract of the same microalga. Components such as polysaccharides, amino acids, cytokinins, auxins and gibberellins affect cellular metabolism processes, led to enhance seed germination, seedling growth and stimulate plant vegetative growth ([11]; [12]; [13]). *Chlorella sp.* cell suspension increased the barley and wheat seed growth compared to those of control (sterilized culture medium). The best treatments were 0.06 g/L and 0.23 g/L of algal suspension for the root and shoot lengths of barley and wheat seeds, respectively [14]. [7] reported that concentration of 2mg/L of *Chlorella sp.* extract could enhance plant length of sugar beet by 500%. Higher dosages (>2mg/L) of the same *Chlorella sp.* extract did not negatively affect plant length and root development of sugar beet. They tested five concentrations and every concentration of applied algal formulations provided enhanced root development. [13] gained enhancement of red radish roots fresh weight by application of seaweed (*Sargassum vulgare*) extract at 1 and 2% by about 11.15 and 24.06%, respectively. Fresh weight of shoot was increased by 35.96 and 70.25% over the control treatment, respectively. On the contrary, [15] reported that brown seaweed (*Ascophyllum nodosum*) extract did not affect yield of two broccoli cultivars.

Conclusion

Germination tests using red radish seeds were conducted to test whether algal suspensions could have stimulating effect on the initial phase of plant growth. Both microalgae strains affected root and shoot of red radish seedlings. Changes of root and shoot length were indicators of promising plant growth promotion based on microalgae. The best results were obtained with 2% concentration of *Chlorella sp.* strain 71. Accordingly, this alga proved to be better for further application as an efficient biostimulator on red radish seed germination.

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