Polish Journal of Microbiology
2020, Vol. 69, No 2, 235–238
https://doi.org/10.33073/pjm-2020-023

SHORT COMMUNICATION

Effect of *Chlorella vulgaris* on Growth and Photosynthetic Pigment Content in Swiss Chard (*Beta vulgaris L.* subsp. *cicla*)

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Submitted 6 March 2020, revised 10 April 2020, accepted 19 April 2020

Abstract

Microalgae application in agriculture is an alternative measure that could be highly beneficial to plants. The application of microalgae *Chlorella vulgaris* S45 and its effect on plant growth and pigment content in Swiss chard were investigated. In the treatments, 5% and 10% algal suspensions were applied by spraying on plants and in soil, respectively. *C. vulgaris* S45 affected the initial growth of Swiss chard and the content of photosynthetic pigments positively. The correlation analysis proved the existence of statistically significant interdependency between chlorophyll a (Chl *a*) content and leaf number ($r = 0.876$ at $p < 0.05$), and chlorophyll b (Chl *b*) content and fresh leaf weight ($r = 0.783$ at $p < 0.05$).

Key words: Swiss chard, soil microalgae, foliar spraying, growth parameters, pigment content

One of the challenges of modern agriculture is to organize production sustainably, applying all the means that are available to farmers in order to minimize the negative influences of agrochemicals on plants and soil. Microbial formulations containing effective microorganisms (EM) with the biofertilizer or biostimulator properties could be an alternative to chemical substances used in agriculture. Many positive effects are attributed to EM. Stimulated plant growth (Ku et al. 2018), and improved plant quality (Khalid et al. 2017) have been reported. Microalgae and their positive effects on plants are getting more attention in recent years because of their multifunctionality. Green algae species (*Chlorella vulgaris, Chlorella sorokiniana,* and *Chlorella pyredoinosa*) are rich sources of proteins, lipids, carbohydrates, pigments, and other metabolites with different antimicrobial, antioxidant, and antitumor properties (Panahi et al. 2019). Moreover, microalgae are used as animal feed and human food. The application of green algae in vegetable production is also well documented (Hajnal-Jafari et al. 2016; Kim et al. 2018). Live algal cells or cell extracts are applied mostly as a soil amendment but also through seed priming or by foliar spraying (Barone et al. 2018). When applied foliarly, a thin algal biofilm is formed on the plant surface, which enables faster nutrients uptake, reduces evapotranspiration, and provides additional protection against pathogenic microorganisms and parasites (Ortiz-Moreno et al. 2019). Swiss chard (*Beta vulgaris* L. subsp. *cicla*) is a leafy vegetable very rich in vitamins K, A, and C but also in fatty acids, phospholipids, glycolipids, polysaccharides, ascorbic acid, folic acid, pectins, saponins, flavonoids, phenolic acids, and betalains (Gao et al. 2009). Swiss chard leaves contain high amounts of photosynthetic pigments such as chlorophylls and carotenoids. Their content in the plant can be enhanced through varying agricultural management practices (Barickman and Kopsell 2016). Although few pieces of researches focused on the investigation of EM application in Swiss chard (Daiss et al. 2008; Mouhamad et al. 2017), there is no information about green microalgae utilization in Swiss chard production for yield enhancement and/or quality improvement. Therefore, the aim of this research was to investigate the application of microalgae *C. vulgaris* and its effect on initial plant growth and photosynthetic pigment content in Swiss chard leaves.

The microalga *C. vulgaris* S45 (Algae Collection, Faculty of Agriculture, University of Novi Sad, Serbia), isolated from soil (Vojvodina, Serbia), was used in the research. It was cultured in liquid BG11 medium

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(1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 0.006 g Citric acid, 0.006 g Ferric ammonium citrate, 0.001 g EDTA, 0.02 g Na_2CO_3 and 1 ml Trace metal solution) at 24°C on an orbital shaker (90 rpm) under two cold white lamps (2 × 950 Lm) and a photoperiod of 12 h light/12 h dark for two weeks. An algal culture containing 14×10^6 CFU/ml was used to prepare the treatments for the seed germination and plant fertilization experiments. The treatments were set as follows: 1) control (BG11 medium without algae – 10% (v/v) water solution); 2) 5% (v/v) algal suspension (applied by spraying on plants); 3) 10% (v/v) algal suspension (applied by spraying on plants); 4) 10% (v/v) algal suspension (applied in soil).

Swiss chard seedlings were grown on humus/sand (3:1) mixture under the controlled condition at room temperature (25 ± 2°C) and natural daylight photoperiod for two weeks. The seedlings were transplanted in pots (800 ml) with the same humus/sand (3:1) mixture. Each treatment contained four repetitions. The first application of microalgae was made one week after seedlings transplantation following the experiment scheme. The second application was performed 30 days later. Each time, 15 ml of algal inoculums were sprayed on plants or added to the soil. Seven days after the second application the plant material was collected for pigments quantification. Leaves number, leaf (stalk + leaf) length and weight, root length, and weight were counted and measured. Chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), and carotenoids contents in leaves were calculated according to von Wettstein (1957). Plant samples (0.2 g) were grounded with 25 ml of 80% acetone in combination with 0.1% $CaCO_3$ to prevent chlorophyllase activity. After grinding, the samples were filtered, and the final volume (25 ml) was transferred to graduate tubes. The absorbance was read at 662, 644, and 440 nm, respectively, according to von Wettstein (1957) and Holm (1954) (Unicam SP600 spectrophotometer Series 2, Cambridge England). The pigment concentrations were calculated using von Wettstein's formula as following: Chl $a = 9.784 \times A662 - 0.99 \times A644$ Chl $b = 21.426 \times A644 - 4.65 \times A662$; Carotenoids $= 4.695 \times A440 - 0.268 \times$ (Chl $a + b$). The concentration of pigments was expressed in mg/g of fresh weight of leaves according to the following formula: mg/g = (mg/l × dilution) / (sample weight × 1,000). All the assays were performed in triplicate. The software Statistica, version 13.3 (TIBCO Software Inc.) was used for statistical analysis. The least significant difference test (Fisher LSD) and the Spearman correlation analysis were performed to compare the results between treatments.

Foliar and soil application of *C. vulgaris* S45 influenced the initial growth parameters of Swiss chard (Table I). The number of leaves per plant increased in both foliar treatments and treatment with soil application in comparison to the control. The highest number was achieved after the treatment with a 10% suspension applied foliarly. Leaf length and weight also differed significantly in treatments where algae were applied. The highest leaf length was obtained when *C. vulgaris* S45 was applied in the soil. The inoculated plants had bigger roots with an increased weight. Root length was not affected significantly by treatments.

Table II
Effect of *Chlorella vulgaris* S45 on pigment content in Swiss chard leaf (mg/g).

Treatments	Chlorophyll a	Chlorophyll b	Carotenoids
Control	0.0012^{b*}	0.3949^{c}	0.0563^{b}
5% foliar	0.0149^{c}	0.3491^{b}	0.0311^{d}
10% foliar	0.0326^{a}	0.5354^{a}	0.1033^{a}
10% soil	0.0126^{c}	0.4000^{c}	0.0488^{c}

* Different letters in subscripts indicate statistically significant difference according to the Fisher LSD test ($p < 0.05$)

C. vulgaris S45 affected the initial growth of Swiss chard positively, which is in accordance with the results of Faheed and Fattah (2008), who studied the effect of green algae on lettuce (*Lactuca sativa*) growth parameters (fresh and dry weight and shoot and root length).

The best results were achieved in treatments where *C. vulgaris* S45 were applied foliarly, particularly after the use of the 10% algal suspension. Microalgae contain different nutrients; produce secondary metabolites like hormones, enzymes, vitamins and/or pigments that could lead to significant increases in crop growth parameters, yield quantity, and its quality. When applied via foliar spraying, plants usually respond more rapidly since foliar uptake and translocation of nutrients and solutes are faster. Our results correlate with the results of Dias et al. (2016), who found positive effects of microalgae products (*Spirulina platensis*) applied on leaves of eggplant. The tomato fertilization with *Nannochloris* sp. 424 leads to better plant development and growth (Oancea et al. 2013). The authors found an increase in the plant height by more than 10% when compared to the control, also better development of root length (108.08% control), leaf number (120.31% control), and leaf area (105.16% control). The weight of fresh lettuce increased by 56.34% after foliar treatment with *C. vulgaris* (Hajnal-Jafari et al. 2016).

The soil application of *C. vulgaris* S45 affected positively the leaf length (24.76 cm) and fresh leaf weight (11.33 g/plant) as well as root length (8.46 cm) and fresh root weight (0.33 g/plant), though the increase was not statistically significant. Microalgae as soil additives can promote plant nutrition, which in turn enhances all physiological reactions that lead to enhanced growth (Faheed and Fattah 2008). In transplanted vegetable crops such as Swiss chard, the application of microalgae

Table I
Effect of *Chlorella vulgaris* S45 on the plant growth parameters.

Treatments	Leaf number	Leaf length cm	Root length cm	Fresh root weight g/plant	Fresh leaf weight g/plant
Control	5^{c*}	16.66^{c}	6.75^{ab}	8.46^{b}	0.17^{b}
5% foliar	7^{bc}	18.43^{bc}	5.36^{b}	6.87^{b}	0.14^{ba}
10% foliar	9^{a}	21.96^{ab}	7.60^{a}	13.04^{a}	0.37^{a}
10% soil	7^{bc}	24.76^{a}	8.46^{a}	11.33^{a}	0.33^{ab}

* Different letters in subscripts indicate statistically significant difference according to the Fisher LSD test ($p < 0.05$)

has particular importance since plants go through a very stressful period. The root system, after transplantation, must be provided with a sufficient supply of nutrients in order to plant development proceeds normally. Barone et al. (2019) found that soil treatment with *C. vulgaris* and their extract increased soil enzymatic activity as well as the growth of tomato plants in treated soil. The soil application of *Acutodesmus dimorphus* biomass (50 and 100 g of dry biomass per 28-cm pot) on tomato seedlings, three weeks before the seedling transplantation resulted in the increased plant growth (higher numbers of branches and flowers), compared to the non-treated control (Garcia-Gonzalez and Sommerfeld 2016).

According to the results (Table II), in Swiss chard leaves, Chl *a* content ranged from 0.0012 mg/g to 0.0326 mg/g. The highest concentration of *C. vulgaris* S45 as foliar treatment led to the highest content of Chl *a* and Chl *b* (0,0326 mg/g and 0.5354 mg/g, respectively). Our results showed higher content of Chl *b* after all treatments when compared to Chl *a* content. It could be because during the plant experiment, although conducted in controlled conditions with natural daylight photoperiod, plants were not exposed to direct sunlight. Goncalves et al. (2001) also found higher Chl *b* concentrations in tonka beans and mahogany grown in the shade. Foliar treatment with 10% algal suspension showed a significant increase in carotenoids content (0.1033 mg/g) compared to the control.

The correlation analysis proved the existence of statistically significant interdependency between Chl *a* content and leaf number ($r = 0.876$ at $p < 0.05$). High correlations were observed between Chl *b* content and leaf fresh weight leaf ($r = 0.783$ at $p < 0.05$). Carotenoids content and fresh leaf weight also correlated positively ($r = 0.720$ at $p < 0.05$)

Plant pigment content is an important quality indicator, which has a great impact on consumer selection. Chlorophylls and carotenoids accumulation is influenced not only by plant physiological, biochemical, and genetic attributes, but also by environmental factors, such as light, temperature, and fertilization (Barickman et al. 2016). Application of fresh microalgal cells increased the pigmentation (Chl *a*, Chl *b*, and carotenoids content) in Swiss chard. The results of this study comply with other studies related to biostimulants application and pigments accumulation in tomato and watermelon (Abdel-Mowgoud et al. 2010; Djuric et al. 2014). Coppens et al. (2016) recorded an increased carotenoid concentration in tomato fruits treated with dry biomass of *Nannochloropsis* spp., *Ulothrix* spp., and *Klebsormidium* spp. Seed soaking and plant treatment with different microbial consortia containing algae led to increased accumulation of chlorophylls and carotenoids in plants (Dineshkumar et al. 2018). The improved photosynthetic activity resulted in an improved yield quality. The stimulation of chlorophyll and carotenoid biosynthesis was associated with enhanced plant growth in the study with lettuce inoculated with *C. vulgaris* (Faheed and Fattah 2008). The correlation analysis showed that higher pigment content could positively influence plant growth and final yield development. The high interdependency between cotton chlorophyll content and yield parameters was also found in the research conducted by Boggs et al. (2003). Blackmer and Schepers (1995) also found a higher correlation between chlorophyll content and the maize grain yield in a later stage of development. On the other hand, Güler and Özcelik (2007) argued that lower leaf chlorophyll values in the early developmental stage of dry bean (38 days after emergence) did not mean that the final yield could be lower.

In conclusion, the results indicated that *C. vulgaris* S45 might be used as an alternative foliar fertilizer that could enhance and improve the growth of Swiss chard, especially after the use of the 10% algal suspension. The application of an appropriate microalgae formulation could be an important measure to achieve a more sustainable and eco-friendly food production.

Acknowledgments

This work was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (Project III 43002).

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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