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Utilization of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] on plant growth and nutrient uptake of garden cress (*Lepidium sativum* L.) grown in different fertilizer applications

Aynur Sadak Turhan[1,*] **Suat Şensoy[2]**

[1]Van Yuzuncu Yil University, The Institute of Natural and Applied Sciences, Horticultural Sciences, Van, Türkiye
[2]Van Yuzuncu Yil University, Agricultural Faculty, Horticulture Department, Van, Türkiye

[*]Corresponding Author: aynuursadak@gmail.com

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Abstract
This study was employed as a pot experiment in the controlled greenhouse conditions in order to investigate the effect of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] application on plant growth and nutrient uptake of garden cress (*Lepidium sativum* L.) grown in different doses of mineral fertilizer applications. Sieved soil in 3-liter pots was used as the growing medium. Equal amount of irrigation was applied to all pots during the period from seed sowing to the end of the experiment. Microalgae application was applied twice (100 ml and 150 ml per pot) to the seedling-growing medium. As chemical fertilizer, 0%, 50% and 100% of NPK (160 mg N kg^{-1}, 80 mg P_2O_5 kg^{-1}, and 100 mg K_2O kg^{-1}) were applied. As the parameters in garden cress, shoot height, shoot fresh weight, total soluble content (TSS), chlorophyll amount (SPAD value) and some nutrients element (K, Ca, Fe, Zn, Cu and Mn) contents were examined. At the end of the study, microalgae applications were found to have a promising effect on plant growth and some nutrient uptake. It was observed that the values of the studied traits generally increased in the microalgae application compared to the control group.

Keywords
Bio-fertilizer, Garden cress, Microalgae, Nutrient content, Plant growth

Introduction

In response to the constantly increasing population in the world, the need for food is also increasing rapidly (Sencar, 1988). Due to the continuous increase in the world population, agricultural areas should also be increased, which is not so feasible. Therefore, the amount of product obtained from the unit area should be increased or the existing areas used should be sustainable (Midmore, 1993). The soil structure of Turkey is generally poor in terms of organic matter (Kaçar and İnal, 2008). It is also known that the intensive use of artificial chemical fertilizers in agricultural lands deteriorates the soil structure and pollutes the underground water resources, and the excessive use of these chemicals disrupts the ecological balance (Turan, 2007). The sustainability of the solutions to be produced in response to the problems encountered in production is an important criterion (Koru and Cirik, 1999). Moreover, it is important to use sustainable resources correctly in terms of environment and human health (Kut et al., 2007).

Garden cress (*Lepidium sativum* L.), is included in the Brassicaceae family. It is a rather fast-growing, edible herb; it is an annual herbaceous plant; it is used as an appetizer with its pleasant peppery, tangy flavor and aroma (Vural et al., 2000). It is also prized as a medicinal herb especially for diabetes (Tahraoui et al., 2007).

Chlorella, a genus of single-celled green algae and defined as the oldest living thing known for 2.5 billion years, is an important food source. *Chlorella* is dark green. Its greenery is high in chlorophyll. It is known as green freshwater algae (Kut et al., 2007). In recent years, different microalgae have been used as plant nutrients in agricultural production (Okur et al., 2001). Today, the widespread use of microalgae fertilizers is getting prominent (Eşiyok et al., 2001). It is known that microalgae have positive effects on plant growth and development. Moreover, it increases the yield and quality of the agricultural crops and is used as a biofertilizer in agriculture (Engin et al., 2019). Schreiber et al. (2018) reported that algal biomass could also support crop growth on marginal soils.

This study aimed to investigate the effects of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] use on plant growth and nutrient uptake in garden cress (*Lepidium sativum* L.) in different doses of mineral fertilizer applications to see if it can compensate for the use of artificial fertilizers to some extent.

Materials and methods

In the present study, the effect of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] use on plant growth in garden cress [*Lepidium sativum* L. cv. Helen (Sim Arzuman Seeds)] was investigated. The study was carried out as a pot experiment in controlled greenhouse conditions (15± 4 Cº night and 28± 4 Cº day). Microalgae application was applied twice (100 ml and 150 per pot) to the seedling growing medium and as mineral fertilizer, 0%, 50% and 100% of NPK (160 mg N $kg^{-1,}$ 80 mg P_2O_5 $kg^{-1,}$ and 100 mg K_2O kg^{-1} as 20:20:20 NPK, Potassium sulfate, and urea) were applied as base fertilizer and 40 mg N kg^{-1} as urea two weeks after seedling emergence (Table 1). The soil properties used in the experiment are given in Table 1.

Sieved soil in 3-liter pots was used as the growing medium, and 40 seeds were sown in each pot. Equal amount of irrigation was applied to all pots during the period from seed sowing to the end of the experiment, is about 6.5 weeks.

Table 1. Applications and soil properties

#	Application	
1	Control (no microalgae and no fertilizer)	
2	%50 NPK fertilizer	
3	%100 NPKfertilizer	
4	Microalgae	
5	%50 NPK fertilizer + Microalgae	
6	%100 NPK fertilizer + Microalgae	
	Soil properties	
Potassium (K_2O) kg ha^{-1}	580.2	High
Phosphor (P_2O_5) kg ha^{-1}	31.2	Little
Lim (%)	11.025	Medium
Organic Matter (%)	1.98	Little
Total salt (%)	0.036	No salt
pH	7.21	Neutral

Cultivation and application of microalgae

The microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] used in the present study were obtained from Cukurova University, Faculty of Fisheries and cultured in a tissue culture laboratory at Van Yuzuncu Yil University. Bold Wynne nutrient medium ($NaNO_3$-0.250 g; $MgSO_4.7H_2O$-0.075 g; K_2HPO_4-0.075 g; KH_2PO_4-0.0175 g; NaCl-0.025 g; $CaCl_2.H_2O$-0.025 g; 1000 ml of Distilled Water) was employed in the production of bulk cultures (Duygu et al., 2017). The nutrient medium prepared in 1000 ml was equally separated into two 500 ml flasks, autoclaved at 121˚C for 20 minutes for sterilization. Microalgae were cultured with 9 ml of medium +1 ml of suspended culture. The sowing of the bulk cultures was initiated with 500 ml and then transferred to 1000 ml nutrient medium. To prevent contamination and for air circulation, the mouth of the flasks was not closed, but the sterilized cotton was placed (Ağırman, 2015). After the first two days, the cultures were aired with an aquarium pump to keep the bulk cultures in suspension. The light needs of the cultures were met artificially with a light source (150 µmol $m^{-2}.s^{-1}$) placed horizontally at a distance of 22 cm from the cultures. Cultures were treated with a 16:8 light/dark period and grown at 22-25 °C. Cell counts were performed using a Thoma slide. *Chlorella vulgaris* Beyerinck (Beijerinck) algae were applied at the rate of $2x10^7$ algae l^{-1} to the half of the application twice (at seed sowing and 20 days after as 100 ml and 150 ml, respectively).

The studied parameters are as follows:

Shoot fresh weight (g)

The shoot weight was determined with a weighing scale with a precision of 0.1 g.

Shoot height (cm)

The shoot length was measured with a ruler with a precision of 1 mm.

Total Soluble Solid Content (TSS brixº)

Garden cress samples were extracted with a blender, then TSS was determined with a hand refractometer (Atago, Tokyo, Japan).

Chlorophyll amount (SPAD value)

Chlorophyll content in garden cress leaves was determined with a SPAD meter (Minolta SPAD-502, Osaka, Japan).

Plant Nutrient analysis

The plant samples were put in an oven (65 ºC) for constant weight. The 0.5 grams of dried and grinded samples were pre-burned with 1 ml of ethyl alcohol in crucibles and the samples were burned in a muffle furnace at 500 ºC for 9-12 hours. 4 ml of 3 N HCl was mixed with the obtained ash. The samples were left on the hot plate and kept on it until they turned yellow. When the yellow color was formed, the crucibles taken from the hot plate were transferred to the volumetric flask with the help of the filtering set and the samples were made ready for reading (Kaçar and İnal, 2008).

Statistical analysis

IBM SPSS 21.0 package program was employed for variance analysis to compare the data obtained from the average of different doses of fertilizer applications and microalgae applications. T-test was applied for the differences between microalgae applications, and Duncan multiple range test was used for fertilizer applications (Duncan, 1955).

Results and Discussion

The effect of different doses of NPK fertilizer and microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] application on the growth of garden cress (*Lepidium sativum* L.) was investigated in the present study. It has been determined that microalgae applications have a positive effect on plant growth and some mineral matter contents in general and some noticeable parameters are discussed below. The data about shoot fresh weight, shoot height, Total Soluble Solid Content (TSS) and chlorophyll amount (SPAD value) are given in Table 2; the values of some nutrients (K, Ca, Fe, Zn, Cu and Mn) were also indicated in Table 3.

NPK fertilizer application had mostly higher and more significant values than the control application on plant growth parameter of garden cress (Table 2). Microalgae application had also largely higher and significant values than the control application on plant growth parameter of garden cress (Table 2). Microalgae application caused a significant increase in the shoot fresh weight and shoot height values compared to control microalgae application. Microalgae application caused significant ($p \leq 0.01$) increases (about 129% and 36%) in the shoot fresh weight and shoot height values in garden cress, respectively (Table 2). Similarly, chlorophyll amount (SPAD value) was affected and increased significantly by NPK fertilizer ($p \leq 0.01$) and microalgae ($p \leq 0.05$) applications. Microalgae application had about 27 % more chlorophyll amount (SPAD value) than the control application. On the other hand, the increases caused by NPK fertilizer and microalgae on TSS content of garden cress were insignificant (Table 2).

Table 2. Effect of microalgae application on plant growth of garden cress grown with different doses of NPK fertilizer

	Shoot fresh weight (g pot^{-1})			Shoot height (cm)		
NPK Fertilizer	Microalgae -	Microalgae +	Mean	Microalgae -	Microalgae +	Mean
0% (Control)	2.21	6.13	4.17 B**	9.5	11.7	10.6 C**
50%	3.84	8.53	6.18 A	12.0	16.2	14.1 B
100%	4.39	9.23	6.81 A	13.0	18.2	15.6 A
Mean	3.48 B**	7.96 A		11.5 B**	15.3 A	

	Chlorophyll amount (SPAD value)			Total Soluble Solid Content (TSS brix^{0})		
NPK Fertilizer	Microalgae -	Microalgae +	Mean	Microalgae -	Microalgae +	Mean
0% (Control)	28.65	39.02	33.83 C**	3.40	3.50	3.45 ^{ns}
50%	34.55	42.70	38.62 B	3.45	4.45	3.95
100%	35.32	43.55	39.43 A	4.10	4.52	4.31
Mean	32.80 B*	41.75 A		3.65 ^{ns}	4.15	

^{ns}: not significant, *: Significant at $p \leq 0.05$, **: Significant at $p \leq 0.01$

In the present study, some nutrient (K, Ca, Fe, Zn, Cu and Mn) contents of garden cress grown with different doses of NPK fertilizer and microalgae application were determined and listed in Table 3. The effect of microalgae use on nutrient content was especially positive for potassium ((K), zinc (Zn), and copper (Cu). Microalgae application caused a significant ($p \leq 0.01$) increase (about 33%) in K content of garden cress (Table 3). Similarly, microalgae application caused a significant ($p \leq 0.01$) increase (about 17%) in Zn content of the plants (Table 3). Likewise, in Cu content, there was about 24% significant ($p \leq 0.01$) increase due to microalgae application (Table 3). Moreover, fertilizer applications significantly increased the contents of K, Cu, and Zn. However, both applications caused no significant differences for calcium (Ca) and manganese (Mn) contents. Bio-fertilizers could possess a significant place in the development of plant growth, soil fertility and environmental factors in sustainable agriculture (Vernieri et al., 2006; Singh et al., 2016; Godlewska et al., 2019). It has been reported that microalgae application in agriculture might play an important role in the amelioration of nutrient content and plant growth (Jardin, 2015).

Table 3. Effect of microalgae application on some nutrient contents of garden cress grown with different doses of NPK fertilizer

	K (ppm)			Ca (ppm)		
NPK Fertilizer	Microalgae -	+	Mean	Microalgae -	+	Mean
0% (Control)	5098	6486	5792 C**	4244	4300	4277 ns
50%	5873	7491	6797 B	4325	4408	4366
100%	7407	9741	8574 A	4506	4696	4601
Mean	6023 B **	8018 A		4358 ns	4468	

	Fe (ppm)			Zn (ppm)		
NPK Fertilizer	Microalgae -	+	Mean	Microalgae -	+	Mean
0% (Control)	441.0	462.9	451.9 ns	5.56 e**	9.79 d	7.67 C*
50%	427.3	475.7	451.5	10.47 c	10.57 c	10.52 B
100%	449.8	489.6	469.7	12.66 b	13.26 a	12.96 A
Mean	439.3 ns	476.0		9.56 B**	11.20 A	

	Cu (ppm)			Mn (ppm)		
NPK fertilizer	Microalgae -	+	Mean	Microalgae -	+	Mean
0% (Control)	2.89	3.30	3.12 B**	25.29	25.66	25.47 ns
50%	3.01	3.94	3.57 AB	26.94	26.08	26.49
100%	3.21	4.26	3.66 A	25.13	24.82	24.97
Mean	3.06 B**	3.78 A		25.78 ns	25.52	

ns: not significant, *: Significant at $p \leq 0.05$, **: Significant at $p \leq 0.01$

Microalgae, as a bio-fertilizer or bio-stimulant, could especially play important role in particular infertile soils. Organic matter produced by microalgae might also enrich the soil structure. The results of Schreiber et al. (2018) studied microalgae on wheat and confirmed that algal biomass can support crop growth on marginal soils. These researchers determined that microalgae fertilization increased the shoot size to 87 and 107% of that of sand supplied with mineral fertilizer. Michalat et al. (2015) suggested that algal extracts might induce stronger seed germination and plant growth parameters in garden cress because they are rich in nutrients and polyphenols. Michalat et al. (2016) also reported that algal extracts enhanced chlorophyll and carotenoid content in the shoots, as well as root thickness and aboveground biomass in garden cress and wheat. It was also indicated that *Chlorella vulgaris* as a bio-fertilizer is effective and cost-effective in improving soil nutrients for greater yield in okra (Agwa et al., 2021). Anitha et al. (2016) studied the effect of microalgae use on the nutrient content in tomato seedlings and determined that microalgae ameliorated the zinc, potassium and calcium contents significantly. Zodape et al. (2011) also examined the effect of microalgae on tomatoes and determined that nutrient contents of the microalgae applied plants improved significantly compared to the control plants.

Similarly, Grouch et al. (1990) investigated the microalgae effect on the lettuce seedlings and reported that microalgae usually improved the yield and the contents of Ca and K. Sayed Ahmed et al. (2021) accounted that *Cholorella* might raise mineral matter content in garden rocket by upregulation of key genes in their biosynthetic pathway.

Conclusion

Parallel to the expanding populace in the world, those require to nourishment also increases. An aftereffect of expanded pressures, the nonstop-escalated utilization of artificial chemical fertilizers might cause pollution in nature and could deteriorate the ecological balance. In the face of this situation, agricultural lands would be destroyed, and therefore, the amount and quality of agricultural crops declines. With the utilization of claiming bio-fertilizers such as microalgae, the employment of artificial chemical fertilizers could be decreased. In the present study, in which the impact of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] utilization on plant growth in garden cress (*Lepidium sativum* L.) was investigated, the effect of microalgae, defined as bio-fertilizer, was determined to be by and large encouraging for plant development and nutrient uptake. It was seen that there were important ameliorations of microalgae applications in many examined traits, and microalgae might compensate for the use of artificial fertilizers to some extent. In the future, it is thought that as the number of investigations on the association of microalgae and agricultural plants increases, newer knowledge will be revealed for more sustainable agriculture.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent form publication

Not applicable.

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