



The Effect of Use of Microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] in Different Fertilizer Applications on Plant Growth of Garden Rocket (*Eruca vesicaria* ssp. *sativa* Mill.)

Aynur Sadak Turhan^{1,a,*}, Büşra Günsan Can^{1,b}, Turgay Kabay^{2,c}, Suat Şensoy^{2,d}

¹Horticultural Sciences, The Institute of Natural and Applied Sciences, Van Yüzüncü Yıl University, 65090 Van, Turkey

²Horticulture Department, Agricultural Faculty, Van Yüzüncü Yıl University, 65090 Van, Turkey

*Corresponding author

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ABSTRACT

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This study was carried out as a pot experiment in controlled greenhouse conditions in order to reveal the effect of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] application on plant growth of rocket salad (*Eruca vesicaria* ssp. *sativa* Mill.) in different doses of 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 per pot) to the seedling growing medium. As chemical fertilizer, 0%, 50% and 100% of NPK (160 mg N kg⁻¹, 80 mg P₂O₅ kg⁻¹, and 100 mg K₂O kg⁻¹) were applied. As parameters in rocket plants, shoot length, shoot fresh weight, shoot dry weight, stem diameter, leaf area, leaf relative water content, membrane damage index, total soluble content, and some nutrients (K, Ca, Na, Zn, Cu, and Mn) contents were examined. According to the data obtained, microalgae applications were found to have a positive effect on plant growth in general. It was observed that the values increased in most parameters examined in algae-applied applications compared to the control group. Among the applications, the best values were found in 50% fertilizer + microalgae and 100% fertilizer + microalgae applications.

^a aynuersadak@gmail.com

^b <https://orcid.org/0000-0002-5865-6497> | ^b busra.gunsan@gmail.com

^d <https://orcid.org/0000-0003-4071-860X>

^c turgaykabay@gmail.com

^d <https://orcid.org/0000-0002-3239-0037> | ^d Suatsensoy@gmail.com

^d <https://orcid.org/0000-0001-7129-6185>



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Introduction

In response to the increasing population in the world, the need for food is increasing rapidly and continuously (Sencar, 1988; Engin et al., 2019). Achieving balanced and adequate diet is gradually becoming a problem (Özdemir, 2014). Despite the continuous increase in the world population, either it is necessary to increase the agricultural areas or to increase the amount of products obtained from the unit area (Midmore, 1993; Ergun et al., 2020).

Eruca vesicaria ssp. *sativa* Mill. commonly known as garden rocket, salad rocket or arugula, is an annual plant, native to Mediterranean Basin and it is cool season salad vegetable with a unique, rich aroma, taste, and nutritious components, as in other members of the Brassicaceae family (Morales and Janick, 2002; Beck, 2005; Salk et al., 2008; Eşiyok, 2012; Barazani and Ziffer-Berger, 2014). Garden rocket, which has a rich content of vitamins, is also important in terms of human nutrition and it is known that

it is good for some diseases (eye infections, night blindness, etc.) due to the vitamins A and C it contains (Balçı, 2019). Garden rocket, which is becoming increasingly important, comprises a wide variety of phytochemicals that promote health (Kusvuran and Ellialtıoğlu, 2021); therefore, garden rocket production and consumption has increased significantly in recent years in the world and Turkey (Temel, 2016; Hassan et al., 2017).

Some soils are poor in terms of organic matter due to the effect of climate or other factors (Kacar, 1997). The use of intensive chemical fertilizers in agricultural areas causes deterioration of soil structure and pollution of underground water resources (Turan, 2007). In agricultural production, it is very important to use sustainable resources correctly in terms of human and environmental health (Cirik and Cirik, 1999). Excessive use of chemicals in agricultural lands disrupts the ecological balance. New solutions to the

problems encountered in agriculture must be sustainable (Koru and Cirik, 1999). Different microalgae used in recent years are considered as sustainable plant nutrient sources (Okur et al., 2001). Fertilization strategies are important for lowering hazardous substances in leafy vegetables such as garden rocket (Santamaria, 1998). Moreover, it is wise to use bio-stimulants for reducing nutrient amount in agricultural production systems (Vernieri et al., 2006).

The positive effect of microalgae on plant growth is becoming noticeable (Kut et al., 2007). Microalgae, which are used as plant nutrients, produce quality products in agricultural crops. Considering the increase in yield and quality, microalgae have been used as bio-fertilizers in agriculture (Engin et al., 2019), and there has been increasing use of microalgae-based fertilizers (Eşiyok et al., 2001). Approximately 1 million tons of microalgae are used in soil enrichment programs in the world (Engin et al., 2019). The use of some bio-fertilizers in terms of sustainability in agricultural lands increases the quality and yield because it is known that it increases nutrient uptake in the soil (Kut et al., 2007). The aim of this study was to reveal the effects of using fewer chemical fertilizers, thus saving fertilizer and protecting the environment, through the use of microalgae in garden rocket (*Eruca vesicaria* ssp. *sativa* Mill.), as well as revealing the effects of microalgae on plant growth and some nutritional content of garden rocket.

Materials and Methods

In the present study, the effect of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] use on plant growth in garden rocket [*Eruca vesicaria* ssp. *sativa* Mill. cv. Rota (Sim Arzuman Seeds)] was investigated. The study was carried out as a pot experiment in controlled greenhouse conditions ($15 \pm 4^\circ\text{C}$ night and $28 \pm 4^\circ\text{C}$ day). Microalgae application was applied twice (100 ml and 150 per pot) to the seedling growing medium and 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} as 20:20:20 NPK, Potassium sulphate, 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 to 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.

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; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0.075 g; K_2HPO_4 -0.075 g; KH_2PO_4 - 0.0175 g; NaCl -0.025 g; $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ -0.025 g; 1000 ml of Distilled Water) was used in the production of bulk cultures (Duygu et al., 2017). The nutrient medium prepared in 1000 ml was equally divided into two 500 ml flasks, autoclaved at 121°C for 20 minutes to ensure sterilization and their cultivation was carried out in a sterile cabinet. Microalgae was cultured with 9 ml of medium +1 ml of suspended culture. The sowing of the bulk cultures was started with 500 ml and then transferred to 1000 ml nutrient medium. In order to prevent contamination and at the same time to ensure air circulation, the mouth of the flasks was not completely closed, but the cotton that was sterilized in an autoclave 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 with artificial lighting with a light source ($150 \mu\text{mol m}^{-2} \text{ s}^{-1}$) was placed at a distance of 22 cm from the cultures, horizontally, from behind. Cultures were treated with a 16:8 light/dark period and grown at 22 - 25°C at room temperature. Cell counts were performed using a Thoma slide. *Chlorella vulgaris* Beyerinck (Beijerinck) algae was applied at the rate of 2×10^7 algae l^{-1} to the half of the application twice (at seed sowing and 20 days after as 100ml and 150 ml, respectively) (Aydoner et al., 2018; Ergun et al., 2020).

The Studied Parameters as Follow:

Shoot fresh and dry weight (g)

The fresh weight was determined by weighing the garden rocket plants in each pot with a weighing scale with a precision of 0.1 g. The same samples were left open for 24 hours, then dried in an oven at 65°C for 48 hours to dry thoroughly, and the dry weight was determined by measuring on a weighing scale with 0.1 g sensitivity.

Table 1. Applications and soil properties

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

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

Chemical Fertilizer	Shoot fresh weight (g pot ⁻¹)			Shoot dry weight (g pot ⁻¹)		
	Microalgae		Mean	Microalgae		Mean
	-	+		-	+	
0% (Control)	8.63 ^{ns}	13.14	10.89 ^{B**}	1.40 ^{ns}	1.56	1.48 ^{B**}
50%	33.84	38.62	36.23 ^A	3.47	3.55	3.51 ^A
100%	36.31	37.20	36.76 ^A	3.26	3.46	3.36 ^A
Mean	26.26 ^{ns}	29.65		2.71 ^{ns}	2.86	
Chemical Fertilizer	Stem diameter (mm)			Shoot height (cm)		
	Microalgae		Mean	Microalgae		Mean
	-	+		-	+	
0% (Control)	1.50 ^{ns}	2.15	1.83 ^{B**}	10.50 ^{ns}	11.50	11.00 ^{B**}
50%	2.03	2.96	2.49 ^A	15.50	19.75	17.63 ^A
100%	1.84	2.10	1.97 ^B	17.00	20.25	18.63 ^A
Mean	1.79 ^{B**}	2.40 ^A		14.33 ^{B**}	17.17 ^A	
Chemical Fertilizer	TSS (Brix ^o)			Leaf area (cm ² plant ⁻¹)		
	Microalgae		Mean	Microalgae		Mean
	-	+		-	+	
0% (Control)	3.25 ^{cd*}	5.97 ^a	4.61 ^{ns}	16.60 ^{d**}	13.90 ^e	15.25 ^{C**}
50%	3.47 ^c	4.52 ^b	4.00	30.63 ^c	41.95 ^a	36.29 ^B
100%	3.57 ^c	4.45 ^b	4.01	36.83 ^{bc}	39.80 ^b	38.31 ^A
Mean	3.43 ^{B**}	4.98 ^A		28.02 ^{B**}	31.88 ^A	

^{ns}: not significant, *: Significant at P<0.05, **: Significant at P<0.01

Shoot height (cm) and Stem diameter (mm)

The shoot length was measured with a ruler with a precision of 1 mm. The stem diameter was determined with a caliper in mm (± 0.5).

Leaf Area (cm²/plant)

Garden rocket leaves with a known circle area were weighed. Then, the leaf area of the plant was calculated by proportioning the leaf weight of the whole plant according to this area and weight relationship (Kuşvuran, 2010).

Determination of Leaf Relative Water Content (LRWC) (%)

The relative water content of the leaves and the turgor weights of the leaf samples taken from the plants were determined after they were kept in distilled water for 4 hours after the leaf fresh weights were weighed on a weighing scale with an accuracy of 0.1 g. Then, after the same leaves were kept in an oven at 65°C for 48 hours, were weighted again and the relative water content of the leaves was determined as following (Kuşvuran, 2010).

$$LTWC = (FW-DW)/(TW-DW) \times 100$$

There;

FW: Fresh Weight

DW: Dry Weight

TW: Turgor Weight

Membrane Damage Index (MDI) (%)

Samples were taken from garden rocket leaves with a disc sample with a diameter of 17 mm, and EC was measured after they were kept in distilled water for 5 hours. Membrane damage index value was obtained by measuring the EC value again after the same disc samples were kept in a boiling water bath at 100 °C for 10 minutes (Kuşvuran, 2010).

$$MDI = (Lt-Lc/1-Lc) \times 100$$

There;

Lt: EC of stressed plant leaf before autoclaving/ EC after autoclaving

Lc: EC of control plant leaf before autoclaving/ EC after autoclaving

Amount of Total Soluble Solid Content (TSS brix^o)

After taking samples from garden rocket plants extracted with a blender, the Then TSS was determined with a hand refractometer (Atago PAL-1, Tokyo, Japan).

Plant Nutrient Analysis

The plant samples were in an oven (65 °C) 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 °C 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 (Kacar, 1997; Kacar and İnal, 2008).

Statistical Analysis

In the study, IBM SPSS 21.0 package program was used for variance analysis in order 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

In the present study, the effect of different doses of chemical fertilizer and microalgae application on the growth of garden rocket (*Eruca vesicaria* ssp. *sativa* Mill.)

was investigated. It has been determined that microalgae applications have a positive effect on plant growth in general and some noticeable parameters are discussed below. The data about shoot fresh and dry weight, shoot length, stem diameter, leaf area, and TSS are given in Table 2; membrane damage index and leaf relative water content values are given in Table 3 and some nutrient (K, Ca, Na, Zn, Cu and Mn) content values are indicated in Table 4.

Chemical fertilizer application had generally higher and significant values than the control application on plant growth parameter of garden rocket (Table 2). Microalgae application had also generally higher and significant values than the nil application on plant growth parameter of garden rocket (Table 2). For the shoot fresh and dry weight values of the 50% and 100% fertilizer application were considerably higher than 0% fertilizer application. Microalgae application caused also insignificant increase in the shoot fresh and dry weight values compared to no microalgae application. Microalgae application caused significant ($P \leq 0.01$) increases (about 34% and 20%) in stem diameter and shoot height in garden rocket, respectively (Table 2). While the 50% and 100% fertilizer application caused significant increase in shoot height, only 50% of fertilizer application significantly increased the stem diameter in garden rocket (Table 2).

Microalgae application caused also significant ($P \leq 0.01$) increases (about 45% and 14%) in TSS and leaf area in garden rocket, respectively (Table 2). Fertilizer application had significant increases in leaf area in garden rocket. The interaction of microalgae and fertilizer application was found significant in TSS and leaf area in garden rocket. The highest TSS value (5.97 brix⁰) was determined in microalgae application with nil fertilizer application, and this were followed by the other microalgae applications. The highest leaf area value (41.95 cm² plant⁻¹) was determined in microalgae application with 50% fertilizer application, and this were followed by the microalgae application with 100% fertilizer application (Table 2). The positive effects of microalgae applications on plant growth have been observed in many vegetable species. Hassan et al. (2017) stated that the foliar spraying with Cyanobacterium *Spirulina platensis*, blue green algae, at 5% had positively significant effects on plant height, numbers of leaves, chlorophyll a and b, rocket comparing to N-P-K control treatment, In a study examining the effects on plant growth by using *Chlorella vulgaris* in tomato, it was determined that positive increases were observed on plant growth, shoot fresh weight, stem diameter, shoot dry weight, shoot length and TSS amount (Özdemir et al., 2016). It has been reported that microalgae applications to lettuce seedlings significantly increase

shoot fresh and shoot dry weight, shoot length and stem diameter (Faheed et al., 2008). It was observed that the plant fresh and dry weight, shoot length, leaf area and stem diameter values of microalgae application to tomato seedlings increased compared to the control group (Anitha et al., 2016). In a study in which different doses of fertilizers and microalgae (*Chlorella vulgaris*) applications were applied in lettuce cultivation, it was reported that microalgae applied groups had increasing effects on leaf area, stem diameter, shoot length, shoot fresh and dry weight values compared to other application groups (Ergun et al., 2020).

Membrane damage index and leaf relative water content values examined in this study, which was conducted to investigate the effects of microalgae and two different fertilizer doses on garden rocket plant growth, are discussed below (Table 3). Considering the membrane damage index values, it was determined that the effect of microalgae applications was generally positive. It was determined that the differences between the values of the membrane damage index microalgae application group and the control group were statistically significant ($P \leq 0.01$). There are also statistically significant differences between microalgae applications and microalgae + fertilizer interaction values.

Microalgae application caused significant ($p \leq 0.01$) decrease (about 59%) in membrane damage index in garden rocket (Table 3). The lowest membrane damage index value (8.33%) was determined in microalgae application with nil fertilizer application, and microalgae applications with the 50% and 100% fertilizer application had also lower membrane damage index values (14.15% and 18.58%, respectively) compared to other applications having no microalgae. There were also some insignificant increases in leaf relative water content in microalgae applied garden rocket (Table 3).

Due to their high protein content, microalgae provide significant advantages for plants in cell growth and development, and enable cell protection and repair, regulation of cellular activities, and the activation of defense mechanisms against chemical signals and negativity in adverse environmental conditions (Solomon, 1999; Koru et al., 2005; Özdemir, 2014). In a study on tomato, it was stated that while abiotic stresses showed a decrease in leaf relative water content values in plants, there was an increase in microalgae application values (Munns, 2005). Again, according to the values obtained from plants exposed to stress conditions, microalgae applications stand out among the application groups with the least damage among the membrane damage index values (Zodape et al., 2011).

Table 3. Effect of microalgae application on membrane damage index and (%) leaf relative water content of garden rocket grown with different doses of chemical fertilizer

Chemical Fertilizer	Membrane damage index (%)			Leaf relative water content (%)		
	Microalgae		Mean	Microalgae		Mean
	-	+		-	+	
0% (Control)	39.63 ^{a*}	8.33 ^f	23.98 ^{ns}	74.61 ^{ns}	83.36	78.98 ^{ns}
50%	29.43 ^c	14.15 ^e	21.79	79.64	85.25	82.44
100%	30.28 ^b	18.58 ^d	24.43	68.95	79.68	74.31
Mean	33.11 ^{A**}	13.68 ^B		74.40 ^{ns}	82.76	

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

Table 4. Effect of microalgae application on some nutrient contents of garden rocket grown with different doses of chemical fertilizer

Chemical fertilizer	K (ppm)			Ca (ppm)		
	Microalgae		Mean	Microalgae		Mean
	-	+		-	+	
0% (Control)	4655 ^{ns}	6082	5368 ^{C**}	3578 ^{ns}	3979	3778 ^{C*}
50%	6048	7300	6674 ^B	3999	5419	4709 ^B
100%	6482	8225	7353 ^A	4018	7945	5981 ^A
Mean	5728 ^{B**}	7202 ^A		3865 ^{B**}	5781 ^A	
Chemical fertilizer	Na (ppm)			Zn (ppm)		
	Microalgae		Mean	Microalgae		Mean
	-	+		-	+	
0% (Control)	2982 ^{ns}	2516	2749 ^{B**}	5.030 ^{ns}	6.887	5.958 ^{ns}
50%	4449	2938	3693 ^{AB}	6.255	6.910	6.567
100%	5531	3026	4278 ^A	6.760	7.390	6.538
Mean	4320 ^{A*}	2826 ^B		6.015 ^{B**}	7.062 ^A	
Chemical fertilizer	Cu (ppm)			Mn (ppm)		
	Microalgae		Mean	Microalgae		Mean
	-	+		-	+	
0% (Control)	3.427 ^{ns}	3.465	3.446 ^{ns}	35.93 ^{ns}	31.86	33.90 ^{ns}
50%	5.190	4.042	4.534	27.64	32.14	30.21
100%	4.530	4.777	4.653	34.17	31.95	33.06
Mean	4.309 ^{ns}	4.095		33.03 ^{ns}	31.98	

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

In the present study, some nutrient (K, Ca, Na, Zn, Cu and Mn) contents of garden rocket grown with different doses of chemical fertilizer and microalgae application were determined and listed in Table 4.

The effect of microalgae use on nutrient content was especially positive for K, Ca, Na, and Zn. Microalgae application caused significant ($P \leq 0.01$) increase (about 36%) in K content of garden rocket (Table 4). Similarly, microalgae application caused significant ($P \leq 0.05$) increase (about 50%) in Ca content of garden rocket (Table 4). However, and fortunately microalgae application caused significant ($P \leq 0.05$) decrease (about 35%) in Na content of garden rocket (Table 4). Moreover, fertilizer applications significantly increased the contents of K, Ca, and Na. Sayed Ahmed et al. (2021) reported that *Chlorella* could increase nutrient content of garden rocket through upregulation of key genes in their biosynthetic pathway. Agwa et al. (2017) stated that *Chlorella vulgaris* as a bio-fertilizer is efficient and economical in improving soil nutrients for greater productivity of okra. In a study, investigating the effect of microalgae use on plant nutrient content in tomato seedlings, the effect on zinc, potassium and calcium content was investigated, and it was stated that the use of microalgae increased significantly these nutrients (Anitha et al., 2016). The effect of microalgae on plant growth and nutrient content of lettuce seedlings was investigated and it was reported that microalgae generally increased yield and Ca and K contents in lettuce seedlings (Grouch et al., 1990). In another study examining the effect of microalgae on tomato plants, nutrient content increased significantly compared to control plants (Zodape et al., 2011). Hassan et al. (2021) reported that seaweed extract could generally stimulate morpho-agronomic and bioactive properties of garden rocket and be used as multifunctional bio-fertilizer. Bio-fertilizers have an important place in the development of plant growth, soil fertility and environmental factors in a sustainable agriculture (Verdieri et al., 2006; Singh et al., 2016; Godlewska et al., 2019). It is also stated that bio-stimulants also reduce harmful

substance for plant and human health (Verdieri et al., 2006). It has been determined that microalgae applied to seedlings and plants play an important role in increasing the nutrient content and improving plant growth characteristics (Jardin, 2015).

The rhizosphere microorganisms could interact with each other over multifold ways and responding of the surrounding environments. Although, we have not studied, it is known that microalgae could also establish mutualistic interactions with AMF and bacteria (Hristozkova et al., 2018; Abinandan et al., 2019; Kang et al., 2021). Hristozkova et al., (2018) reported that dual inoculation with both microalgae and AMF stimulated mycorrhizal function (concentration of glomalin-related proteins) and improved plant performance both directly and indirectly through mycorrhizal stimulation. Kang et al. (2021) reviewed that microalgae and plant growth-promoting bacteria (PGPB) were recognized as substitutes to chemical fertilizers for improving soil fertility due to their bio-fertilizing properties, through the production of bioactive compounds (e.g., phyto-hormones, amino acids, and carotenoids) and also their ability to prevent plant pathogens. Abinandan et al. (2019) reviewed that microalgae could have potential soil traits as carbon fixation, extracellular polysaccharides and initiation of biological soil crust, and dual inoculation with both microalgae (*Chlorella* sp.) and cyanobacteria was a very helpful factor for soil fertility especially in marginal soil by improving nitrogen and soil enzyme activities, and microbial biomass.

Conclusion

In the present study, in which the effect of microalgae [*Chlorella vulgaris* Beyerinck (Beijerinck)] use on plant growth in garden rocket (*Eruca vesicaria* ssp. *sativa* Mill.) was investigated, the effect of microalgae, defined as bio-fertilizer, was found to be generally positive for plant growth. There were significant differences between the

values of microalgae applications and non-microalgae applications in many examined parameters. Microalgae application together with 50% fertilizer application also gave better results than 100% fertilizer application in terms of some studied traits (such as leaf area, membrane damage index, LRWC and Na content). In parallel with the increasing population in the world, the need for food also increases, and as a result of increased pressures, the continuous and intensive use of chemical fertilizers causes the consumption of natural resources and the deterioration of the ecological balance. In the face of this situation, agricultural lands are destroyed, the amount of the product produced decreases and also the quality of the product decreases. With the use of bio-fertilizers, both the use of chemical fertilizers will be reduced and the destruction of agricultural lands will be prevented. Thus, there will be no problems in sufficient food production and resources will not be consumed. It is thought that as the number of studies on the relationship between microalgae and plants increases, newer insight will be discovered for much more sustainable agriculture. In future studies, it will be useful to study the mutualistic relationships of other beneficial microorganisms such as AMF and PGPR with microalgae and rhizosphere in detail.

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