International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 14(1): 33-46, 2021
Research Article

MICROALGAE (*Chlorella vulgaris*)-MEDIATED SECONDER METABOLITES AND ANTIOXIDATIVE DEFENSE SYSTEM IMPROVE PLANT GROWTH AND SALT TOLERANCE IN MELON

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(Received 22th March 2021; accepted 25th May 2021)

ABSTRACT. Microalgae, such as *Chlorella vulgaris* (green algae) are beneficial microscopic organisms that may result in a plant having improved nutrient uptake, growth, and abiotic stress tolerance. In this study, an investigation of tolerant and sensitive melon genotypes, grown under salt-stress conditions, with regards to the impact of microalgae on physiological, morphological, and enzymatic activity was performed. Microalgae applications significantly increased shoot length, and fresh and dry weight, and leaf number and area, and photosynthetic pigments and of melon plants compared to the only salt stress treatment. In addition, following the above-mentioned procedures, there were significant increases in the relative water content, total phenolic and flavonoid contents, K and Ca ion contents, and superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutation reductase (GR) activity. However, the malondialdehyde (MDA), Na and Cl ions contents were significantly decreased. Hence, the results support the administration of a foliar application to the microalgae in order to increase the melon plant's defense system, enabling it to tolerate the negative effects resulting from salinity.

Keywords: *Antioxidative enzyme activity, green algae, ion regulation, oxidative stress, SOD*

INTRODUCTION

Salinity is a significant abiotic stress factor that threatens agriculture in both arid and semiarid environments, affecting over 20% of the world's irrigated land. Salt stress results in changes in a plant's biochemical, physiological, and morphological responses and so reduced growth, yield, biomass, and quality of crop plants.

Salinity in growth mediums is cause unfavorable impact on plant growth and development, which could presumably be due to the existence of salt in the soil, thus reducing the plant's water uptake (low osmotic potential), resulting in high levels of salt entering the plant via transpiration, which causes damage to the transpiring leaf cells (specific ion toxity) [1, 2]. Under salt stress, plants often experience plant-water relation disturbances and develop a build-up of toxic ions. In response, there are three adaptation methods that plants can employ: 1) develop an osmotic stress tolerance, 2) exclude Na or Cl, and 3) develop a tolerance to the accumulated Na or Cl in the tissues [2]. Salinity is causes excessive reactive oxygen species (ROS) accumulation, which may result in lipid peroxidation, protein oxidation, enzyme inactivation, damage to DNA, and/or interaction with other essential plant cell components. High concentrations of salt may result in stomatal closure, reducing the availability of carbon dioxide in the leaves and causes carbon fixation inhibition, resulting in chloroplasts being exposed to high levels of excitation energy, which then brings about increased ROS generation, such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radical (OH), and singlet oxygen

($^1O_2$) [1]. In order to minimize the toxic affects caused by ROS, plants possess various kinds of enzymatic and non-enzymatic antioxidative systems. The enzymatic system comprises superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase, and glutathione reductase (GR), where as non-enzymatic antioxidants comprise ascorbate, reduced glutathione (GSH), carotenoids, and tocopherol. SOD comprises enzymes that expedite O_2^- to H_2O_2 conversion. Then, H_2O_2 is scavenged further by catalase (CAT) and ascorbate peroxidase (APX), resulting in H_2O and O_2. After oxidization, the ascorbate is reduced by the glutathione (GSH) caused by the oxidized glutathione (GSSG), which is then catalyzed by GR, resulting in the loss of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) [3].

Microalgae are a biofuel that is superior and photosynthetic, as well as the world's largest oxygen-producing organisms, which are crucial for planetary functions and sustainability of the ecosystem. They may potentially be an alternative that is sustainable for enhancing and protecting agricultural crops. Following the application microalgae products, plants have been reported to have had different responses, such as robust growth, increased yield, improved nutrient uptake, and increased biotic and abiotic stresses resistance (fungal infections, pest attacks, and frost), increased quality, and fruit with a longer shelf life [4-6]. It was reported by Markou and Nerantzis [7] that species of microalgae cultured under saline stress cumulate with lipids and carbohydrate-specific secondary metabolites (like pigments and vitamins), which are highly value substances that can be utilized in cosmetics, foods, and pharmaceuticals. However, microalgae polysaccharides have been reported as having a good ability to improve plant growth, which means they may potentially be used as biostimulants. Algae are comprised of active compounds, like organic and free amino acids, enzymes, and phytohormones, as well as bioactive secondary metabolites, vitamins, and vitamin precursors [4, 8] essential nutrients and plant growth regulators like auxins and cytokinins [6]. Approximately 40,000 microalgae species have been categorized thus far, and among those, Chlorella vulgaris has drawn the attention of scientists due to its high levels of protein, and biomass totaling more than 55% dry weight (DW). The high concentration of protein, lipids (5%–40% DW), and carbohydrates (15%–55% DW) also allows it to be used as animal feed, in human nutrition and cosmetics, as well as in biofertilizers [9]. Melon is a significant crop in arid and semi-arid areas under saline stress conditions [10]. Generally, even though melon is known for its moderate tolerance to salt stress, Kusvuran et al. [11] reported that tolerance to salt in melons depends on the cultivars used; some are sensitive, while others are tolerant. Though several researchers reported the effects of *Chlorella vulgaris* on plants growth and yield only limited studies were carried on salt tolerance in melon. Hence the objectives of this study was investigated the effect that microalgae has on plant growth and the biochemical and physiological responses of melon seedlings under salt stress; in addition determined the relationship between of microalgae application and improve salt tolerance.

MATERIALS AND METHODS

Experimental Conditions and Treatments

Salinity responses of the genotypes were previously reported two salt - tolerant (T1 and T2) and two salt - sensitive (S1 and S2) melon genotypes were used as plant materials [12, 13]. Seeds of four genotypes melon were germinated in a mixture of peat:perlite substrate (2:1) ration in a greenhouse (temperature: 26±2°C and 18±2°C were the day and night temperatures ±2 and relative humidity: 65%± 5). After 18 days of sowing (DAS), the uniformity seedlings were transferred to plastic pots containing 12 L of vermiculite. The plants were grown in growth chamber and irrigated with nutrient solution. The composition of the nutrient solution used was as follows (M): $Ca(NO_3)_2$ $4H_2O$, 3.0x10-3; K_2SO_4, $0.90x10^{-3}$; $MgSO_47H_2O$, $1.0x10^{3}$; KH_2PO_4, $0.2x10^{-3}$; H_3BO_3, $1.0x10^{-5}$; 10^{-4} FeEDTA, $MnSO_4$ H_2O, $1.0x10^{-6}$; $CuSO_45H_2O$, $1.0x10^{-7}$; $(NH)6Mo_7O_24H_2O$, $1.0x10^{-4}$; $ZnSO_4.7H_2O$, $1x10^{-4}$ [14]. Four plants were grown per pot and 5 pots were included in each replicate (4 replications). Starting from 32 DAS, salt treatment has been started by 50 mM NaCl concentration and increased by the increments of 50 mM NaCl per day until a final concentration of 200 mM NaCl was achieved at the end of 4th day. Applied amount of water in the experiment was calculated according to the ratio of "drained water / applied water" [15]. Under the control conditions, without stress, this ratio was around 30%. The plants were subject to treatment for 16 days. Control plants were grown under non-stress conditions for the same period of time. Six treatments were formed in each experiment as follows: control [C: irrigation with nutrient solution free from NaCl], salt treatment [S: irrigation with nutrient solution contained 200 mM], salt treatment + microalgae (5%) [S+MA: irrigation with nutrient solution contained 200 mM NaCl + foliar microalgae treatment].

Chlorella vulgaris is unicellular non halotolerant algae have fast growth rate, cosmopolitan distribution and ability to grow in different harsh environment [5] and so this microalga was selected. As microalgae was used liquid commercial product in this experiment (Natural Bioplasma®, Denge Tarim, Turkey) (number of viable algae 2x107alg/ml; pH:7; density: 1; content of vitamins and amino acids: lysin, methionine, cystin, tryptophan, histidine, leucine, phenylanine, vailin, arginine, biotin, A, B1, B2, C, E). The microalgae was sprayed on the foliage of plants to run off at three days apart.

Growth characteristics measurements

The end of the experiment, shoot fresh and dry weight, shoot length, shoot diameter, number of leaves per plant, leaf area per plant were estimated.

Relative water content (RWC%) and photosynthetic pigments

Relative water content (RWC% was estimated by following the method as prescribed by Sanchez et al. [16] and Türkan et al. [17]. RWC was calculated as: RWC (%) = [(FW–DW)/ (TW – DW)] × 100, were TW is turgid weight after floated on deionized water for 5 h.

Chlorophyll (Chl) a, b and total carotenoids contents were determined by Arnon [18]. Leaf pigment was extracted with 80% (v/v) acetone and absorbance of the extraction was measured at 663, 645 and 470 nm with a spectrophotometer (Shimadzu UVmini-1240).

Determination of Malondialdehyde (MDA) Content

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction [19]. Frozen samples were homogenized in a prechilled mortar with 2 volumes of ice-cold 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged for 15 min at 15000 × *g*. An assay mixture containing a 1 mL aliquot of the supernatant and 2 mL of 0.5% (w/v) TBA in 20% (w/v) TCA was heated to 95°C for 30 min and then rapidly cooled in an ice bath. After centrifugation (10000 × *g* for 10 min at 4°C), the supernatant absorbance (532 nm) was read and values corresponding to nonspecific absorption (600 nm) were subtracted.

Determination of Total Phenolic and Total Flavonoid Contents

The total phenolic content was determined using a Folin-Ciocalteu reagent. The phenolic content of leaves was expressed in milligrams. Gallic acid was used as a standard [20]. Flavonoid content was determined by colorimetric assay [21, 22]. Total flavonoids were expressed on a fresh weight (fw) basis as milligrams of quercetin equivalents per gram.

Determination of Ion Contents (Na^+, K^+, Ca^{++}, and Cl^-)

For ion determination, the plants were harvested and dried at 65°C for 48 h. The shoot samples of were burned at 550°C, and dissolved in 1% (v/v) hydrochloric acid, and then analysed for Na, K, and Ca using an atomic absorption spectrometer (Varian Spectra AA 220 FS). The shoot Cl concentration was determined using the Mohr method [14].

Determination of Antioxidative Enzyme Activites

Enzymes were extracted from 0.5 g of leaf tissue using a mortar and pestle, with 5 mL of extraction buffer containing 50 mM of potassium-phosphate buffer (pH 7.6) and 0.1 mM of disodium ethylenediaminetetraacetate. The homogenate was centrifuged at 15 000 × *g* for 15 min, and the supernatant fraction was used to assay for the enzymes. All of the operations for the preparation of the enzyme extractions were performed at 4°C. The SOD was assayed according to Karanlik [23], by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. The CAT activity was determined by monitoring the disappearance of H_2O_2. APX activity was determined by measuring the consumption of ascorbate from its the absorbance at 290 nm. One unit of APX activity was defined as the amount of enzyme required to consume 1 μmole of ascorbate min^{-1} [24]. The GR activity was determined by measuring the enzyme-dependent oxidation of NADPH from it's the absorbance at 340 nm. One unit of GR activity was defined as the amount of enzyme that oxidized 1 μmole of NADPH min^{-1}.

Statistical analysis

The plot design used for the experiment was completely randomized, comprising 4 replicates. The mean values were compared using the Tukey multiple range test. Statistical significance was accepted as $P < 0.05$ using SPSS v.13.0 software for Windows (SPSS Inc., Chicago, IL, USA). Data were presented as the mean ± standard

deviation (SD) and error bars represent the standard errors of the means in all of the figures.

RESULTS AND DISCUSSION

Growth Characteristics

An improvement value in growth parameters of the melon plants grown on a saline condition by using microalgae is showed in Table 1. Melon genotypes treated with NaCl, these traits were reduced by 49.7, 41.3, 28.7, 43.3, and 33.2%, respectively compared to the control. However, microalgae application under salt stress significantly enhanced growth components such as, shoot fresh and dry weight, shoot length, shoot diameter, number of leaves per plant, leaf are per plant of melon compared to those salt stressed groups. These reactions were changed among to 8-33% rations. When SMA compared to S; SMA enhanced to amelioration for growth by 28-77%. *Chlorella vulgaris*, the green algae, is a photosynthetic organism that is regarded as a crucial biofertilizer. It has mainly been studied because it is commercially significant due to its high protein levels, vitamins, essential amino acids, and fatty acids [25]. It has been shown; microalgae application was successful in limiting the effects of 200 mM NaCl on the growth of melon seedlings and development in this study. The favorable effect of microalgae might be predicated to its success providing the plants with necessary nutrients and phytohormones. Tarraf et al. [26] showed that fenugreek plants treated with a foliar application of algae extract had a significantly increased the number of leaves and branches, plant height, and fresh and dry weight during the vegetative growth and flowering stages. Sharara et al. [27] reported that microbial inoculation such as microalgae stimulated growth in the plants in 2 ways: 1) directly, through the production of plant hormones and an improved uptake of nutrients, or 2) indirectly, via microbial balance changes in the rhizosphere towards beneficial micro-organisms. These improvements occurred clearly in salt sensitive genotypes, S1 and S2, and SMA provided to recovery for growth parameters average 42-145% rations. Using of Chlorella vulgaris with NaCl could be enhancing the nutrient uptake of plant which means the status may be help amelioration at growth parameters. Thus, compared to control condition, growth parameters decreased by 33-50%; however, when microalgae were applied, these impulses were determined by 8-33% rations. In fact, this hypothesis were reported that in corn [28, 29], wheat, maize, bean and lettuce [30]. In addition to this, the growth medium and cellular extracts of some species of microalgae have been reported as containing phytohormones (auxins, abscisic acid, cytokinins, gibberellins, and salicylic acid), all of which play a significant role in the development of plants. Plant hormones are vital for a variety of plant development and growth aspects. Cell division, the regulation of root and shoot development, stimulation of leaf growth, and formation of flowers, fruit, and seeds are the result of cytokinins, as a result of their stabilizing effect on photosynthetic machinery, ability to suppress senescence, and improve sink strength and nitrogen acquisition [4]. Such as other growth parameters, leaf number and area decreased at salt treatment 14-24% and 12-17% in T1 and T2; 42-64% and 58-71% in S1 and S2, respectively. With application of Chlorella vulgaris, protection of loss at leaf number and area values showed increasing 7-8% and 8-18% in T1 and T2 under salt stress, respectively. Especially, microalgae provide recovery in S1 and S2. Compared to salt treatment; in these parameters, improvement was shown among to 29-86%. This situation may be the result of an increased access to nutrients

responsible for augmenting protein synthesis, leading to an increased accumulation of carbohydrate by means of *C. vulgaris* [25].

Table 1. *Effects of microalgae application on growth parameters of melon genotypes under saline condition*

		Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Shoot length (cm plant^{-1})	Shoot diameter (mm plant^{-1})	Leaf Number (number plant^{-1}plant)	Leaf Area (cm^2 plant^{-1})
C	T1	132.50 ±2.45^{c}	20.46 ±1.18^{c}	22.00 ±1.56^{f}	8.60 ±0.49^{a-c}	18.00 ±0.29^{b}	477.02 ±2.80^{ab}
	T2	105.00 ±2.08^{d-f}	20.82 ±1.30^{c}	56.33 ±2.04^{b}	7.55 ±0.38^{c-e}	16.00 ±0.61^{bc}	411.33 ±1.68^{bc}
	S1	193.25 ±3.46^{b}	36.47 ±1.12^{b}	50.00 ±2.21^{bc}	9.29 ±0.20^{a}	27.33 ±0.93^{a}	541.88 ±2.07^{a}
	S2	228.25 ±2.29^{a}	47.70 ±1.75^{a}	65.66 ±2.08^{a}	8.98 ±0.37^{ab}	28.33 ±0.34^{a}	296.17 ±1.88^{e}
S	T1	98.25 ±2.73^{ef}	16.83 ±1.92^{cd}	16.66 ±1.06^{f}	6.55 ±0.48^{ef}	13.66 ±0.25^{cd}	406.43 ±2.72^{cd}
	T2	93,25 ±3.43^{f}	17.87 ±1.52^{cd}	45.66 ±1.53^{c}	7.48 ±0.33^{c-e}	14.00 ±0.65^{c}	341.82 ±2.92^{de}
	S1	36.50 ±3.28^{g}	6.33 ±1.04^{e}	20.66 ±1.43^{f}	5.65 ±0.54^{f}	7.66 ±0.35^{e}	225.53 ±2.42^{f}
	S2	45.00 ±4.08^{g}	7.48 ±1.91^{e}	24.00 ±1.32^{ef}	4.42 ±0.27^{g}	10.00 ±0.40^{de}	170.09 ± 3.06^{f}
S+MA	T1	120.00 ±3.12^{cd}	17.48 ±1.04^{cd}	19.00 ±0.75^{f}	8.33 ±0.39^{a-d}	14.66 ±0.53^{bc}	442.73 ±2.57^{bc}
	T2	108.50 ±4.93^{d-f}	20.14 ±1.58^{c}	52.83 ±2.47^{bc}	7.74 ±0.55^{c-e}	15.66 ±0.58^{bc}	405.32 ±2.24^{cd}
	S1	112.25 ±2.21^{c-f}	15.00 ±1.99^{d}	32.00 ±1.00^{d}	7.95 ±0.24 $^{b-d}$	14.33 ±0.48^{bc}	383.56 ±1.84^{cd}
	S2	119.25 ±2.78^{c-e}	21.34 ±0.57^{c}	31.00 ±1.46^{de}	7.21 ±0.29^{de}	13.00 ±0.65^{cd}	220.18 ±2.15^{f}

*Each value represents the mean of four replicates. For each parameter, different superscripted letters represent statistically significant differences at P< 0.05 according to the Tukey test (C: control; S: salt stress (200 mM NaCl); S+MA: 200 mM NaCl + microalgae)

Relative Water Content (RWC) and Photosynthetic Pigments

Melon seedling treated with salt stress showed decreased RWC at 20-24% in T1 and T2 and 43-56% in S1 and S2 as compared to control, respectively (Table 2). However, a significant improvement in the RWC of microalgae treated plants was observed increasing by 5-13% in T1 and T2 and 6-54% in S1 and S2 when compared with the plant treated with 200 mM NaCl alone ($p \leq 0.05$). Under salt (200 mM) salt stress, photosynthetic pigments such as total chl a, chl b and total carotenoid were reduced by 10-37%, 9-28%, and 4-47%, respectively in melon genotypes (Table 2). These reactions were shown clearly in sensitive genotypes (23-37%, 27-28%, 34-47% decrease). Auxiliary addition of microalgae to the salt stressed melon plants induced significant increase photosynthetic pigments by 28-46%, 30-56% and 23-154% compared to salt condition, respectively. Under salt stress or an irrigation deficit caused to plants display mild dehydration, which is seen through decreased water capacity, due to greater water uptake difficulties or a lower substrate water level. The most negative leaf water and stem water potential values were observed in plants subjected to water and salinity stress, because passive dehydration as well as the accumulation of salt play a role in decreasing the water potential in leaves [31]. One of the most effortless agricultural variables used for screening plants for a tolerance to salt or drought is the relative water content [32]. While RWC values decreased under salt stress among to 12-26% in T1 and T2 and 41-53% in S1 and S2, with microalgae application RWC shown increase 9-17% in T1 and T2 and 6-54% in S1 and S2 in the same conditions. Increased leaf RWC by microalgae under salt stress suggests that microalgae application could enhance leaf melon water relations and support maintaining cell turgor pressure. Growth inhibition in plants is the result of decreased chlorophyll content, possibly due to ROS-induced chlorosis, photo-reduction, and triplet chl formation, which causes serious damage to photosystems I and II, and the formation of chlorophyll in plants [33]. In this study, the favorable effects of the microalgae was identified on chlorophyll components and increased among to 28-46%, 30-56% compared to salinity. Moreover, this advances

especially adjusted in S1 and S2 which in these genotypes, Chla and Chlb increased by 50-56% and 109-154% (Table 2). The other component carotenoid content was increased in melon plants treated with microalgae under salt stress (23-154% increase). Carotenoids essentially play a critical part in light harvesting and oxidative damage protection through the deactivation of singlet oxygen, satisfying the excited triplet state in chlorophyll, and the enhancement of carotenoid synthesis as a way of protecting itself from photo damage caused by cell division arresting when exposed to salt stress [5, 34]. Carotenoids play a significant role in the photosynthesis process, including light harvesting, photo-protection, scavenging of 1O2, the dissipation of excess energy, and stabilization of the structure [7]. Dineshkumar et al. [25] reported that increased chlorophyll accumulation in organic fertilizers, such as microalgae, even at a decreased rate, could be the result of the cooperative effects of consortium, which better facilitates plant N, P, and K uptake resulting in increased chlorophyll accumulation.

Table 2. *Effects of microalgae application on relative water content (RWC) and photosynthetic pigments of melon genotypes under saline condition*

		RWC (%)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Total carotenoids (mg g^{-1} FW)
C	T1	87.66 ±1.80^{a}	0.26 ±0.02^{d}	1.17 ±0.03^{c}	0.63 ±0.05^{cd}
	T2	82.56 ±1.79^{ab}	0.28 ±0.03^{cd}	1.01 ±0.06^{e}	0.56 ±0.02^{de}
	S1	84.67 ±1.28^{ab}	0.27 ±0.01^{cd}	1.18 ±0.05^{c}	0.59 ±0.08^{de}
	S2	84.96 ±1.13^{ab}	0.26 ±0.02^{d}	1.09 ±0.04^{d}	0.63 ±0.04^{cd}
S	T1	68.87 ±1.51^{cd}	0.23 ±0.02^{ef}	1.01 ±0.06^{e}	0.60 ±0.04^{de}
	T2	67.32 ±1.09^{d}	0.25 ±0.03^{de}	0.92 ±0.05^{f}	0.48 ±0.03^{ef}
	S1	37.19 ±1.86^{g}	0.17 ±0.02^{g}	0.86 ±0.04^{f}	0.31 ±0.02^{g}
	S2	48.68 ±1.00^{f}	0.20 ±0.04^{fg}	0.78 ±0.03^{g}	0.41 ±0.03^{fg}
S+MA	T1	77.26 ±1.53^{bc}	0.34 ±0.04^{a}	1.32 ±0.04^{a}	0.74 ±0.03^{bc}
	T2	70.00 ±1.82^{cd}	0.31 ±0.01^{ab}	1.05 ±0.06^{de}	0.64 ±0.09^{cd}
	S1	57.58 ±1.08^{e}	0.30 ±0.03^{bc}	1.29 ±0.05^{ab}	0.79 ±0.04^{ab}
	S2	51.64 ±1.18^{ef}	0.28 ±0.03^{cd}	1.22 ±0.04^{bc}	0.86 ±0.05^{a}

*Each value represents the mean of four replicates. For each parameter, different superscripted letters represent statistically significant differences at P< 0.05 according to the Tukey test (C: control; S: salt stress (200 mM NaCl); S+MA: 200 mM NaCl + microalgae)

Malondialdehyde (MDA) Content

To confirm the salinity induced oxidative stress conditions, intercellular levels of stress biomarker MDA was evaluated (**Table 3**). The MDA content was the lowest in control plants and increased significantly under 200 mM NaCl conditions. When compared with the control groups, the MDA levels increased different rations in melon genotypes (9.53-22.53 μmol g^{-1} FW). The highest MDA levels were determined in sensitive genotypes (290-547% increase). But, microalgae mitigated the stress effects on plants and further decreased the contents of MDA. In point of fact, through microalgae treatment, MDA content was decreased at 9-34% rations. Salt stress results in the formation of free radicals in plants, which cause irreversible lipid and protein damage. Cell membrane integrity is destroyed by lipid peroxidation, eventually resulting in cellular death [35]. The lipid peroxidation increase is due to compounds like hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), and superoxide radical (O_2^-) in chloroplasts. Malondialdehyde (MDA), which plays the role of a cellular toxicity bio-

indicator, is a well-known oxidation resulting from lipid peroxidation during oxidative stress [33]. In this study, lipid peroxidation of melon genotypes increased with salt stress. Besides, MDA content was significant in sensitive genotypes (290-547% increase) compared to tolerant genotypes (157-158% increase). The results showed that microalgae treatment reduced the MDA levels, presenting a favorable effect in reducing the oxidative stress resulting from salt stress.

Total Phenolic and Total Flavonoid Contents

Under salt stress, total phenolic and flavonoid contents decreased in melon genotypes (7-29% decreases) except T1 genotype (17-47% increases), compared to control (**Table 3**). Contrary, microalgae treatment proved to result in significant increase in the mean values of total phenolic and flavonoid contents compared with both control and individually 200 mM NaCl (25-103% and 19-65% increase). The maximum mean values were obtained in microalgae application (72-102 µgGAE/ml of total phenolic; 24-41 mgQE/100g) and with microalgae treatment in S1 and S2 total phenolic and flavonoid contents showed increase between 46% and 99% compared to non-treatment salinity condition.

During extreme environmental conditions, microalgae multiply due to stress and a variety of secondary metabolites are synthesized and produced, which is assumed to be an endeavor by microorganisms at retaining their rate of growth or increasing their likelihood of survival [7]. Our results indicated that usage of microalgae maintained an important increase in total phenolic and flavonoid contents compared to untreated plants under salt stress (25-103% and 19-65% increase). These results clearly indicate that the microlagae plays a stimulatory role in phenolics accumulation in melon. Similarly, Abd El-Baky et al. [36] reported that microalgae is a source of unique compounds that are biologically active, like phenols, polysaccharides, phycobiline, steroids, and terpenoids. The elevated total phenol level is believed to be a result of significant photosynthesis rates, which is predicated by a large photosynthetic area and high photosynthetic pigment levels using a treatment of yeast as a biofertilizer [25].

***Table** 3. Effects of microalgae application on MDA, total phenolic and total flavonoid Contents of melon genotypes under saline*

		MDA ($\mu mol\ g^{-1}$ FW)	Total phenolic ($\mu g\ GAE\ ml^{-1}$)	Total flavonoid ($mgQE\ 100g^{-1}$)
C	T1	3.69 ± 0.51^{d}	$50.26 \pm 1.51^{f-h}$	26.34 ± 1.16^{cd}
	T2	4.02 ± 0.89^{d}	63.67 ± 1.13^{de}	24.52 ± 1.54^{de}
	S1	3.48 ± 0.25^{d}	64.72 ± 1.10^{de}	21.27 ± 1.57^{ef}
	S2	4.36 ± 0.25^{d}	$57.34 \pm 1.84^{e-g}$	17.21 ± 1.67^{fg}
S	T1	9.53 ± 1.18^{c}	74.16 ± 1.61^{c}	30.82 ± 1.69^{c}
	T2	10.36 ± 0.66^{c}	58.64 ± 1.26^{ef}	21.97 ± 1.15^{de}
	S1	22.53 ± 0.68^{a}	45.45 ± 1.12^{h}	16.84 ± 1.26^{fg}
	S2	17.03 ± 1.07^{b}	49.37 ± 1.27^{gh}	14.76 ± 0.92^{g}
S+MA	T1	8.67 ± 0.88^{c}	102.09 ± 2.64^{a}	$41.06\ 1.37^{a}$
	T2	9.43 ± 0.62^{c}	80.50 ± 1.98^{c}	36.29 ± 1.27^{b}
	S1	15.18 ± 0.97^{b}	90.83 ± 1.02^{b}	25.33 ± 0.67^{de}
	S2	11.17 ± 0.62^{c}	72.11 ± 1.07^{cd}	24.20 ± 1.35^{de}

*Each value represents the mean of four replicates. For each parameter, different superscripted letters represent statistically significant differences at $P< 0.05$ according to the Tukey test (C: control; S: salt stress (200 mM NaCl); S+MA: 200 mM NaCl + microalgae)

Ion Contents (Na^+, K^+, Ca^{++}, and Cl^-)

Compared to with control, salt stress increased Na and Cl contents of all genotypes (800-1667% in Na and 918-1781% increase in Cl) (**Table 4**). Nevertheless, these traits were more significant in S1 and S2 (Na: 7.07 and 6.41%; Cl: 5.24 and 4.41%, respectively). With microalgae application, salt stressed plants accumulated less Na and Cl and these ion accumulations reduced 18-45% compared to only salt application. On the contrary of toxic Na and Cl ion, K and Ca ion accumulation decreased in salt condition. These reaction was determined as 43-9%, 3-15%, 65-40%, and 56-47%, T1, T2, S1, and S2, under salt stress condition, respectively. Foliar microalgae application alleviated the stress effects on these parameters and significantly increased K and Ca contents compared to the NaCl salt stressed. Microalgae application ensured increasing the content of K by 11-37% and the content of Ca by 19-53%. Ion toxicity in plant cells is the result of salt stress caused by a significant Na and Cl cell influx, as well as the reality that the majority of plants amass a high concentration of Cl and Na ion in their shoots when cultivated under salt stress, which is a significant cause of decreased growth [1, 37]. While under salt (200 mM) stress toxic ions contents significantly increased, used of microalgae inhibited these ions accumulation (18-45% decrease) under salt stress (Table 3). The uptake of K and Ca was reduced by Na in melon genotypes under salinity conditions. A decreased K level is a response commonly observed under salt stress because it directly competes for charge-dependent binding sites with Na [38]. Our results showed that microalgae limited toxic ion accumulation so enable to K and Ca accumulations (11-53% increase). Microalgae may potentially prevent the loss of nutrients as the result of N, P, and K being released slowly, as an organic fertilizer, which is based on the plant's needs. Phototrophic microorganisms comprise trace elements and substances that promote plant growth, like phytohormones, amino acids, antifungal substances, and macronutrients [25]. Plaza et al. [4] found that the foliar application of scenedesmus hydrolysates was also seen to increase the leaf and shoot number, and improve foliar concentrations of P, K, Ca, and Mg. Decrease in leaf Ca levels under salt stress indicates an improvement in membrane stability and decrease in chlorophyll levels, respectively [1].

***Table 4**. Effects of microalgae application on Na, K, Ca and Cl contents of melon genotypes under saline condition*

		Na (%)	K (%)	Ca (%)	Cl (%)
C	T1	0.47 ± 0.05^{f}	5.59 ± 0.23^{ab}	$5.95 \pm 0.22^{a-d}$	0.32 ± 0.06^{g}
	T2	0.53 ± 0.08^{f}	5.08 ± 0.49^{bc}	6.49 ± 0.17^{ab}	0.33 ± 0.09^{g}
	S1	0.40 ± 0.06^{f}	5.90 ± 0.48^{ab}	$5.89 \pm 0.20^{a-d}$	0.28 ± 0.05^{g}
	S2	0.52 ± 0.05^{f}	6.22 ± 0.56^{a}	$6.31 \pm 0.49^{a-c}$	0.22 ± 0.06^{g}
S	T1	4.23 ± 0.17^{d}	$3.18 \pm 0.23^{d-f}$	$5.39 \pm 0.53^{c-e}$	3.26 ± 0.15^{de}
	T2	4.65 ± 0.24^{cd}	$3.47 \pm 0.19^{d-f}$	$5.48 \pm 0.34^{b-e}$	3.76 ± 0.40^{cd}
	S1	7.07 ± 0.37^{a}	2.54 ± 0.21^{fg}	3.10 ± 0.43^{g}	5.24 ± 0.16^{a}
	S2	6.41 ± 0.16^{ab}	2.16 ± 0.10^{g}	3.75 ± 0.54^{fg}	4.41 ± 0.22^{b}
S+MA	T1	2.29 ± 0.20^{e}	4.13 ± 0.24^{cd}	$6.46 \pm 0.25^{a-c}$	2.28 ± 0.45^{f}
	T2	2.32 ± 0.28^{e}	3.86 ± 0.15^{de}	6.95 ± 0.29^{a}	2.73 ± 044^{ef}
	S1	5.49 ± 0.14^{bc}	$3.40 \pm 0.18^{d-f}$	4.76 ± 0.33^{ef}	3.97 ± 0.28^{bc}
	S2	5.24 ± 0.23^{c}	$2.96 \pm 0.12^{e-g}$	5.01 ± 0.32^{de}	3.09 ± 0.16^{e}

*Each value represents the mean of four replicates. For each parameter, different superscripted letters represent statistically significant differences at $P< 0.05$ according to the Tukey test (C: control; S: salt stress (200 mM NaCl); S+MA: 200 mM NaCl + microalgae)

Antioxidative Enzyme Activities

Antioxidative enzyme activity (SOD, CAT, GR and APX) levels were evaluated in C, S and SMA treatments (**Fig. 1**). Salt stress caused increase SOD, CAT, GR and APX activities different levels. Under 200 mM NaCl condition, the highest increase was determined in T1 and T2 (13-77%, 228-376%, 282-321%, 138-232% increase) compared the control. But, these increases were by 16-67%, 131-139%, 41-84%, and 19-43% in S1 and S2. It is evident from Fig. 1. that microalgae treatments had a serious effect on antioxidative enzyme activities such as SOD, CAT, GR, and APX of the melon genotypes under salt stress. At S+MA application, enzyme activities increased by 28-41%, 21-35%, 48-67%, 54-72% in tolerant genotypes and in sensitive genotypes by 37-60%, 45-49%, 92-128%, and 116-172% in comparison with 200 mM treatment.

ROS in plant cells are generated via normal cellular metabolism or stressful environmental conditions like drought, heavy metals, herbicides, nutrient deficiency, radiation, or salinity. Straight a consequence of salinity is the induction of stress antioxidant enzymes by exposed plants to minimize damage caused by reactive oxygen species [39]. Enzymatic antioxidant defense systems, such as APX, CAT, DHAR, GR, MDHAR, POX, SOD and non-enzymatic antioxidant defense systems, like ascorbate, carotenoids, glutathione, glycine betain, phenolic compounds, polyamines, proline, and sugar [40, 41]. Photosynthetic photosystems I (PSI) and PSII reaction centers are the central chloroplastic ROS production locations under light conditions. O_2^- is the result of the Mehler reaction in the PSI systems in the antenna pigments. Next, it is converted by SOD to H_2O_2, which acts as a main ROS scavenger and is the most efficient metallozyme. It is then reduced even further by APX in the water-water cycle [42]. H_2O_2 is significantly scavenged by catalase into H_2O and O_2 in peroxisome. Under stress, GR sustains the cytoplasm's GSSG to GSH pool, preserving the balance of redox in the cells via the inter-conversion of the reduced GSH and oxidized GSSG that was catalyzed by the GR. In here, in which response to microalgae application in salt-stressed medium were examined, researchers reported increased antioxidative enzyme activity such as SOD, CAT, GR, and APX. These increases were statistically significant and determined by 21-172% rations. Sing et al. [43] reported that plants have defenses against oxidative damage including physiological and biochemical status changes using PGPRs to facilitate protection against losses due to pathogens or abiotic stress, and improved plant tolerance to abiotic stress as a result of physical and chemical changes is an approach that is rather new and overlaps a great deal with the process of systemically-induced resistance in plants. Since microalgae *Chlorella vulgaris* a colonial green microalgae [9], it may be assists plants in plants to nurture growth under salt stress due to the evocation of the generated systemic responses in plants as PGPR.

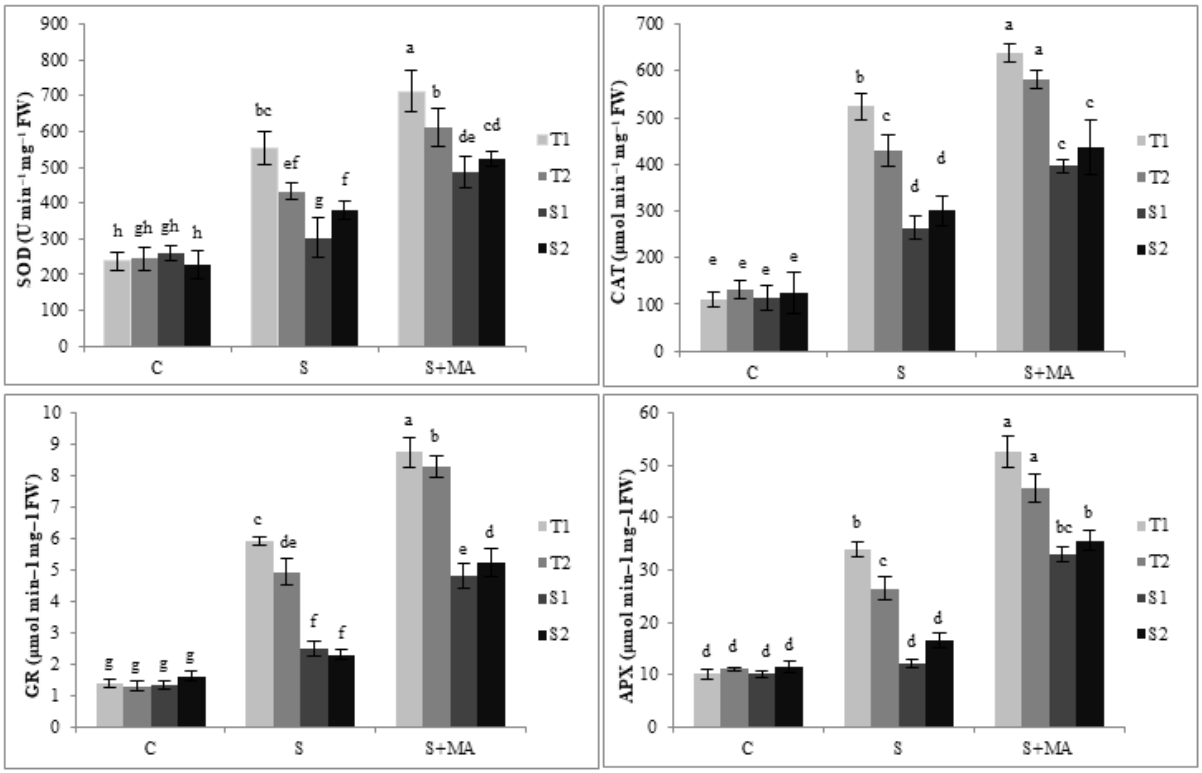

***Fig.1**. Effects of microalgae application on SOD, CAT, GR, and APX enzyme activities of melon genotypes under saline condition (C: control; S: salt stress (200 mM NaCl); S+MA: 200 mM NaCl + microalgae). Each value represents the mean of four replicates. For each parameter, different letters represent statistically significant differences at $P < 0.05$ according to the Tukey test.*

CONCLUSION

Microbiological fertilizers are important to environment favorable and renewable cheaper source for agricultural practices. One of the most significant features that make microalgae valuable; contain high percentage of macronutrients, considerable amount of micronutrients and amino acids. The application of microalgae to salinity condition appeared to be favorable to growth and development as well as the physiological and biochemical processes of the melon. These effects were observed clearly in salt sensitive genotypes and the integrative microalgae treatment supported to salt tolerance in these genotypes. Therefore, microalgae application has been achieved to be helpful strategy for enhancing the tolerance of the melon plants when grown under salinity conditions.

Acknowledgement. Authors gratefully acknowledge Denge Tarim (Turkey) and would like to thank Cankiri Karatekin University, Scientific Research and Project Unit for their support part of this study.

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