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The Use of Halophytic Companion Plant (*Portulaca oleracea* L.) on Some Growth, Fruit, and Biochemical Parameters of Strawberry Plants Under Salt Stress

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Abstract

Strawberry is a saline sensitive plant adversely affected under slightly or moderately saline conditions. Growth and biochemical parameters of strawberry plants grown under NaCl (0-, 30-, 60-, and 90 mmol L^{-1}) conditions with or without a halophytic companion plant (*Portulaca oleracea* L.) were investigated in a pot experiment. Salt stress negatively affected the growth, physiological (stomatal conductance, electrolyte leakage, total soluble solids) and biochemical parameters such as chlorophyll contents (chl-*a* and chl-*b*), proline, hydrogen peroxide, malondialdehyde, catalase, and

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peroxidase enzyme activities, lycopene, vitamin C contents along with the mineral uptake of strawberry plants. The companionship of *P. oleacea* increased fresh weight, dry weight, and fruit average weight and total fruit yield of strawberry plants along with the improvement of physiological and biochemical parameters. This study showed that cultivating of *P. oleracea* with strawberry plants under salt stress conditions effectively increased strawberry fruit yield and quality. We, therefore, that approaches towards the use of *P. oleracea* could be an environmentally friendly method that should be commonly practised where salinity is of great concern**.**

Keywords: abiotic stress, strawberry, companion plants, phytoremediation

1. Introduction

Salinity is one of the most significant environmental issues limiting plant productivity, especially in arid and semi-arid climates. Due to Na^{+} and Cl^{-1} ion accumulation, many physiological and biochemical processes are disturbed in plants. The NaCl stress results in the development of leaf chlorosis and necrosis, thus reducing the photosynthetic capability of the plants. As a consequence, the assimilation of carbohydrates available for fruit production is significantly reduced [1, 2].

Strawberry is a small fruit crop of great importance throughout the world. Strawberry belongs to the family Rosaceae in the genus Fragaria, containing 23 species [3, 4].

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Strawberry represents an important commercial fruit crop with increasing cultivation areas in the world and rising consumption, but it is considered sensitive to NaCl salinity although the sensitivity criteria and causes of the injuries due to toxic Na^+ or Cl^- ions are important points of discussion. NaCl salinity not only reduces the crop yield but also deteriorates the quality parameters in many crops including strawberry [5, 6].

Strawberry cultivation has become an important greenhouse and open field crop in the Mediterranean basin although drought and salinity are often major limiting factors [7, 8].

In the present study, we evaluated the effects of different levels of salt stress on strawberry plants grown with or without the halophytic companion plant *P. oleracea* L. to remediate the physiological and biochemical parameters of strawberry plants.

2. Material and Methods

2.1. Experimental design and Plant growth

The experiment was conducted in September- January 2018-2019 periods in a semi-controlled greenhouse at the University of Harran, Sanlıurfa, Turkey. Fresh strawberry (Rubygem variety) plants were grown alone or in combination with *P. oleracea* seedlings in 8-L pots containing torf soil under natural light conditions. The

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average day and night temperatures were 35±2/28±2 °C during the course of experiment. A trial was performed in a randomized block design. The first group of strawberry plants grown under differing NaCl (0-, 30-, 60-, and 90 mmol L^{-1}) conditions were designated as S_0, S_{30}, S_{60}, and S_{90}; the second group of plants grown with *P. oleracea* under the same NaCl conditions were designated as SP_0, SP_{30}, SP_{60}, and SP_{90}. Treatments in each group were replicated five times. Seedlings were transplanted individually to the pots. Strawberry seedlings following one-week growth of establishment in pots were accompanied with *P. oleracea* seeds which were sown and germinated at a rate of 25 companion plants per pot. After germinations, the pots were irrigated with or without salt to the full pot capacity throughout the experimental period. The plants were fertigated with Hoagland's nutrient solution once a week. The treatment continued for five months and the plants were harvested at the optimum stage of physiological maturity for the evaluation of salinity responses (Figure 1).

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Figure 1. Strawberry plants grown with or without *P. oleracea* under different NaCl conditions.

2.2. Plant growth and fruit properties

Strawberry fruits were harvested when 90% of the fruit surface had reached a fully red color. At the end of the experiment, total fruit weights were determined, and the average fruit yield was calculated.

Plant crown and root fresh weight (Fwt) were analyzed immediately after the harvest. Dry weight (Dwt) of plant organs was determined after drying samples at 70° C to weight constancy.

Total soluble solids (TSS) was determined from the fruit juice with a hand refractometer. The results are expressed in percent (%) Catania et. Al. [9].

A determination of the lycopene content was obtained according to the method of Barrett and Anthon [10] with minor modifications Karakas [11]. One gram of strawberry fruit was extracted with 10 mL of ethanol: hexane solution (4:3). The mixture was centrifuged at 10,000 *g* for 10 min at room temperature than 100 mL of the supernatant was added to 7 mL of ethanol: hexane solution (4:3) mixture and vortexed. Following one-hour incubation at room temperature, 1 mL of H_2O was added to the tubes and vortexed. The tubes were then kept in the dark to form different phases. The top phase was obtained and measured at 503 nm against a hexane blank with a UV

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microplate spectrophotometer (Epoch, SN: 1611187, made in USA). Lycopene content was calculated according to the following formula, (Eq. 1).

$$\mu g\ \text{Lycopene}\ \ g^{-1}\ \text{Fwt} = \frac{A_{503}\ \text{x}\ 2.7}{172\ \text{x}\ 0.1}\text{x}537 \qquad \text{(Eq. 1)}$$

where 537 g/mole is the molecular weight of lycopene, 2.7 ml is the volume of the hexane layer, 0.1 g is the weight of tomato added, and 172 $mmol^{-1}$ is the extinction coefficient for lycopene in hexane.

The vitamin C content of fruits was determined according to the methods of Oz [12] with small modifications Karakas [11]. Five grams of strawberry fruit were homogenized in 25 mL of oxalic acid. The mixture was then centrifuged at 10,000 *g* for 10 min at room temperature. One mL of this mixture was added to 7 mL of a 1% oxalic acid solution and 8 mL of dye reagent. The dye reagent was prepared by dissolving 84 mg of $NaHCO_3$ in 80 mL of boiling distilled H_2O containing 100 mg of 2,6-dichloro phenol indophenol (2,6-DCPIP). The mixture was filtered and cooled and diluted to 100 mL with deionized H_2O. Then, 25 mL of this solution was obtained, diluted to 500 mL with deionized H_2O, vortexed, and kept at 4°C until use. The mixture was then vortexed and measured at 518 nm against the oxalic acid and dye mixture with a UV microplate spectrophotometer (Epoch, SN: 1611187, made in USA).

Electrolyte leakage (EL) was assessed as described by Lutts et al. [13] using leaf discs for each treatment. Fully expanded young leaf samples were washed three times

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with deionized water to remove surface-adhered electrolytes and dusts. Leaf discs were placed in closed vials containing 10 mL of deionized water and incubated at 25 °C on a rotary shaker for 24 h; subsequently, the electrical conductivity of the solution (EC_1) was determined. Samples were then autoclaved at 120 °C for 20 min and the final electrical conductivity (EC_2) was obtained after equilibration at 25 °C. The EL was calculated as follows, (Eq. 2.).

$$EL\ (\%) = \frac{EC1}{EC2} x100 \qquad \text{(Eq. 2.).}$$

Stomatal conductivity (SC) was determined on the youngest fully expanded leaves on upper branches of the plants with leaf promoter (SC-1) at midday. Measurements were conducted by clamping the leaves in the leaf chamber. The actual vapor flux from the leaf through the stomata is expressed as mmol $m^{-2}s^{-1}$ Karlidag et al. [14].

2.3. Biochemical parameters

Chlorophyll content (Chl-*a*, Chl-*b*) of strawberry plants were determined based on the method of Arnon [15] with minor modifications Karakas [11]. Leaf samples (0.5 g) were homogenized in a 10 mL acetone: water (80/20, v/v) mixture and filtered through Whatman No.2 filter paper then placed in the dark tubes. Chl-*a*, Chl-*b* of the plant samples were read at UV microplate spectrophotometer (Epoch, SN: 1611187, made in

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USA) at 663 nm, 645 nm, respectively against 80% acetone blank. The results were expressed as mg L^{-1} and calculated as mg g^{-1} Fwt.

The proline (pro) measurement was conducted according to the method of Bates et al. [16] with slight modifications Karakas [11]. Leaf material (0.5 g) was homogenized in 10 mL of 3% w/v sulphosalicylic acid using a mortar and a pestle. The homogenate was filtered through Whatman No. 2 filter paper. Then, 2 mL of filtrate was mixed in a test tube with 2 mL of acid-ninhydrin reagent (1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid) and boiled at 100°C for one hour. The reaction was terminated in an ice bath. The reaction mixture was then extracted using 5 mL of toluene. The tubes were thoroughly shaken for 15-20 seconds and left for 20 min at room temperature to achieve separation for two layers. The chromophore containing toluene was removed and allowed to warm to room temperature, and the absorbance of the solution was measured at 515 nm using a toluene blank. Proline concentration was determined using the calibration curve made with L-proline (Sigma-Aldrich 81202-06-4) as µmol g^{-1} Fwt.

Hydrogen peroxide levels (H_2O_2) were determined according to Velikova et al. [17] with slight modifications Karakas et al. [18]. Fresh plant tissue (0.5 g) was homogenized in an ice bath with 5 mL 0.1% (w:v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 g for 15 min at 4 ^{o}C and 0.5 mL of the

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supernatant was added to 0.5 mL 10 mmol L^{-1} potassium phosphate buffer (pH 7.0) and 1 mL of 1 mol L^{-1} potassium iodide. The absorbance was read at 390 nm using a UV microplate spectrophotometer (Epoch, SN: 1611187, made in). The H_2O_2 content was expressed as µmol g^{-1} Fwt.

The malondialdehyde (MDA) content was determined according to the method of Sairam and Sexena [19] with slight modifications Karakas et al. [18]. Leaf material (0.5 g) was homogenized in 10 ml of 0.1% (w/v) TCA solution. The homogenate was centrifuged at 10,000 *g* for five minutes. Four mL of 20% v/v TCA containing 0.5% v/v thiobarbituric acid (TBA) was added to one milliliter of the supernatant. The mixture was incubated in boiling water for 30 min, and the reaction was stopped by placing the reaction tubes in an ice bath. The mixture was centrifuged again at 10,000 *g* for 5 min and the absorbance of the supernatant was read at 532 and 600 nm. Here, the MDA content of leaves is expressed as nmol g^{-1} Fwt, (Eq. 3).

$$MDA(nmol\ g^{-1}) = \frac{Extract volume(ml)\ \mathrm{x}\left[(A_{532} - A_{600})/(155\,mM^{-1}\,cm^{-1})\right]}{Sample\ amaunt\ (g)}\ \mathrm{x}10^{3} \qquad \text{(Eq. 3)}$$

Catalase enzyme activity (CAT, EC. 1.11.1.6) was determined by monitoring the decomposition of H_2O_2 according to the method of Milosevic and Slusarenko [20] with minor modifications Karakas et al. [18]. Fresh leaf tissue (0.5 g) was homogenized in 10

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mL of a 50 mmol L^{-1} Na-phosphate buffer solution, then 50 mL of plant extract was added to a 2.95 mL (50 mmol L^{-1} Na-phosphate buffer, 10 mmol L^{-1} H_2O_2, and 4 mmol L^{-1} Na_2EDTA) reaction mixture and measured for 30 seconds at 240 nm with a UV microplate spectrophotometer (Epoch, SN: 1611187, made in USA). One CAT activity unit (U) is defined as a change of 0.1 absorbance unit per minute. Activity is expressed as enzyme units per gram Fwt.

Peroxidase enzyme activity (EC.1.11.1.7) was determined according to the method by Cvikrova et al. [21] with slight modifications Karakas et al. [18]. For the analysis, 100 mL of extract (obtained as above) was added to 3 ml of the reaction mixture (5 mmol L^{-1} H_2O_2, 50 mmol L^{-1} Na-phosphate, 13 mmol L^{-1} guaiacols, and pH 6.5). The reaction was initiated with an H_2O_2 addition and was measured at 470 nm using a UV microplate spectrophotometer (Epoch, SN: 1611187, made in USA) at a one-minute interval until 3rd minute. One unit of POX activity was defined as a change of 0.1 absorbance unit per minute at 470 nm. Activity is expressed as enzyme units per gram of Fwt.

Leaf mineral (K^+, Na^+, Ca^{+2}, Mg^{+2}, Cl^{-1}) contents were determined according to the procedure of Chapman and Pratt [22] with slight modifications Karakas [11]. Dry plant samples (0.5) g were ground in porcelain crucibles. The porcelain crucibles were placed into a muffle furnace, and the temperature was gradually increased up to 500 ^oC.

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The cooled ash was then dissolved in 5 mL 2 N hydrochloric acid. After 30 minutes, the volume was made up to 50 mL with distilled water, and the supernatant was filtered through Whatman No. 42 filter paper. Then resulting supernatant containing Na^{+}, K^{+}, Ca^{+2}, and Mg^{+2} ions were determined by Inductively Coupled Plasma (ICP, Perkin Elmer). Chloride was determined by ion chromatography after the filtration through filter paper.

Data were analyzed by SPSS 22 (ANOVA test) at a significance level of $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT). Data are presented as a mean value ± the standard error.

3. Results

Strawberry plant growth, fruit properties, biochemical parameters, and mineral contents were significantly affected by all salinity levels. Crown fresh and dry weights of strawberry plants in saline conditions were significantly lower in plants grown alone in saline conditions when compared to those of plants grown in combination with *P. oleracea* under the same conditions. For example, the crown fresh weight of the plants was 55.16, 37.62, and 35.16 g $plant^{-1}$, grown alone in S_{30}-, S_{60}-, and S_{90} mmol L^{-1} NaCl conditions. When plants were grown in combinations with *P. oleracea*, the conditions of plants were significantly improved at all NaCl conditions. Fresh weight of plants increased %64.38, %44.76, %44.49, at SP_{30}, SP_{60} and SP_{90} mmol L^{-1} NaCl conditions

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(Figure 2A). Similar improvements were recorded at dry weight of plants (Figure 2B). In general, the combination of companion plants (*P. oleracea*) was found to be effective in increasing the Fwt and Dwt under each NaCl conditions.

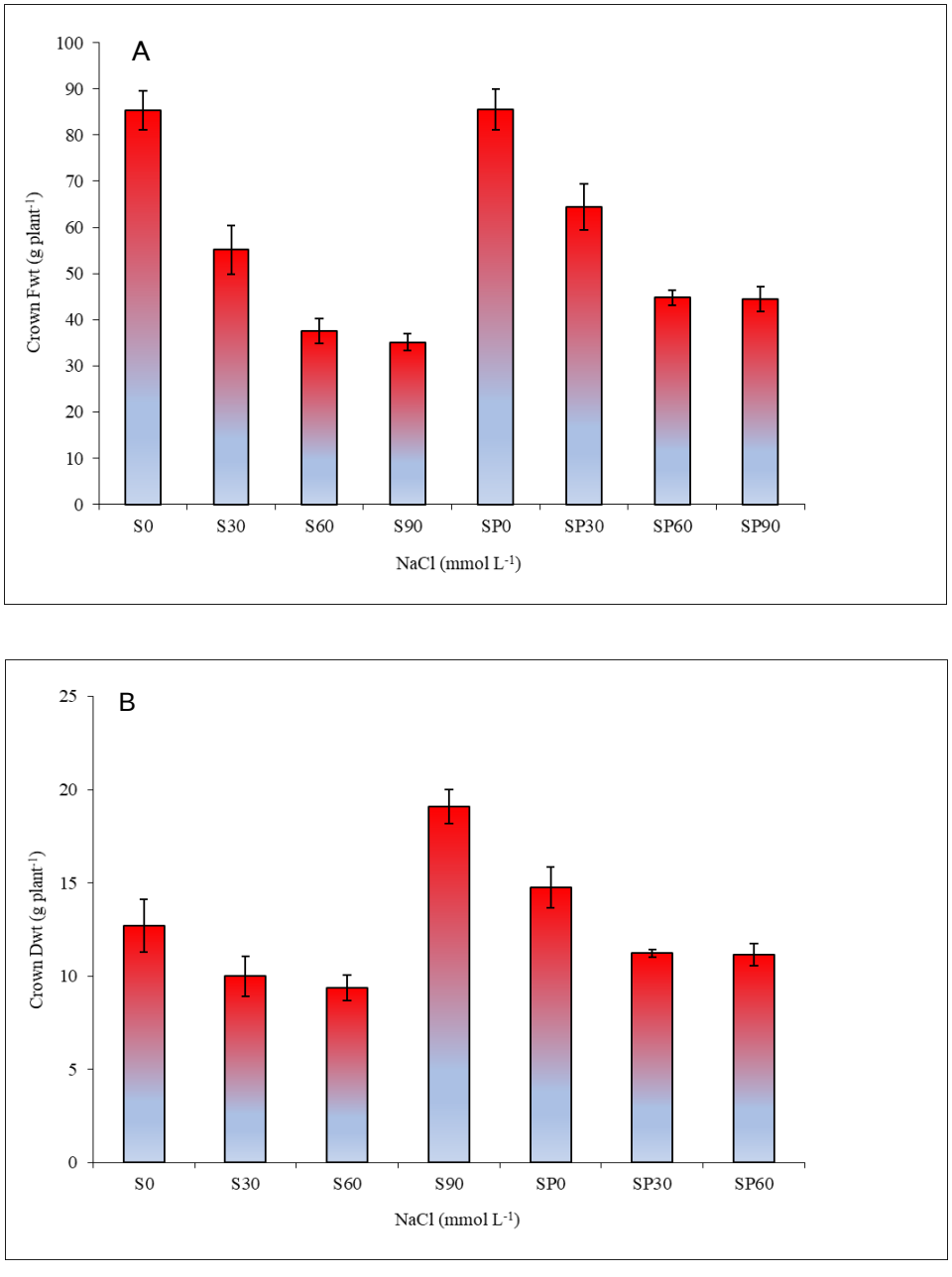

Figure 2. Crown Fwt (A) and Dwt (B) of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

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EL is considered an important criterion for salt stress parameters. EL was increased with increasing levels of salt. For example, leaf EL was found as 11.90 and 11.07, at S_0 and SP_0 respectively. EL was significantly increased from 15.61 to 25.34% with respect to conditions from S_{30} to S_{90}. When P. oleracea accompanied to these rowing at the NaCl conditions, the increase of EC was so minimal that only 11.10 to 15.32 % was recorded SP_{30} -SP_{90}, Figure 3.

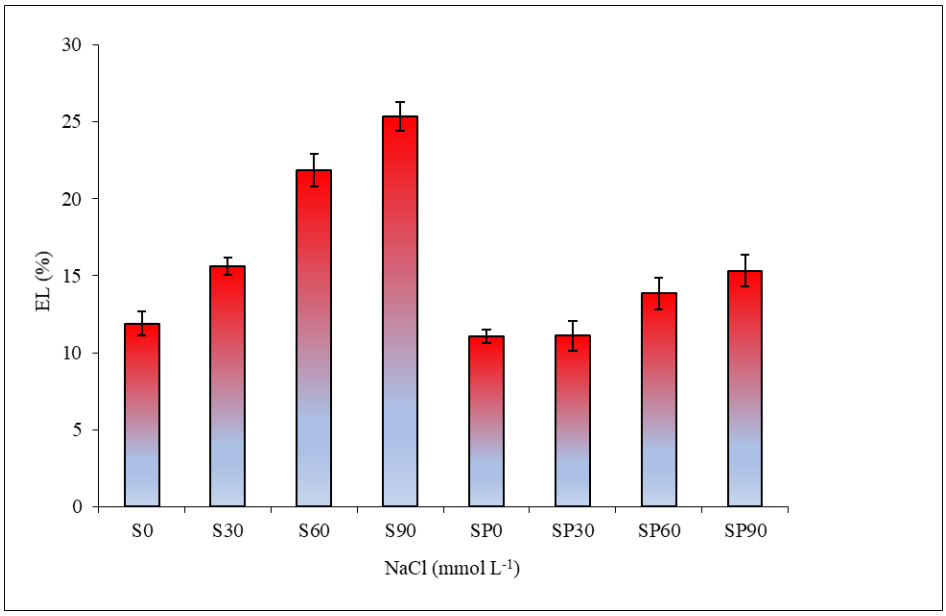

Figure 3. Leaf EL of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

Stomatal conductivity in saline conditions was gradually decreased as the concentrations of NaCl levels increased in plants grown alone in saline conditions (Figure 4). However, the cultivation of *P. oleracea* improved the SC of strawberry plants under all NaCl conditions when compared to those grown alone in saline

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conditions. The improvement of SC increased by $S_{30,}$ SP_{30} 183.26%, 230.80%, $S_{90,}$ SP_{90} 94.10%, 131.08% in mixed cultivation.

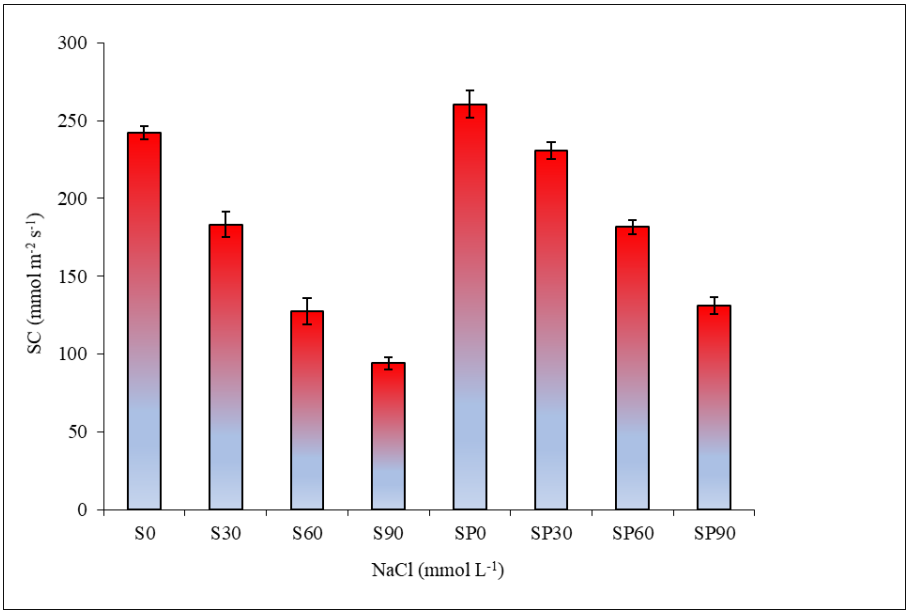

Figure 4. SC of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

Average fruit weight and yield of strawberry plants under NaCl conditions were reduced in plants grown alone, however, cocultivation of strawbbery plants with *P. oleracea* increased average fruit weight as well as total fruit weight (Figure 5A and 5B).

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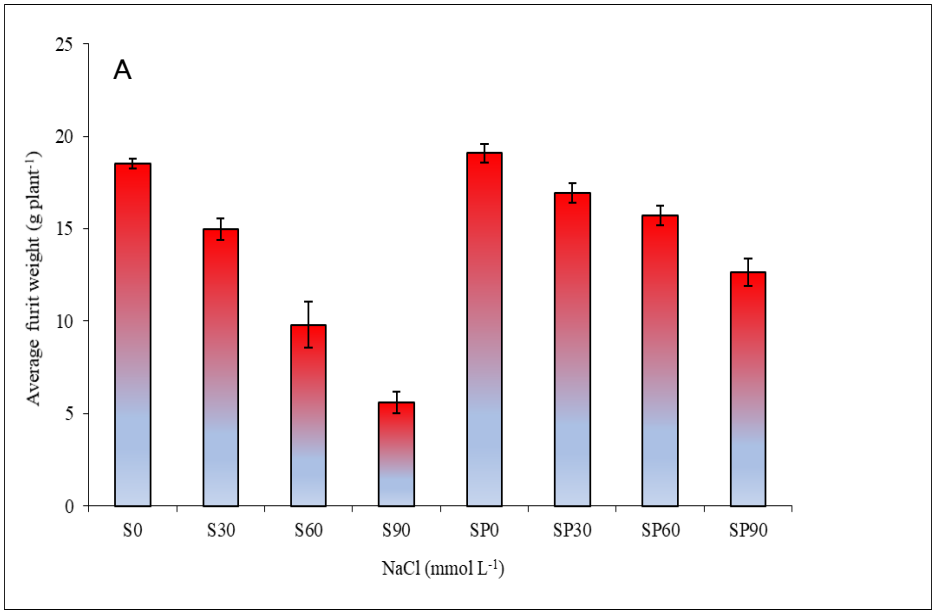

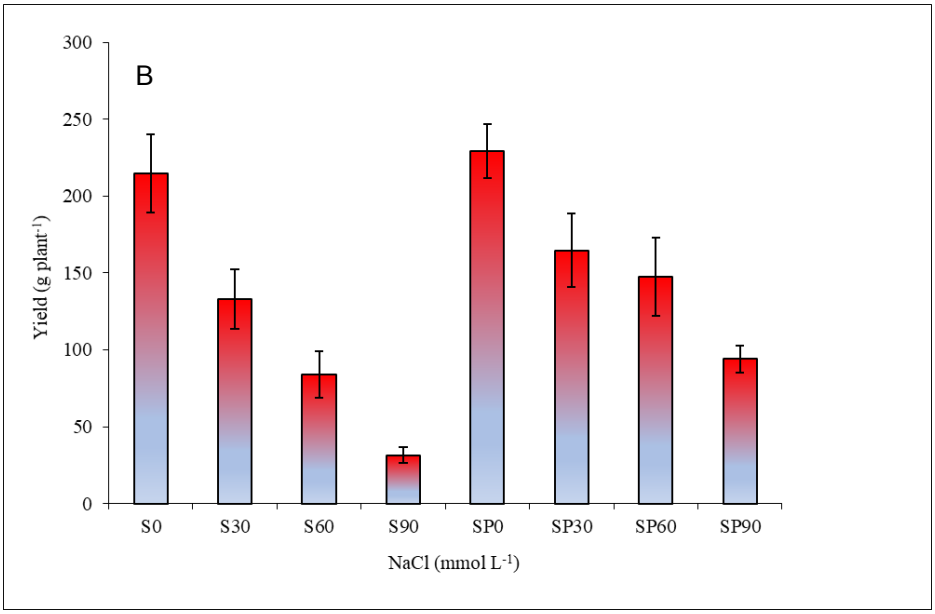

Figure 5. Strawberry average fruit weight (A) Strawberry yield (B) of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

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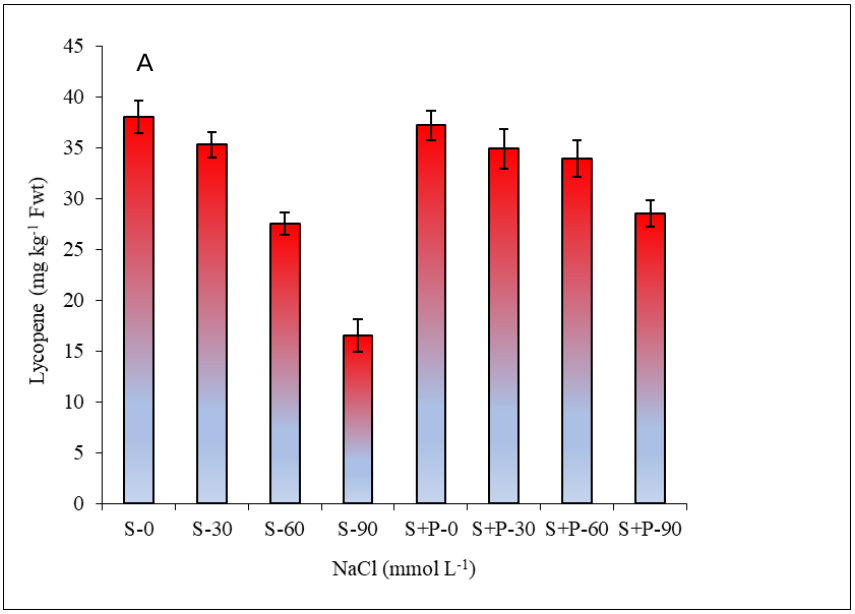
The employement of *P. oleracea* not only increased the crop yield and physiological parameters but also improved the quality of fruits in terms of lycopene and vitamin C contents. Lycopene and vitamin C contents were gradually decreased as the concentrations of NaCl increased. Again, the employement of *P. oleracea* increased the lycopene and vitamin C at all NaCl levels (Figure 6A and 6B). For example, the remarkable effect was more evident at 90 mmol L^{-1} NaCl conditions both lycopene and vitamin C contents which increased 16.56-, 28.55 mg kg^{-1} Fwt, and 32.53-, 43.12 mg 100 g^{-1} Fwt, respectively.



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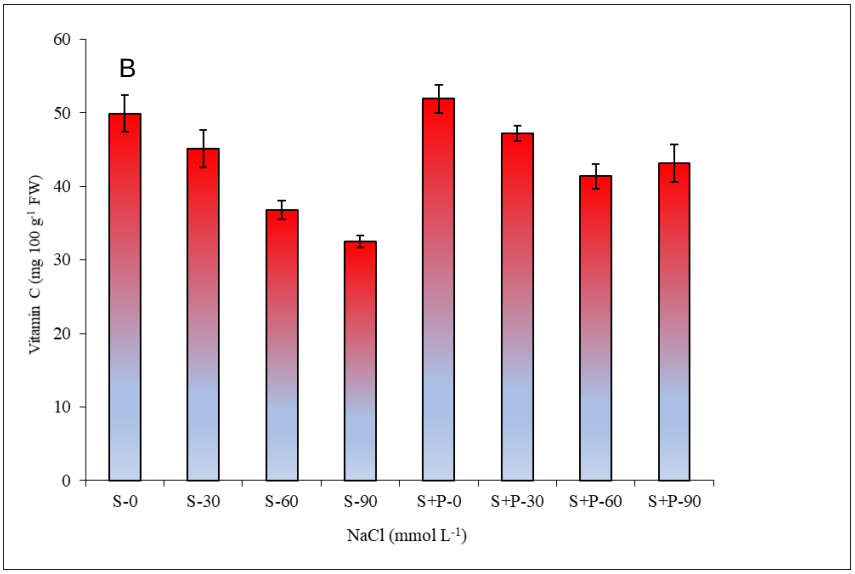

Figure 6. Lycopene contents (A) and Vitamin C (B) of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

TSS contents of the fruits in saline conditions were significantly lowered. Cocultivation of *P. oleracea* unlike often parameters did not improve the conditions of strawberry plants (Figure 7).

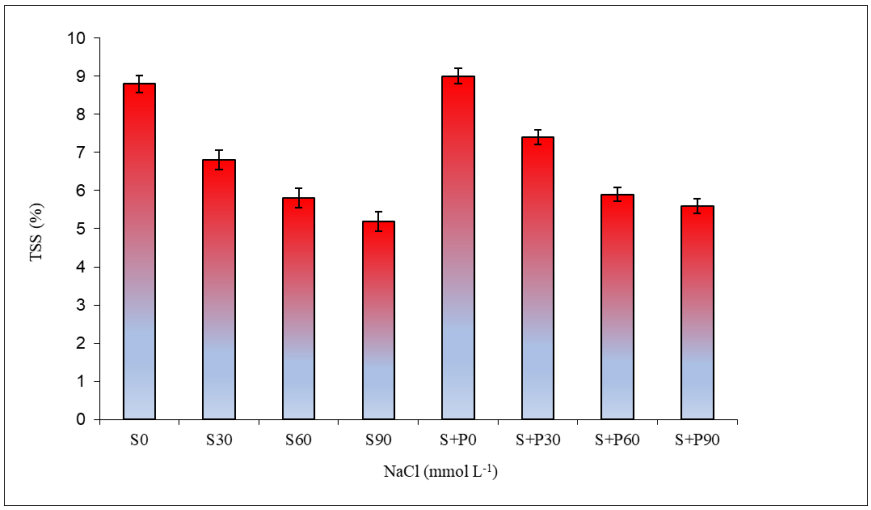

Figure 7. TSS contents of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

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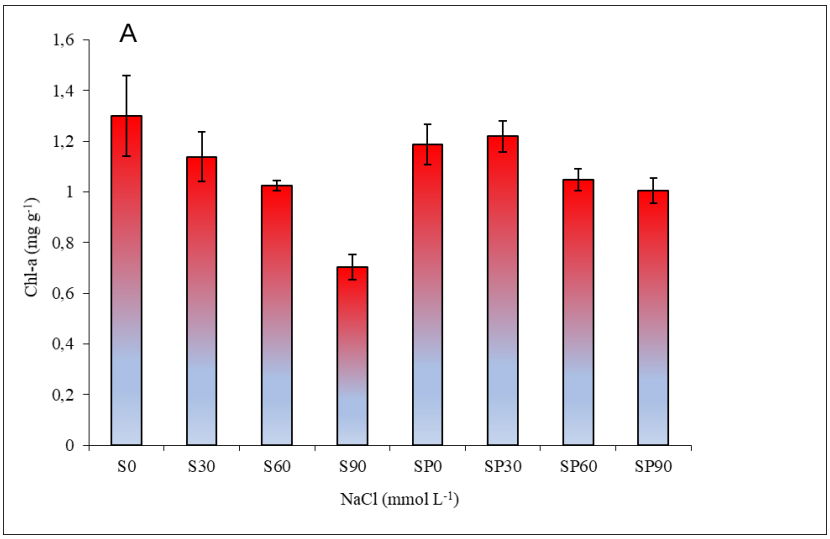
Chl-*a* and Chl-*b* were significantly affected by salinity at S_{60} and S_{90} mmol L^{-1} NaCl levels ($P \leq 0.05$). for example, the Chl-*a* and Chl-*b* were determined as 0.70 mg g^{-1}, 0.36 mg g^{-1} Fwt, respectively at S_{90} mmol L^{-1} NaCl levels in strawberry plants. The positive effects of *P. oleracea* on Chl-*a* and Chl-*b* contents at SP_{90} mmol L^{-1} NaCl were evident in which Chl-*a* and Chl-*b* contents were 1.01 mg $g^{-1}$and 0.51 mg g^{-1} Fwt in strawberry plants (Figure 8A and 8B).



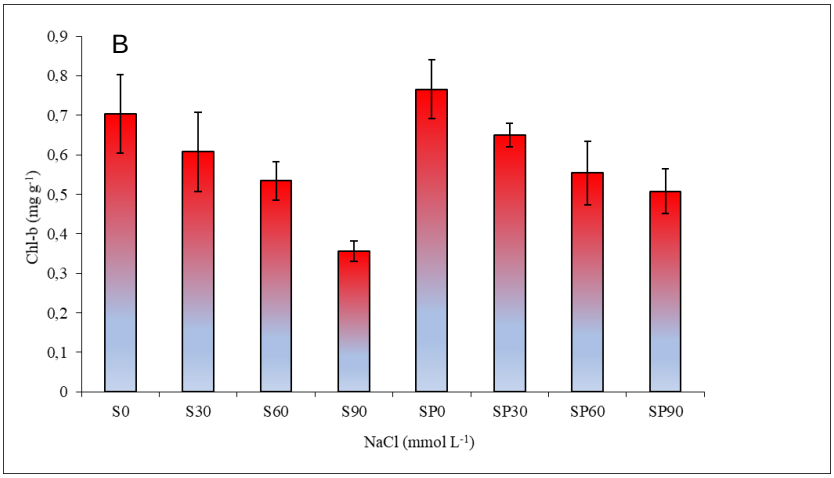


Figure 8. Chl-*a* (A), Chl-*b* (B) contents of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

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Leaf proline content significantly increased as the concentrations of NaCl levels increased as a response to salinity stress. ($P \leq 0.05$), Figure 9. The highest proline level was determined as 13.77 µmol g^{-1} Fwt at S_{90} mmol L^{-1} NaCl treatment whereas, at SP_{90} condition, the proline level decreased down to 4.40 µmol g^{-1}. Therefore, the combination of *P. oleracea* not only improved the physiological and biochemical conditions of strawberry plants but also reduced the stress metabolite levels.

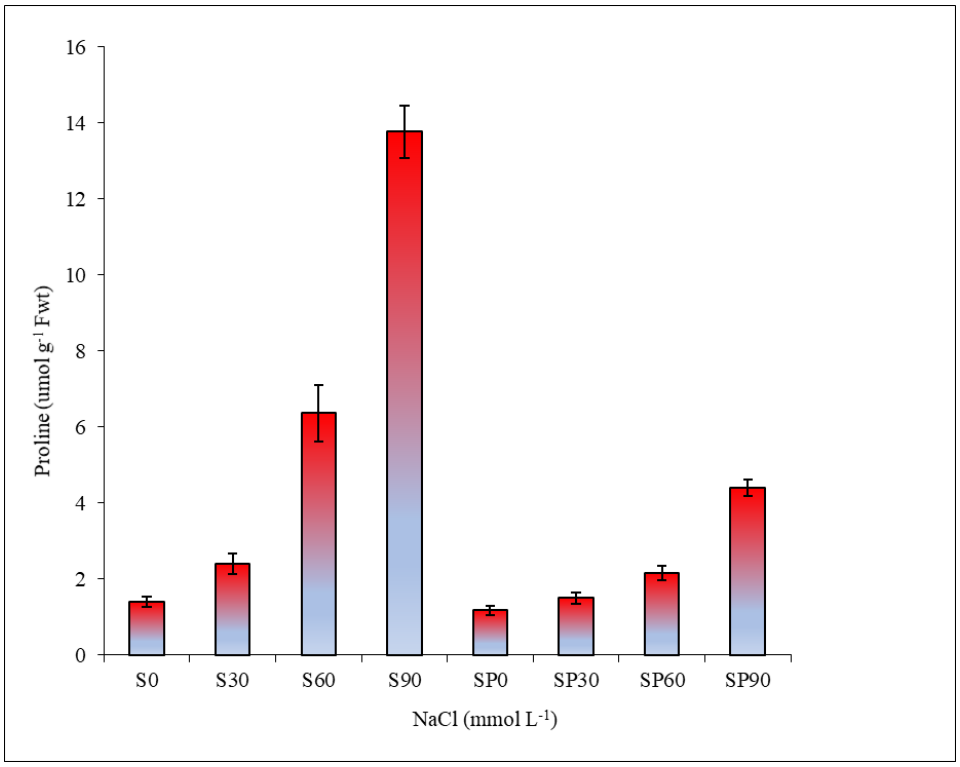

Figure 9. Leaf proline content of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

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The companion of *P. oleracea* had a remarkable effect to reduce the impact of NaCl stress in strawberry plants. Again, leaf H_2O_2 and MDA contents increased with the increasing levels of salt stress. The highest H_2O_2 and MDA levels were determined as 74.72 nmol g^{-1} Fwt and 13.58 nmol g^{-1} Fwt at S_{90} mmol L^{-1} NaCl level. Cocultivation of *P. oleracea* with strawberry plants reduced the contents of H_2O_2 and MDA levels down to 31.56 nmol g^{-1} Fwt and 4.15 nmol g^{-1} Fwt (Figure 10A and 10B).

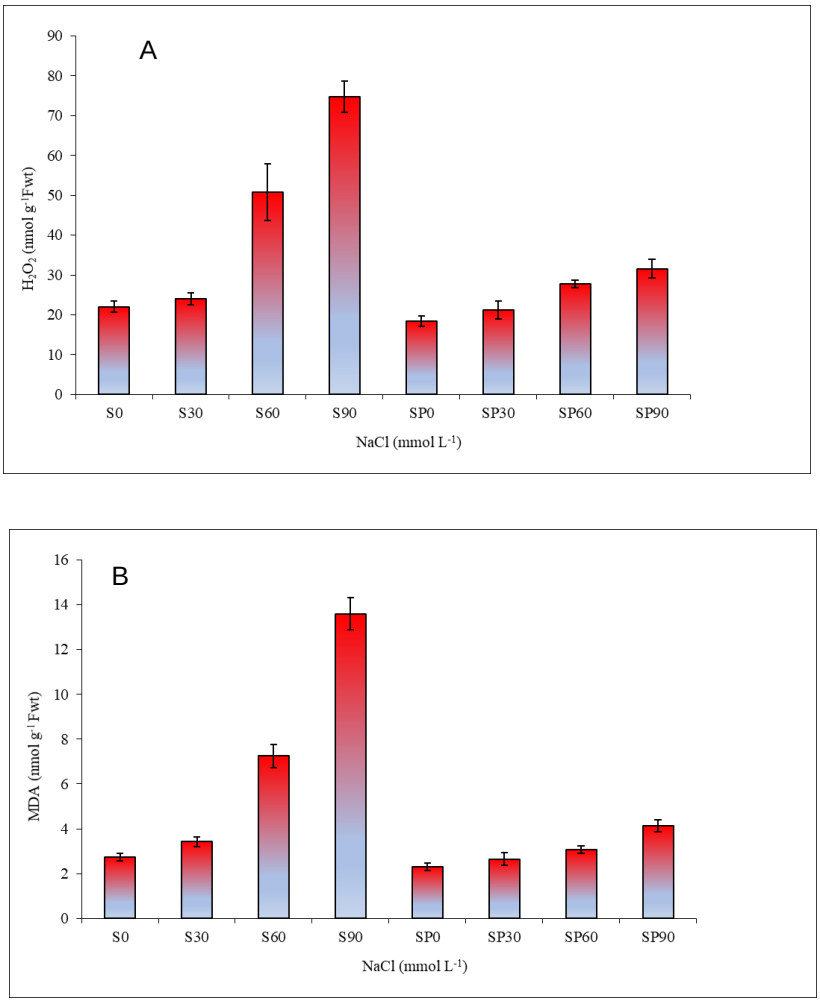

Figure 10. Leaf H_2O_2 (A) and MDA (B) contents of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

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POX and CAT antioxidant enzymes showed a parallel pattern as those of previous parameters. Cocultivation of *P. oleracea* significantly decreased antioxidant enzyme levels at 60-and 90 mmol L^{-1} NaCl conditions, (Figure 11 A and 11 B).

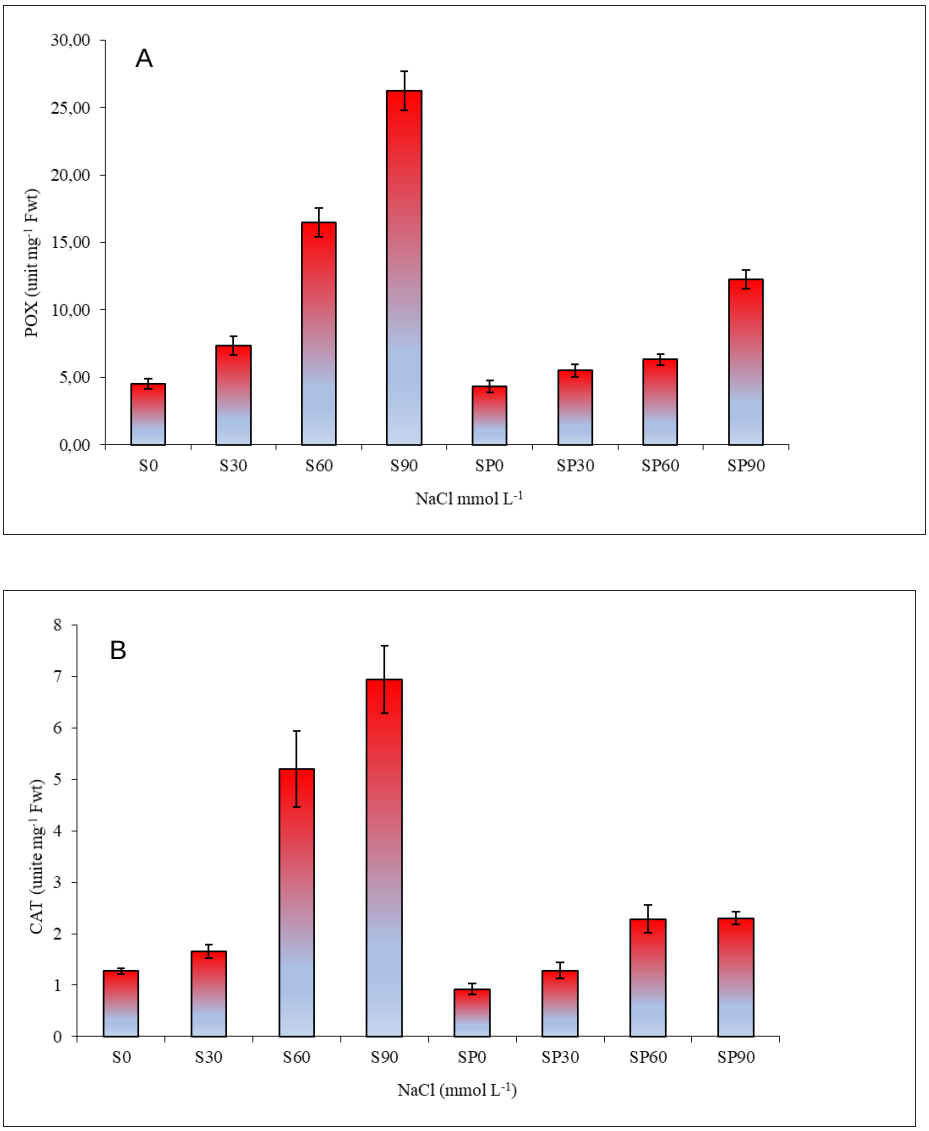

Figure 11. POX (A) and CAT (B) antioxidant enzymes contents of strawberry plants grown alone or in combination with *P. oleracea* in differing NaCl levels (0, 30, 60, 90 mmol L^{-1}).

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Leaf mineral contents

Concentrations of beneficial ions such as K^+, Ca^{2+} ions decreased with the increases in salinity levels in strawberry plants. The lowest K^+ and Ca^{2+} ions were determined at the S90 level. Leaf Mg^{2+} content was not affected significantly upon NaCl stress. *P. oleracea* cocultivation with strawberry plants enhanced the Mg^{2+} ion level at NaCl treatments, Table 1. Under saline conditions, gradual increases of Na^+ and Cl^- ions were evident in strawberry plants grown at increasing NaCl salinity, however, employment of *P. oleracea* significantly decreased the Na^+ and Cl^- ions, Table 1.

Table 1. Strawberry leaf mineral contents.

Treatment	K^+ (%)	Ca^{2+} (%)	Mg^{2+} (%)	Na^+ (%)	Cl^- (%)
S_0	2.32^a	2.39^a	0.32^a	0.17^e	0.35^d
S_{30}	1.82^b	1.97^a	0.28^a	0.32^d	0.67^d
S_{60}	1.41^c	1.86^b	0.29^a	0.69^b	2.09^b
S_{90}	1.02^e	1.72^b	0.28^a	1.09^a	3.69^a
SP_0	2.42^a	2.25^a	0.35^a	0.09^e	0.25^d
SP_{30}	2.27^a	2.15^a	0.33^a	0.19^e	0.46^d
SP_{60}	1.78^b	2.15^a	0.34^a	0.31^d	1.16^c
SP_{90}	1.66^b	2.14^a	0.31a	0.49^c	1.29^c

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The P≤0.05 significance level for various plant combinations in each NaCl levels (0-, 30-, 60- and 90 mmol L^{-1}) was indicated with different letters using Duncan's Multiple Range Test. A strawberry grown alone is expressed as S, the strawberry *P. oleracea* companionship is expressed as SP.

4. Discussion

Salinity stress is one of the most important and damaging abiotic stresses that globally affect plant growth and yield. Increased salinity levels damage plants during vegetative and reproductive stages and therefore, reduce biomass and crop yield and negatively affects crop quality. Under salt stress conditions, more Na^+ accumulated in the shoot and root of cultivars while K^+ content decreased [23]. Quality parameters such as vitamin contents and aromatic substances and pigments are significants reduced. Leaf proline content increased with an increase in salinity stress. Increasing proline content under salinity conditions indicates the adverse effects of osmotic stress on the plant. Proline and soluble carbohydrates (also known as compatible solutes) are expected to be accumulated under salinity in strawberry [24]. The increase in proline and soluble carbohydrates can be considered as a criterion for tolerance capacity against salinity [25]. This study showed that *P. oleracea* combination with strawberry decreased the proline levels under salinity along with the reduction of Na^+ and Cl^- ion levels by reducing toxic levels of salt ions. *P. oleracea* gave promising results on strawberry plants grown at different NaCl stress levels (0-, 30-, 60-, and 90 mmol L^{-1}).

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Mozafari et al. [26] stated that salinity negatively affected the growth parameters, pigment content, and membrane stability and disturbed the ionic exchange in plants. For example, Saied et al. [27] stated that strawberry was considered a saline-sensitive plant, and chlorophyll and other metabolites significantly deteriorated. This both, directly and indirectly, led to diminished productivity in plants [28]. We determined that fresh weight, dry weight, stomatal conductance, fruit average weight and total yield, chlorophyll (Chl-*a* and Chl-*b*), total soluble solids, lycopene, vitamin C, leaf mineral content (K^+ and Ca^{2+}) of strawberry plants decreased with increasing level of NaCl stress. Strawberry plants grown in companion with *P. oleracea* improved the condition of plants and much fewer reductions in terms of total yield and quality were evident. The positive effect on strawberry growth was quite remarkable. Leaf electrolyte, proline, malondialdehyde, H_2O_2 contents, catalase and peroxidase enzyme activities, leaf mineral contents (Na^+ and Cl^-) of strawberry plants increased with increasing level of salinity. The companion plants helped strawberry plants via reducing toxic ions and antioxidant enzyme levels as well as stress metabolites. With the improvement of those parameters, electrolyte leakage and stomatal conductance were also improved, and this was reflected in the quality of fruits in terms of lycopene and vitamin C contents. This study showed that mixed planting with *P. oleracea* in high salinity levels might be an effective phytoremediation technique that might significantly increase the yield production and quality of strawberry. Similar findings were also reported for *S. soda*

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plants by Karakas [29] who suggested that the improvement of tomato plants by companion plants under saline conditions (1.3 and 6.5 dS m^{-1}) was achieved via the synthesis of substances used for fruit development instead of building up substances for mechanisms of stress tolerance. It is important to note that synthesizing stress metabolites and antioxidant enzymes and quite costly for plants to cope with abiotic or biotic stress factors [29]. Instead of generating crop plants combating against stress factors, the strategy which involves removing stress factors would be much appreciated. Any genetic modifications or biochemical approaches that increase the removing capacity of toxic ions or compounds from the soil habitat would be an environmentally - friendly approach and a safe strategy. For example, Grafienberg et al. [30] and Karakas et al. [31] stated that reductions in stress metabolites and the uptake of toxic ions allowed tomato plants to use more energy to build up organic components such as lycopene and proteins instead of producing substances for defense mechanisms. In this study, salinity stress resulted in a reduction in vitamin C content and lycopene contents in strawberry. Jamalian et al. [32] showed that salinity reduced the vitamin C content of strawberry, which is in line with the results of the present study. The decrease in vitamin C content of fruit at high salinity levels was be attributed to the decrease in carbohydrate (sugar) production caused by the decrease in photosynthesis required for vitamin C biosynthesis.

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Yaghubi et al. [33] reported that MDA concentration was also high in strawberry plants at salt stress conditions. They reported that reactive oxygen species (ROS) production was higher than the scavenging capacity of antioxidant enzymes. The dismutation of O_2^- [2] into H_2O_2 and O_2 was reported to increase H_2O_2 concentration [34]. This was observed by higher H_2O_2 content in salt-stressed strawberry plants than control plants. Since H_2O_2 was accompanied by an increase in the key antioxidant enzymes such as CAT, POD, and superoxide dismutase (SOD) therefore, the reduction of H_2O_2. Thus it appeared that, in strawberry leaves and roots, SOD, should be achieved to prevent further damages to cell components. In our methodology, we achieved the decrease of stress metabolites while supressing the antioxidant enzymes via the use of *P. oleracea* plants. Although antioxidant enzymes such as SOD, CAT, and POD are known to substantially reduce the levels of superoxide and hydrogen peroxide in plants and play a vital role in plant defense against oxidative stress [35], the increase of these enzymes might interfere with the chemical compounds involved in quality parameters such as lycopene and vitamin C. with the use of *P. oleracea*, we achieved to reduce stress metabolites and toxic ions and increased the quality-related compounds without increasing levels of antioxidant compounds that also saved the energy to be used for defense responses, increased, the energy saved by this approach was used to increase metabolite functions and quality parameters.

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5. Conclusion

Strawberry cultivation has become popular recently, however, this led to an increase of cultivated areas. These areas have become saline-polluted or saline-prone or saline-prevalent areas. Since strawberry in a salt-sensitive plant, it is easily affected by a mild or moderate level of salinity. A very low level of NaCl could reduce the crop yield and reduce the quality of fruits.

In this study, strawberry seedlings were grown alone or in combination with *P. oleracea* under differing NaCl concentrations. Strawberry seedlings under increasing NaCl salinity were negatively affected in terms of physiological, morphologycal and biochemical parameters. Defending plants synthesized various stress metabolites such as proline, MDA, H_2O_2, and antioxidant enzymes to ease the negative effects of NaCl toxicity. However, increases of these metabolites were negatively correlated with the quality-related metabolites such as vitamin C and lycopene contents. Cultivation of strawberry plants with *P. oleracea* plants reduced the concentrations of stress metabolites and antioxidant enzyme levels and contributed to increases of vitamin C and lycopene contents indirectly.

We suggest that employment of P. oleracea would remediate the conditions of strawberry parameters via accumulating Na^+ and Cl^- ions and thus causing reductions in the synthesis of stress metabolites. Use of *P. oleracea* is a qute practical and

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environmentally - friendly approach where salinity is prevalent. This plant could also be used in high saline conditions.

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