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OPTION: Production Végétale

Thème

**USE OF PLANT EXTRACTS TO
MITIGATE SALT STRESS IN TOMATO
(SOLANUM LYCOPERSICUM L.)**

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List of Abbreviations

AC: Absorbance of control.

AS: Absorbance of sample.

CL: Cloves (*Syzygium aromaticum*).

CL50: plants treated with *S. aromaticum* (cloves) extract and NaCl.

CON: control = plants irrigated only with water.

GP: Germination percentage.

GS: Germination seeds.

LDW: Leaf dry weight.

LA: Leaf area.

MPE's: Medicinal plant extracts.

NaCl: Sodium chloride.

OD: Optical density.

RWC: Relative water content.

RM50: plants treated with *Rhamnus lycioides* extract and NaCl.

RM: *Rhamnus lycioides* subsp. *Oleoides*.

SWE: Seaweed extract.

SS: Soluble sugars.

Salt: plants irrigated with NaCl.

SW (0.1%): plants irrigated with 0.1% of SWE and NaCl.

TF: Tafgha.

TW: Turgid weight.

TPC: Total polyphenol content.

TF50: plants irrigated with of *R. lycioides* (Tafgha) and NaCl.

الأطروحة : استخدام المستخلصات النباتية للتخفيف من الإجهاد الملحي في الطماطم (**SOLANUM LYCOPERSICUM L.**)
الإسم و اللقب : زميلي أية و لشخب مريم
المؤطر : د. فرشة عزالدين

ملخص :

يهدف هذا البحث إلى دراسة تأثير المعالجة المسبقة أو الري باستخدام مستخلصات من ثلاثة نباتات طبية (*Syzygium aromaticum*; *Rhaponticum acaule*; *Rhamnus lycioides*) على إنبات ونمو صنف طماطم هاينز 1530 .

أشارت النتائج إلى أن الإجهاد الملحي المعتدل (أثناء الإنبات 100mMNaCl و خلال مرحلة الشتلات 50mMNaCl) أثر بشكل كبير وسلبى على معظم المعايير المدروسة. ومع ذلك ، فإن تطبيق المستخلصات النباتية إما كمعالجة مسبقة أو من خلال الري خفف من الآثار السلبية للملوحة ، مما عزز تحمل شتلات الطماطم للملوحة .

في حين أن النتائج واعدة ، إلا أنه من الضروري إجراء مزيد من الدراسات لتوضيح الآليات التي تؤثر بها هذه المحفزات الحيوية على تحمل الملوحة ونمو النبات.

الكلمات المفتاحية : .

Thesis : Use of Plant Extracts to Mitigate Salt Stress in Tomato (*Solanum lycopersicum L.*)

First name and Name : Zemali Aya & Lachekhab Meriem

Directed by : Dr. FERCHA Azzedine

Abstract:

This research aims to investigate the impact of pre-treatment or irrigation using extracts from three medicinal plants (*Syzygium aromaticum*, *Rhaponticum acaule*, and *Rhamnus lycioides*) on alleviating the adverse effects of salt stress on the germination and growth of the Heinz 1530 tomato variety.

The findings indicated that moderate saline stress (100 mM NaCl during germination and 50 mM NaCl during the seedling stage) significantly and negatively affected most studied parameters. However, the application of plant extracts either as a pre-treatment or through irrigation mitigated the negative impacts of salinity, enhancing the salinity tolerance of tomato seedlings.

While the results are promising, further studies are necessary to elucidate the mechanisms by which these biostimulators influence salinity tolerance and plant growth.

Key words: Salt stress, Salt tolerance, Seed germination, Seedling growth, Medicinal plant extracts.

Exposure of plants to salinity in irrigation water or soil can limit plant growth by disrupting various physiological and biochemical processes (Munns & Tester, 2008). The use of chemicals like fertilizers, pesticides, etc. to enhance crop resistance to biotic and abiotic stresses exerts a significant environmental impact (Zhang *et al.*, 2018). Nowadays, one of the biggest challenges for scientists is the development for more eco-friendly systems for manage plant stress (Ali *et al.*, 2021). In this context, research on natural resources as alternatives to conventional chemical approaches, such as plant biostimulants, has been increasing in recent years (Verma *et al.*, 2020).

The European Biostimulants Industry Council defines plant biostimulants as substances and/or microorganisms that, when applied to plants or the rhizosphere, stimulate natural processes to enhance nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality (Brown & Saa, 2015). These products, which contain *bioactive molecules*, have a beneficial effect on morphological, biochemical, and physiological processes, thereby promoting plant tolerance to adverse environmental conditions (Alzahrani & Rady, 2019). Numerous studies have shown that the use of *plant extracts* increases plant growth and yield of many crops, under both favorable and non-favorable conditions (Attia *et al.*, 2023; Hernández-Herrera *et al.*, 2022; Krid *et al.*, 2023; Yadav *et al.*, 2024).

Tomato (*Solanum lycopersicum* L.) ranks as the second most valuable vegetable crop grown globally, following potato, due to its substantial nutritional value for human diet and its economic importance (Erba *et al.*, 2013; Liu *et al.*, 2022). According to the latest FAOSTAT report (2022), world tomato production exceeded 189 million tons in 2022, with China, India, and Turkey being the largest producers. Tomato plays a vital role in keeping people safe and vigorous (Ali *et al.*, 2020; Boulaajine & Hajjaj, 2024; Ouattara & Konate, 2024). Tomatoes are an important source of β -carotene, lycopene, lutein, flavonoids like quercetin, vitamin C and E (Dorais *et al.*, 2008). However, in arid and semi-arid areas, increasing salinity negatively affects tomato production performance (Latef & Chaoxing, 2011). Therefore, developing cost-effective and environmentally friendly strategies to improve tomato plant tolerance to salinity is highly recommended in the agricultural sector.

In line with this, previous studies have demonstrated that applying various biostimulants to crop plants makes them more productive, promotes their growth and

improves their tolerance to biotic and abiotic stresses (for review see [Johnson et al., 2024](#), [Martínez-Lorente et al., 2024](#) and references therein).

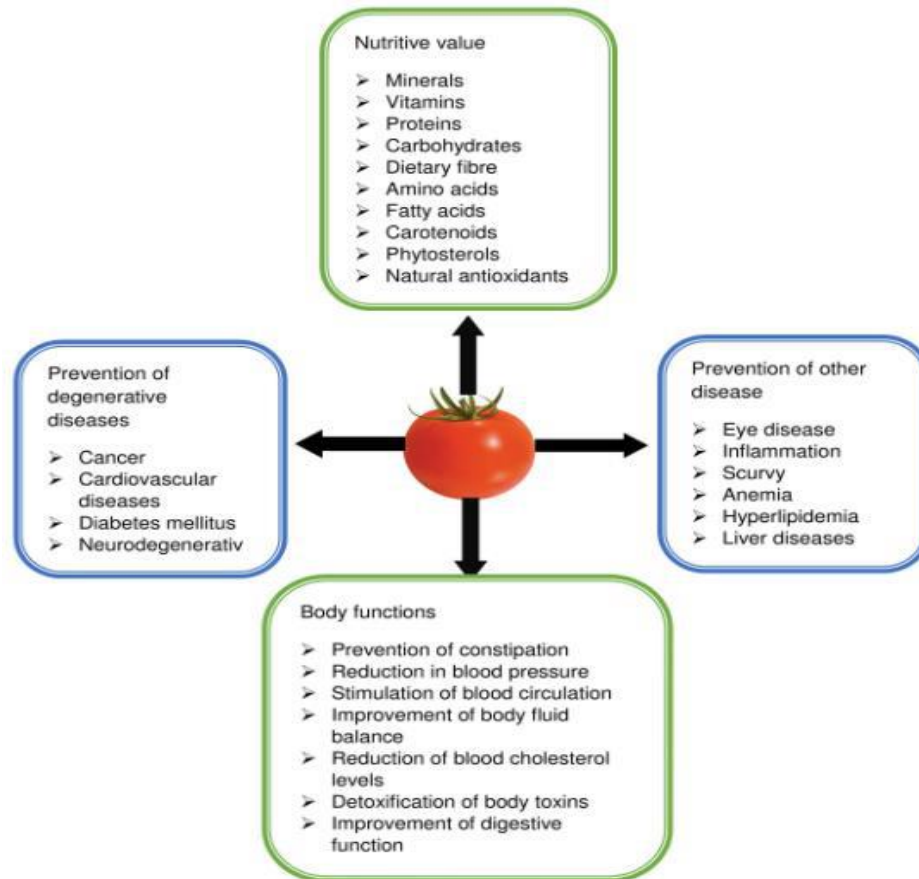


Figure 1. Summary of health benefits of tomato (after [Ali et al., 2020](#))

Biostimulant formulations may contain humic acids, fulvic acids, protein hydrolysis products (amino acids), algae extracts, and beneficial soil microorganisms such as mycorrhizal fungi and plant growth promoting bacteria ([Ahmed et al., 2024](#); [Calvo et al., 2014](#); [Mrid et al., 2021](#)).

The main objective of the current work is to evaluate the effect of some medicinal plant extracts (MPEs) on tomato (var. Heinz1350) germination and seedling growth under salt stress conditions.

1. Medicinal plant extracts preparation

In this study, in addition to Seaweed (see [Attia et al., 2023](#)) and *Syzygium aromaticum* ([Yadav et al., 2024](#)), two local medicinal plants, namely: *Rhaponticum acaule* L (DC) (roots) and *Rhamnus lycioides subsp. oleoides* (leaves) were used. These plants are widely distributed across the Est north of Algeria, especially in the BLEZMA National Park (https://en.wikipedia.org/wiki/Belezma_National_Park) from where they were collected. The plant's samples were crushed after drying at room temperature. Five milligrams of plant material were mixed with 50 mL of methanol (80%) for 72h. Then, the mixture was filtered through Whatman filter paper to remove debris, evaporated (40°C) and the residue was restored in 5 mL of DMSO. This filtrate represented the 100% crude extract. The 50 and 100 ppm concentrations of each extract were subsequently prepared by adding distilled water.



Seaweed
(SW)



Syzygium aromaticum
(Cloves, CL)



Rhaponticum acaule
(Roots, TF=Tafgha)



Rhamnus lycioides subsp. Oleoides
(Leaves, RM)

Figure 2. Photos of the seaweed and the plant extracts used in the present study.

2. Plant material and sterilization

Seeds of tomato (*Solanum lycopersicum* L, cv. Heinz1350) were obtained from a local Market.



Tomato Heinz 1350. Introduced in 1963 by H J Heinz Co.

The variety delivers very high yields of uniform bright red 3in fruits which are perfect in salads or for sauces which they were originally grown for. A robust variety delivering heavy leafed plants with good disease and crack resistance. A good performer in lighter sandy soils and drier locations. ¹

Figure 3. Characteristics of tomato cultivar used in this study.

3. Seeds pretreatment:

Tomato seeds were placed in open containers (for ventilation) containing aqueous solutions of *Syzygium aromaticum* (cloves extract at 50 ppm), *Rhaponticum acaule* L (roots extract = 50 ppm), and *Rhamnus lycioides subsp. oleoides* (leaves extract at 50 ppm) and left for 24 hours in a dark and ventilated place at room temperature (around 24°C). The tap water was used for control. The used concentrations were selected based on preliminary screening test (data not showed).

¹ <https://premierseedsdirect.com/product/tomato-heinz-1350-new/#:~:text=Tomato%20Heinz%201350.,good%20disease%20and%20crack%20resistance.>

4. Petri dish experiment:

Treated or untreated Seeds were surface sterilized with 2% sodium hypochlorite solution, followed by a thorough rinse with distilled water. Then, seeds were soaked for 24 h at room temperature in different herbal extract concentrations; distilled water serving as the control. Ten seeds were spread on a Petri dish containing double layers of moist filter paper with water (control) or 100 mM NaCl (stressed). Petri dishes were kept in complete darkness at room temperature for germination. After 15 days of germination, the final germination percentage was calculated according to the following formula:

$$\text{FGP} = (\text{GS}/\text{TS}) * 100$$

with GP representing the germination percentage, GS the number of germinated seed, and TS the number of sown seeds. For each germination condition, three replicates of each treatment were performed.

5. Pot experiment:

Tomato plants were grown under semi-controlled conditions; 16-h light and 08-h dark regimes at 24+/-2 °C. After disinfection with sodium hypochlorite (2%) for 10 min and rinsing with distilled water, the seeds were planted in pots containing peat moss. Three seedlings of *Solanum lycopersicum* (Heinz 1350) were planted in a medium plastic pot (1Kg), and three repetitions were present in each condition.

We based our choice of NaCl concentration used to induce salinity stress on previous investigations where it has been mentioned that tomato plants are moderately tolerant to salt stress.

After 2 weeks, the plants were subdivided into four classes:

- *plants irrigated only with water (Con),*
- *plants irrigated with 50 mM of NaCl (Salt),*



Propagating Substrate A 240

Composition:
40% white peat (fine)
60% black peat (fine)
NPK 14-10-18
Trace elements
Wetting agent
pH approx. 5,3 - 5,8

Packing:
80 L Bags

Advantages:
Fine propagation substrate with increased water holding capacity due to the black peat.

Application:
Vegetable young plants in trays
Ornamental plants in trays

- plants irrigated with 0.1% of SWE and 50 mM of NaCl (S0.1%),
- plants irrigated with 50ppm of *R. lycioides* (Tafgha) and 50 mM of NaCl (T50),
- plants treated with 50ppm of *R. acaule* and 50 mM of NaCl (R50),
- plants treated with 50ppm of *S. aromaticum* (cloves) and 50 mM of NaCl (C50),

The irrigation was performed two times per week. Four weeks after initiating treatments (when plants irrigated with 50 mM of NaCl presented a noticeable diminution in weight and length), all plants were harvested.

6. Measured parameters

In addition to plant length and fresh/dry weight, leaf area, the following physiological/biochemical characteristics were measured. All the experiments and preparations were performed using fresh leaves of the harvested plants.

- **Relative water content**

Measurements of relative water content (RWC) were performed on leaves collected at the end of experiment (30 DAS). FW of the leaves selected was immediately measured after cutting. In order to obtain the turgid weight (TW), the leaves were immersed in distilled water in a closed test tube and incubated under normal room temperature and dim light for 12 h. At the end of the imbibition period, the leaves were taken out, properly wiped to remove the water on the surface and weighed. Afterwards, the leaves were put in a convection oven for 48 h at 80 °C to obtain DW. RWC was calculated according the equation:

$$\text{RWC in \%} = [(\text{FW}-\text{DW})/(\text{TW}-\text{DW})] \times 100$$

- **Chlorophylls and carotenoids content**

Photosynthetic pigments were determined by the method of Lichtenthaler (1987). Two hundred milligrams of freshly cut leaves were homogenized in 20 mL 80% acetone. Homogenates were centrifuged at 3,000 × g for 10 min in a refrigerated centrifuge at 4°C. Absorbance of the supernatant was determined at 646, 663, and 470 nm, respectively. Chlorophylls and total carotenoids concentrations (µg/mL) were calculated by the following formulas:

$$\text{Chlorophyll a } (\mu\text{g. mL}^{-1}) = 12,25 \times \text{DO}_{663} - 2,79 \times \text{DO}_{647}$$

$$\text{Chlorophyll b } (\mu\text{g. mL}^{-1}) = 21,5 \times \text{DO}_{647} - 5,10 \times \text{DO}_{663}$$

$$\text{Carotenoid } (\mu\text{g. mL}^{-1}) = (1000 \times \text{DO}_{470} - 1,82 \times \text{chla} - 85,02 \times \text{chlb}) / 198$$

- **Proline content**

The proline amounts accumulated by leaves were determined by colorimetric method as described by [Monneveux & Nemmar \(1986\)](#). The extraction was carried out from a composite mixture of 100 mg of fresh leaf segments from three plants per treatment. The concentration of proline was determined with a spectrophotometer (Shimadzu Model UV-1900i, Japan) by measuring the optical density (OD) at $\lambda = 520$ nm. The proline contents were determined according to a calibration curve prepared using pure proline ($A = 1.6129[\text{Pro}]$, $R^2=0.99$).

- **Total soluble sugars content**

Total soluble sugars are determined by the method of [Dubois *et al.* \(1956\)](#). It consists in taking 100 mg of plant material, in test tubes, is added 3 ml of 80% ethanol in order to extract the sugars and then left at room temperature for 48 hours. At the time of dosing, the tubes are placed in the oven at 80°C, to evaporate the alcohol. In each tube 20 mL of distilled water is added to the extract, 2 mL of the test solution are placed in clean glass tubes, with one mL of 5% phenol added; And 5 mL of concentrated sulfuric acid are then rapidly added while avoiding the pouring of acid against the walls of the tube. After obtaining an orange-yellow solution on the surface, the vortex is passed to homogenize the solution. The tubes are then left for 10 minutes and placed in a water bath for 10 to 20 minutes at a temperature of 30°C. The optical density is read at a wavelength of 585 nm. The sugar contents were determined according to a calibration curve prepared using pure glucose. ($A=38.9[\text{TSS}] + 0.404$, $R^2=0.9635$).

- **Total phenolic content**

The Folin–Ciocalteu method was used to measure the total phenolic content, according to the methods described in the literature ([Chavan *et al.*, 2013](#)). One gram of dry biomass of tomato leaves was taken and extracted with 10 mL of 80% methanol. A test tube containing about 0.5 mL of 80% methanolic extract and 0.5 mL of methanol were taken and 300 μL of 50% diluted Folin–Ciocalteu's phenol reagent was added. The reaction mixture was left at room temperature for 10 min in the dark, after which twenty percent aqueous sodium carbonate solution was added. Distilled water was then added to keep the sample's final volume at 3 mL. At 650 nm, the absorbance of the reaction mixture was measured after standing for 60 min at room temperature. As a control, a standard curve was created using gallic acid at various concentrations ($A = 0.0178 [\text{GAE}]+0.0427$, $R^2=0.9904$).

- **2,2-Diphenyl-1-picrylhydrazyl radical assay (DPPH)**

The leaves samples were homogenized in 80% methanol and then centrifuged at 5000 rpm for five minutes. The supernatant was collected and used for the measurement of antioxidant activity by DPPH radical scavenging assay (Chen et al., 2017). In a test tube, 0.5 mL of extract was taken and diluted with 2.5 mL of methanol. The sample mixture was combined with 1 mL of 0.004% DPPH solution and incubated for 30 min in the dark. Ascorbic acid was used as standard. The absorbance was measured at 517 nm and the proportion of antioxidant activity was determined using the following formula:

$$\text{Radical scavenging activity} = \frac{(AC - AS)}{AC} \times 100.$$

AC representing absorbance of control and AS representing absorbance of sample.

7. Statistical analyses

The experimental design adopted in this experiment is full randomization. The results were subjected to an ANOVA one-way at a threshold of 5% and comparisons were made between the different treatments using the LSD test (5%).

These analyses were performed using Statgraphics Centurion (v.19, Statgraphics Technologies, Inc. The Plains, VA, USA).

1. Seeds germination and seedlings growth

Figure 4 illustrated the effect of pre-treatment of tomato seeds with various plant extracts on their germination ability under salt stress conditions (100mM NaCl), compared to the non-stressed control seeds.

Tomato seed germination occurred in most of the treatments after 3 days. As expected, salt stress significantly ($P < 0.001$) decreased the final germination percentage of Heinz1530 tomato seeds. The control group exhibited a FGP of 70%, whereas the stressed group showed a markedly lower final germination rate of 16.67%, indicating a reduction of over 76%.

In contrast, seed pre-treatment improved the FGP of tomato seeds despite salt stress conditions (100 mM).

In this regard, the highest positive effects on seed germination were recorded for the T50, R100, T100 treatments with FGP of 45, 43 and 35% respectively.

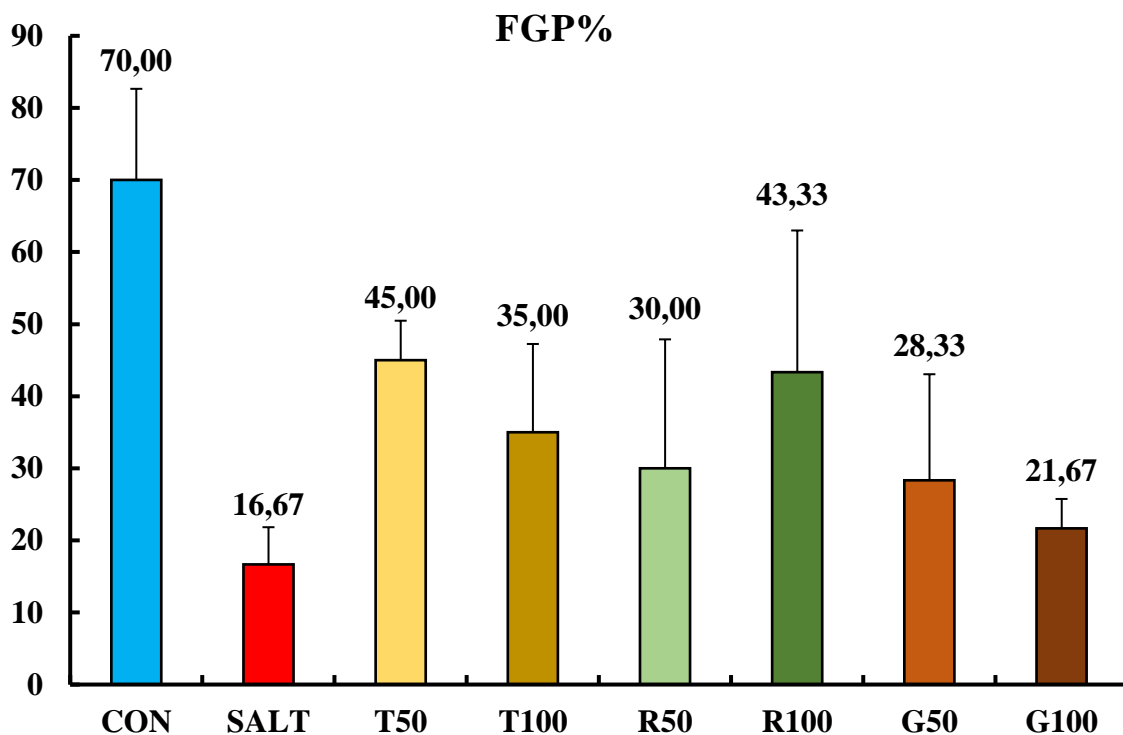


Figure 4. Effect of tomato seed pretreatment (Heinz1350) with medicinal plant extracts on germination under salt stress conditions (100mM, NaCl). Each value represents the average of three repetitions \pm SD.

2. Growth parameters

- *Plant Length*

Figure 5 represents the impact of the different plant extracts on the height of tomato plants (45 days old) in the presence of salt stress (50mM NaCl) compared to the control group (untreated and unstressed plants).

Compared to the control (24.90 cm), salt stress (50mM) negatively affected plant height (20.83cm) inducing a loss of more than 16%.

In contrast, the treatment of plants with the addition of algae and medicinal plants extracts to the irrigation water not only reversed the effect of salinity on the weight growth of tomato plants, it even induced a beneficial effect and thus improved the length of the plants (24.5-26.13 cm).

In this respect, the best treatments were C50, T50 and R5 with 26.13; 25.97 and 25.70 cm respectively.

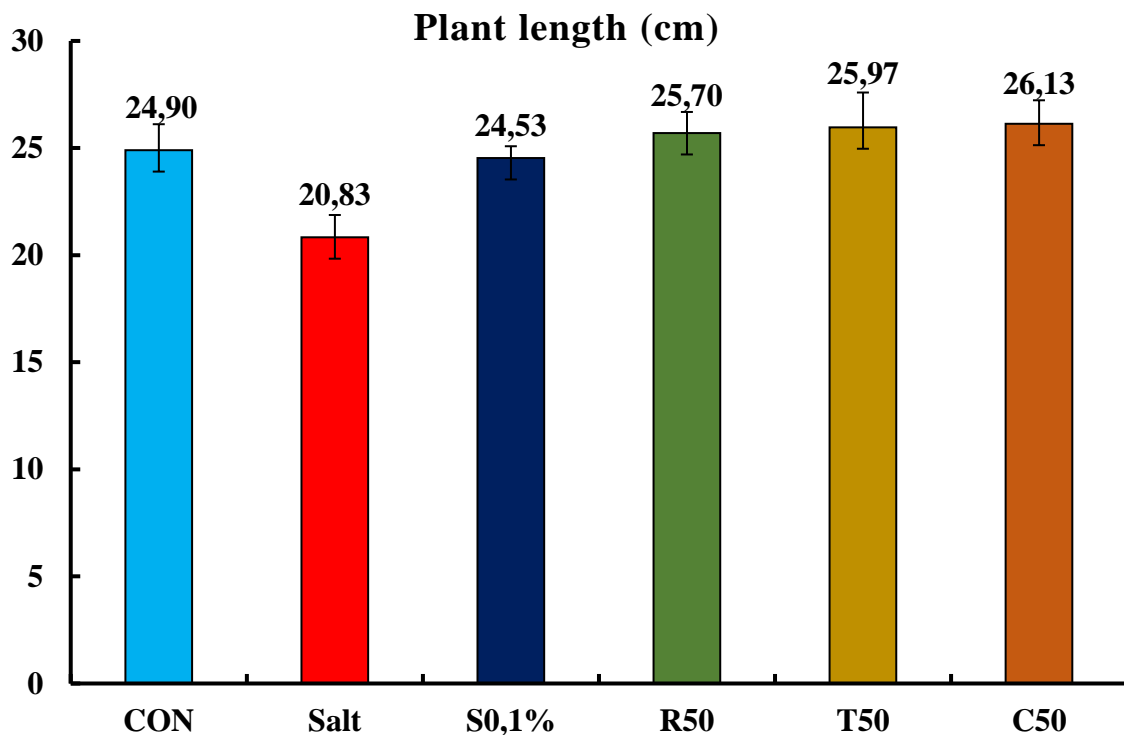


Figure 5. Effect of the addition of Seaweed (0,1%) and Medicinal Plant (50ppm) extracts to irrigation water on the plant length (cm) of tomato plants grown under salt stress conditions (50mM, NaCl). Each value represents the average of three repetitions \pm SD.

- *Leaf dry weight*

As shown in Figure 6, salinity (50 mM) does not appear to have significantly (Table 1) affected the dry weight of tomato leaves, despite the reduction recorded (0,058 g) compared to the control (0,074 g).

On the other hand, the addition of the extracts to the irrigation water not only counteracted the effect of salinity on leaf biomass, but induced an improving effect on this parameter.

The best score was obtained by treatment with R50 where the recorded leaf biomass was 0,115 g, which represents a 55% improvement rate compared to the control.

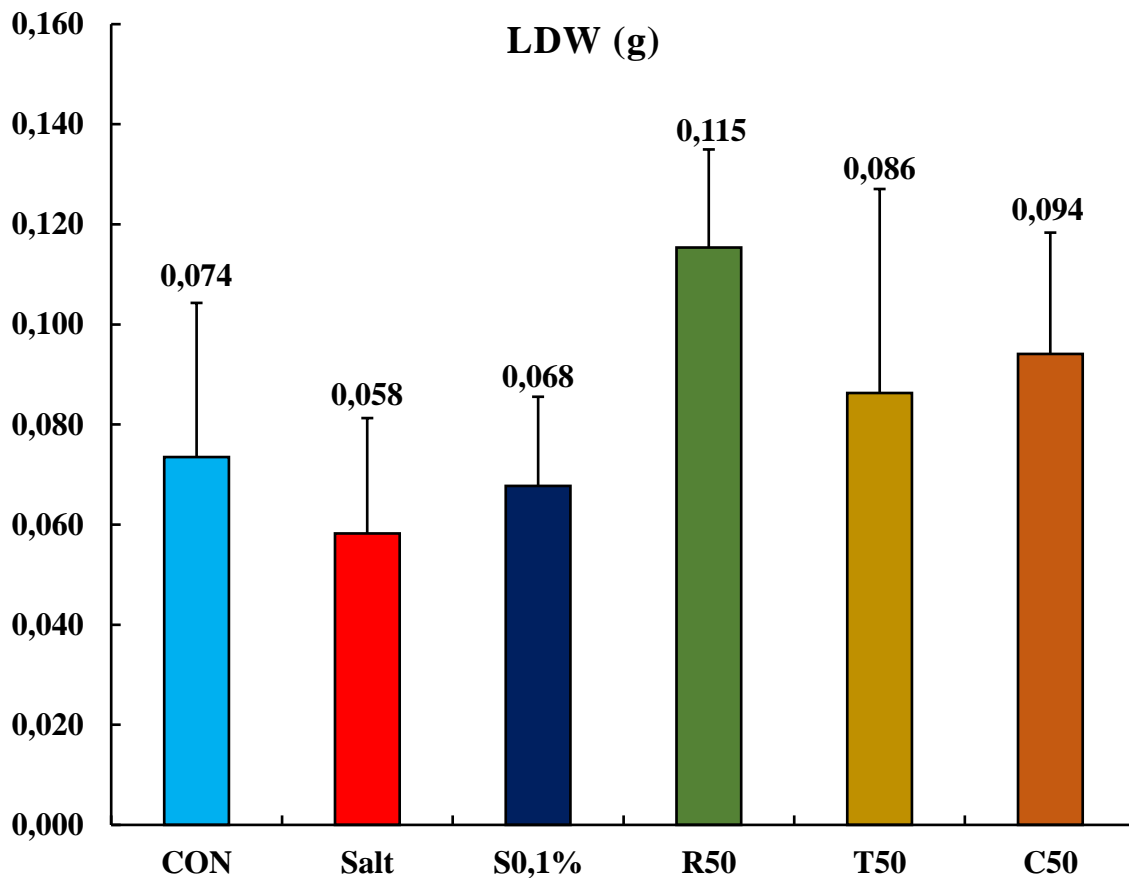


Figure 6. The effect of the addition of Seaweed (0.1%) and Medicinal Plant (50ppm) extracts to irrigation water on the leaf dry weight (biomass in g) of tomato plants grown under salt stress conditions (50mM, NaCl). Each value represents the average of three repetitions \pm SD.

- *Leaf area*

The leaf area (LA) was significantly affected by salinity (50mM) where we recorded a low of almost 50% of the leaf area (Figure 7). On the contrary, the exogenous application of algae and plant extracts improved this parameter enormously despite the stress condition. The best treatments were respectively, R50 (51.08cm²), T50 (46.2cm²) and C50 (43.01cm²).

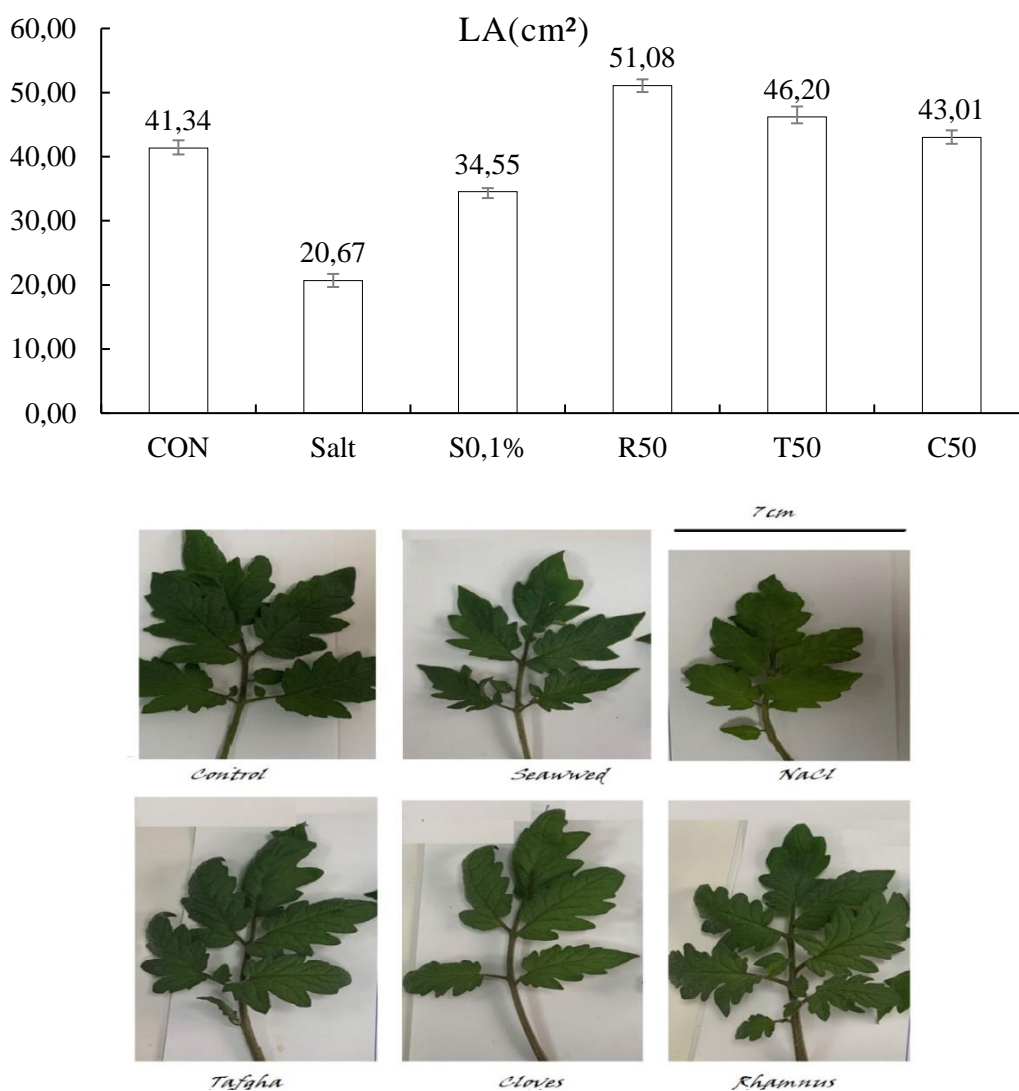


Figure 7. a) -Effect of the addition of the effect of the addition of Seaweed (0.1%) and Medicinal Plant (50ppm) extracts to irrigation under saline stress conditions (50mM) on the leaf surface (LA=cm²). B) - Photos of the 3rd leaf of tomato plants under different growing conditions (Con = control; salt: NaCl 50mM; S: seaweed at 0.1% + NaCl 50 mM; T = Tafgha 50ppm + NaCl 50mM; R: rhamnus lycioides 50 ppm + NaCl 50mM. Each value represents the average of three repeats \pm SD.

3. Physiological and biochemical parameters

▪ *Relative water content (RWC):*

Salt stress had a significant effect ($P < 0.01$, Table 1) on the relative moisture content of tomato leaves (Figure 8). However, the exogenous application of plant extracts significantly improved ($P < 0.05$) the RWC of the leaves. In comparison, treatment with the R50 extract gave the best recovery for RWC (90.70%), while under stress conditions RWC achieved the lowest (67.87). T50, C50 and S0.1 treatments also offer significant effects, but to a lesser extent than R50.

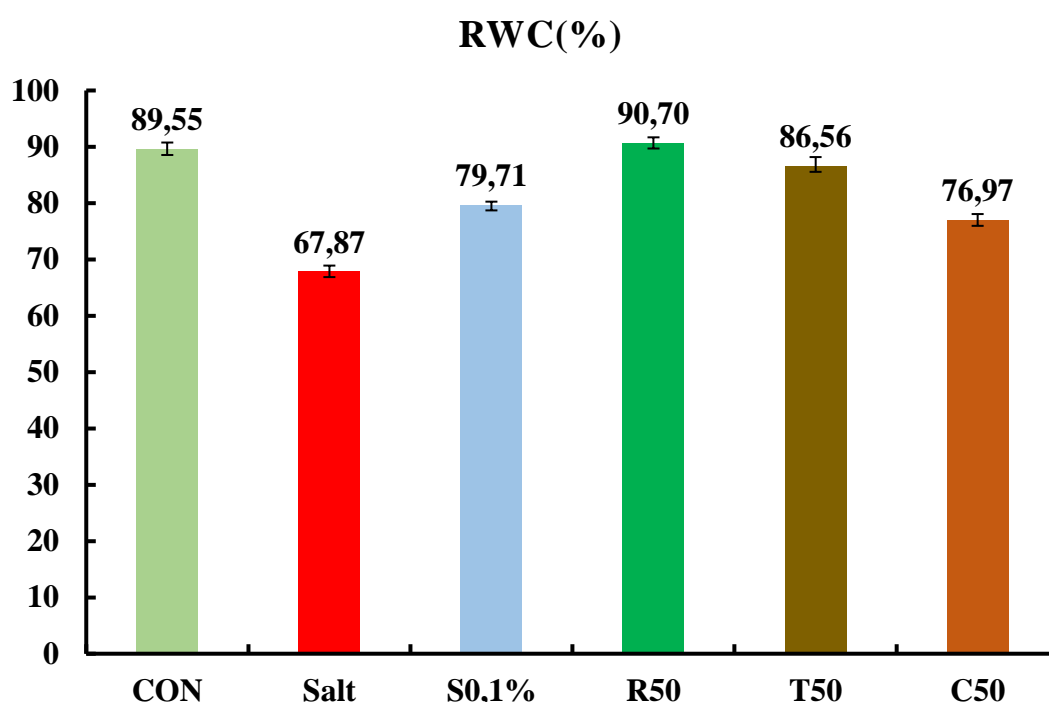


Figure 8. Effect of Seaweed (0.1%) and Medicinal Plant (50ppm) extracts added to irrigation water under saline stress conditions (50mM) on the RWC (%).

▪ *Chlorophylls a and b:*

According to the Figure 9, the salt stress caused a significant ($P < 0.05$) decrease in the Chl-a content of the tomato leaves. On the contrary, the application of the plant extracts, in particular those of T50 & R50, counteracted the effect of salt stress on Chl-a.

In similar way, the Figure showed that chlorophyll *b* content decreased in the leaves of tomato plants that treated with salt stress.

Overall, the treatments of stressed plants with 50ppm of the different medicinal plant extracts appears to significantly reduced the salt stress effect on chlorophyll *a* and *b*.

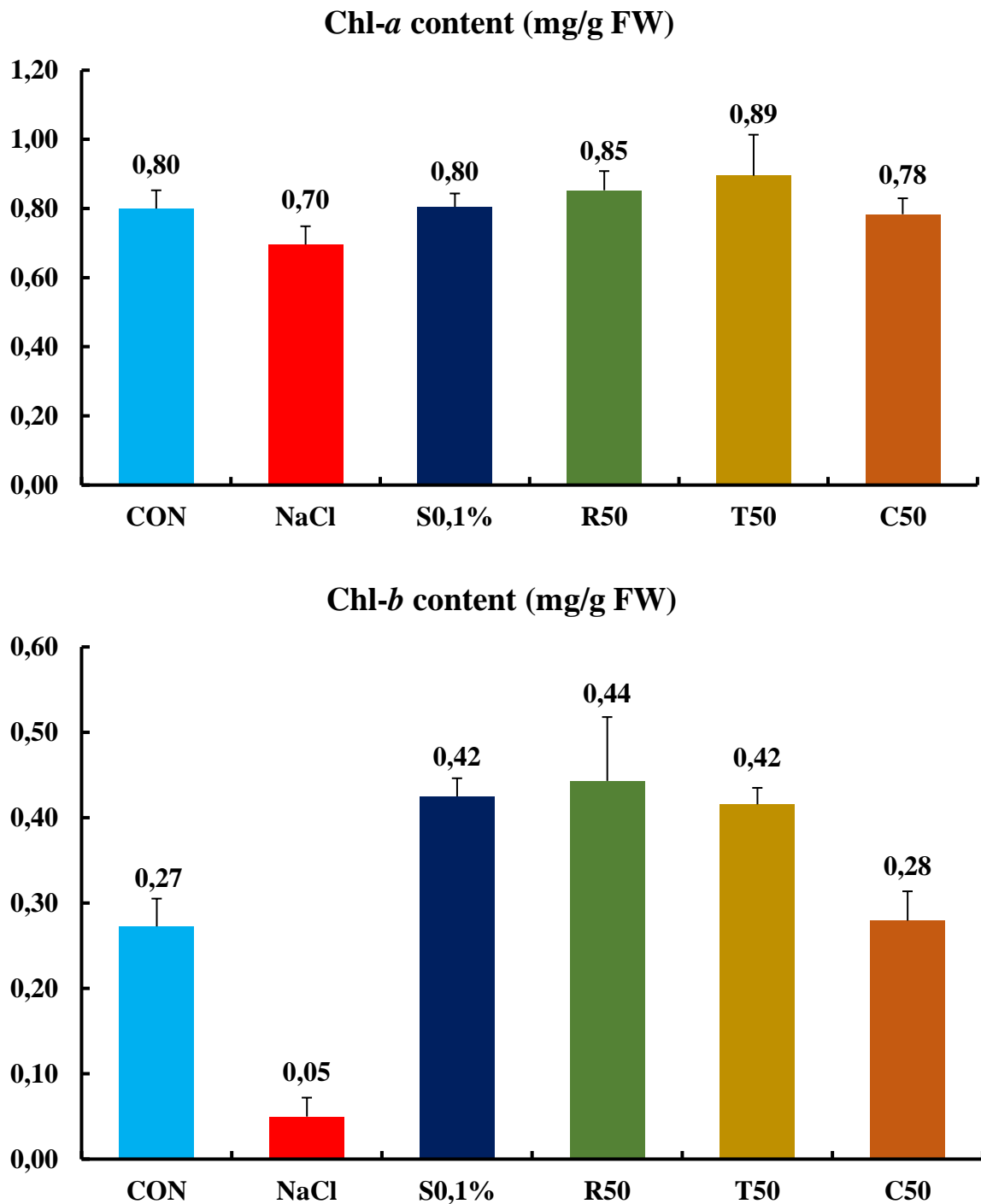


Figure 9. Effect of Seaweed (0.1%) and Medicinal Plant (50ppm) extracts added to irrigation water under saline stress conditions (50mM) on the chlorophylls content. Each value represents the average of three repeats \pm SD.

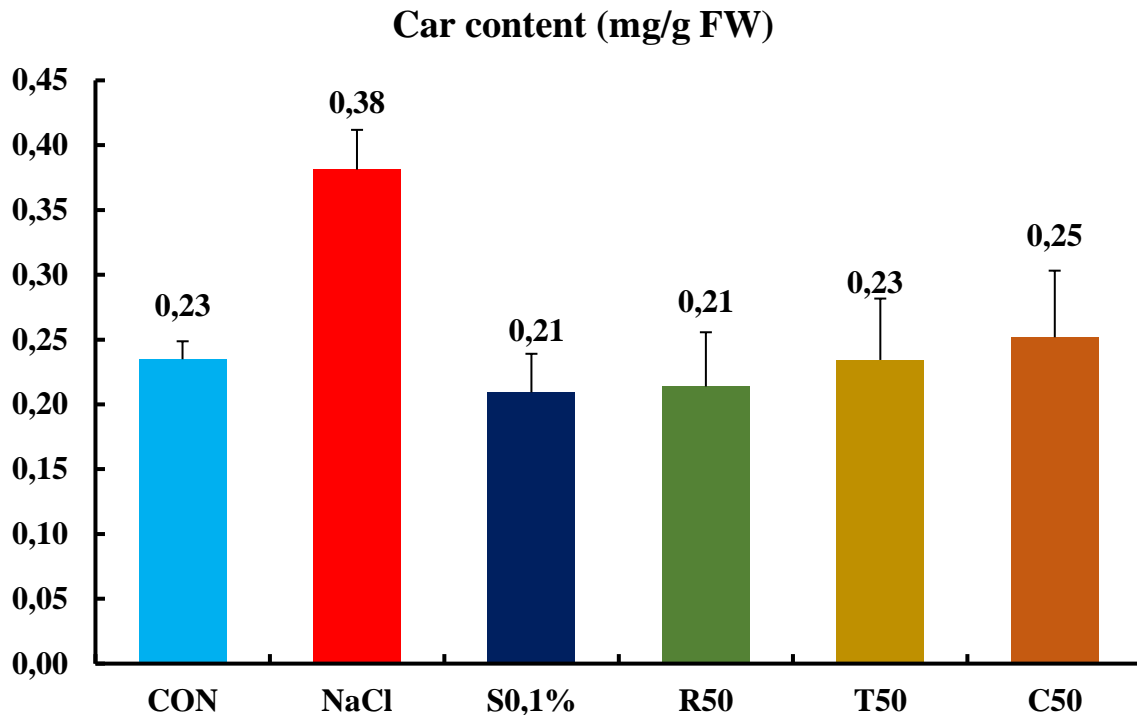


Figure 10. Combined effect of seaweed/plant extracts and salinity on the leaf carotenoids content (mg/g FW). Each value represents the average of three repetitions \pm SD.

▪ ***Carotenoid's content***

The Figure 10 represented the carotenoids content (measured in mg/g dry weight) across the different treatments showed that there was a significant in the carotenoid content.

The control group, has a carotenoid content of 5.67 mg/g FW, and the salt treatment, represented in red, shows a slight increase in carotenoid. Content to 5.89 mg/g FW. Treatments S0.1% and R50 had an equal effect, giving the same percentage of carotenoid content, while treatment T50 gave a percentage of 5.80. However, C50 treatment proved to be the most effective with a score of 5.9

▪ ***Proline content***

Proline content in leaves of the tomato plants increased in all treatments (Figure 11, Table 1).

The highest concentration was recorded in case of plant treated with R50 extract. (0.94 μ g/g FM). In contrast, the lowest concentration was obtained in plants treated with seaweed extract (0.66 μ g/g FM).

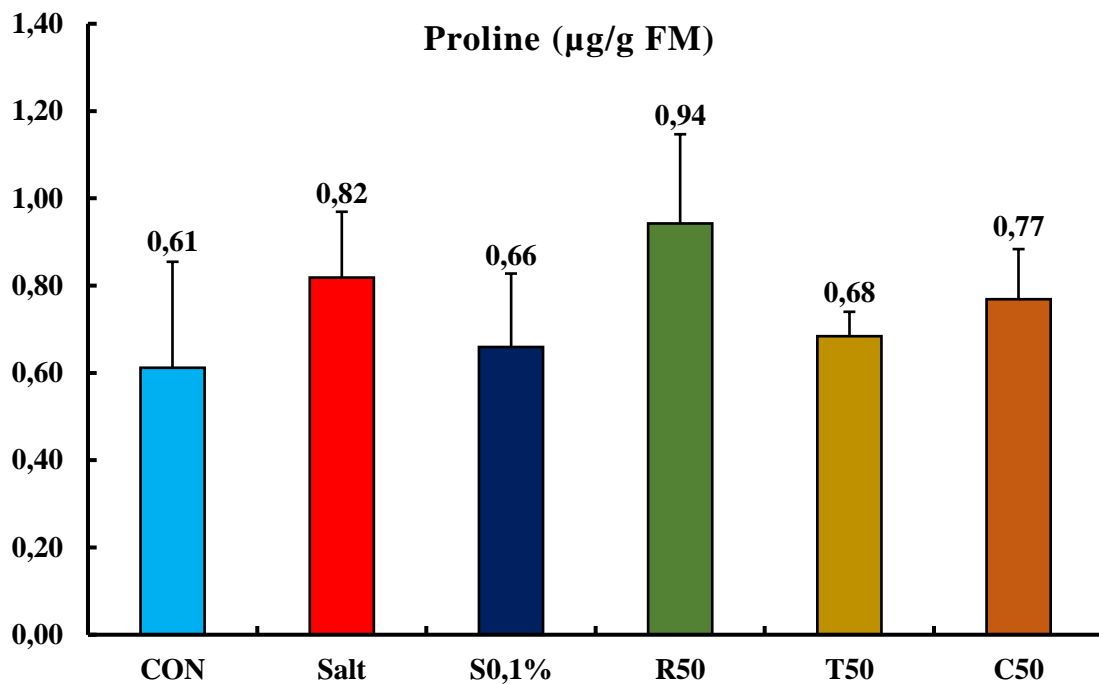


Figure 11. Combined effect of plant extracts and salinity on the leaf proline content (µg/g FW). Each value represents the average of three repetitions ± SD.

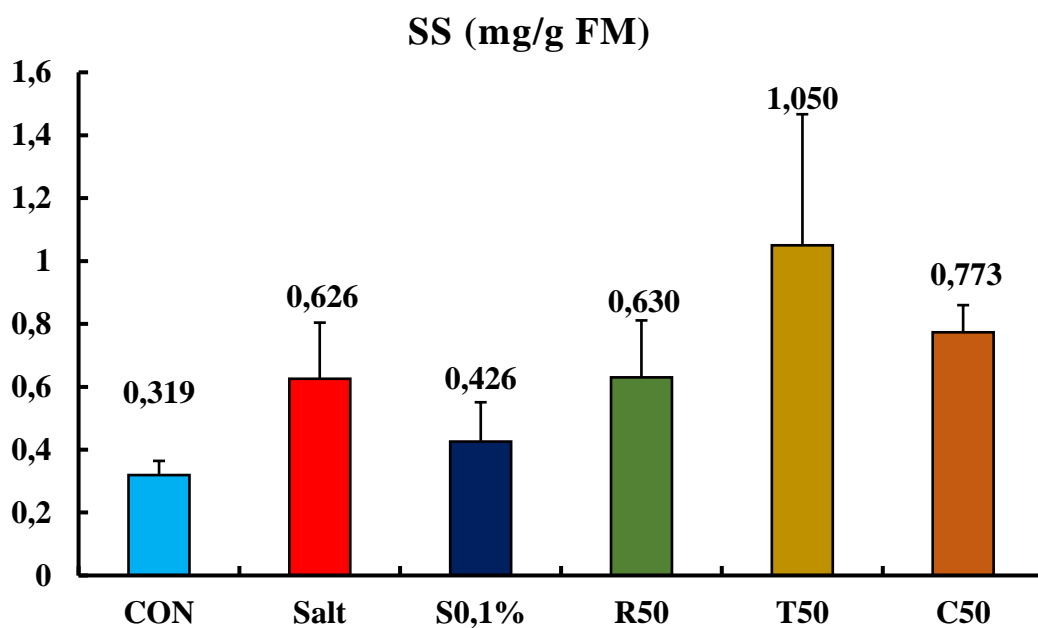


Figure 12. Combined effect of plant extracts and salinity on the leaf soluble sugars content (mg/g FW). Each value represents the average of three repetitions ± SD.

■ ***Soluble Sugars content***

As shown in Figure 12, compared to the control (CON), salt stress (50 mM NaCl) induced the accumulation of soluble sugars in tomato leaves, so that the SS content increased from 0.42 mg/mL to 0.63 mg/mL. Statistical analysis revealed that this response is significant ($P < 0.05$, Table 1). Compared to control, the C50 treatment results in a SS of approximately 2,0 $\mu\text{g/mL}$, indicating a significant increase in sugar content, although not as high as the T50 treatment.

■ ***Total phenols content***

As shown by Figure 13, the total phenols content (TPC) in leaves of tomato plants were found to significantly increase under all the treatments by extracts, when subjected to salinity stress.

Plants treated with both R50 and S0,1% showed higher TPC with 5.35 mg EGA/g DW each. On the other hand, plants treated with T50 and C50 have recorded TPC of 2,64 and 2,59 mg EGA/g DW respectively. The highest TPC recorded in CON (2,71 mg EGA/g DW).

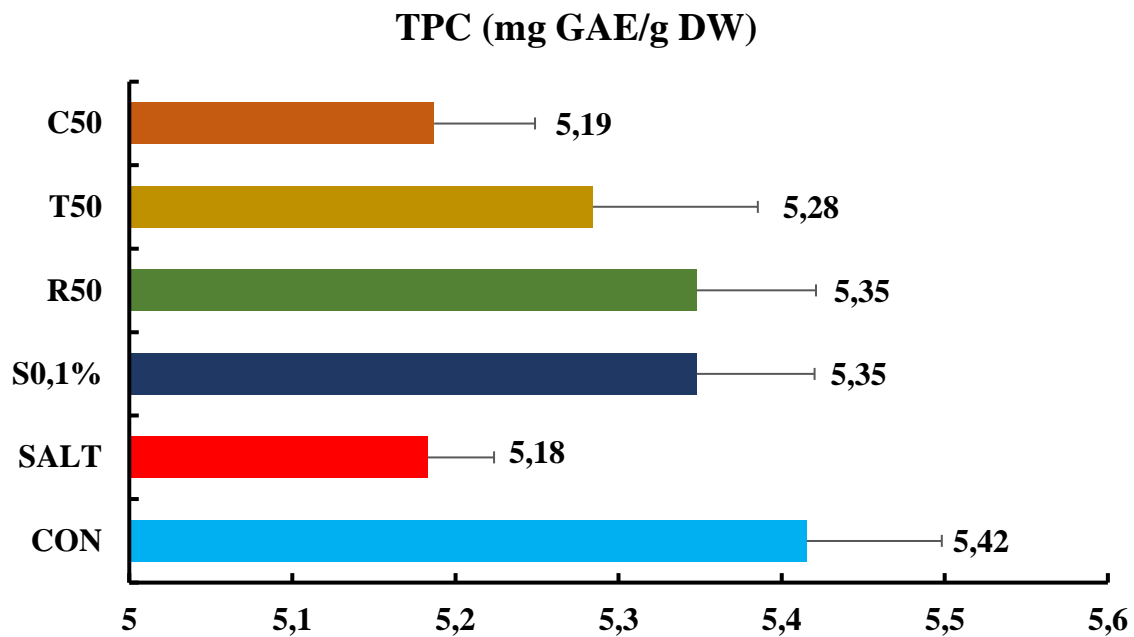


Figure 13. The interactive effect of plant extract and salinity on the total phenols content of tomato leaves extract. Each value represents the average of three repetitions \pm SD.

■ ***Antioxidant activity***

Figure 14, showed the antioxidant activity of tomato leaves extract, across different treatments, measured as scavenging activity of DPPH.

The highest antioxidant activity was recorded in plant treated with seaweed extract (19.52%), followed by R50 extract (16.6%).

Salt treatment showed lower DPPH scavenging activity with only 12,73%, indicating a decrease in antioxidant activity compared to control (17.87%).

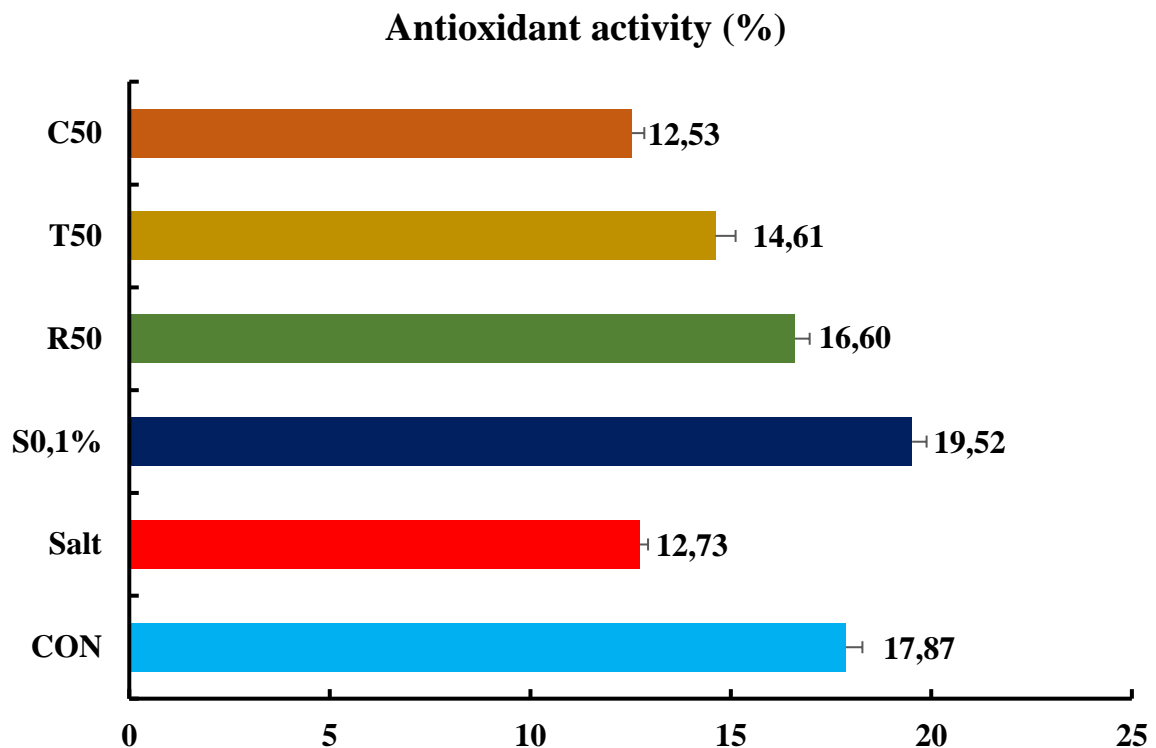


Figure 14. The interactive effect of plant extract treatment and salinity on the antioxidant activity (%) of tomato leaf extract. Each value represents the average of three repetitions \pm SD.

Table 1. Measured parameters with their average values, standard deviation and statistical analysis.

	RWC	PL	LA	SS	PRO	CHL(a)	CHL(b)	CAR	TPC	AA
CON	89,55 ±7,22	24,90 ±1,21	41,34 ±1,06	0,319 ±0,045	0,498 ±0,159	0,800 ±0,053	0,273 ±0,033	0,235 ±0,014	5,42 ±0,08	17,87 ±5,25
SALT	67,87 ±4,98	20,83 ±1,04	20,67 ±3,02	0,626 ±0,179	0,818 ±0,151	0,696 ±0,053	0,050 ±0,022	0,381 ±0,031	5,18 ±0,04	12,73 ±5,87
S0,1%	79,71 ±3,98	24,53 ±0,551	34,55 ±3,499	0,426 ±0,125	0,659 ±0,168	0,805 ±0,039	0,425 ±0,021	0,209 ±0,03	5,35 ±0,07	19,52 ±2,07
R50	90,70 ±3,221	25,70 ±0,985	51,08 ±1,436	0,630 ±0,181	0,942 ±0,204	0,853 ±0,056	0,443 ±0,075	0,214 ±0,042	5,35 ±0,07	16,60 ±0,22
T50	86,56 ±6,209	25,97 ±1,626	46,20 ±4,007	1,050 ±0,417	0,684 ±0,769	0,895 ±0,119	0,416 ±0,019	0,234 ±0,047	5,28 ±0,10	19,93 ±8,21
C50	76,97 ±4,226	26,13 ±1,097	43,01 ±4,696	0,773 ±0,086	0,769 ±0,115	0,783 ±0,046	0,279 ±0,034	0,252 ±0,051	5,19 ±0,062	19,93 ±8,21
F	8.66	9.23	32.97	4.58	3.09	3.11	44.04	8.56	4.88	0.70
P value	0.0011	0.0008	0.0001	0.0144	NS	0.0498	0.0001	0.0012	0.0119	0.633
LSD5%	9.58	3.7	8.07	0.42	NS	0.157	0.136	0.129	0.161	NS

DISCUSSION

Salinity is one of the main abiotic stresses that significantly reduces crop growth and productivity (Machado & Serralheiro, 2017). The harmful effects of salinity occur due to the plant's difficulty absorbing water from the root environment and its inability to feed itself (Marschner, 1995). In fact, growing tomato plants under salt stress reduces their yield in terms of quantity and quality (Zhang *et al.*, 2016). These phenomena can be mitigated by the use of biostimulants (Maach, *et al.*, 2020). Studies have shown that the application of various biostimulants to crop plants makes them more productive, promotes their growth and improves their tolerance to biotic and abiotic stresses (Calvo *et al.*, 2014; Sharma *et al.*, 2014).

The results showed that seed pre-treatment had a significant effect on the tomato germination performances (Figure 3). These results are consistent with those of Aymen *et al.* (2014) and Ali *et al.* (2018) who used algae extracts to improve tomato seed germination under saline stress conditions. However, the response of tomato seeds was not the same for all treatments. The highest germination performance was achieved in the seed groups treated with T50 followed by R100. In line with this, many studies have shown that the difference in response to pretreatment is likely due to the chemical composition of the applied extracts, as plants do not have the same chemical composition (Hernández-Herrera *et al.*, 2023).

It is widely accepted that exposure to NaCl induced reduction in plant growth and development (Munns & Tester, 2008). Our results corroborate this finding (Figures 4-6). The inhibition of leaf growth or leaf elongation appears to be one of the first effects of salt stress (Beartiz *et al.*, 2001; Khelil *et al.*, 2007). Leaves, which are the main organs for photosynthesis and transpiration, play an important role in the growth and development of higher plants, especially under stress conditions (Munns, 2002). Thus, the leaf elongation rate and final leaf size seems to be a key element in plant tolerance to abiotic stresses (Fercha, 2011). The reduction in leaf area can be caused either by reduced cell division or expansion, since both of them are equally sensitive to environmental stresses. In contrast, treatment of tomato plant with plant extracts mainly with R5 and T50 seem to have markedly improved the leaf growth. Similar results have been reported by Naboulsi *et al.* (2022).

Chlorophyll is the main pigment found in most oxygenated photosynthetic organisms. The chlorophyll content of plants is one of the main factors that reflects their rate of

photosynthesis (Mao *et al.*, 2017, cited by Naboulsi *et al.*, 2022). Numerous studies have demonstrated that variations in pigment content can provide valuable information on the physiological performance of leaves and indicate their photosynthetic capacity and the general state of plant health (stress or disease) (Zulkarnaini, *et al.*, 2007). The results of the present study showed that the chlorophyll a and b content increased significantly in tomato leaves treated with the extracts, in particular, the treatments with the R50 and T50 extracts.

Similar results have been reported by previous studies (Hernández-Herrera *et al.*, 2022; and reference therein), suggesting that optimized photosynthetic performance and antioxidant defense systems (proline, total phenols, and flavonoids) appear to be major factors modulating SE responses to salinity tolerance in tomato plants.

Carotenoids are accessory pigments in photosynthetic systems and also protect chlorophylls from oxidative destruction under stress (Gadi & Goswami, 2016). In the present study, we found that treatments with used MPEs affected the carotenoids content of tomato leaves in a contradictory way. In contrast to the T50 and C50 treatments, the S0.1% and R50 treatments significantly reduced the carotenoid content of tomato leaves.

Proline, especially in water and salt stress, has an osmoprotective role and is usually increased under stressed conditions, improving the antioxidant system of plants (Rady *et al.*, 2016). Under these conditions, R50 and C50 had the highest proline content, while S0.1% and T50 had the lowest values. Soluble sugars have been shown to act as functional osmolytes in many plant species, accumulating at high levels in response to high salinity and other abiotic stress treatments in current experiments, sugar contents actually decrease with increasing soil salinity, as soluble sugar results have shown that response to treatments was recorded in seeds treated with T50 and C50 compared to control and salt stressed plants.

Plant-based phenols are a broad family of secondary metabolites that range from basic phenolic acids to more complex compounds such as stilbenes, flavonoids, and tannins (Szabo *et al.*, 2022). In response to salinity, plant increase their content on antioxidant molecules such as phenolic compounds to protect the cellular constituents against the ROS that accumulate during salt stress (Metwally & Soliman, 2023). In contrast, in another study it has been reported that water deficit treatment of tomato generally resulted in increased carotenoids concentration and decreased polyphenols concentration (Stoleru *et al.*, 2020) our results showed that salt stress induced a slight but significant increase in phenolic compounds content. Conversely, the application of extracts, in particular those of S0.1%, R50 and T50. For

antioxidant activity, treated tomato plants grown under salt stress showed the lowest antioxidant activity which is consistent with the results of similar studies such that of [Metwally & Soliman \(2023\)](#).

CONCLUSION

This experiment aimed to assess the impact of treating seeds and plants with seaweed and medicinal plant extracts on the germination and growth of tomato plants under saline stress conditions.

The results clearly indicate that:

- Salt stress, even at moderate levels, significantly affects the germination, growth, and metabolic parameters of tomato plants.
- Pre-treating seeds and plants with seaweed and medicinal plant extracts is an effective method to mitigate the adverse effects of salinity on tomato germination and growth (var. Heinz1530).
- Plant extracts have the potential to enhance the salt tolerance of tomatoes, likely through different mechanisms due to their unique chemical compositions.
- The use of plant extracts, particularly *Rhamnus lycioides*, is recommended as an excellent biostimulant, suggesting its adoption by farmers as an effective and cost-efficient strategy for managing salinity.
- However, further research is necessary to optimize the application of plant extracts to better alleviate salt stress in crops.

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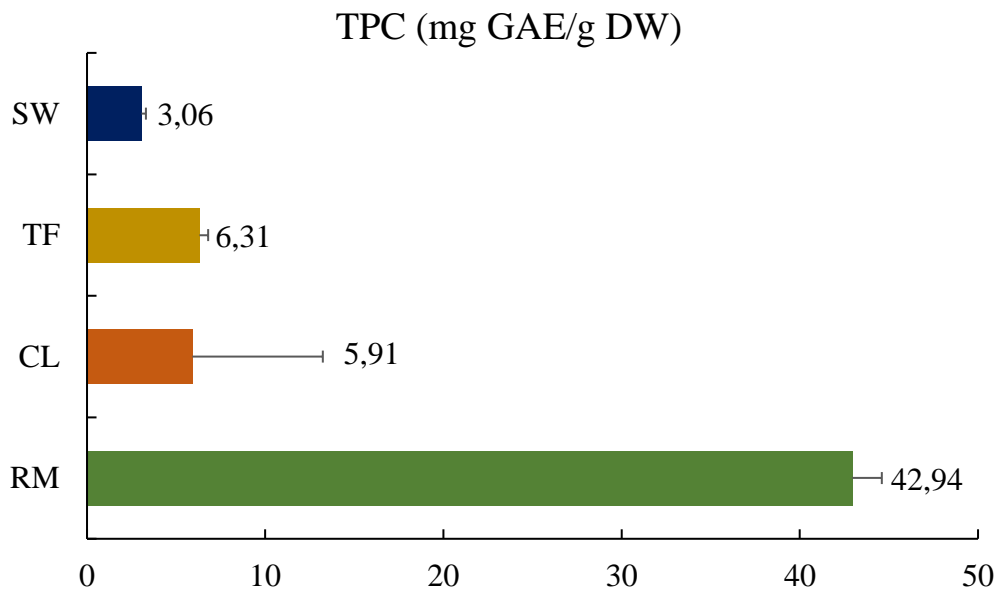
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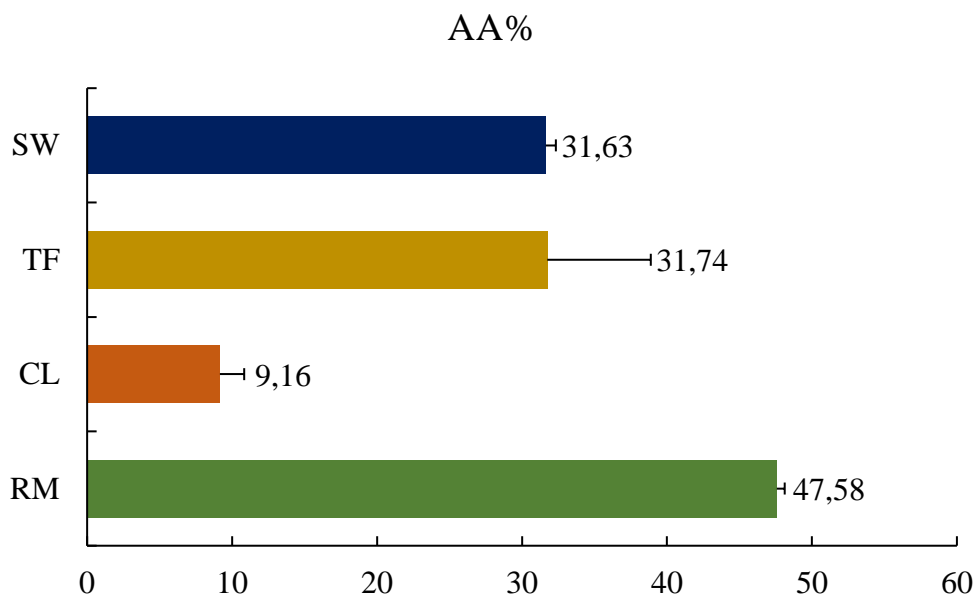
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Appendix 1

Total polyphenol content of the different extracts used in the present study.



Antioxidant activity of the different extracts used in the present study.



Appendix 2

Statistical analyses

ANOVA Table for FGP by TREATMENT

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0,696146	4	0,174037	13,05	0,0000
Within groups	0,573574	43	0,0133389		
Total (Corr.)	1,26972	47			

Multiple Range Tests for FGP by TREATMENT

Method: 95,0 percent LSD

TREATMENT	Count	Mean	Homogeneous Groups
SALT	6	0,403552	A
G50	12	0,490144	AB
R50	12	0,58312	BC
T50	12	0,62694	C
CON	6	0,833608	D

Contrast	Sig.	Difference	+/- Limits
CON - G50	*	0,343463	0,116458
CON - R50	*	0,250487	0,116458
CON - SALT	*	0,430056	0,134475
CON - T50	*	0,206667	0,116458
G50 - R50		-0,092976	0,095088
G50 - SALT		0,0865925	0,116458
G50 - T50	*	-0,136796	0,095088
R50 - SALT	*	0,179569	0,116458
R50 - T50		-0,04382	0,095088
SALT - T50	*	-0,223388	0,116458

* denotes a statistically significant difference.

ANOVA Table for PROLIN by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0,345979	5	0,0691958	3,09	0,0507
Within groups	0,268696	12	0,0223913		
Total (Corr.)	0,614675	17			

ANOVA Table for SS by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1,00469	5	0,200937	4,58	0,0144
Within groups	0,527027	12	0,0439189		
Total (Corr.)	1,53171	17			

Multiple Range Tests for SS by Treatment

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
CON	3	0,319309	A
S0,1%	3	0,425779	AB
SALT	3	0,625519	AB
R50	3	0,629976	AB

C50	3	0,773309	BC
T50	3	1,05003	C

Contrast	Sig.	Difference	+/- Limits
C50 - CON	*	0,454	0,372822
C50 - R50		0,143332	0,372822
C50 - S0,1%		0,347529	0,372822
C50 - SALT		0,14779	0,372822
C50 - T50		-0,276721	0,372822
CON - R50		-0,310668	0,372822
CON - S0,1%		-0,106471	0,372822
CON - SALT		-0,30621	0,372822
CON - T50	*	-0,73072	0,372822
R50 - S0,1%		0,204197	0,372822
R50 - SALT		0,0044577	0,372822
R50 - T50	*	-0,420053	0,372822
S0,1% - SALT		-0,199739	0,372822
S0,1% - T50	*	-0,62425	0,372822
SALT - T50	*	-0,424511	0,372822

* denotes a statistically significant difference.

ANOVA Table for Chla by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0,0682809	5	0,0136562	3,11	0,0498
Within groups	0,0526743	12	0,00438952		
Total (Corr.)	0,120955	17			

Multiple Range Tests for Chla by Treatment

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
SALT	3	0,695793	A
C50	3	0,783437	AB
CON	3	0,799717	AB
S0,1%	3	0,804539	AB
R50	3	0,852643	B
T50	3	0,894841	B

Contrast	Sig.	Difference	+/- Limits
C50 - CON		-0,01628	0,117865
C50 - R50		-0,0692053	0,117865
C50 - S0,1%		-0,0211013	0,117865
C50 - SALT		0,087644	0,117865
C50 - T50		-0,111404	0,117865
CON - R50		-0,0529253	0,117865
CON - S0,1%		-0,00482133	0,117865
CON - SALT		0,103924	0,117865
CON - T50		-0,095124	0,117865
R50 - S0,1%		0,048104	0,117865
R50 - SALT	*	0,156849	0,117865
R50 - T50		-0,0421987	0,117865
S0,1% - SALT		0,108745	0,117865
S0,1% - T50		-0,0903027	0,117865
SALT - T50	*	-0,199048	0,117865

* denotes a statistically significant difference.

ANOVA Table for Chlb by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
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Between groups	0,335969	5	0,0671938	44,04	0,0000
Within groups	0,0183073	12	0,00152561		
Total (Corr.)	0,354276	17			

Multiple Range Tests for Chlb by Treatment

Method: 95,0 percent LSD

<i>Treatment</i>	<i>Count</i>	<i>Mean</i>	<i>Homogeneous Groups</i>
SALT	3	0,0498067	A
CON	3	0,272608	B
C50	3	0,279315	B
T50	3	0,415556	C
S0,1%	3	0,424931	C
R50	3	0,443059	C

<i>Contrast</i>	<i>Sig.</i>	<i>Difference</i>	<i>+/- Limits</i>
C50 - CON		0,00670667	0,0694859
C50 - R50	*	-0,163744	0,0694859
C50 - S0,1%	*	-0,145616	0,0694859
C50 - SALT	*	0,229508	0,0694859
C50 - T50	*	-0,136241	0,0694859
CON - R50	*	-0,170451	0,0694859
CON - S0,1%	*	-0,152323	0,0694859
CON - SALT	*	0,222801	0,0694859
CON - T50	*	-0,142948	0,0694859
R50 - S0,1%		0,018128	0,0694859
R50 - SALT	*	0,393252	0,0694859
R50 - T50		0,0275027	0,0694859
S0,1% - SALT	*	0,375124	0,0694859
S0,1% - T50		0,00937467	0,0694859
SALT - T50	*	-0,365749	0,0694859

* denotes a statistically significant difference.

ANOVA Table for Car by Treatment

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Between groups	0,0616597	5	0,0123319	8,56	0,0012
Within groups	0,0172957	12	0,0014413		
Total (Corr.)	0,0789554	17			

Multiple Range Tests for Car by Treatment

Method: 95,0 percent LSD

<i>Treatment</i>	<i>Count</i>	<i>Mean</i>	<i>Homogeneous Groups</i>
S0,1%	3	0,209308	A
R50	3	0,213807	A
T50	3	0,23429	A
CON	3	0,23485	A
C50	3	0,251798	A
SALT	3	0,381203	B

<i>Contrast</i>	<i>Sig.</i>	<i>Difference</i>	<i>+/- Limits</i>
C50 - CON		0,0169482	0,0675388
C50 - R50		0,0379916	0,0675388
C50 - S0,1%		0,0424902	0,0675388
C50 - SALT	*	-0,129405	0,0675388
C50 - T50		0,0175087	0,0675388
CON - R50		0,0210434	0,0675388
CON - S0,1%		0,025542	0,0675388
CON - SALT	*	-0,146353	0,0675388
CON - T50		0,000560509	0,0675388

R50 - S0,1%		0,00449859	0,0675388
R50 - SALT	*	-0,167396	0,0675388
R50 - T50		-0,0204829	0,0675388
S0,1% - SALT	*	-0,171895	0,0675388
S0,1% - T50		-0,0249815	0,0675388
SALT - T50	*	0,146913	0,0675388

* denotes a statistically significant difference.

ANOVA Table for RWC by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1150,67	5	230,134	8,66	0,0011
Within groups	319,057	12	26,5881		
Total (Corr.)	1469,73	17			

Multiple Range Tests for RWC by Treatment

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
SALT	3	67,8699	A
C50	3	76,9678	AB
S0,1%	3	79,7122	BC
T50	3	86,556	CD
CON	3	89,5491	D
R50	3	90,6984	D

Contrast	Sig.	Difference	+/- Limits
C50 - CON	*	-12,5813	9,17316
C50 - R50	*	-13,7306	9,17316
C50 - S0,1%		-2,7444	9,17316
C50 - SALT		9,0979	9,17316
C50 - T50	*	-9,58824	9,17316
CON - R50		-1,14933	9,17316
CON - S0,1%	*	9,83691	9,17316
CON - SALT	*	21,6792	9,17316
CON - T50		2,99306	9,17316
R50 - S0,1%	*	10,9862	9,17316
R50 - SALT	*	22,8285	9,17316
R50 - T50		4,1424	9,17316
S0,1% - SALT	*	11,8423	9,17316
S0,1% - T50		-6,84384	9,17316
SALT - T50	*	-18,6861	9,17316

* denotes a statistically significant difference.

ANOVA Table for PL by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	59,0244	5	11,8049	9,23	0,0008
Within groups	15,3467	12	1,27889		
Total (Corr.)	74,3711	17			

Multiple Range Tests for PL by Treatment

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
SALT	3	20,8333	A
S0,1%	3	24,5333	B
CON	3	24,9	B
R50	3	25,7	B

T50	3	25,9667	B
C50	3	26,1333	B

Contrast	Sig.	Difference	+/- Limits
C50 - CON		1,23333	2,01183
C50 - R50		0,433333	2,01183
C50 - S0,1%		1,6	2,01183
C50 - SALT	*	5,3	2,01183
C50 - T50		0,166667	2,01183
CON - R50		-0,8	2,01183
CON - S0,1%		0,366667	2,01183
CON - SALT	*	4,06667	2,01183
CON - T50		-1,06667	2,01183
R50 - S0,1%		1,16667	2,01183
R50 - SALT	*	4,86667	2,01183
R50 - T50		-0,266667	2,01183
S0,1% - SALT	*	3,7	2,01183
S0,1% - T50		-1,43333	2,01183
SALT - T50	*	-5,13333	2,01183

* denotes a statistically significant difference.

ANOVA Table for LA by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1721,6	5	344,32	32,97	0,0000
Within groups	125,315	12	10,4429		
Total (Corr.)	1846,91	17			

Multiple Range Tests for LA by Treatment

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
SALT	3	20,6714	A
S0,1%	3	34,5453	B
CON	3	41,3435	C
C50	3	43,0087	C
T50	3	46,2031	CD
R50	3	51,0828	D

Contrast	Sig.	Difference	+/- Limits
C50 - CON		1,66527	5,74891
C50 - R50	*	-8,07412	5,74891
C50 - S0,1%	*	8,46347	5,74891
C50 - SALT	*	22,3374	5,74891
C50 - T50		-3,19434	5,74891
CON - R50	*	-9,73939	5,74891
CON - S0,1%	*	6,79821	5,74891
CON - SALT	*	20,6721	5,74891
CON - T50		-4,85961	5,74891
R50 - S0,1%	*	16,5376	5,74891
R50 - SALT	*	30,4115	5,74891
R50 - T50		4,87978	5,74891
S0,1% - SALT	*	13,8739	5,74891
S0,1% - T50	*	-11,6578	5,74891
SALT - T50	*	-25,5317	5,74891

* denotes a statistically significant difference.

ANOVA Table for TPC by TREATMENT

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0,133204	5	0,0266409	4,83	0,0119
Within groups	0,0662374	12	0,00551979		
Total (Corr.)	0,199442	17			

Multiple Range Tests for TPC by TREATMENT

Method: 95,0 percent LSD

TREATMENT	Count	Mean	Homogeneous Groups
SALT	3	5,18315	A
C50	3	5,18689	A
T50	3	5,28427	AB
R50	3	5,34794	B
S0,1%	3	5,34794	B
CON	3	5,41536	B

Contrast	Sig.	Difference	+/- Limits
C50 - CON	*	-0,228464	0,132171
C50 - R50	*	-0,161049	0,132171
C50 - S0,1%	*	-0,161049	0,132171
C50 - SALT		0,00374532	0,132171
C50 - T50		-0,0973783	0,132171
CON - R50		0,0674157	0,132171
CON - S0,1%		0,0674157	0,132171
CON - SALT	*	0,23221	0,132171
CON - T50		0,131086	0,132171
R50 - S0,1%		0	0,132171
R50 - SALT	*	0,164794	0,132171
R50 - T50		0,0636704	0,132171
S0,1% - SALT	*	0,164794	0,132171
S0,1% - T50		0,0636704	0,132171
SALT - T50		-0,101124	0,132171

* denotes a statistically significant difference.

ANOVA Table for AA by TREATMENT

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	117,506	5	23,5012	0,70	0,6330
Within groups	402,055	12	33,5046		
Total (Corr.)	519,561	17			

Multiple Range Tests for AA by TREATMENT