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# Melatonin and arginine combined supplementation alleviate salt stress through physiochemical adjustments and improved antioxidant enzymes activity in *Capsicum annuum* L.

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ABSTRACT

Effects of NaCl stress, melatonin (MT), arginine (ARG), and their combined treatment on morphological, physiological, and biochemical features of *Capsicum annuum* L. were estimated. Antioxidants (total phenolics), total soluble protein and antioxidant enzymes were analyzed in this study. Quantum yield of photosystem II (Y[II]), inorganic ions ($Na^+$, $K^+$, $Ca^{++}$ and $Cl^-$) and yield characters of *C. annuum* were also studied in this research work. Salt stress was applied using 50 and 100 mM sodium chloride. Concentrations selected for exogenous MT and ARG treatments were 100 μM and 2 mM, respectively. Results showed that NaCl salt stress significantly reduced morphological characters. Foliar application of MT, ARG and their combined treatment improved morphological characters in control as well as salt stress plants. There was negligible change in chlorophyll *a* and *b* contents of *C. annuum* under NaCl salt stress. Exogenous MT and ARG treatments slightly improved chlorophyll *a, b* contents in control and stress plants. ROS, MDA and $H_2O_2$, antioxidants (phenolics) and antioxidant enzymes were increased under salt stress. Foliar spray of MT, ARG and their combined treatment tends to decrease MDA and $H_2O_2$ but enhanced total phenolics and antioxidant enzymes under NaCl stress. Salt stress also decreased Y[II] and overall yield of *C. annuum*. Exogenous MT and ARG treatments not only improved photosynthetic capacity of *C. annuum* by improving Y[II] but also enhanced overall yield of *C. annuum* plants in control and stress conditions. It is concluded that exogenous MT, ARG, and their combined treatment alleviates salt stress in *C. annuum* through improving antioxidant enzymes activities, chlorophyll *a* and *b* contents, Y[II] and by scavenging effects of ROS. This resulted in improved plant growth and overall yield of *C. annuum*.

## 1. Introduction

Soil salinity had damaged 1/3rd of the farmland and is now most serious challenge. By an estimation after 28 years from now half of the irrigated land would be salinized (Yan et al., 2021b). Salinity had negative effects on crop production that makes it most curious global issue (Negrão et al., 2017). In this era of modern agriculture, salinity is most challenging issue to cope with as it inhibited or slow down the growth and development of crop. Salinity caused water stress, ions/minerals imbalance and oxidative stress thus inhibiting the growth and developments in plants (Isayenkov and Maathuis, 2019).

Humans had been facing salinity for centuries. According to a report about 6% of world's land is now salinized (Kamran et al., 2020). About 230 million hectares are irrigated land of which 19.5% is saline affected. Dry agriculture land is nearly 1500 million hectares of which thirty-two million hectares is saline affected (Qadir et al., 2014).

Main reasons for increased salinity are poor irrigation practice, silting, seepage, and rising water table (Naorem et al., 2023). Most of countries of arid and/or semiarid region are disturbed by salinity because of low quality water and malpractices of countinous irrigation (Etikala et al., 2021). Low annual rainfall is also a contributing factor to the spread of salt affected soil (Shahid et al., 2018). Most commonly

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salinity is due to clearing of land and replacement of forests; Irrigation practices using salt rich water (Song et al., 2018).

Growth of plants reduced due to ionic imbalance in saline conditions. It is an immediate reaction of plants to salt stress. It resulted in stomatal closure and inhibition of cell expansion that reduced growth of plants (Safdar et al., 2019). Other way of plants reaction to salinity is to slow down their metabolic processes (Liu et al., 2020). It is not an immediate response, but it takes weeks or months to take place. It's effects includes premature leaf senescence resulting cell death (Zhao et al., 2014). Plants also developed ionic tolerance by activating various signal cascades triggered by the entry of salt in root system. Plants reduced net influx of $Na^+$in root and translocation of $Na^+$ toward shoot (Munns et al., 2020).

MT is estimated to be produced endogenously in chloroplast and mitochondria of plants (Tan and Reiter, 2020; Tiwari et al., 2022). MT is present in variety of plants such as herbs, fruit trees and crops (Li et al., 2019b). One may not be able to estimate the exact level of MT in plants as its synthesis depend on seasonal and circadian rhythms. Most of published data about level of MT in plants are approximate values (Li et al., 2019b). Not only the seasonal and circadian rhythms but also the stress conditions and developmental stages affects the MT level in plants.

MT scavenged ROS under stress, thus MT work as antioxidant in plants. It clears that under stress conditions increased level of ROS resulted in increase of MT content (Zhang et al., 2021; Altaf et al., 2022). Grapevine seedlings when grown under salt stress, MT level in its leaves and root enhanced very greatly and got higher with increasing level of salt stress (Xu et al., 2019). In barley and lupin MT content increased under osmotic stress (Arnao and Hernández-Ruiz, 2009). Similar results were obtained in rice when exposed to higher temperature stress (Byeon and Back, 2014). These findings clearly showed that endogenous MT level increases under abiotic stress in plants (Li et al., 2019b; Altaf et al., 2023).

Melatonin played significant role in developing stress resistance in plants as shown by increased MT level under stress conditions. Exogenous MT application diminished deleterious effects of salt stress. MT is multifunctional and can maintain growth and stress resistance. In *Arabidopsis* exogenous MT treatment promoted growth and maintain liveliness of plants (Ismaél Gatica et al., 2015). MT reduced unwanted ROS, regulated ion balance, modulated antioxidant enzyme, and promoted growth and salt resistance of plants (Abdelhamid et al., 2021; Chen et al., 2018; Farouk and Al-Huqail, 2022). Exogenous MT increased antioxidant enzymes activity (Saqib et al., 2023).

ARG, predecessor for the synthesis of polyamines and nitric oxide is multifunctional and most diverse amino acid. ARG affected nitric oxide, polyamine, and proline synthesis (Liu et al., 2006). Polyamines regulates various biological processes in plants, mainly cellular defense against oxidative damage by inhibition of lipid peroxidation and removal of free radicals (Liu et al., 2015).

It is integral part of protein and serves as predecessor for synthesis of various biomolecules, polyamines, and proline (Chen et al., 2019). ARG, whether endogenous or exogenous, plays an important part in plant stress retaliation, such as drought stress (Nasibi et al., 2011). ARG alleviated inhibitory effects on plant growth due to stress (Khalil et al., 2009). In faba bean ARG application increased root weight, length, chlorophyll contents, carotenoid levels and growth of plants (Nassar et al., 2003). ARG is found to be involved in maintaining quality and antioxidants for post-harvest in mango fruit (Li et al., 2019a; Pakkish and Mohammadrezakhani, 2021; Shu et al., 2020; Zhang et al., 2017).

*C. annuum* locally known as "Red pepper" is member of *Solanaceae* family. *C. annuum* has remarkably high economic and cultural values. It is cultivated throughout the world and frequently used as a fundamental spice in many dishes, in cosmetics and pharmaceuticals (Arimboor et al., 2015). *C. annuum* adds striking colors and delicious tastes to diet. It is rich with vitamin A, B, C, E, and vital minerals. Chili pepper is perennial crop and can survive with ease in temperate and tropical areas. It may be planted at any time of year (Saxena et al., 2016; Sousa et al., 2015). Globally *C. annuum* is cultivated over an area of approximately 3.69 million hectares with total production of 40.30 million tons in 2020 (Dong et al., 2023). In Pakistan, *C. annuum* is cultivated as cash crop more importantly in Sindh province, then Punjab, Baluchistan and KPK. Total area under *C. annuum* crop cultivation is 64,800 hectares with total production of 142,100 tones contributing 1.5% to country's GDP (Arain and Sial, 2022).

MT and ARG have antioxidant activities. MT and ARG are produced by plants in response to various biotic and/or abiotic stresses. Thus, exogenous foliar treatment of MT and ARG might also relieve the stress caused by salinity. Aims of the study includes to investigate the effect of NaCl stress on morphology, physiology and yield of *C. annuum*; to exploit role of MT and ARG in eliminating salt stress and to evaluate role of MT and ARG in modulating Y[II] of *C. annuum* under stress. This study will enable us to find ways for reducing effects of salt stress and clear our understanding about MT and ARG role in enhancing growth and yield of crops.

## 2. Materials and methodology

### 2.1. Experimental design and plant material

The experiment was performed at Botanical Garden of University of Education, Lahore. This experimental work was done to evaluate the role of MT and ARG as antioxidants especially against salt stress, when applied separately as well as in combination on *C. annuum* grown under NaCl stress. Three levels for salt (0, 50, and 100 mM), two levels for MT (0 and 100 μM) and two levels for ARG (0 and 2 mM) were used. It was a completely randomize design experiment with 4 replicates and 48 pots in total. Seeds were taken and sown directly in soil. After that mature seedling at four leaves stage were shifted to plastic pots filled with prewashed sand. Pots were kept under shade to prevent wilting and transpiration during transplant. Then after two weeks when seedlings got settled their roots, thinning was performed and four plants of uniform size at equal distance from each other were maintained in every pot. Hoagland solution (Table 1), was prepared using a composition mentioned in Plant Physiology and Development (Taiz et al., 2015). Hoagland's nutrient solution was used at regular interval to fulfill nutrient requirement of plants (Table 1).

### 2.2. Treatment of melatonin, arginine and salt

The 48 pots used in the experiment were divided into three groups as $S_0$, $S_1$ and $S_2$ based on NaCl stress applied. Each of these group was further divided into four sub-groups as $T_0$, $T_1$, $T_2$ and $T_3$ based on MT and ARG treatments applied (Table 2).

Three different concentrations of NaCl were used in this experiment (0, 50, and 100 mM) (Table 2). NaCl stress was given stepwise and the final concentrations for the treatments were achieved in two

**Table 1**
Recipe of Hoagland nutrient solution.

| Compound | Concentration of stock solution ($gL^{-1}$) | Stock solution taken to make 1 liter for final use (mL) |
|---|---|---|
| Macronutrients | | |
| $KNO_3$ | 101.10 | 6.0 |
| Ca $(NO_3)$ $\cdot 4H_2O$ | 236.16 | 4.0 |
| $NH_4H_2PO_4$ | 115.08 | 2.0 |
| $MgSO_4 \cdot 7H_2O$ | 246.49 | 1.0 |
| Micronutrients | | |
| KCL | 1.864 | 2.0 |
| $H_3BO_3$ | 0.773 | |
| $MnSO_4 \cdot H_2O$ | 0.169 | |
| $ZnSO_4 \cdot 7H_2O$ | 0.288 | |
| $CuSO_4 \cdot 5H_2O$ | 0.062 | |
| $H_2MoO_4$ | 0.040 | |
| NaFeDTPA | 30.0 | 0.5 |

**Table 2**
Formulation of treatments applied.

| Groups | Sub-Groups | Treatments Applied | | |
|---|---|---|---|---|
| | | NaCl | Melatonin | Arginine |
| **$S_0$** | $T_0$ | 0 mM | 0 μM | 0 mM |
| | $T_1$ | 0 mM | 100 μM | 0 mM |
| | $T_2$ | 0 mM | 0 μM | 2 mM |
| | $T_3$ | 0 mM | 100 μM | 2 mM |
| **$S_1$** | $T_0$ | 50 mM | 0 μM | 0 mM |
| | $T_1$ | 50 mM | 100 μM | 0 mM |
| | $T_2$ | 50 mM | 0 μM | 2 mM |
| | $T_3$ | 50 mM | 100 μM | 2 mM |
| **$S_2$** | $T_0$ | 100 mM | 0 μM | 0 mM |
| | $T_1$ | 100 mM | 100 μM | 0 mM |
| | $T_2$ | 100 mM | 0 μM | 2 mM |
| | $T_3$ | 100 mM | 100 μM | 2 mM |

installments. In the first installment half of the NaCl concentrations were applied along with Hoagland nutrient solution. On one day interval second installment of NaCl was applied with Hoagland nutrient solution in which final concentrations for the NaCl treatments (50 and 100 mM) were achieved. MT and ARG were foliar sprayed on the same day when final concentrations for NaCl treatments were achieved. Two distinct levels of concentrations (0 & 100 μM) and (0 & 2 mM) were used in the experiment for both MT and ARG, respectively (Table 2).

### 2.3. Sampling and growth parameters assesment

A week after the treatment, one replicate plant from every treatment was uprooted, washed with tap water, and then dried using soft fabric. Shoots and roots were separated using sharp edge blade. Root and shoot fresh weight, root and shoot length and number of leaves were recorded using weighing balance and measuring scale. After that, samples were dried at 65 °C in oven (Pol-Eko SLN 32 Smart SN32200069) to assess dry weight of root and shoot.

### 2.4. Determination of chlorophyll and carotenoid content

Chlorophyll and carotenoid content were assessed following Arnon (1949) protocols. Fully matured and fresh leaf samples were taken from each replicate. Leaves were weighed to 0.5 g and then grinded using pestle and mortar. The grinded sample was then homogenized in 80% acetone (10 mL). Mixture was then filtered using 42 Whatman filter paper. Following that, samples were kept in fridge at 4 °C for 24 h. Absorbance was read at three wavelengths i.e., 480, 645 and 663 nm using UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.). The following formulas were used to calculate the chlorophyll ***a*** and ***b***.

$$\text{Chl.}a\left(\text{mgg}^{-1}\text{FW}\right) = [12.7(\text{OD}_{663}) - -2.69(\text{OD}_{645})]\text{xV} / 1000\text{xW}$$

$$\text{Chl.}b(\text{mgg}^{-1}\text{FW}) = [22.9(\text{OD}_{645}) - -4.68(\text{OD}_{663})]\text{xV} / 1000\text{xW}$$

where, $V$ = Volume of extract (mL); $W$ = Weight of fresh leaf; OD = Optical density.

Carotenoid contents were measured using following formula as mentioned by Lichtenthaler (1987).

$$\text{Carotenoids}\left(\text{mgg}^{-1}\text{FW}\right) = (1000\text{xOD}_{480}) - -(1.9\text{xChl}a - -63.14\text{xChl}b)/214$$

### 2.5. Determining relative water content (RWC) and relative membrane permeability (RMP)

From each replicate leaves of same size were collected, weighed, and quickly floated over distilled water. For three hours, these leaves were immersed in distilled water at 25–26 °C. After three hours leaves became turgid, and their weight was noted. After drying leaves by keeping them in oven at 80 °C for 24 h their dry weights were measured. RWC of the samples was calculated using formula as mentioned by (Jones and Turner, 1978).

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

where: FW = Fresh Weight, DW = Dry Weight, TW = Turgid Weight

For RMP, fully matured and young leaves of uniform size were collected from each replicate. Leaves were chopped and added to tubes having deionized water (20 mL). Mixture was vortex for 5 s and electrical conductivity ($EC_0$) was noted using EC meter (Milwaukee MW805 MAX). The tubes were stored in the refrigerator at 4 °C for 24 h and then $EC_1$ was recorded. Same samples were incubated in autoclave (SH-AC-60 M) at 120 °C for 20 min to take readings for $EC_2$. The percentage RMP was determined using the formula as mentioned (Yang et al., 1996).

$$\text{RMP}(\%) = [(\text{EC}_1 - \text{EC}_0) / (\text{EC}_2 - \text{EC}_0)]\text{x}100$$

### 2.6. Hydrogen peroxide ($H_2O_2$) determination

It was estimated using protocol as mentioned (Velikova et al., 2000). Fresh leaves from each replicate were collected, weighed (0.5 g) and then mixed thoroughly with trichloroacetic acid (0.1%, 5 mL) using pre-chilled pestle and mortar. The extracts were transferred to conical tubes. The centrifugation of the extracts was performed at 12,000 g (15 min and 4 °C) using centrifuge machine (Hermle Z 326 K). Potassium phosphate buffer (0.5 mL, pH 7), potassium iodide (1 mL) and supernatant (0.5 mL) obtained on centrifugation, were taken in test tubes. The mixture was vortexed and absorbance at 390 nm was checked with UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.).

### 2.7. Determination of malondialdehyde (MDA) content

It was determined following the protocol as mentioned by Cakmak and Horst (1991) with few changes. Fresh leaves (0.5 g) were plucked from every replicate and homogenized in 1.0% trichloroacetic acid (3 mL) at 4 °C. After that, homogenate was transferred to conical tubes, it was centrifuged at 20,000 g for 15 min. Following centrifugation, 0.5 mL of the filtrate was extracted in test tubes containing a 0.5% thiobarbituric acid (3 mL), made in 20% trichloroacetic acid. All the samples were then heated at 95 °C for 50 min using shaking water bath (Glas-Col model HSW-1/06). Reaction was terminated by cooling immediately using ice water bath. All samples were centrifuged once more at 10,000 g for 10 min and then absorbance on 532 and 600 nm was measured with a UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.). MDA level (nmol) was calculated for every sample using following formula.

$$\text{MDAlevel}(\text{nmol}) = \left[(\text{A}_{532\text{nm}} - -\text{A}_{600\text{nm}})/1.56\text{x}10^5\right]\text{xV} / \text{Wx}1000000$$

### 2.8. Determining total soluble protein

Fresh leaf samples from each replicate were collected, weighed (0.5 g), and blended in 10 mL of 50 mM pre-cooled phosphate buffer (pH 7.8). The extracts were collected in conical flask and then centrifuged at 6,000 g for 20 min at 4 °C. Supernatant was separated from the residue and stored in freezer (for later use). Protein level in samples was measured using protocol as mentioned by Bradford (1976). Bradford solution was made by dissolving 100 mg of Coomassie Brilliant Blue in 50 mL of ethanol (95%), then adding the solution to 100 mL of phosphoric acid (85%). Distilled water was added to make total volume 1 L. Bradford solution (5 mL) was added to 0.1 mL of already prepared leaf extracts (frozen in deep freezer ~−20 °C). Optical density was measured at wavelength of 595 nm using UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.).

### 2.9. Determination of catalase (CAT) and peroxidase (POD) activity

Catalase reaction solution was prepared by adding these components; 1 mL of 50 mM phosphate buffer (pH 7.0); 1.9 mL of 5.9 mM $H_2O_2$ and 0.1 mL of sample extract (discussed in Section 2.9). Absorbance at wavelength of 240 nm was measured for every 30 s in the time interval of 120 s using UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.) (Chance and Maehly, 1955). Similarly, POD reaction solution was prepared by mixing; 0.7 mL of 50 mM phosphate buffer (pH 5.0); 0.6 mL of 20 mM guaiacol; 0.6 mL of 40 mM $H_2O_2$ and 0.1 mL of sample extract (discussed in Section 2.9). Absorbance was measured at wavelength 470 nm for every 30 s in time interval of 150 s using UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.) (Nickel and Cunningham, 1969).

### 2.10. Ascorbate peroxidase (APX) and superoxide dismutase (SOD) activity analysis

For each replicate, the APX reaction mixture (3 mL) was made, which included 2.7 mL of 50 mM phosphate buffer, 0.1 mL of 7.5 mM ascorbic acid, 0.1 mL of 300 mM $H_2O_2$, and 0.1 mL sample extract (discussed in Section 2.9). APX reaction mixture was then run in UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.) and absorbance were measured at wavelength of 290 nm for every 30 s in time interval of 60 s (Nakano and Asada, 1981). For SOD, reaction mixture was prepared by mixing 130 mM methionine (0.3 mL); 50 μM nitro blue tetrazolium (0.3 mL); 100 μM EDTA-$Na_2$ (0.3 mL) and 20 μM riboflavin (0.3 mL). Sample extract 0.05 mL was added to reaction mixture and then subjected to 4000 lx light for twenty minutes. After that samples were checked for absorbance at 560 nm using UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.) (Charles and Irwin, 1971; Yan et al., 2021a).

### 2.11. Total phenolics determination

Dried leaf samples (0.50 g) were weighed and placed in centrifuge tubes. After homogenizing the samples in 10 mL of 80% aqueous acetone, for one minute, mixture was centrifuged at 4000 g (15 min, 4 °C). Supernatant was removed and dried solid residue was taken for further proceedings. Dried solid residue was mixed in 10 mL of methanol. Folin-Ciocalteu protocol (Lamuela-Raventós, 2018) was followed to determined amount of total phenolics in prepared samples. Prepared samples (2 mL from each replicate) were taken in tubes, 1 mL Folin-Ciocalteu reagent and 0.8 mL sodium carbonate (7.5%) were mixed vigorously and left to stand for 30 min. Absorbances at 765 nm were obtained using UV/VIS spectrophotometer (Shanghai Metash Instruments Co., Ltd.). Total phenolic content was calculated as gallic acid equivalents per gram dry material.

### 2.12. Measuring quantum yield of PS II

Quantum yield of PS II was measured using *Fluor Pen* FP-100 (Photon system Instruments, Czech Republic). Readings were taken after one weak of treatment, on daytime in plentiful sunlight. Mature third leaf from each replicate was placed between clips of device and florescence was measured at 3000 μmol $m^{-2}$ $s^{-1}$ efflux of light (Ayyaz et al., 2020).

### 2.13. Inorganic ions determination

Dried shoot and root samples were taken and grinded well, using pestle and mortar. $H_2SO_4$ was used to digest 0.1 g of shoot and root samples, separately. Samples were taken in test tubes and 2 mL of $H_2SO_4$ was added in these tubes. After 24 h, samples were heated on hot plate and $H_2O_2$ was added drop by drop until mixture was colorless. After that, distilled water was added. Final volume was raised to 50 mL. After filtering this mixture readings for $Na^+$, $K^+$ and $Ca^{++}$ were taken using flame-photometer (Sherwood model 360). For $Cl^-$ ions estimation Mohr's titration method was used (Sezey and Perihan, 2019).

### 2.14. Yield parameters

On ripening of fruits, following yield parameters were recorded; fresh weight (g) of fruits; dry weight (g) of fruits; weight (g) of hundred seeds and number of fruits per plant.

### 2.15. Statistical analysis

CoStat version 6.303, CoHort Software, statistics program was used to perform ANOVA of all parameters studied during the research work. LSD mean compare tests were performed at 0.05 significance level. MS Excel 365 was used to make graphs. R Studio software was used for Pearson correlation and principle component analysis.

## 3. Results

### 3.1. Effects of MT and ARG treatment on morphological characters of C. annuum under salt stress

Shoot fresh weight of *C. annuum* plants was decreased by 12% and 26% under 50 and 100 mM sodium chloride stress, respectively. Shoot fresh weight of control plants was increased by 69%, 76% and 49% with foliar spray of MT, ARG and their combined treatment, respectively, as compared to untreated control. Similar to this, foliar sprays of MT, ARG, and their combination treatment boosted shoot fresh weight in plants under 50 mM NaCl stress by 62%, 76%, and 46%, respectively, and in plants under 100 mM NaCl salt stress by 67%, 74%, and 36%, respectively (Fig. 1-A).

With 50 and 100 mM NaCl stress, the shoot dry weight of *C. annuum* was reduced by 25% and 27% as compared to control, respectively (Fig. 1-B). In comparison to the untreated control, foliar sprays of MT, ARG, and their combination treatment raised shoot dry weight in control plants by 66%, 55%, and 60%, respectively. Similarly, foliar sprays of MT, ARG, and their combination treatment enhanced shoot dry weight by 77%, 99%, and 68%, respectively, in plants subjected to 50 mM NaCl salt stress and by 30%, 51%, and 34%, respectively, in plants subjected to 100 mM NaCl stress.

Root fresh weight of *C. annuum* plants was lowered by 6% and 11% under 50 and 100 mM sodium chloride stress, respectively. Root fresh weight was increased by 56%, 47%, and 50% with foliar spray of MT, ARG and their combined treatment, respectively, as compared to untreated control. Similarly, foliar spray of MT, ARG, and their combined treatment increased root fresh weight by 41%, 80%, and 57%, respectively, in plants under 50 mM NaCl salt stress and by 19%, 63%, and 17%, respectively, in plants under 100 mM NaCl stress (Fig. 1-C).

Under 50 and 100 mM NaCl stress, root dry weight was reduced by 14% and 35%, respectively, compared to the control (Fig. 1-D). When compared to the untreated control, foliar sprays of MT, ARG, and their combination treatment improved root dry weight by 31%, 60%, and 68%, respectively. Similarly, foliar sprays of MT, ARG, and their combination treatment increased root dry weight by 62%, 103%, and 62%, respectively, in plants exposed to 50 mM NaCl salt stress and by 44%, 73%, and 38%, respectively, in plants exposed to 100 mM NaCl salt stress.

Shoot length (Fig. 1-E) of *C. annuum* plants was reduced by 12% and 26% under 50 and 100 mM sodium chloride stress, respectively, in relation to control. Shoot length was increased by 69%, 76%, and 49% with foliar spray of MT, ARG and their combined treatment, respectively, as compared to untreated control. Similarly, foliar spray of MT, ARG, and their combined treatment increased shoot length by 62%, 76%, and 46%, respectively, in plants under 50 mM NaCl salt stress and by 67%, 74%, and 36%, respectively, in plants under 100 mM NaCl stress.

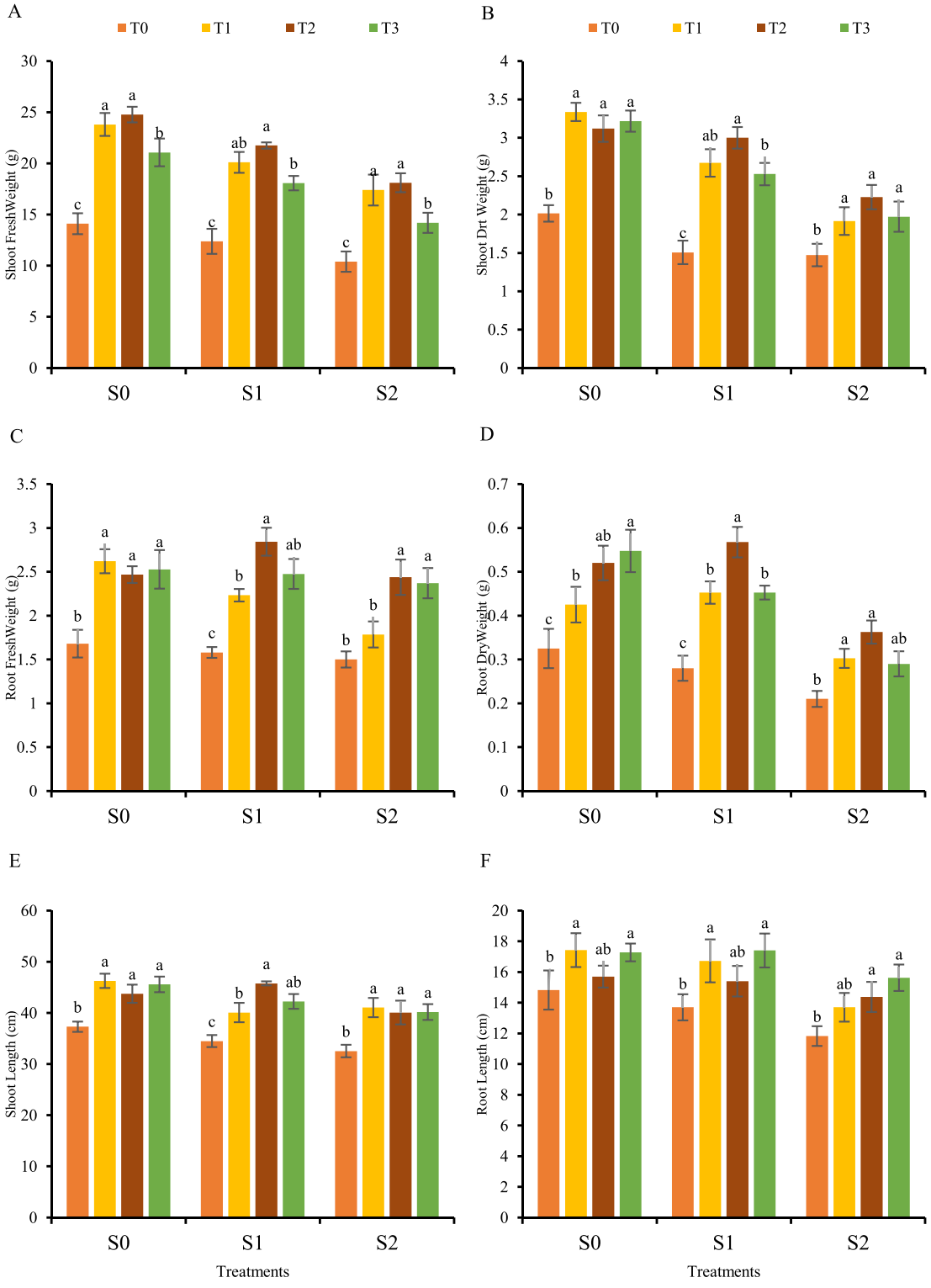


**Fig. 1.** Morphological parameters of *C. annuum* under salt stress and foliar spray of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 μM MT, T2 = 2 mM ARG, T3 = 100 μM MT + 2 mM ARG). *A* = Shoot Fresh weight, *B* = Shoot Dry weight, C = Root Fresh weight, *D* = Root Dry weight, *E* = Shoot Length, *F* = Root Length. Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

Root length (Fig. 1-F) of *C. annuum* plants was decreased by 8% and 20% under 50 and 100 mM sodium chloride stress, respectively, in comparison to control. Root length was increased by 18%, 6%, and 17% with foliar spray of MT, ARG and their combined treatment, respectively, as compared to untreated control. Similarly, foliar spray of MT, ARG, and their combined treatment increased root length by 22%, 12%, and 27%, respectively, in plants under 50 mM NaCl salt stress and by 16%, 22%, and 32%, respectively, in plants under 100 mM NaCl stress in relation to untreated stress plants.

Number of leaves (Fig. 2) of *C. annuum* plants was lowered by 17% and 19% with 50 and 100 mM sodium chloride stress, respectively, in comparison to control. Number of leaves were increased by 65%, 50%, and 50% with foliar spray of MT, ARG and their combined treatment, respectively, as compared to untreated control. Similarly, foliar spray of MT, ARG, and their combined treatment increased number of leaves by 65%, 66%, and 57%, respectively, in plants under 50 mM NaCl salt stress and by 65%, 49%, and 42%, respectively, in plants under 100 mM NaCl stress in relation to untreated stress plants.

### 3.2. Effects of MT and ARG treatment on Chlorophyll a, b, a/b ratio and Carotenoid contents of C. annuum under salt stress

Chlorophyll *a* content in *C. annuum* did not change much under NaCl salt stress. Foliar spray of MT, ARG and their combined treatment too had no impressive impact on chlorophyll *a* content in *C. annuum*. Chlorophyll *a* content in *C. annuum* slightly decreased by foliar spray of MT, ARG and their combined treatment as compared to untreated plants. These results are also demonstrated in Fig. 2-A.

Salt stress did not change chlorophyll *b* (as shown in Fig. 2-B) content in *C. annuum* plants. Foliar spray of MT and combined treatment of MT and ARG increased chlorophyll *b* content by 15% and 19%, respectively in control plants. In contrast, foliar spray of ARG decreased chlorophyll *b* content by 12% in control plants in relation to untreated control. Under 50 mM NaCl salt stress foliar spray of MT increased chlorophyll *b* content by 10%, while both ARG and combined treatment of MT and ARG decreased chlorophyll *b* content by 5% as compared to untreated stressed plants. Under 100 mM NaCl salt stress foliar spray of MT and ARG decreased chlorophyll *b* content by 10% and 4%, respectively, while combined treatment of MT and ARG increased chlorophyll *b* content by 10% as compared to untreated stress plants.

Carotenoid content (Fig. 2-C) was reduced by 8% and 14% with 50 and 100 mM sodium chloride stress, respectively, in comparison to control. Foliar application of MT, ARG and their combined treatment slightly decreased carotenoid contents in both control and stress plants as compared untreated plants. Maximum carotenoid content was present in control plants. Fig. 5-D illustrate ratio between chl *a* and *b* contents under control, 50 and 100 mM NaCl salt stress with and without foliar treatment of MT and ARG.

### 3.3. Effects of MT and ARG treatment on MDA, H2O2, RMP and RWC of C. annuum under salt stress

Malondialdehyde (MDA) level in *C. annuum* plants (Fig. 4-A) was increased by 47% and 25% under 50 and 100 mM NaCl stress in relation to control. Foliar spray of MT, ARG, and their combined treatment increased MDA level by 6%, 3%, and 18%, respectively in control plants as compared to non-sprayed plants. In contrast, foliar spray of MT, ARG and their combined treatment decreased MDA level by 14%, 17%, and 13%, respectively, in plants under 50 mM NaCl salt stress and by 17%, 16%, and 14%, respectively, in plants with 100 mM NaCl stress.

In comparison to the control, hydrogen peroxide ($H_2O_2$) levels in *C. annuum* plants (Fig. 4-B) rose by 10% and 23% under 50 and 100 mM sodium chloride stress, respectively. Foliar spray of MT, ARG and their combined treatment had negligible effect on $H_2O_2$ level in control plants as compared to non-sprayed plants. In contrast, foliar sprays of MT, ARG, and their combination treatment reduced $H_2O_2$ levels by 12%, 12%, and 10% in plants under 50 mM NaCl salt stress, and by 21%, 20%, and 19% in plants under 100 mM NaCl stress, respectively, compared to non-sprayed under stress plants.

Relative membrane permeability (RMP) in *C. annuum* plants (Fig. 4-C) was increased by 16% and 32% under 50 and 100 mM sodium chloride stress, respectively, in relation to control. Foliar spray of MT, ARG and their combined treatment decreased RMP by 6%, 5%, and 10%, respectively, in control plants as compared to non-sprayed plants. Similarly, foliar spray of MT, ARG and their combined treatment decreased RMP by 26%, 32%, and 21% in plants under 50 mM NaCl stress and by 23%, 29%, and 36% in plants under 100 mM NaCl salt stress, respectively, compared to untreated stressed plants.

Relative water content (RWC) of the leaves (Fig. 4-D) was decreased significantly by 10% under stress of 100 mM NaCl, compared to control.

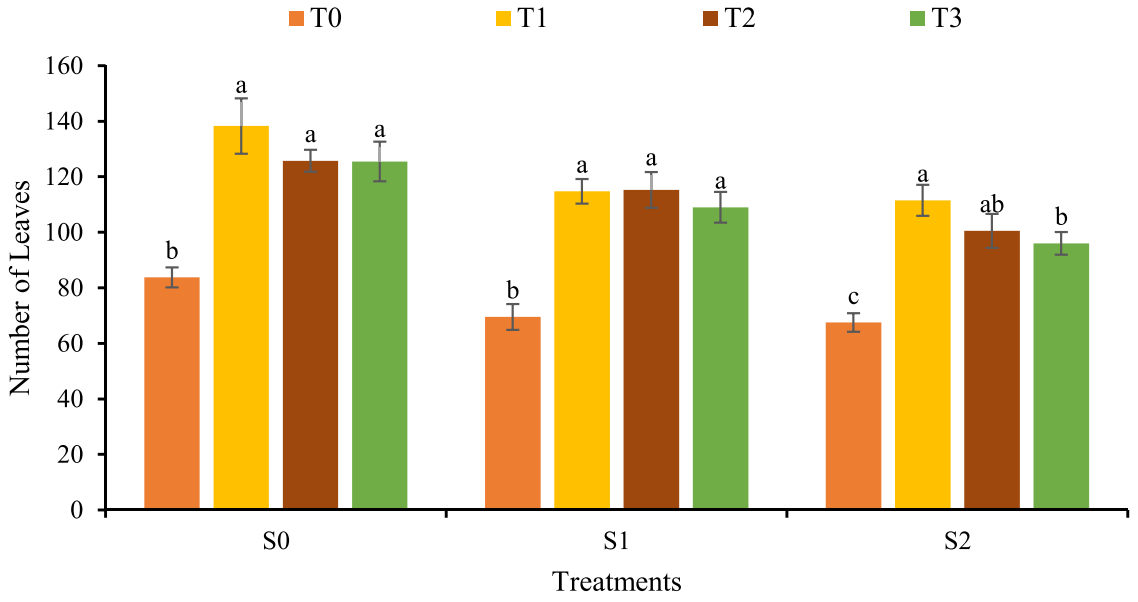


**Fig. 2.** Number of Leaves of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 μM MT, T2 = 2 mM ARG, T3 = 100 μM MT + 2 mM ARG). Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

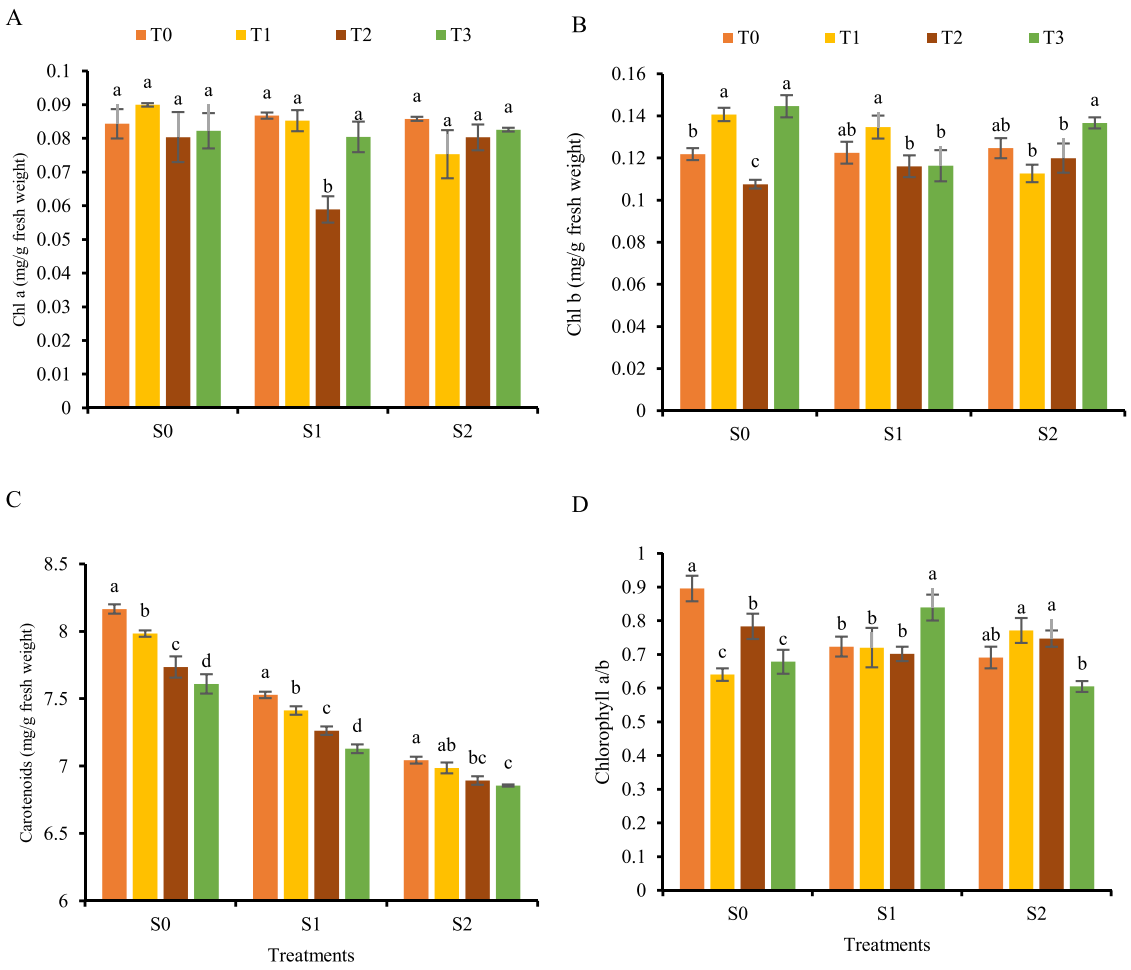


**Fig. 3.** Physiological parameters of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 μM MT, T2 = 2 mM ARG, T3 = 100 μM MT + 2 mM ARG). Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

Foliar treatment of MT 100 μM, ARG 2 mM and their combined treatment restores the decreased level of RWC. In comparison to non-sprayed stress plants, foliar applications of MT 100 μM, ARG 2 mM, and their combination treatment improved RWC by 12%, 14%, and 21% under 50 mM NaCl, and by 17%, 27%, and 15% under 100 mM NaCl, respectively. With increasing levels of salt stress, foliar applications of MT and ARG significantly improve RWC.

### *3.4. Effects of MT and ARG treatment on total soluble protein content in C. annuum under salt stress*

Total soluble protein in *C. annuum* plants (shown in Fig. 5) were increased by 13% and 26% under 50 and 100 mM sodium chloride stress in relation to control, respectively. Foliar spray of MT, ARG, and their combined treatment further increased total soluble protein in plants under stress condition in comparison to untreated stress plants. Exogenous MT, ARG and their combined treatment increased total soluble protein by 7%, 17%, and 17%, respectively, in plants under 50 mM NaCl salt stress and by 10%, 19%, and 14%, respectively, in plants under 100 mM NaCl stress in relation to untreated plants.

### *3.5. Effects of MT and ARG treatment on antioxidant enzymes (CAT, POD, SOD, APX) activity of C. annuum under salt stress*

Salt stress increased antioxidant enzymes activities. Foliar application of MT, ARG, and their combined treatment further enhanced activity of antioxidant enzymes. Under 50 and 100 mM sodium chloride stress, catalase enzyme activity (Fig. 6-A) increased by 8%, and 16%, respectively. Foliar spray of MT, ARG, and their combine treatment improved catalase enzyme activity by 14%, 22%, and 21%, respectively, under 50 mM NaCl and by 18%, 29%, and 24%, respectively, under 100 mM NaCl stress, compared to untreated stressed plants. Catalase levels were highest in plants conditioned to 100 mM NaCl salt stress and treated with ARG foliar spray.

In comparison to the control, salt stress treatments raised POD enzyme levels (as shown in Fig. 6-B) by 35% and 56% under 50 mM and 100 mM NaCl salt stress, respectively. Foliar application of MT, ARG and their combined treatment further enhanced POD enzymes contents under salt stress conditions and control as well as compared to untreated stressed and control plants.

Under 50 mM and 100 mM sodium chloride stress, the levels of ascorbate peroxidase (APX) enzymes were reduced by 13% and 17%, respectively, in comparison to the control. Exogenous MT, ARG, and their combined treatment increased APX enzyme contents by 8%, 6%, and 5%, respectively, under 50 mM NaCl salt stress and by 9%, 8%, and 7%, respectively, under 100 mM NaCl salt stress. These results are shown in Fig. 6-C.

In relation to the control, superoxide dismutase (SOD) levels (Fig. 6-D) in *C. annuum* plants rose by 6% and 20% under 50 and 100 mM sodium chloride stress, respectively. Exogenous MT, ARG, and their combination treatment increase SOD contents by 28%, 24%, and 28%, respectively, under 50 mM NaCl stress, whereas under 100 mM NaCl stress, SOD contents rise by 29%, 36%, and 27%, respectively, in contrast to untreated plants.

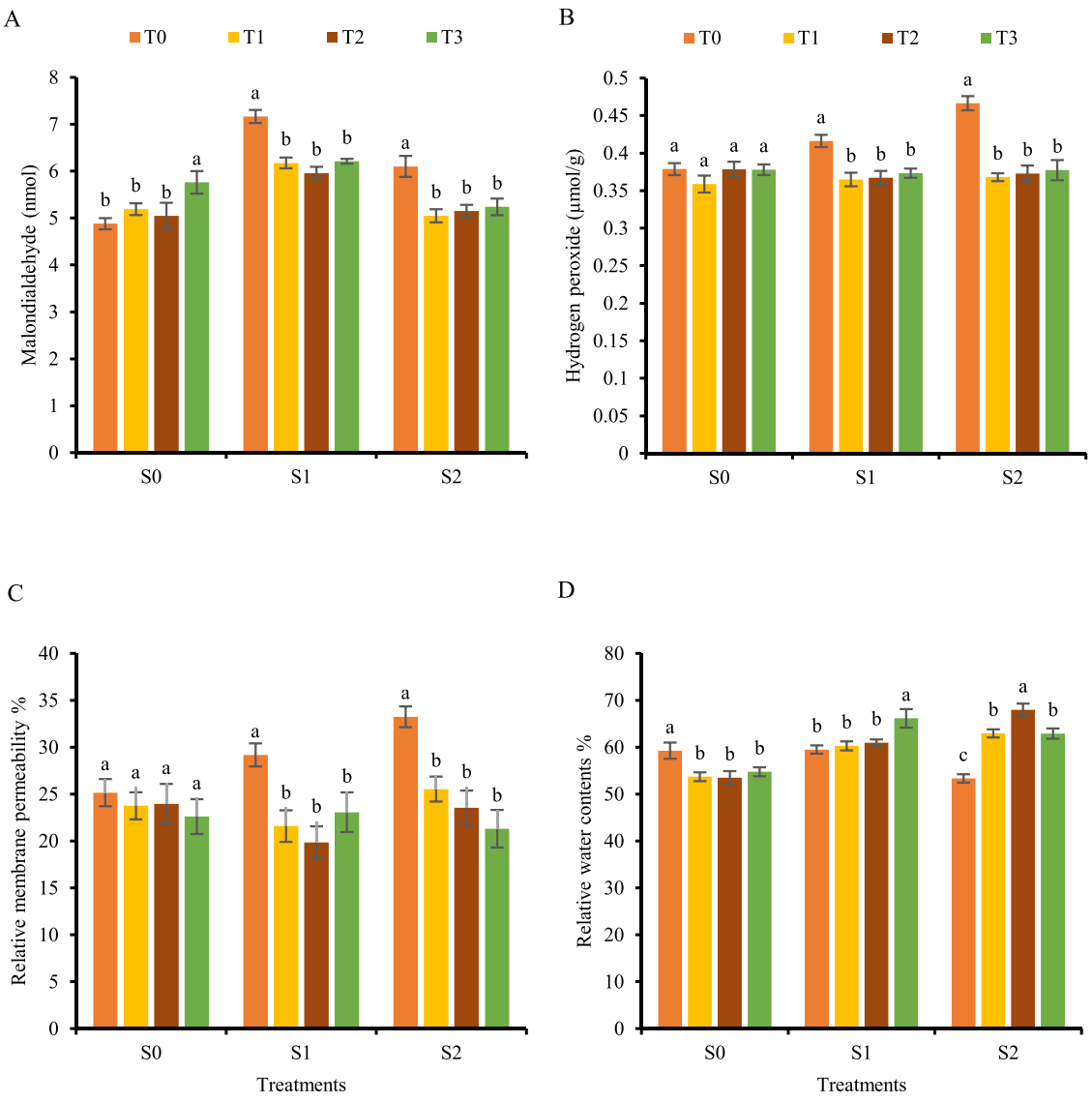


**Fig. 4.** Malondialdehyde (MDA), Hydrogen peroxide ($H_2O_2$), RMP and RWC of *C. annuum* under NaCl stress and foliar spray of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 µM MT, T2 = 2 mM ARG, T3 = 100 µM MT + 2 mM ARG). *A* = MDA, *B* = $H_2O_2$, C = RMP, *D* = RWC. Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

### *3.6. Effects of MT and ARG treatment on total phenolics content in C. annuum under salt stress*

The total phenolic content of *C. annuum* plants (as shown in Fig. 7) was raised by salt stress. Under 50 mM and 100 mM sodium chloride stress, total phenolics increased by 18% and 29%, respectively. Exogenous MT, ARG and their combined treatment increased total phenolics in *C. annuum* by 32%, 35%, and 31% with 50 mM NaCl stress and 20%, 25%, and 31% 100 mM NaCl stress, respectively, in comparison to untreated stress plants.

### *3.7. Effects of MT and ARG treatment on quantum yield of PS II (Y[II]) in C. annuum under salt stress*

In respect to the control, the Y[II] of *C. annuum* plants (as illustrated in Fig. 8) was reduced by 5% and 15% under 50 and 100 mM sodium chloride stress, respectively. Y[II] was increased in by 10%, 6%, and 5% in non-stressed plants (control) with foliar spray of MT, ARG, and their combined treatment, respectively. Similarly, foliar spray of MT, ARG, and their combined treatment increased Y[II] by 20%, 16%, and 16%, respectively, in plants under 50 mM NaCl salt stress and by 21%, 17%, and 21%, respectively, in plants subjected to 100 mM NaCl salt stress.

### *3.8. Effects of MT and ARG treatment on $Na^+$, $K^+$, $Ca^{++}$ and Clions in shoot of C. annuum under salt stress*

#### *3.8.1. $Na^+$, $K^+$, $Ca^{++}$ and Clions in shoot*

$Na^+$ions in shoot of *C. annuum* plants (Fig. 9-A) increased by 16% and 35% under 50 and 100 mM NaCl stress. Foliar sprays of MT, ARG, and their combined treatment lowered $Na^+$ ions by 13%, 9%, and 24%, respectively, in shoots of plants under 50 mM NaCl stress, and by 27%, 19%, and 38%, respectively, in shoots of plants under 100 mM NaCl salt stress.

$K^+$ ions in shoot of *C. annuum* plants (Fig. 9-B) were lowered by 17% and 31% under 50 and 100 mM NaCl stress, respectively. $K^+$ions in shoot of control plants were increased by 10%, 8%, and 8% with foliar spray of MT, ARG and their combined treatment, respectively, as compared to untreated control. Similarly, foliar spray of MT, ARG, and their combined treatment increased $K^+$ions by 13%, 12%, and 30%, respectively, in shoot of plants under 50 mM NaCl salt stress and by 23%, 30%, and 32%, respectively, in shoot of plants with stress of 100 mM NaCl in relation to non-sprayed stress plants.

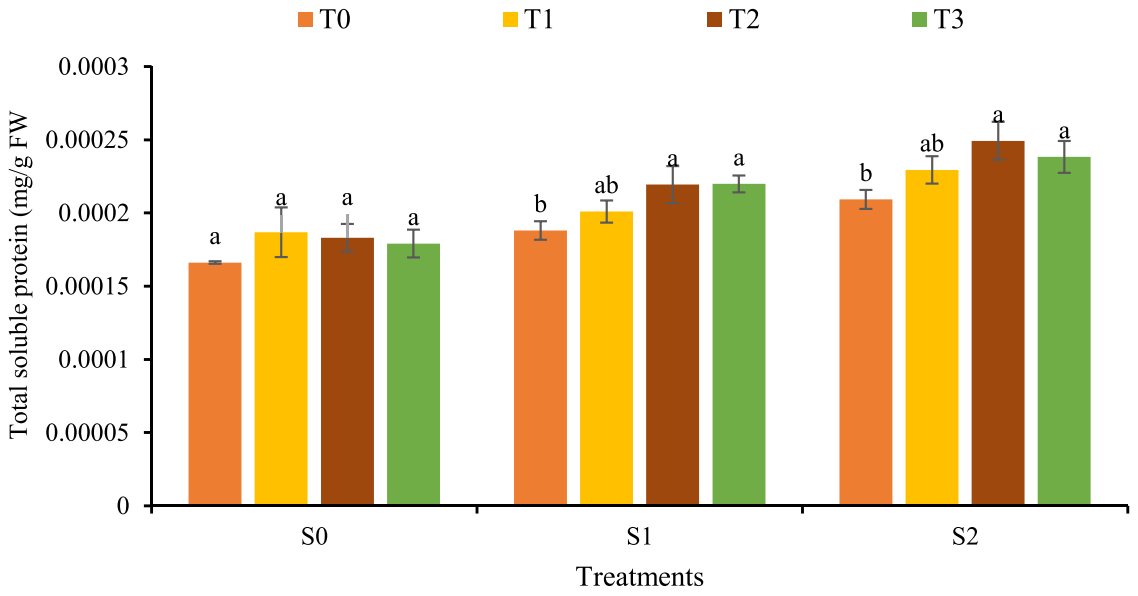


**Fig. 5.** Total soluble protein of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 µM MT, T2 = 2 mM ARG, T3 = 100 µM MT + 2 mM ARG). Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

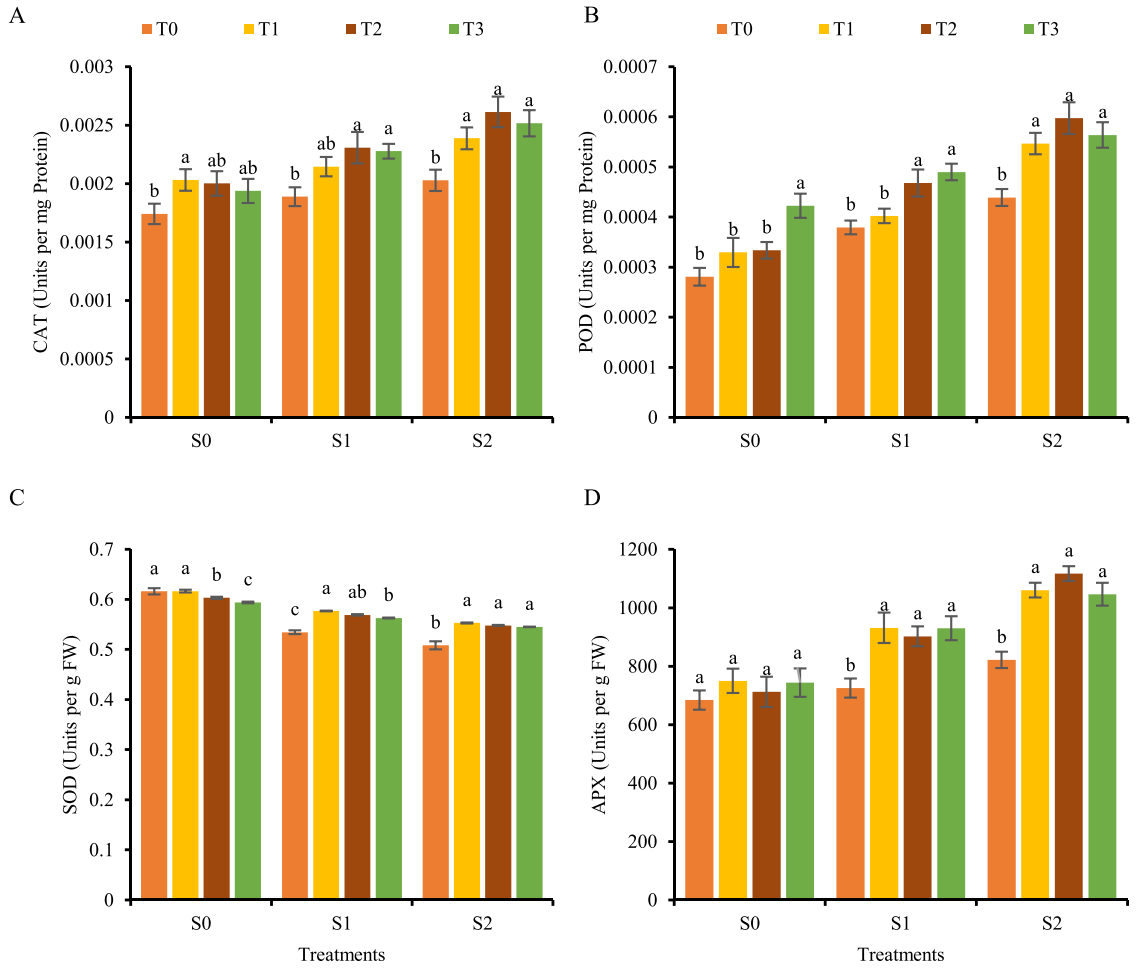


**Fig. 6.** Antioxidant enzymes of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 µM MT, T2 = 2 mM ARG, T3 = 100 µM MT + 2 mM ARG). Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

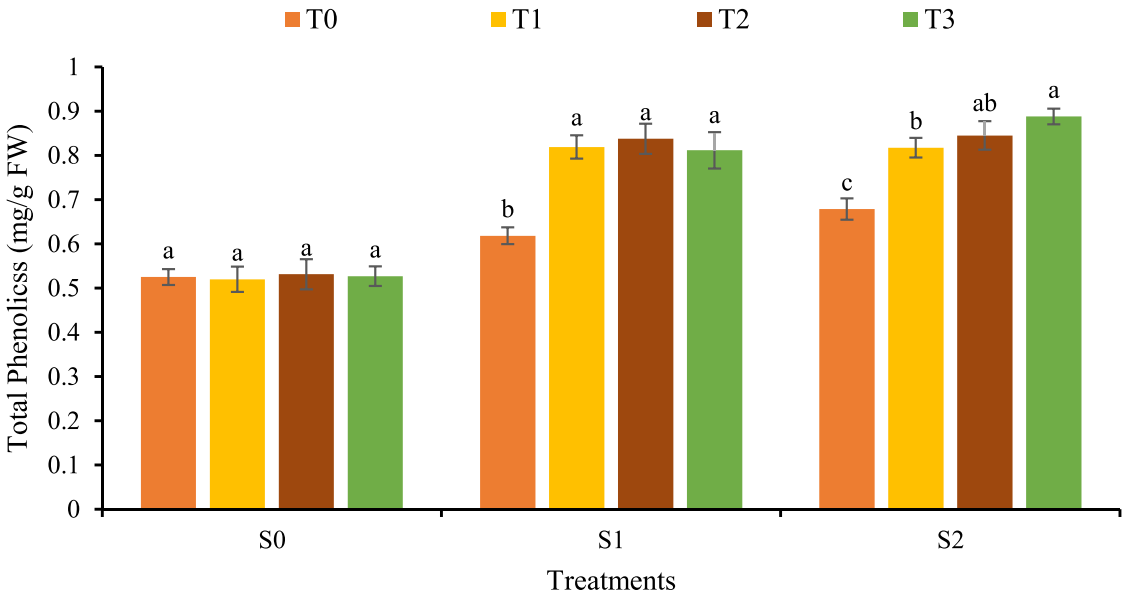


**Fig. 7.** Total phenolics of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 μM MT, T2 = 2 mM ARG, T3 = 100 μM MT + 2 mM ARG). Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

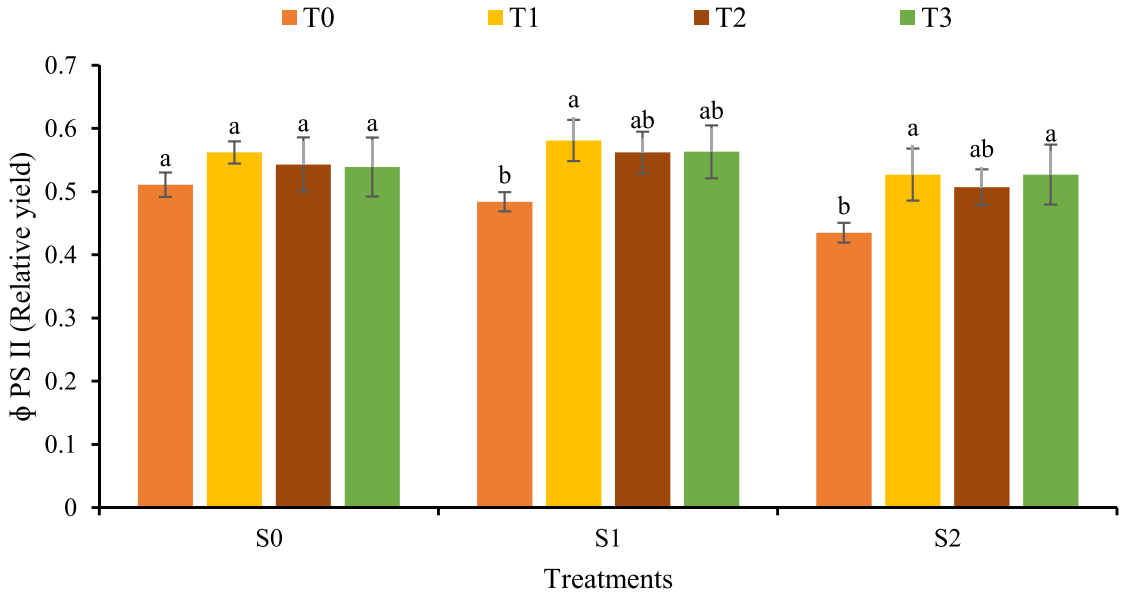


**Fig. 8.** Quantum yield of photosystem II of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 μM MT, T2 = 2 mM ARG, T3 = 100 μM MT + 2 mM ARG). Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

Salt stress decreased $Ca^{++}$ ions (Fig. 9-C) by 13% and 20% with 50 mM and 100 mM sodium chloride treatment, respectively, in shoot of *C. annuum* plants as compared to control. Foliar spray of MT, ARG, and their combined treatment increased $Ca^{++}$ ions by 17%, 9%, and 19%, respectively, in shoot of control plants in respect to non-sprayed control plants. Under 50 mM NaCl salt stress foliar spray of MT, ARG, and their combined treatment increased $Ca^{++}$ ions in shoot of plants by 47%, 38%, and 50%, respectively, in respect to untreated stress plants. Under 100 mM NaCl salt stress foliar spray of MT, ARG, and their combined treatment increased $Ca^{++}$ ions in shoot of plants by 49%, 44%, and 50%, respectively, as compared to untreated stress plants.

$Cl^{-}$ ions in shoot of *C. annuum* plants (Fig. 9-D) increased by 15% and 30% with 50 and 100 mM NaCl stress, respectively, compared to control. Foliar sprays of MT, ARG, and their combined treatment reduced $Cl^{-}$ ions by 33%, 13%, and 29%, respectively, in shoots of plants under 50 mM NaCl salt stress, and by 26%, 19%, and 29%, respectively, in shoots of plants with 100 mM NaCl salt stress, compared to non-sprayed under stress plants.

### *3.9. Effects of MT and ARG treatment on $Na^{+}$, $K^{+}$, $Ca^{++}$ and $Cl^{-}$ ions in root of C. annuum under salt stress*

In comparison to the control, $Na^{+}$ ions in the root of *C. annuum* plants (Fig. 10-A) rose by 16% and 35% under 50 and 100 mM NaCl stress, respectively. Foliar sprays of MT, ARG, and their combined treatment decreased $Na^{+}$ ions by 13%, 9%, and 24%, respectively, in the roots of plants under 50 mM NaCl salt stress, and by 27%, 19%, and 38%, respectively, in the roots of plants under 100 mM NaCl salt stress,

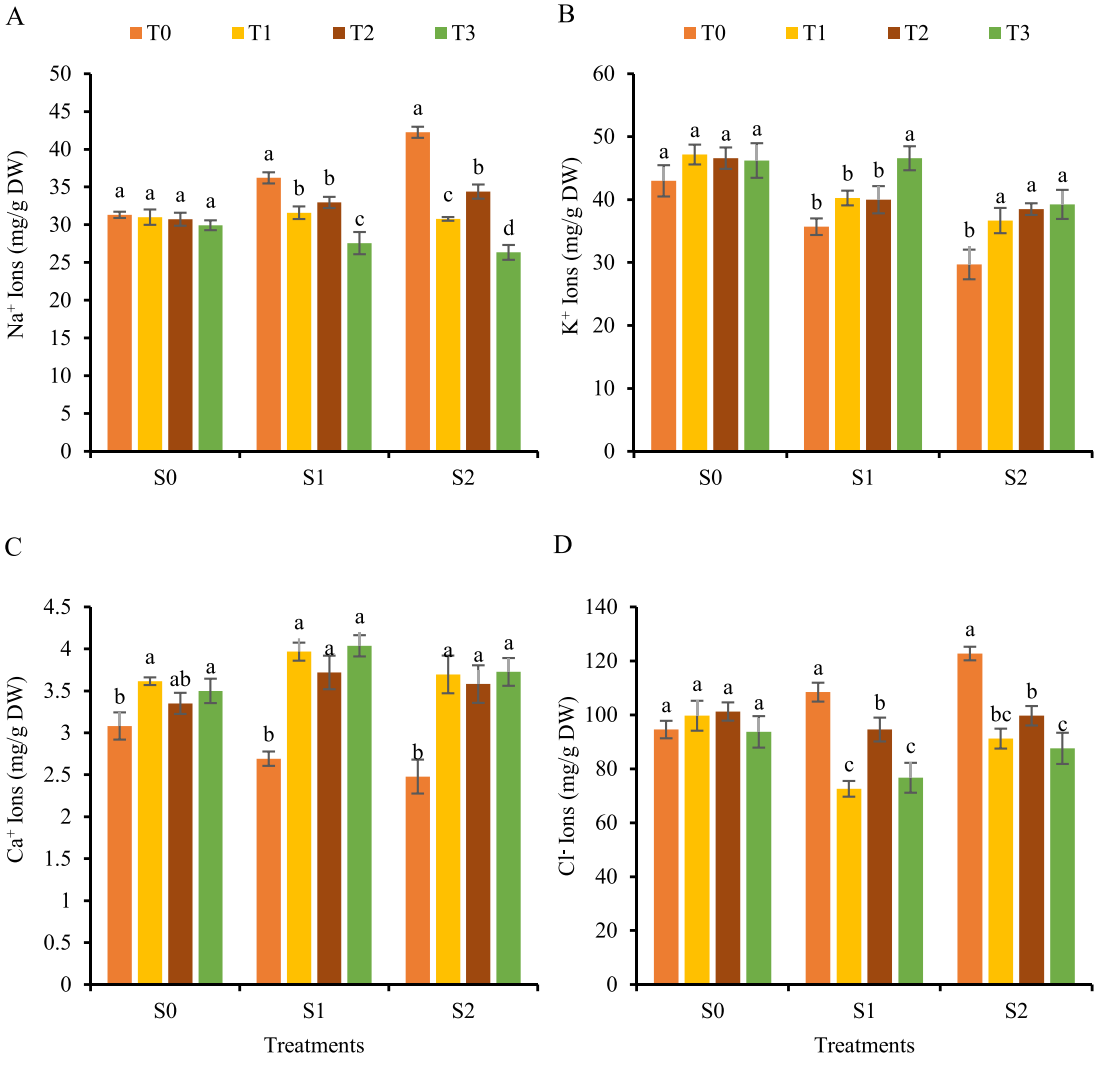


**Fig. 9.** $Na^+$, $K^+$, $Ca^{++}$ and $Cl^-$ ions in shoot of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 µM MT, T2 = 2 mM ARG, T3 = 100 µM MT + 2 mM ARG). *A* = $Na^+$, *B* = $K^+$, C = $Ca^{++}$ and D = $Cl^-$. Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

respectively, compared to non-sprayed under stress plants.

Compared to the control, under 50 and 100 mM NaCl stress, $K^+$ ions in the root of *C. annuum* plants (Fig. 10-B) were reduced by 17% and 31%, respectively. $K^+$ions in root of control plants increased by 10%, 8%, and 8%, respectively, with foliar spray of MT, ARG and their combined treatment as compared to untreated control, respectively. Similarly, foliar spray of MT, ARG, and their combined treatment increased $K^+$ions by 13%, 12%, and 30%, respectively, in root of plants under 50 mM NaCl salt stress and by 23%, 30%, and 32%, respectively, in root of plants under 100 mM NaCl stress, compared to non-sprayed stress plants.

In the root of *C. annuum* plants, salt stress reduced $Ca^{++}$ ions (Fig. 10-C) by 13% and 20%, respectively, under 50 mM and 100 mM NaCl stress. Foliar sprays of MT, ARG, and their combined treatment raised $Ca^{++}$ ions in the roots of control plants by 17%, 9%, and 19%, respectively, compared to non-sprayed control plants. Under 50 mM NaCl stress, foliar sprays of MT, ARG, and their combination raised $Ca^{++}$ ions in plant roots by 47%, 38%, and 50%, respectively, compared to untreated stress plants, while under 100 mM NaCl stress, these treatments raised $Ca^{++}$ ions in plant roots by 49%, 44%, and 50%, respectively, compared to untreated stress plants.

In comparison to the control, $Cl^-$ ions in the root of *C. annuum* plants (Fig. 10-D) rose by 15% and 30% under 50 and 100 mM NaCl stress, respectively. Foliar spray of MT, ARG and their combined treatment decreased $Cl^-$ ions by 33%, 13%, and 29%, respectively, in root of plants under 50 mM NaCl salt stress, while with 100 mM NaCl reduced by 26%, 19%, and 29%, respectively, compared to non-sprayed under stress plants.

### *3.10. Effects of MT and ARG treatment on yield of C. annuum under salt stress*

Salt stress had significant effects on yield of *C. annuum*. With increasing level of NaCl stress fresh weight of fruit (Fig. 11-A) increased. Highest increase in fruit fresh weight (25%) was found in plant with 100 mM NaCl stress in relation to control plants. Lowest mean value recorded for fruit fresh weight was 3.14 g in plants under 50 mM NaCl stress with foliar treatment of MT.

Fruit dry weights (Fig. 11-B) were 0.47, 0.52, and 0.54 g for 0, 50, and 100 mM NaCl stress, respectively. In comparison to the control, fruit dry weight increases by 10% and 14% at 50 and 100 mM NaCl stress, respectively. Foliar treatment of MT, ARG and their combined treatment

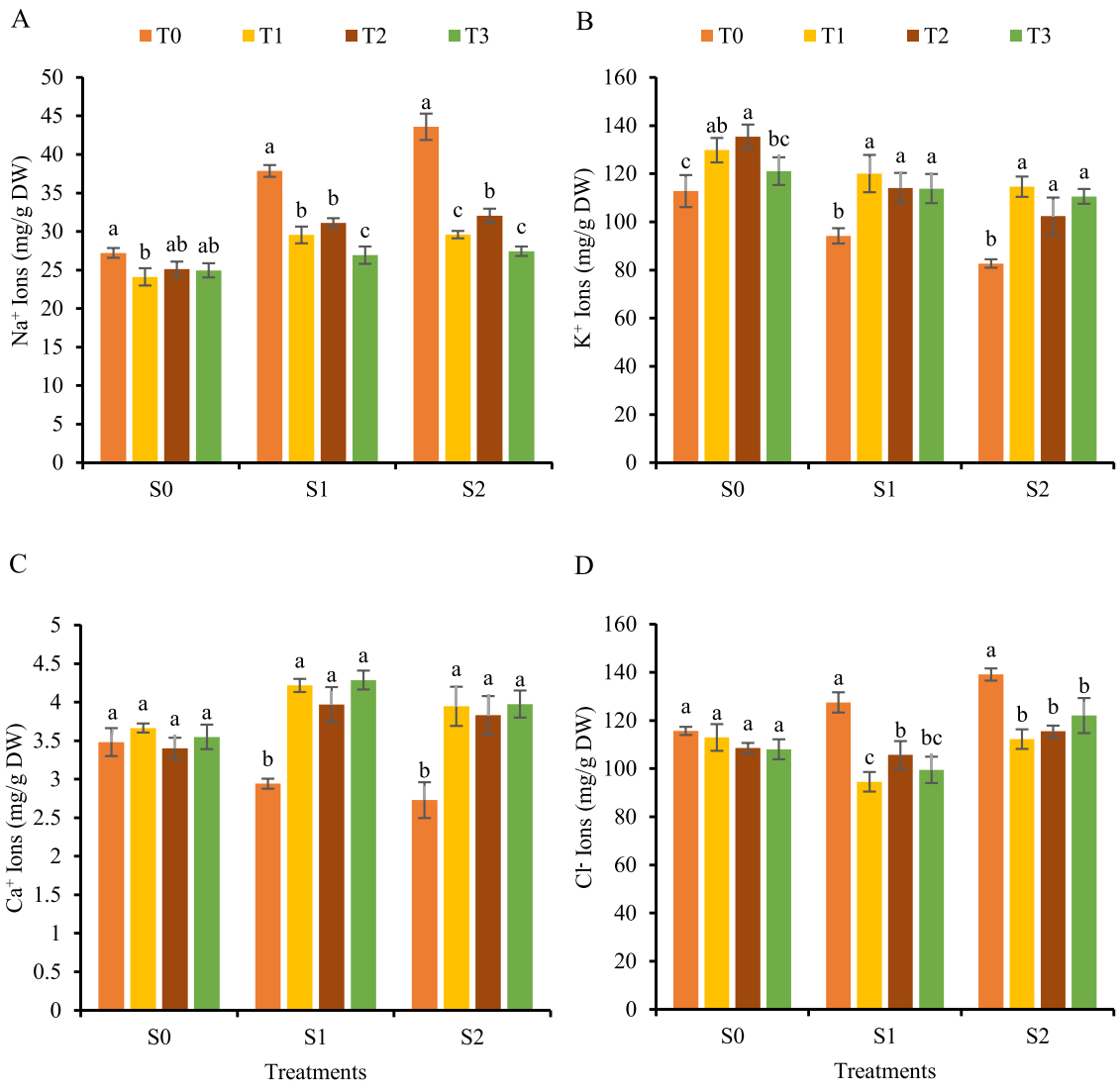


**Fig. 10.** $Na^+$, $K^+$, $Ca^{++}$ and $Cl^-$ ions in root of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 μM MT, T2 = 2 mM ARG, T3 = 100 μM MT + 2 mM ARG). *A* = $Na^+$, *B* = $K^+$, C = $Ca^{++}$ and D = $Cl^-$. Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

did not affect fruit dry weight.

Number of fruits (Fig. 11-C) were lowered by 14% and 19% with 50 mM and 100 mM NaCl salt stress, respectively. Number of fruits per plant increased with foliar spray of MT, ARG and their combined treatment by 22%, 17%, and 28%, respectively, with 50 mM NaCl stress and 24%, 18%, and 29%, respectively, with 100 mM NaCl stress, compared to untreated stressed plants.

Under salt stress, the weight of 100 seeds (Fig. 11-D) reduced considerably. Under 50 and 100 mM NaCl stress conditions, the weight of 100 seeds decreased by 9% and 20%, respectively. When compared to their respective controls, MT, ARG and their combined foliar application increased the weight of 100 seeds by 27%, 21%, and 32%, respectively, in control plants, 24%, 21%, and 32%, respectively, under 50 mM NaCl stress, and 23%, 21%, and 29%, respectively, under 100 mM NaCl salt stress.

### 3.11. Pearson correlations and principle component analysis

Pearson's correlation graphs were shown in Fig. 12 to compute the connection between salt uptake and their effect on various physio-biochemical attributes of *C. annuum* plants. Sodium in roots was positively correlated with, RMP, MDA, $H_2O_2$, S. Na, R. Cl, S. Cl, release of antioxidants, while negatively correlated with PFW, PDW, shoot L, root length, NOL, Chl b, Car, [YII], NOF, 100 seed weight, APX, plant potassium and calcium contents.

The scores (a) and loading plots (b) of principal component analysis (PCA) to assess the influence of different levels of NaCl stress on the growth and physio-chemical parameters of are shown in Figs. 13a, b. They showed that all (12 treatments) were distributed successfully by first two principal components for *C. annuum* plant where increasing level of salt treatments without foliar application (5, 9) were more separated from the treatments such controls (1) and other treatments (2, 3, 4, 6, 7, 8, 10, 11, 12). These treatments gave a clear suggestion that increasing the level of salt (S1, S2) had significant effects on physio-biochemical attributes of *C. annuum* while application (foliar treatment of Mel, ARG, and their combined treatment) has an ameliorative role when compared to non-treated plants (5, 9). Among all the principal components for *C. annuum* plant, first two components, i.e., PC1 (Dim 1) and PC2 (Dim 2) exhibited maximum contribution and accounted for 80% of the total variance in the dataset of which, PC1 contributed 50.8%, while PC2 contributed 28.6%, respectively (Fig. 13). The first group of variables with which PC1 is positively correlated includes: $H_2O_2$, RMP, MDA, FFW, FDW, root and shoot (Na, Cl) concentrations, and release of antioxidants. While a significant negative correlation of

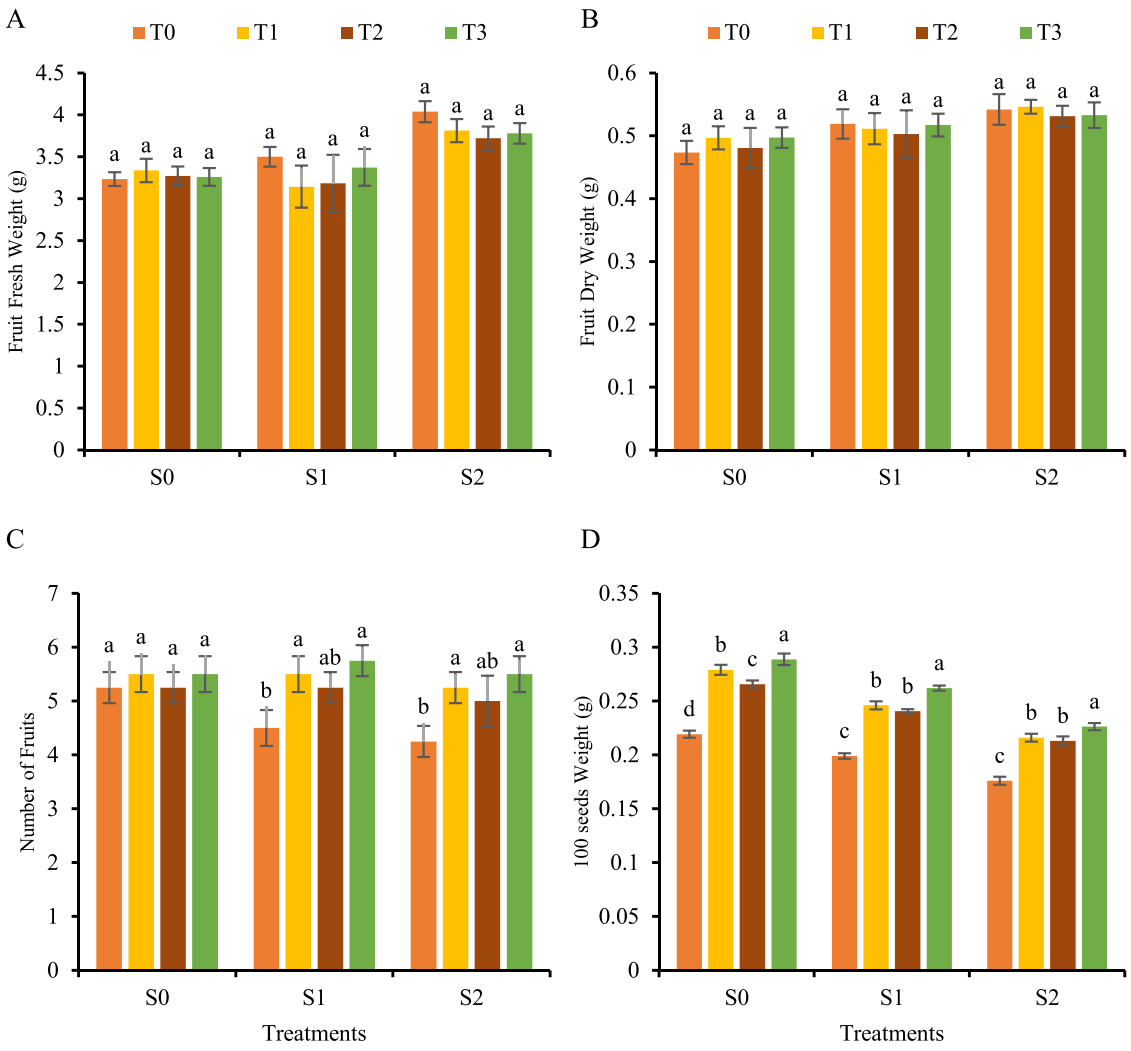


**Fig. 11.** Yield parameters of *C. annuum* under NaCl stress and foliar application of MT, ARG, and their combined treatment (S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl, T0 = control, T1 = 100 μM MT, T2 = 2 mM ARG, T3 = 100 μM MT + 2 mM ARG). *A* = Fresh weight of fruits, *B* = Dry weight of fruits, C = Number of fruits per plant and *D* = Weight of 100 seeds. Graph bars with same letters in each group shows that mean values do not differ at significance level of 5%.

PC1 variables was found with the variables aligned with PCA2, Car, PFW, APX, PDW, shoot L, root length, NOL, Car, [YII], P-Ca, P-K, number of fruits, and 100 seed weight.

## 4. Discussion

Salinity is rapidly growing worldwide, poses serious threats to production and yield of crops. Severe conditions results inhibition of growth and cell injuries as water uptake capability of plants is reduced (Smoleń et al., 2020). Cell division and expansion is effectively hampered due to salinity (Arif et al., 2019). Effect of NaCl stress on growth, morphology, physiology, biochemistry, Y[II], inorganic mineral ions and yield of *C. annuum* and counter effect of foliar treatment of exogenous MT, ARG, and their combined treatment to relieve stress were analyzed, in this study. For this purpose, *C. annuum* seedlings were grown and given 50 and 100 mM NaCl stress. Concentrations selected for foliar spray of MT and ARG were 100 μM and 2 mM, respectively. These concentrations were selected, based on literature cited by different researchers (Feng et al., 2019; Kaya and Doganlar, 2019; Kaya et al., 2020b; Shakhsi--Dastgahian et al., 2022).

*C. annuum* plants were affected adversely when exposed to NaCl salt stress. A clear-cut reduction in growth was noted with 50 and 100 mM NaCl stress. In the meanwhile, foliar application of MT, ARG and their combined treatment not only retrieve severe effects of NaCl stress but also promoted growth of plants under stress. Results justify findings of previous work done by different researchers who revealed that foliar application of MT and ARG promotes growth of plants and improve salt stress tolerance (Imran et al., 2021; Kaya and Doganlar, 2019; Shakhsi-Dastgahian et al., 2022). Salt stress had a very significant influence on the morphological characteristics of *C. annuum* evaluated in this study. All morphological characters were decreased under NaCl stress. Such findings are also reported in literature. Hahm et al. (2017) revealed that NaCl salt stress significantly lowered pepper plant growth. In current study, foliar application of MT, ARG, and their combined treatment alleviated NaCl stress and promoted growth of pepper plants. In cotton all growth parameters were reduced significantly under salt stress, while exogenous MT treatment eliminate inhibitory effect of NaCl stress (Shen et al., 2021). Wang et al. (2021) described that ARG improved growth of aerial parts and promoted root growth. Surprisingly, NaCl stress had no influence on chlorophyll *a* and *b* levels in *C. annuum* plants. These results are contradictory to previous findings reported in research articles that revealed negative impact of NaCl stress on chlorophyll contents (Sofy et al., 2022). In this study, salt stress reduced carotenoids contents which is also found in rice when given 150 mM NaCl stress (Yan et al., 2021b). MT, ARG, and their combined foliar spray treatment increased chlorophyll *a, b*, and carotenoid levels in control and stressed plants. Same findings are also described by Chen et al. (2018) and (Freitas et al., 2022).

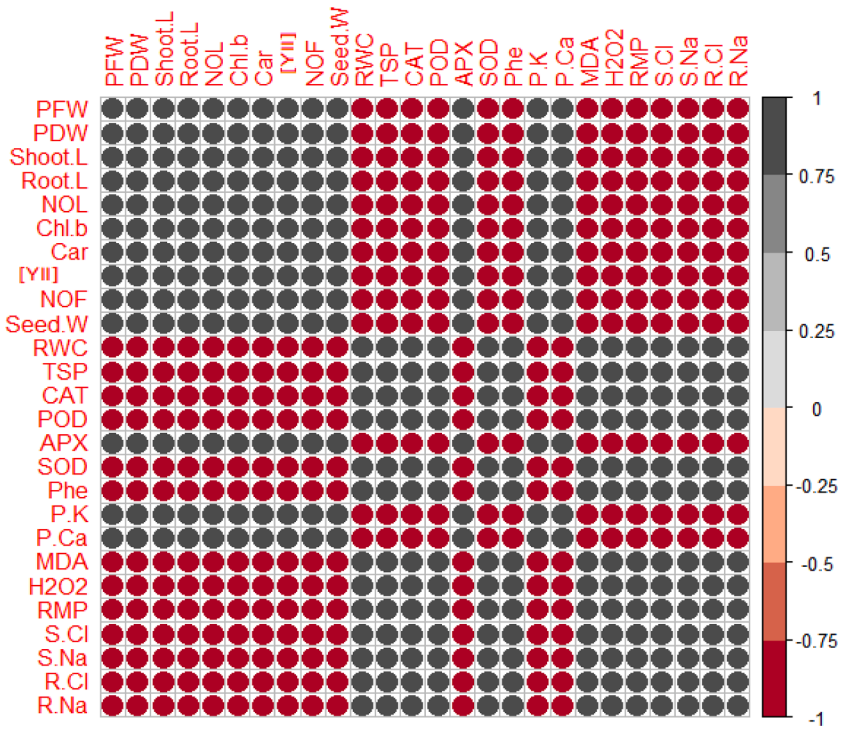


**Fig. 12.** Relationship between salt uptake and plant eco-physiological attributes of *C. annuum* grown under salt stress. Different abbreviations PFW (plant fresh weight), PDW (plant dry weight), Shoot L (shoot length), Root L (root length), NOL (no. of leaves), Chl *b* (chlorophyll *b*), Car (carotenoids), [YII] (Quantum yield of photosystem II), MDA (malondialdehyde), $H_2O_2$ (hydrogen peroxide), RMP (relative membrane permeability), RWC (Relative water contents), TSP (total soluble protein), CAT (catalase), POD (peroxidase), APX (ascorbate peroxidase), SOD (super oxidase), Phe (phenolics contents), R. Na (Root sodium contents), S. Na (shoot sodium contents), R. Cl (root chloride contents), S. Cl (shoot chloride contents), P.K (plant potassium contents), P.Ca (plant calcium contents), FFW (Fruit fresh weight), FDW (Fruit Dry weight) P. Cl (plant chloride contents), NOF (no. of fruits), Seed W (100-ssed weight).

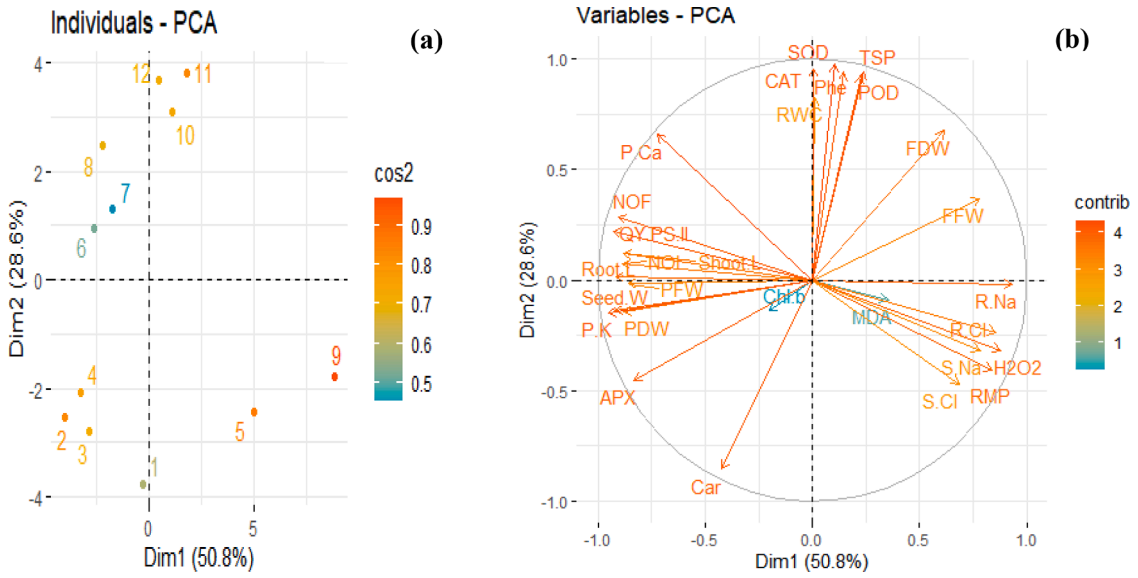


**Fig. 13.** Scores (a) and loading plots (b) of principal component analysis on various studied attributes of *C. annuum* grown under various levels of salt stress with or without the foliar treatment of MT, ARG, and their combined treatment. Score plot (a) represents separation of treatments as (1) S0 (0 mM NaCl); (2) S0+MT; (3) S0+ARG; (4) S0+MT+ARG; (5) S1 (50 mM NaCl); (6) S1+MT; (7) S1+ARG; (8) S1+MT+ARG; (9) S2 (100 mM NaCl); (10) S2+MT; (11) S2+ARG; (12) S2+MT+ARG. Various abbreviations used in the figure are similar as in Fig. 12.

Malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$) and relative membrane permeability (RMP) of *C. annuum* were decreased significantly with 50 and 100 mM sodium chloride stress. Foliar application of MT, ARG and their combined treatment resulted in decreased value for MDA, $H_2O_2$ and RMP significantly irrespective of conditions either control or stress. Jiang et al. (2020) observed same trend when they exposed cotton plants to salt stress with 50 μM MT treatment. Exogenous ARG also decreased MDA contents in *Salicornia europaea* when applied in combination with salt (Shakhsi-Dastgahian et al., 2022). MT treatment scavenged ROS directly or through activating antioxidative enzymes (Jiang et al., 2022; Liu et al., 2021). Compared to control seedlings, root and leaf tissue of Ni stressed tomato had increased level of MDA and EL. However, MT-pretreated tomato seedlings showed evidently reduced MDA content and EL, compared to Ni stress only (Altaf et al., 2021).

Relative water content (RWC) of *C. annuum* plants did not change much under 50 mM NaCl salt stress but 100 mM NaCl salt stress decreased RWC significantly. Foliar spray of MT, ARG and their combined treatment decreased RWC in control plants as compared to non-sprayed plants while it increased RWC significantly under stress. In rice seedlings when exposed to salt stress RWC was decreased significantly and exogenous MT treatment reduced inhibitory effects of sodium

chloride stress on rice seedlings resulting in recovery of RWC (Yan et al., 2021b).

The effects of salt stress and foliar sprays of MT, ARG, and their combination treatment on total soluble proteins and antioxidant enzymes were extremely significant. Total soluble proteins and antioxidant enzymes (CAT, POD and SOD) were enhanced under 50 and 100 mM NaCl stress in this research. The activity of enzymes mentioned previously, was effectively improved in L. *scindicus* under NaCl stress (Ahammed et al., 2020). Under salt stress, APX enzymes were modestly reduced. MT, ARG, and their combination foliar spray boosted total soluble proteins and antioxidant enzymes. Mango fruits treated with exogenous ARG also showed higher antioxidant enzymes activity as compared to control (Pakkish and Mohammadrezakhani, 2021). Exogenous MT also significantly increased antioxidant enzymes activity in rice seedlings under stress (Yan et al., 2021a).

In this study, total phenolics in *C. annuum* plants were raised under 50 and 100 mM sodium chloride stress, and foliar treatment with MT, ARG, and their combination treatment significantly boosted phenolics under stress. Although foliar spray of MT, ARG and their combined treatment did not affect phenolics compound in *C. annuum* in control plants. In rice seedling, phenolics compound were increased in stress and exogenous MT further enhanced phenolics in *C. annuum* (Yan et al., 2021a). Mango fruits treated with exogenous ARG had a considerable increased level of total phenolics, compared to control (Pakkish and Mohammadrezakhani, 2021).

Salt stress significantly decreased quantum yield of photosystem II while foliar application of MT, ARG, and their combined treatment significantly increased Y[II] in control as well as stress plants. Y[II] of green bean was also decreased under salt stress but in plants pretreated with exogenous MT a significant increase in Y[II] was observed (Elsayed et al., 2021).

$Na^+$ and $Cl^-$ ions were increased while $K^+$ and $Ca^{++}$ ions were decreased significantly under salt stress in this study. Foliar spray of MT, ARG and their combined treatment resulted in decreased level of $Na^+$ and $Cl^-$ ions and increased level of $K^+$ and $Ca^{++}$ ions in under stress plants. These results showed that MT and ARG treatments maintain $Na^+/K^+$ ions homeostasis in *C. annuum* under stress. These results coincide with previous work done by various researchers. Yan et al. (2020) described that exogenous MT maintain $Na^+$ and $K^+$ ions balance in rice seedlings.

Salt stress and foliar spray of MT, ARG and their combined treatment had significant effects on yield of *C. annuum*. Salt stress slightly increased fruit fresh and dry weight but it significantly decreased number fruits per plant. Results showed overall decreased in yield of *C. annuum* plants under stress. Weight of 100 seeds was decreased under NaCl stress. Foliar spray of MT, ARG and their combined treatment promoted all tested yield characters of *C. annuum* in this study. Results of this study are in accordance with findings of previous research work (Kaya et al., 2020a; Pakkish and Mohammadrezakhani, 2021).

All studied morphological parameters in the experiment were positively corelated with each other and with Y[II], $K^+$ions in the shoot and roots and yield. That means increase in shoot fresh and dry weight, root fresh and dry weight, shoot and root length, number of leaves and Y[II] ultimately resulted in improved yield of *C. annuum* plants. Antioxidant enzymes (CAT, POD, and SOD) were positively highly corelated with total soluble protein contents in pepper plants and with fresh and dry weight of fruits. This revealed that with increased level of total soluble protein content antioxidant enzymes were also increased in pepper plant that ultimately resulted in improved yield of plants. $Na^+$and $Cl^-$ ions contents in shoot and root of plants were positively corelated with MDA, $H_2O_2$ and RMP that showed their role in imposing oxidative stress to plants. $Na^+$and $Cl^-$ ions were negatively corelated with shoot and root fresh weight, shoot and root dry weight, shoot and root length, number of leaves, Y[II], number of fruits and weight of 100 seeds. These findings showed that increasing level of $Na^+$and $Cl^-$ ions in shoot and root of plants resulted in reduced morphological characters, Y[II] and ultimately yield of pepper plants.

## 5. Conclusion

Effects of NaCl stress and foliar treatment of exogenous MT, ARG, and their combined treatment on morphological, physiological, and biochemical features of *C. annuum* (*C. annuum*) were estimated. Antioxidants, total soluble proteins, and antioxidant enzymes were analyzed in this study. Quantum yield of photosystem II, inorganic ions and yield characters of *C. annuum* were also studied in this research work. Results showed that 50 and 100 mM sodium chloride stress significantly lowered morphological characters of *C. annuum*. Foliar application of MT, ARG and their combined treatment improved all these morphological character in control as well as salt stress plants. Under NaCl salt stress, the chlorophyll *a* and *b* contents of *C. annuum* changed insignificantly. Exogenous MT and ARG treatments enhanced chlorophyll *a* and *b* content marginally in control and stressed plants. ROS, MDA, $H_2O_2$, antioxidants (phenolics) and antioxidant enzymes were increased significantly under NaCl stress. Under stress, foliar spray of MT, ARG, and their combined application reduced MDA and $H_2O_2$ but enhanced phenolics and antioxidant enzymes. Salt stress also decreased Y[II] and overall yield of *C. annuum*. Exogenous MT and ARG treatments not only improved photosynthetic capacity of *C. annuum* by improving Y[II] but also enhanced overall yield of *C. annuum* plants in control and stress conditions. It is concluded that exogenous MT, ARG, and their combined treatment alleviates salt stress in *C. annuum* through improving antioxidant enzymes activities, chlorophyll *a* and *b* contents, Y[II] and by scavenging effects of ROS. This ultimately improved plant growth and overall yield. As results showed, foliar spray of MT, ARG and their combined treatments were significantly effective in alleviating NaCl stress in *C. annuum* plants. On basis of results obtained, it is recommended that effects of foliar treatment of MT and ARG should be assessed on large scale in fields (saline areas) to get real time results. This could be helpful in improving per hectare yield of *C. annuum*, especially in saline areas.(Fig. 3).

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## CRediT authorship contribution statement

**Sheeraz Usman:** Validation, Formal analysis, Methodology, Investigation. **Ghulam Yaseen:** Visualization, Methodology, Investigation, Supervision, Data curation. **Zahra Noreen:** Project administration, Supervision, Data curation, Validation. **Muhammad Rizwan:** Visualization, Software, Writing – original draft, Writing – review & editing. **Hafeez Noor:** Formal analysis, Writing – original draft, Resources, Software, Funding acquisition. **Hosam O. Elansary:** Formal analysis, Writing – original draft, Resources, Software, Funding acquisition.

## Declaration of Competing Interest

All the authors declare no conflict of interest

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