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Verdant Legacy

Research article

Role of $(NH_4)^+$, (NO_3), K^+ and $(SO_4)^-$ to Tackle Salt Sensitivity of *Allium Cepa* L. under Saline Conditions

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Abstract

Allium cepa L., an economically important bulbous crop, is a glycophytic plant species and is sensitive to mild concentrations of salt. A large area of Pakistan is affected by salinity, as it is in a semiarid and arid territory with a subtropical continental climate that is currently experiencing salinization. Therefore, this project was designed to explore the salinity tolerance in onions mediated by individual and combination of fertilizers (NH_4NO_3 and K_2SO_4) as source of $(NH_4)^+$, (NO_3), K^+ and $(SO_4)^-$ at two growth stages (vegetative, bulb maturation stage). For this purpose, three salt treatments (4.5, 8.5 and 12.5 dS m^{-1} NaCl) were applied at vegetative and bulb maturation stages. The fertilizer treatments included T1) 250 mg/kg NH_4NO_3, T2) 250 mg/kg K_2SO_4, T3) 125mg/kg NH_4NO_3 + K_2SO_4, T4) 150 mg/kg NH_4NO_3 + 100 mg/kg K_2SO_4, T5) and 100 mg/kg NH_4NO_3 + 150 mg/kg K_2SO_4. It was observed that salinity affected onion's growth and yield. Whereas combined and individual effect of fertilizers improved the growth and yield of onion by increasing the chlorophyll level, leaf and bulb relative water content, enzymatic antioxidant activities, proline and soluble sugar content. However, the combination of NH_4NO_3 +K_2SO_4 showed improvement in growth and osmolytes production even at 12.5 dS m^{-1}. Therefore, it was concluded that nitrogen fertilizer and combination of N+K can improve *A. cepa* production.

Keywords: Ammonium nitrate, Morphological parameters, Physio-biochemical parameters, Potassium sulphate, Salinity, Salt tolerance

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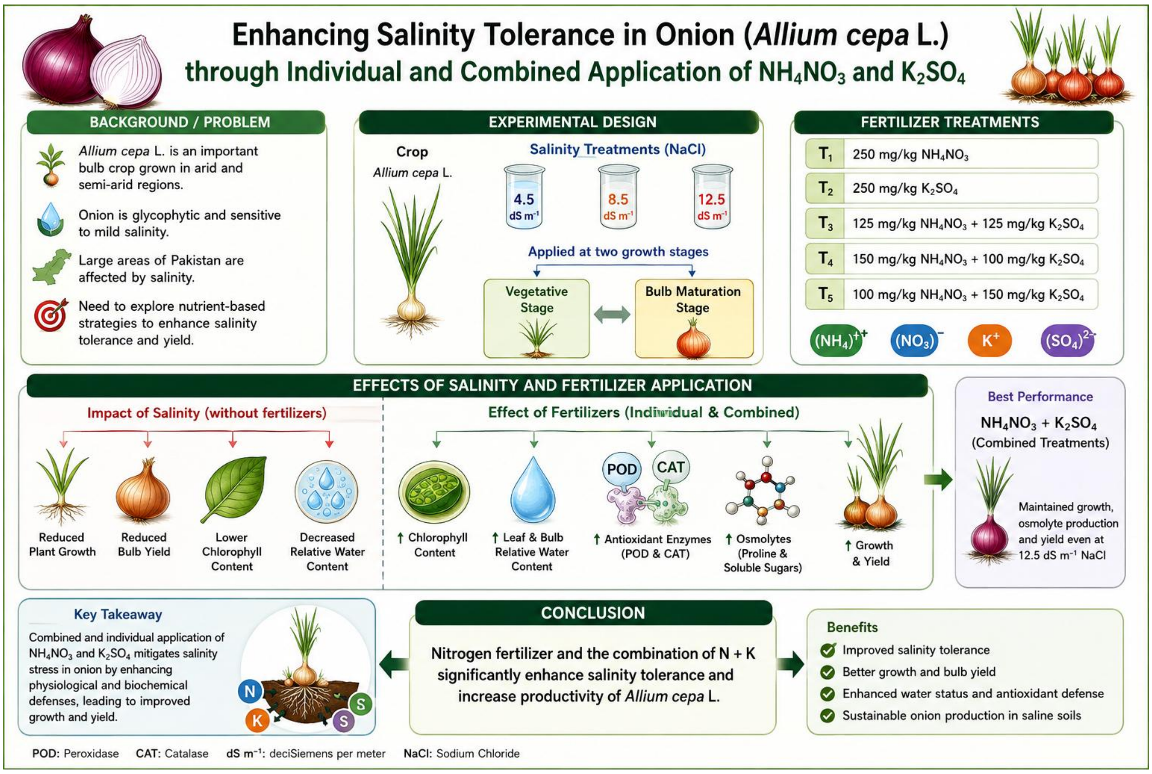
Graphical Abstract



Introduction

Allium cepa L. commonly known as onion, is a bulbous seasonal vegetable of the family Alliaceae. This crop has long been cultivated by farmers as a source of income and contributed considerably towards the economic development (Khan et al., 2020). *A. cepa* contain high nutrition and essential oils, also used in food as flavoring agent. The onion bulbs contain allyl propyl disulfide which causes tanginess in it. In addition, the phytochemical composition of *A. cepa* contributes bactericidal and fungicidal properties to this vegetable and makes it efficacious as traditional medicine. Onion bulbs have been reported for diuretic properties and beneficial for digestive tract. Onions are good for eyes, act as a heart stimulant and useful as anti-rheumatic remedies (Bibi et al., 2021). Globally, onion is cultivated on approx.5.2 million hectares with average production of 19 tons per acre. Whereas, in Pakistan its cultivation is about 1.8 million tons, harvested from 138 thousand hectors. Annually, this production rate is decreasing owing to different factors affecting it. Onion growth and productivity across the world is usually affected by various environmental stressors. Among those, salinity stress is the most threatening and limiting factor for plant growth and yield (Amiri et al., 2022). Besides climatic factors, quality of irrigation water and improper application of fertilizers are among the serious causes of salinity in arid and semi-arid areas (Kumar et al., 2015; Pitman and Lauchli, 2002). Salinity interrupt plant's morpho-physiological and biochemical processes such as, plant growth, development, reproduction, plant water potential, ion homeostasis, root mass, absorption of nutrients, leaf coloration, decreasing green pigments, and activity of various enzymes (Ahmed et al., 2021). Salinity impact on plants mainly depends upon plant's species and salt type (Vaishnau et al., 2015). Among all salts, sodium chloride (NaCl) is the most toxic (Ahmadi and Souri, 2019). Worldwide, the recorded yearly losses in agricultural output from salt-affected lands are more than US$12 billion and getting higher (Abbasi et al., 2016)

Fertilizer application is an adaptive management practice for improving soil fertility, maintaining soil pH, preventing and controlling soil degradation (Semida et al., 2021). Various fertilizer applications i.e., biofertilizers, nano fertilizers and conventional fertilizers are practiced nowadays. Nano fertilizers release nutrition steadily and gradually. It takes more time for plants to fully absorb them (Etana et al., 2019). However, this technology has the drawback of retaining nanoparticles in soil. Nano fertilizer application also requires more funding and adequate knowledge to be effective at field level. Bio fertilizers are organic fertilizers that trigger an increase in crop productivity up to a certain point (Limeneh et al., 2020; Mazumder et al., 2019). Conventional fertilizers are inorganic fertilizers that have been used by the farmers from decades. They are available in different chemical compositions. They provide nutrients instantly to plants when dissolved in water. These fertilizers contain nutritional elements for plant growth and helps in reclamation of saline soil (Agnieszka et al., 2017; Mageshen et al., 2022) Among these conventional fertilizers, urea, DAP and NPK are rigorously practiced. These fertilizers have higher N content (46% N), which is a valuable source for plants. On the other side, excessive NPK supply on soil provides a surplus of nitrogen, compared to phosphorus resulting in mosaic infections and destruction of beneficial soil microbes. Such fertilizers are much expensive and often face shortage due to insufficient availability. However, the combined effect of different fertilizers has great potential to improve the soil composition, soil structure, prevent erosion, and increase soil organic carbon, humus, and nutrient contents, which are the most growth-constraining factors in saline soils (Wang et al., 2013).

Nitrogen application in the form of ammonium (NH_4^+) and nitrate (NO_3^-) is beneficial for the productivity of all plants particularly onion. It positively effects the absorption of phosphorus from the soil as well as increases root growth (IFC, 1982). Nitrogen and potassium are often referred to as the primary macronutrients absorbed by the plants in large quantities from the soil and taken up by plant as essential elements, increasing crop yield by 70-80%. Hence, nitrogen and potassium fertilizers (ammonium nitrate (NH_4NO_3) and potassium sulphate (K_2SO_4)) help in developing various parameters like plant growth and biomass, leaf relative water content, gas exchange and chlorophyll fluorescence, photosynthetic pigments, reactive oxygen species (ROS) and antioxidant enzyme activities to survive and maintain pH in saline soil (Wenxue et al., 2003). The use of sophisticated technologies over conventional technologies creates serious knowledge gap.

Improvements in the adoption of conventional fertilizers globally is both economical and beneficial for food security goals. Thus, our study provides novel strategies for usage of conventional fertilizers such as ammonium nitrate (NH_4NO_3) and potassium sulphate (K_2SO_4). Ammonium nitrate application as a fertilizer aid in even distribution of nitrogen over the time (Huang et al., 2020). Application of potassium sulphate fertilizer can enhance the nutrient uptake mechanism of lants under salinity stress (Gubb and Tavis, 2002). Under saline and drought stress, application of optimum concentration of potassium fertilizer can enhance the total dry mass of plants. Therefore, in this study ammonium nitrate (NH_4NO_3) and potassium sulphate (K_2SO_4) were applied separately and in combined form to enhance salt tolerance in *Allium cepa* L. cultivated under various salt stress conditions.

2. Materials and Methods

2.1: Plant Material

Seed of *A. cepa* were collected from vegetable research institute, Ayub Agricultural Research Institute (VRI, AARI), Faisalabad.

2.2: Soil Analysis

Moist soil samples were oven dried and homogenized Soil was examined for EC (electric conductivity), pH, and texture, in Molecular Taxonomy Laboratory, LCWU, Lahore, Pakistan. The amount of organic matter %, available phosphorus, available potassium and nitrogen were determined through soil testing services of Soil and Water Testing Laboratory, Lahore, Pakistan.

2.3: Field Preparation

The experiment was conducted in Botanical garden of Lahore College for Women University Lahore. The experimental design was split block RCBD. Soil was loamy. Healthy onion tillers were selected and planted in each Block.

2.4: Salt Stress Conditions

Three different concentrations of NaCl (4.5 (C1), 8.5 (C2) and 12.5 dS m^{-1} (C3)) was used as salt stress agent in the soil. Stress was initiated after one week of transplantation and treatment was applied at two growth stages by following the respective protocol (Wang et al., 2013).

2.5: Fertilizer Treatments

After first salt stress treatment, fertilizer application was initiated at an interval of one week. Five treatments were made (T1 = Ammonium nitrate (NH_4NO_3) 250 mg/kg of soil as nitrogen source. T2 = Potassium sulphate (K_2SO_4) 250 mg/kg, T3 = combination of NH_4NO_3 (125 mg) and K_2SO_4 (125 mg), T4 = combination of NH_4NO_3 (150 mg) and K_2SO_4 (100 mg), T5 = combination of NH_4NO_3 (100 mg) and K_2SO_4 (150 mg)) along with a control.

2.5.1: Germination Assay

Speed of germination (SOG), percentage of germination energy (GE %), final germination percentage (FG %) and germination inhibition index (GII) were calculated by following formulas.[35-37]

$$\textbf{Speed of germination} = \frac{\text{(Number of germinated seed)}}{\text{(Days of first count)}} + \cdots + \frac{\text{(Number of germinated seed)}}{\text{(Days of final count)}}$$

$$\textbf{Germination energy (\%)} = \frac{\text{(Number of germinated seed at DAS)}}{\text{(Total number of seed)}} \text{x}100$$

$$\textbf{Final Germination percentage (\%)} = \frac{n}{N} \text{x}100$$

$$\textbf{Germination inhibition index} = \frac{(\text{SGPcontrol} - \text{SGPsalt})}{(\text{SGPcontrol})} \times 100$$

2.5.2: Evaluation of Morphological Parameters

Plant morphological parameters like leaf length, leaf diameter, bulb length and bulb diameter were measured according to the IPGRI growth descriptor (Anonymous, 2001). Plant growth and biomass at early, vegetative and bulb stage were calculated by the respective protocols (Kausar, 2014).

2.5.3: Determination of physiochemical Parameters of soil and onion plant

2.5.2.1: Relative Soil Water Content

Relative soil water content (RSWC) was measured by following Hura et al., 2007, before and after stress at vegetative and bulb maturation stages.

2.5.2.2: Relative Water Content of leaf and bulb of onion

The relative water content of leaf and bulb was calculated by following formulas:

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

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

Where FW is fresh weight, DW is dry weight and TW is turgor weight (Xu et al., 2009; Gholami and Ehsanzadeh, 2017).

2.5.2.3: Photosynthetic Pigments

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, xanthophyll and carotenoids were determined following the method of Hand et al., 2017 and calculated by following equations.

$$\boldsymbol{Chlorophyll\ a}\ (\mu g/ml) = 12.21\ (A663) - 2.81\ (A645)$$

$$\boldsymbol{Chlorophyll\ b}\ (\mu g/ml) = 20.13\ (A645) - 5.03\ (A663)$$

$$\boldsymbol{Carotene}\ (\mu g/ml) = (1000\ (A470) - 3.27\ (Chl-a) - 104(Chl-b))/227$$

$$\boldsymbol{A} = A(x+c) + A(a) + A(b)$$

$A_{(x+c)}$ absorbance of xanthophyll and carotenoids, $A_{(a)}$ absorbance of chlorophyll a and $A_{(b)}$ absorbance of chlorophyll b.

2.5.3: Determination of biochemical parameters

2.5.3.1: Soluble sugar content

The soluble sugar content was measured in accordance with Dubois et al., 1951 and spectrophotometer absorbance was checked at 420 nm wavelength.

2.5.3.2 Proline content

Proline content was evaluated at 520nm spectrophotometerically by ninhydrin technique. Pure proline (sigma) was used as a standard (Liu et al., 2014)

2.5.3.3: Antioxidant enzyme activities

Antioxidant enzyme reactions such as

catalase (CAT) and peroxidase (POD) were determined according to the method of Alici and Arabaci (2016)

2.5.3.3.1 CAT activity

For CAT activity determination, 500 µl of extracted plant material was mixed with 1 mL of 30 mM H_2O_2. The absorbance was checked by spectrophotometer at 240 nm after 5 minutes of H_2O_2 addition in it. The decomposition of H_2O_2 with 1 g tissue in 1 minute expressed CAT activity (Kaminska et al., 2022).

2.5.3.3.2 POD activity

For POD determination 500 µl of plant extract was mixed with 1 mL of 1 mM H_2O_2 and 0.5 mL of 8 mM guaiacol. The absorbance was checked with spectrophotometer at 420 nm after one or two minutes of H_2O_2 addition. Peroxidase activity was expressed in units of enzyme that causes the absorbance increase of 0.1 per minute in U g^{-1} FW (unit per gram fresh weight) (Arzani and Ashraf, 2016)

2.6: Statistical Analysis

The data was presented as mean (n=3) and ±Standard deviation. Effect of different treatments and concentrations on salt tolerance of *Allium cepa* L. was subjected to two-way ANOVA (analysis of variance) using IBM SPSS statistics v 20. Pearson correlation coefficient (*r*) was used to demonstrate whether two variables were positively or negatively correlated to each other through Origin pro-2022 software.

3. Results and discussion:

3.1 Soil Physiochemical Analysis

The texture of the experimental soil was loamy, containing mixture of sand, silt, and clay. Table 1 indicates the chemical properties of soil. Electrical conductivity (EC) of soil was 0.64 dS/m and pH value between 7.84-8.14, indicated as alkaline (basic) soil. The mineral content was calculated to be 0.042 mgkg^{-1} for nitrogen, 5.6 mgkg^{-1}, 102 mgkg^{-1} and 0.9% for phosphorus, potassium, and organic matter (Table 1).

Table 1: Soil Physiochemical Attributes

pH	7.84-8.14
Electrical Conductivity	0.64dS/m
Texture	Loamy soil
Available N	0.042 mgkg^{-1}
Available P	5.6 mgkg^{-1}
Available K	102 mgkg^{-1}
%Organic Matter	0.9%

3.2 Germination Assay

The effect of different concentrations of salt (4.5, 8.5, 12.5 dS m^{-1}) and fertilizers on germination parameters including speed of germination (SG), germination energy percentage (GEP), final germination percentage (FGP) and germination inhibition index (GII) are presented in table 2. The results showed that seed treated with treatment T6 under C3 (12.5 dS m^{-1}) stress condition had the maximum speed of germination (4.9±1.4). Whereas seed treated with T1 treatment under C3 stress observed to have minimum speed of germination (1.006±0.5). The maximum energy percentage was obtained by C1T4 treated seed (NH_4NO_3 and K_2SO_4). Whereas minimum energy percentage was observed in C1 for T5 and T7 treatments, followed by T3 and T7 in C2 soil condition. T2 treated seed grown in C1 (4.5 dS m^{-1}) stress condition were observed to have the highest FG (53.3% ± 5.7) and T7 treated seed with minimum FG (2.5 dS m^{-1}). The obtained results also showed that under C3 saline stress, T7 treated seed had maximum germination inhibition index (GII) (73.33±11.5). While in contrast, T6 treated seed observed to have minimum GII. Analysis of variance of the obtained data showed that water, salinity, fertilizers, and their combinations had significant effects on seed germination (p<0.05) (Supplementary Information Table 1).

Table 2: Effect of treatments and saline stress on *Allium cepa* L. seed germination parameters (speed of germination, germination energy percentage, final germination percentage, germination inhion index) biti

Concentration	Treatments	SG	GE%	FG%	GII%
C1	**T1**	3.8±0.46^{a}	6.6±5.7^{d}	40±15.2^{e}	21.6±2.8^{c}
	T2	3.9±1.1^{b}	6.6±5.7^{d}	53.3±5.7^{d}	13.3±23.09^{g}
	T3	3.7±1.2^{b}	6.6±5.7^{d}	33.3±5.7^{d}	26.6±23.09^{g}
	T4	4.9±1.2^{b}	10±0^{a}	46.6±5.7^{d}	0±0^{a}
	T5	3.09±0.5^{a}	0±0	43.3±5.7^{d}	6.6±11.5^{d}
	T6	4.9±1.4^{b}	6.6±5.7^{d}	46.6±5.7^{d}	133±28.8^{h}
	T7	2.4±0.6^{a}	0±0	20±0	56.6±5.7^{d}
C2	**T1**	3.8±1.2^{b}	6.6±5.7^{d}	43.33±5.7^{d}	6.6±11.54^{f}
	T2	3.5±1^{b}	10±0	40±10^{e}	15±13.2^{e,f}
	T3	2.378±0.9^{a,b}	0±0	33.33±5.7^{d}	28.33±10.4^{d}
	T4	3.7±0.7^{a}	10±0^{a}	36.6±5.7^{d}	21.6±2.8^{c}
	T5	3.06±0.6^{a}	6.66±5.7^{d}	36.66±5.7^{d}	21.6±2.8^{c}
	T6	4.94±1.4^{b}	6.6±5.7^{d}	46.6±5.7^{d}	216±160.7^{i}
	T7	2.1±0.9^{a}	0±0	16.66±5.7^{d}	66.6±11.5^{d}
C3	**T1**	1 ±0.5^{a}	0±0	26.66±11.5^{e,f}	35±21.7^{e}
	T2	1.06±0.6^{a}	0±0	26.66±5.7^{d}	41.666±17.5^{g}
	T3	3.56±1.7^{b}	6.6±5.7^{d}	33.33±5.7^{d}	28.33±10.4^{d}
	T4	3.2±1.4^{b}	3.33±5.7^{d}	43.3±15.2	6.6±30.5^{h}
	T5	3.3±0.1^{a}	6.7±5.6^{c,d}	23.3±5.7^{d}	50±10^{d}
	T6	4.9±1.4^{b}	6.6±5.7^{d}	46.6±5.7^{d}	283±125.8^{j}
	T7	1.3±0.4^{a}	0±0	10±0	73.3±11.5^{e}

The Pearson correlation coefficients analysis was evaluated among different germination traits and presented in Table 3. Speed of germination observed to exhibit strong, positive correlation with germination energy percentage and final germination percentage. However, a negative correlation was calculated with germination inhibition percentage.

3.3 Morphological Parameters

3.3.1 Plant Growth and Biomass

Figure 1 and 2 showed significant increase in the leaf length (cm), bulb length (cm), bulb diameter (cm), fresh and dry weight of leaf (g) at both growth stages as compared to the control. Results of morphological parameters indicated the maximum salt tolerance at vegetative stage by C2T1 treated plants followed by C1T2, compared to the control. Whereas, at bulb maturation stage, C1T2 and C1T4 plants showed maximum salt tolerance.

The maximum leaf length was observed in plants grown under C1T3 at vegetative stage (34.3±0.6) and at bulb maturation stage (24.4±0.2). However, the minimum leaf length was observed in control plants at both growth stages (13.6±0.3 and 12.166±1.3), respectively. A significant reduction in bulb length of onions was observed in control as shown in figure 1b. The highest bulb length (3.6±0.2) was observed in plants grown under C2 (8.5 dS m^{-1}) condition, treated with T1 at bulb maturation stage.

Significant ($p<0.05$) effect of different treatments, concentrations, and stages on leaf diameter and bulb diameter was also observed (figure 1c and 1d). An increased leaf diameter in response to salt stress and fertilizers was observed at vegetative stage in C1T3 (1.633±0.153) while, reduced leaf diameter was observed in C3T2 (0.933±0.057) treated plants. At bulb maturation stage, C1T1 (3.367±0.153) treated plants exhibited thicker diameter (cm) and C3T7 plants had thin diameter (1.1±0.05). Plants grown under C1T1 conditions exhibited maximum fresh weight (g) at vegetative stage (11.3±0.02) and bulb maturation stage (42.9±9.04) as compared to other treatments and control. Whereas, C2T4 treated plants showed minimum fresh weight at vegetative (4.9±0.2) and bulb stage (3.867±0.430) (Supporting Information figure 2a , 2b).

3.3.2 Physiological Parameters:

No significant change in relative water content (RWC) of onion was observed in leaves or bulbs, at both growth stages (figure 3c, d). The maximum leaf relative water content (LRWC) was observed in C1T5 (97.7±0.7) and lower in C3T7 (57.6±0.2) at vegetative stage. While at bulb maturation stage, the maximum RWC was observed in C1T4 treatment (96.2±0.9) and minimum in C3T7 plants (77.7±1.4) respectively.

The chlorophyll content of onion bulbs at vegetative stage had highest values than at bulb maturation stage (figure 4a and 4b). Saline regimes caused reduction in chlorophyll content of leaves mainly at bulb stage as compared to the vegetative stage. The highest chlorophyll content was measured in C1T3 plants (12.464±0.154) at bulb maturation stage and C1T4 (16.254±0.219) at vegetative stage. While the lowest chlorophyll content was calculated in C1T2 plants (2.793±0.378) at vegetative stage and C2T7 plants (0.211±0.013) at bulb maturation stage.

3.3.3 Biochemical Parameters:

The soluble sugar and proline content (µg/ml) of onion was determined and presented in figure 5. An increased level of soluble sugar content was observed for every increase in the salinity stress (figure 5a and 5b). The highest level of soluble sugar among all treatments was observed in C3T7 plants (5.9±0.06) at vegetative stage and lowest by T6 treatment (1.6±0.1) at all salinity stress conditions. A remarkable increase in soluble sugar content of onion leaves was observed at bulb stage under C2T4 soil condition (5.4±0.3) and decline with C1T3 treatment (3.1±0.1).

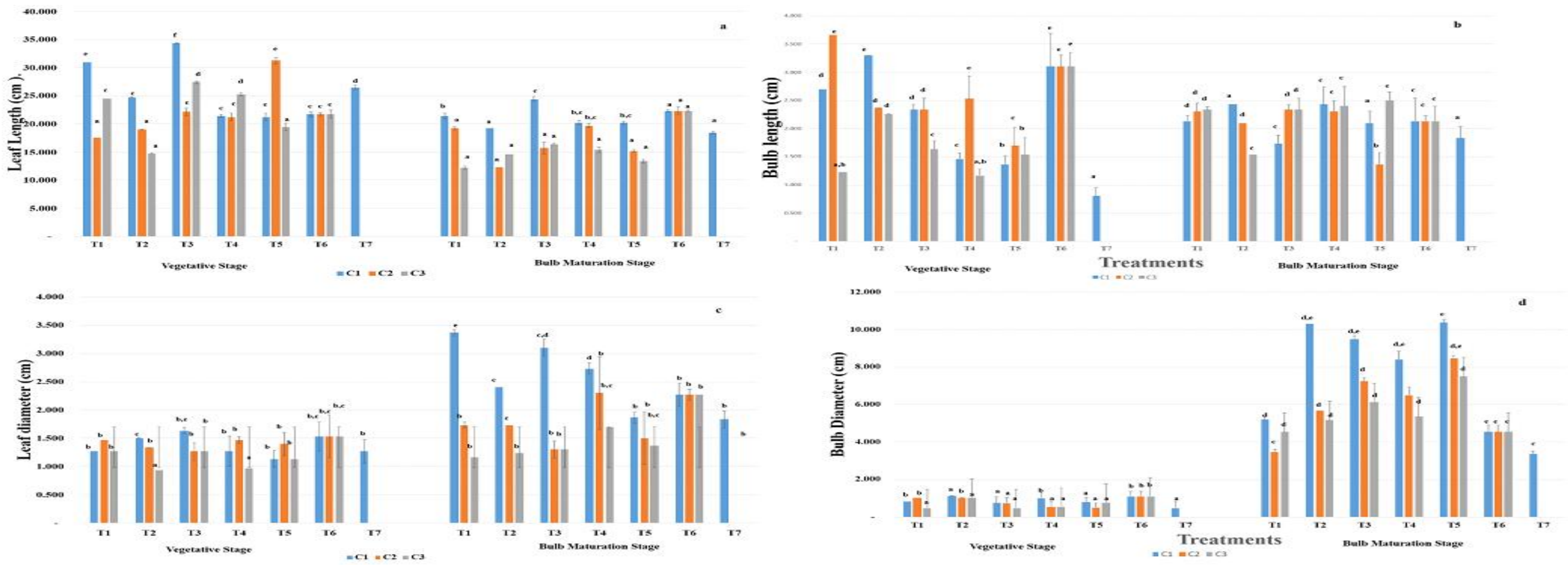


Figure 1. Plant growth and biomass parameters of *Allium cepa* L. observed at vegetative stage and bulb maturation stage under salt stress and fertilizer treatments. (a) Leaf length under salt stress (b) Bulb length after fertilizer treatments (c) Leaf diameter under salt stress (d) Bulb diameter after fertilizer treatments **Keynotes:** C1= 4.5dS/m, C2= 8.5dS/m, C3= 12.5dS/m, T1= NH_4NO_3, T2= K_2SO_4, T3= NH_4NO_3: K_2SO_4, T4= $K_2SO_4 < NH_4NO_3$, T5= $NH_4NO_3 < K_2SO_4$, T6= control, T7= NaCl

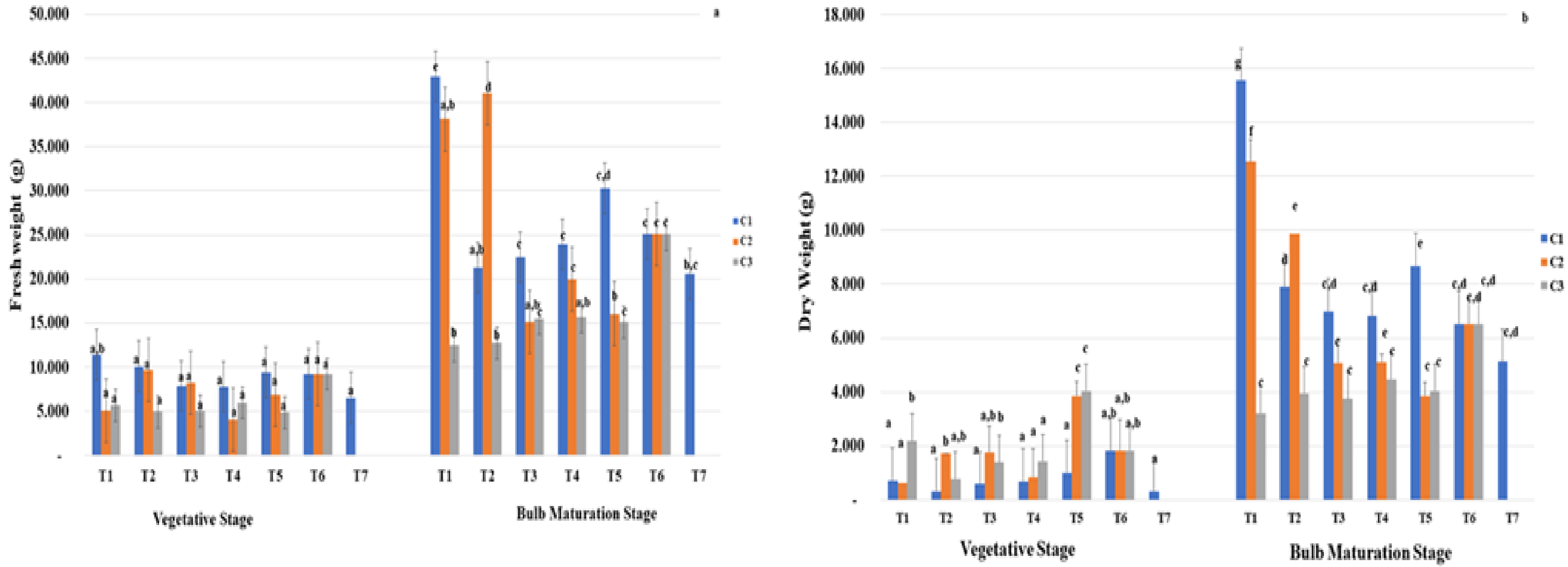


Figure 2. Fresh and dry weight (g) of *Allium cepa* L. observed at vegetative stage and bulb maturation stage under salt stress and fertilizer treatments (a) fresh weight (g) (b) dry weight (g). **Keynotes:** C1= 4.5dS/m, C2= 8.5dS/m, C3= 12.5dS/m, T1= NH_4NO_3, T2= K_2SO_4, T3= NH_4NO_3: K_2SO_4, T4= K_2SO_4< NH_4NO_3, T5= NH_4NO_3< K_2SO_4, T6= control, T7= NaCl.

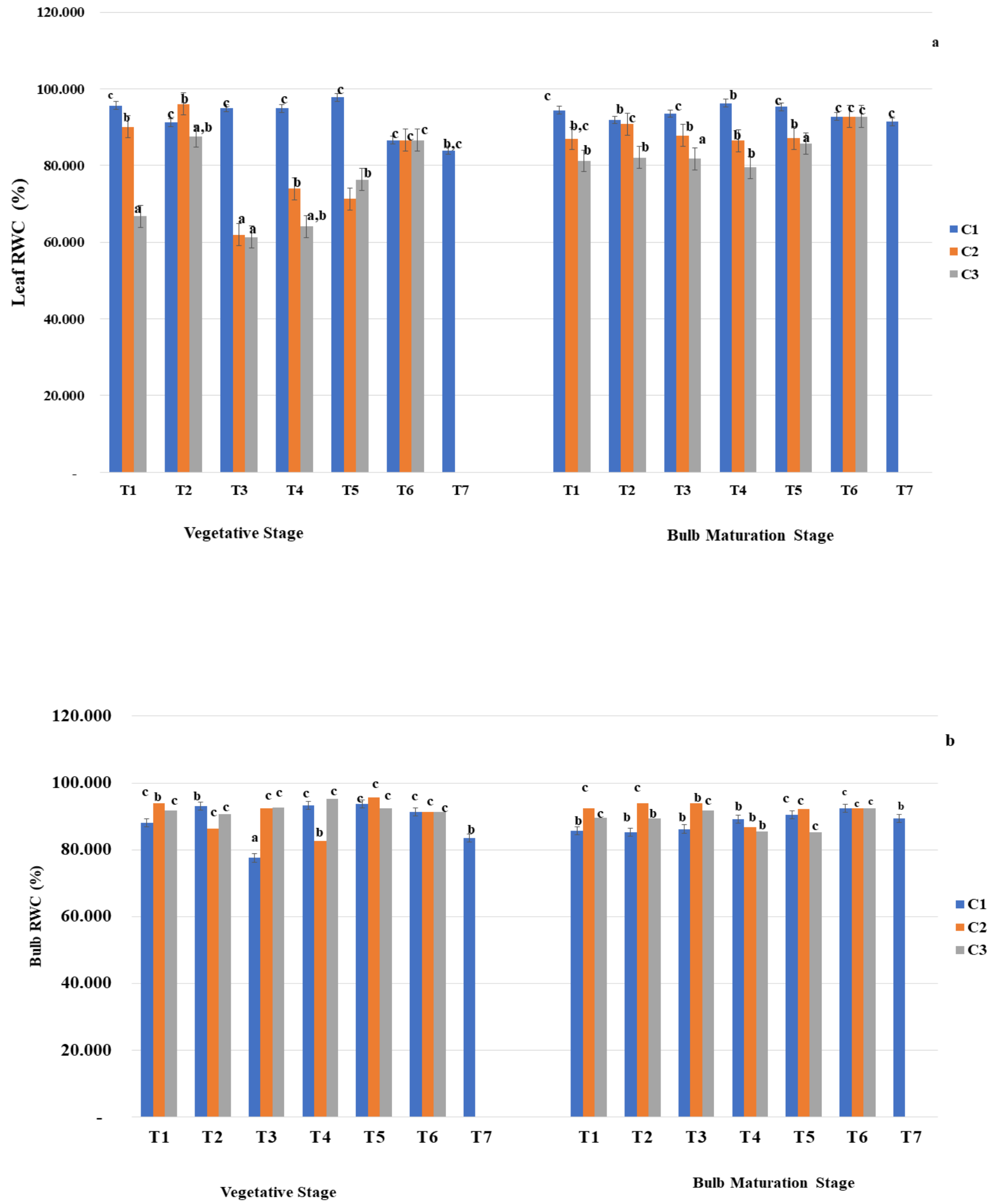
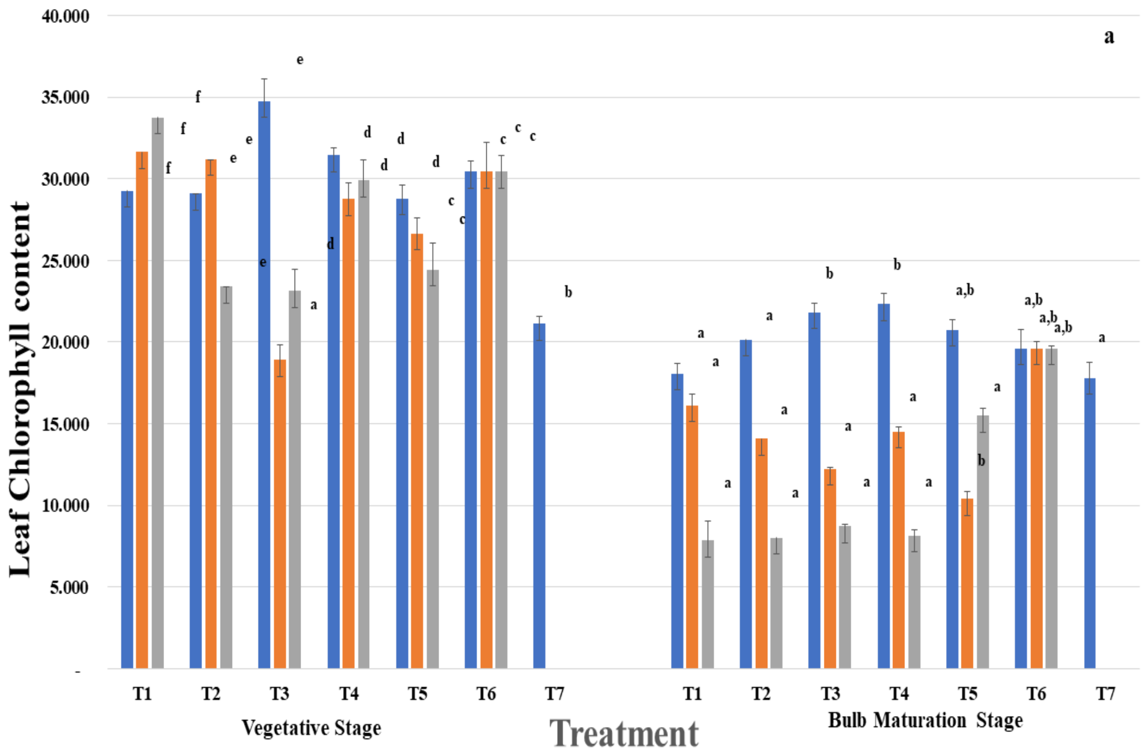


Figure 3 Leaf relative water content (RWC) and Bulb relative water content of *Allium cepa* L. observed at vegetative stage and bulb maturation stage under salt stress and fertilizer treatments (a) Leaf RWC (b) bulb RWC

Keynotes: C1= 4.5dS/m, C2= 8.5dS/m, C3= 12.5dS/m, T1= NH_4NO_3, T2= K_2SO_4, T3= NH_4NO_3: K_2SO_4, T4= K_2SO_4< NH_4NO_3, T5= NH_4NO_3< K_2SO_4, T6= control, T7= NaCl



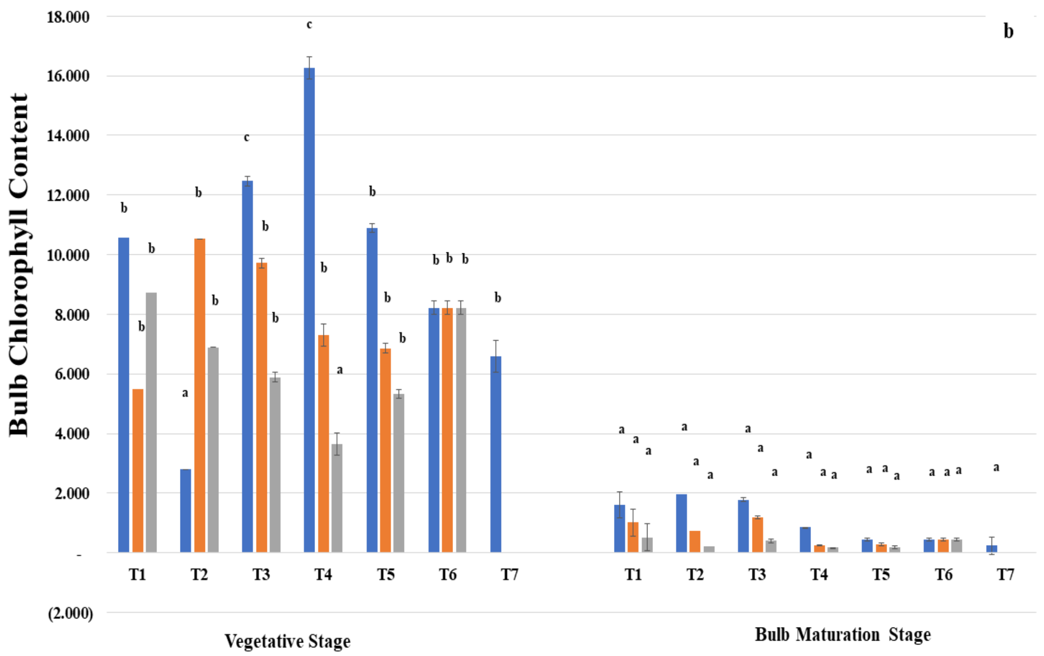


Figure 4. Chlorophyll content of *Allium cepa* L. observed at vegetative stage and bulb maturation stage under salt stress and fertilizer treatments (a) leaf chlorophyll content (b) bulb chlorophyll content.**Keynotes:** C1= 4.5dS/m, C2= 8.5dS/m, C3= 12.5dS/m, T1= NH_4NO_3, T2= K_2SO_4, T3= NH_4NO_3: K_2SO_4, T4= K_2SO_4< NH_4NO_3, T5= NH_4NO_3< K_2SO_4, T6= control, T7= NaCl.

Similarly, proline content was calculated at vegetative and bulb maturation stage of onion (figure 5c and 5d). The maximum proline content was calculated in C3T4 treated plants at bulb stage (15.9±0.08) and at vegetative stag (14.3±0.5) compared to the control treatments

Increase in salinity level resulted in highly significant (p<0.05) increase in catalase (CAT) enzyme activity with increased accumulation of H_2O_2 at both growing stages. The maximum accumulation of H_2O_2 in onion leaves was measured with C3T3 plants (0.348±0.057) at vegetative stage and C2T2 plants (0.269±0.061) at the bulb stage. Lower level of H_2O_2 was observed with C1T4 treated plants (0.055±0.018) at vegetative stage and C1T6 plants (0.115±0.010) at bulb stage. Variations in CAT activity were observed at both growth stages (Figure 6a and 6b).

3.4 Correlation Analysis for Morpho-Physiological and Biochemical Attributes

The two tailed test of Pearson correlation was computed at significance level p < 0.05% (Figure 7). Salinity stress and fertilizers (ammonium nitrate and potassium sulphate) application positively contributed towards the enzymatic antioxidant activities (peroxidase and catalase). The maximum leaf peroxidase (POD) activity was observed by C2T1 treated plants at vegetative stage and minimum by T6 treated plant during bulb maturation stage. Conversely, a significant increase in bulb POD content was observed at bulb maturation stage compared to the vegetative stage (figure 6c and 6d). The positive correlation of leaf chlorophyll content with leaf peroxidase enzyme content and a negative correlation with leaf and bulb soluble sugar content was observed. Leaf and bulb diameter had positive correlations with plant fresh weight and dry weight showing that salt stress coupled with fertilizer treatments had significant effects on these morphological traits. However, leaf length was negatively correlated with bulb diameter. Similarly leaf relative water content showed highly significant positive correlation with bulb peroxidase activity (POD) but negative correlation with bulb catalase activity (CAT) and soluble sugar content.

Salinity is among the major environmental stressors affecting agriculture productivity in arid and semi-arid regions of the world. Physiological responses to excess salt in plant cells, which has adverse effects on the plant metabolism is known as salt stress. Thus, for the enhancement and development of salt tolerance it is important to systematically analyze crop behavior and tolerance mechanisms which helps in the utilization of agricultural lands in saline ecosystems (Farissi et al., 2011).

Plant life cycle is highly susceptible to abiotic stressors, such as salinity, which simultaneously induces osmotic stress and ionic toxicity (Peng et al., 2016). Under salt stress, the soil solution's osmotic pressure elevates than that of the plant cells. Therefore, plants are unable to acquire adequate water as a result (Ibrahim et al., 2019). Accumulation of salt in plant tissue has a detrimental effect on numerous metabolic processes, decrease seed germination rate, seedling growth and plant development. Ibrahim et al., 2019 revealed that germination percentage of wheat seed was positively affected by nitrogen application at moderate salinity level and remained ineffective at increased salt concentration (12 dS m^{-1}). According to Gou et al., 2020 studies, 200 mM NaCl concentration reduces the germination percentage, germination index and seedling vigor of cucumber seeds. The present study observed the effect of different germination parameters on seeds grown under salinity stress. It was observed that fertilizer application mitigates the adverse effect of salinity stress and significantly increases the

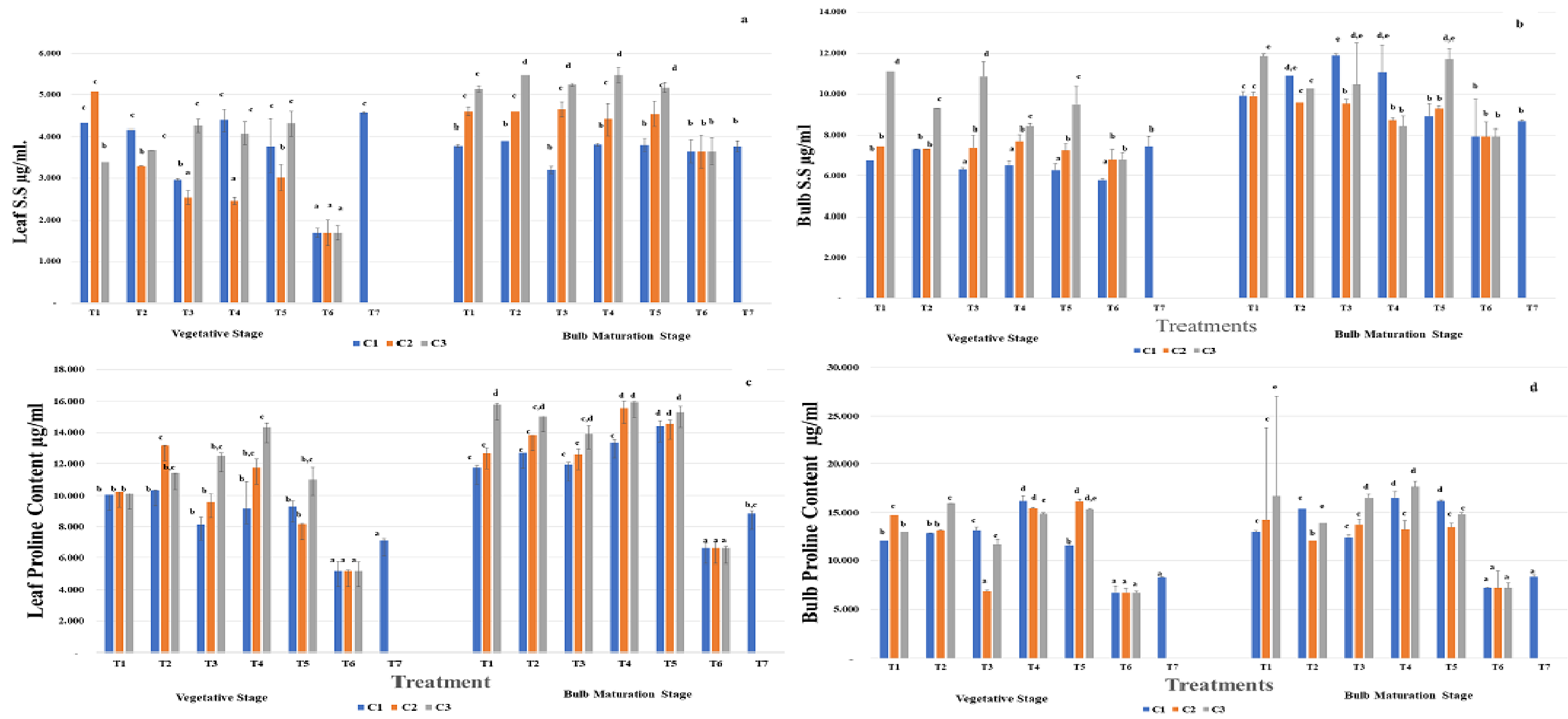


Figure 5. Soluble Sugar and proline content (µg/mL) of *Allium cepa* L. (a) leaf soluble sugar content (b) Bulb soluble sugar content (c) Leaf proline content (d) bulb proline content Keynotes: C1= 4.5dS/m, C2= 8.5dS/m, C3= 12.5dS/m, T1= NH_4NO_3, T2= K_2SO_4, T3= NH_4NO_3:K_2SO_4, T4= K_2SO_4> NH_4NO_3, T5= NH_4NO_3 > K_2SO_4, T6= control, T7= NaCl.

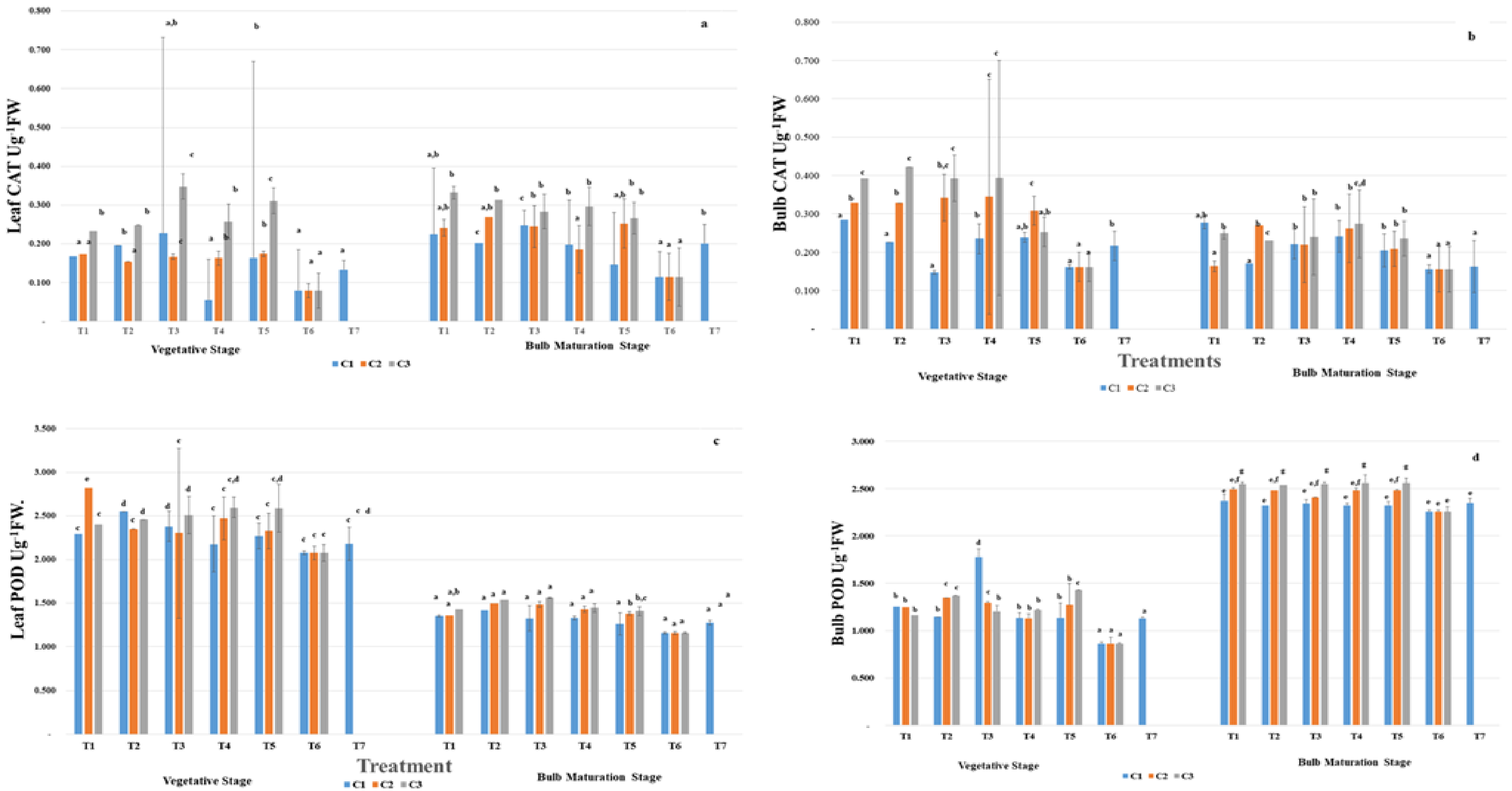


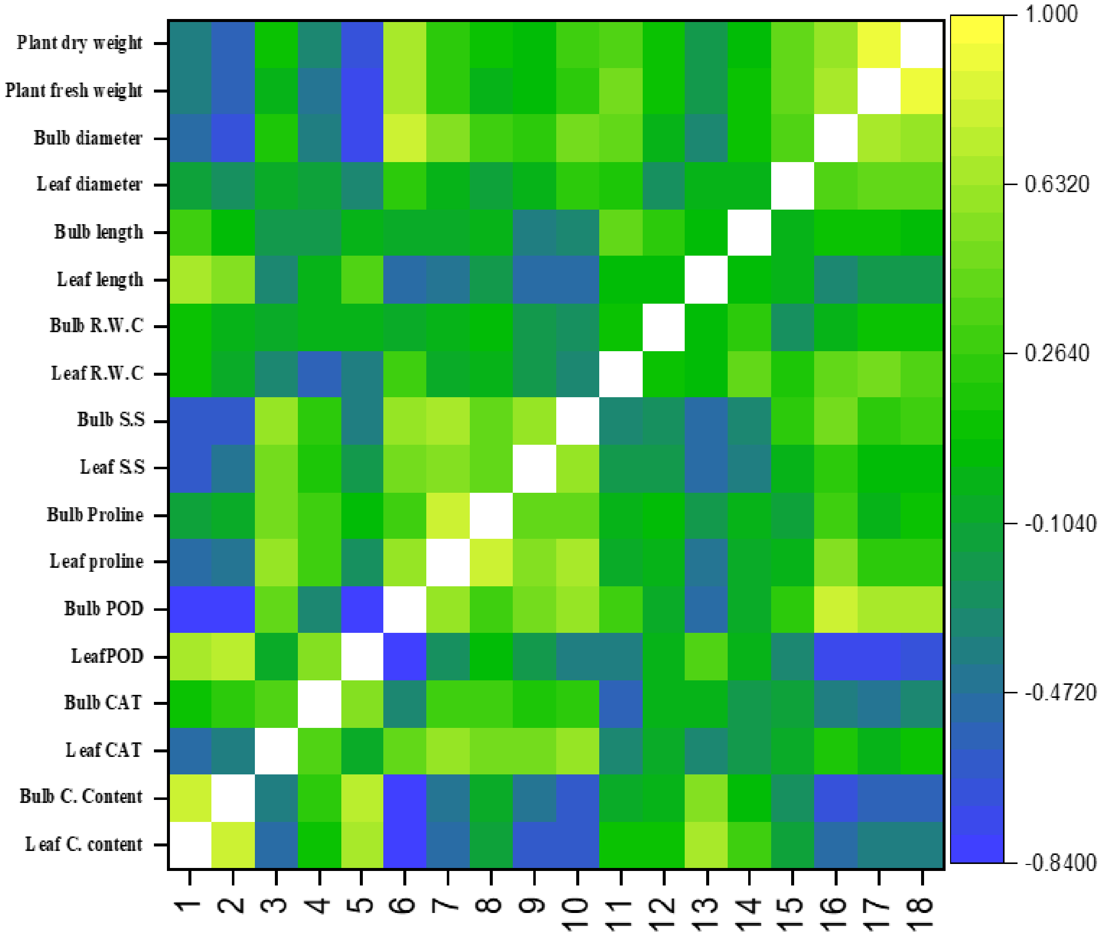
Figure 6. Enzymatic antioxidant activity of *Allium cepa* L. measured at vegetative stage and bulb stage (a) leaf catalase activity (b) bulb catalase (c) leaf peroxidase (d) bulb peroxidase.Keynotes: C1= 4.5dS/m, C2= 8.5dS/m, C3= 12.5dS/m, T1= NH_4NO_3, T2= K_2SO_4, T3= NH_4NO_3:K_2SO_4, T4= K_2SO_4> NH_4NO_3, T5= NH_4NO_3 > K_2SO_4, T6= control, T7= NaCl.

speed of germination, final germination percentage and germination energy percentage. These findings indicated that ammonium nitrate and potassium sulphate regulated positive comeback of seed germination in response to stress conditions. Potassium played a considerable vital role in nitrate metabolism in dwarf apples (Zhu et al., 2020). Fertilizer application amidst the most important factors affecting crop development and productivity. Use of optimum concentration of fertilizer may increase plant Production, reduce salt stress, and stimulate plant development (Zhu et al., 2020). In the present study plants treated with ammonium nitrate and potassium sulphate fertilizer under 4.5 (dS m^{-1}) salinity treatment showed maximum increase in plant growth and biomass as compared to the plants with no fertilizer treatment. Research had shown that the appropriate application of fertilizer can reduce the growth inhibition and negative effects brought on by salt stress (Jayakumar et al., 2014).

The presence of nitrogen reduces the accumulation of Na^+ and increases the absorption of nitrates from soil into the plant tissues. Results of physiological parameters demonstrated that soil relative water content before and after stress increased in soil irrigated with saline water (4.5 dS m^{-1}) after application of NH_4NO_3. Nitrate assisted hydraulic capacity of soil and water enhanced plant growth under salt stress condition (Hussain et al., 2022) The increase of the level of salts in the soil can affect water and nutrient absorption, resulting in a metabolic imbalance that can lead to the reduction of plant growth and production (Azeem et al., 2023). In the present study, maximum increase in leaf and bulb relative water content was observed by potassium fertilizer than nitrogen at early vegetative stage. Whereas treatment containing equal ratio of both fertilizers (ammonium nitrate and potassium sulphate) showed prominent effectiveness at bulb stage. Thus, it can be assumed that potassium acts as the main activator of several enzymes involved in sugar transport, protein synthesis, photosynthesis, and N metabolism. Thus, play an important role in growth of cell with improved development (Oosterhuis et al., 2014).

Photosynthetic pigments mainly chlorophyll content of leaf and bulb increased in T3 (NH_4NO_3= K_2SO_4) treatment at both growing stages. However, plants growing in T7 treatment with different salt levels illustrated low level increase in all physiological parameters. Ahmad et al., 2019 reported that inorganic nutrients supplementation like K^+ helps to mitigate detrimental effects of the salt on growth of plant by increasing photosynthetic activities thus improving salt tolerance in *Brassica oleracea* L.

Fertilizers could enhance the positive osmotic potential of soil, that results in enhanced stomatal aperture, photosynthetic rate, osmotic chemicals (Proline, Malondialdehyde (MDA), soluble sugar) and improves the water absorption Kadavifci et al., 2005. The biochemical parameters showed an upsurge in salt concentration caused increase in soluble sugar and proline content of *Allium cepa* L. leaf and bulb. According to Shoaib et al., 2018 among the onion growth phases, bulb phase is the most vulnerable to water and salt stress, with detrimental effects on both fresh weight and product quality. Moreover, in addition to weakening the plant and impacting its physiology directly, salt can make the plant more susceptible to infections (Abdissa and Tekalign, 2011). Salinity has a detrimental impact on the average bulb's morphology, weight, diameter, and consistency. In the present study fertilizer application helps to regulate these non-enzymatic metabolites. salt stressed soils, leads to the accumulation of soluble sugars compared to nitrogen deficient. An increased proline content was also observed in the present study with increased



According to Hussain et al., 2022 sufficient supply of nitrogen alter the detoxification of ratio of nitrogen fertilizer under stress conditions.

Figure 7: Pearson Correlation analysis among different morpho-physiological and biochemical parameters under different salt concentrations and fertilizer treatments.

Keynote: LCC-leaf chlorophyll content, BCC-bulb chlorophyll content, L(CAT)-leaf catalase, B(CAT)-bulb catalase , LP-leaf peroxidase (POD), BP-bulb peroxidase (POD), LP-leaf proline, BP-bulb proline, LSS-leaf soluble sugar, BSS-bulb soluble sugar, LRWC-leaf relative water content, BRWC-bulb relative water content, LL-leaf length, BL-bulb length, LD-leaf diameter, BL-bulb diameter, PFW-plant fresh weight, PDW-plant dry weight, SWRCBS-soil relative water content after stress, SWRCAS-soil relative water content after stress

Fertilizer treatments considerably enhanced the levels of osmotic substances and anti-oxidative enzymes. Likewise, Ibrahim et al., 2019 observed an increment in POD and CAT activities of wheat seedlings in response to salinity stress and nitrogen fertilizer level. It can be concluded that elevated antioxidant enzymes may reduce the harm of reactive oxygen species (ROS) and other metabolic activities.

In addition to the morphological, physiochemical, and biochemical parameters, fertilizers also boosted biomass yield of onion in the saline soils. Messina et al., 2021 observed that use of N fertilization on onion enhanced onion crop production. Nitrogen application reduces Na^+ ion and increase accumulation of K^+ increasing crop production (Sikder et al., 2022) Potassium competes with excessive sodium (Na^+), maintain water balance, enhance antioxidant enzymatic attributes, increase chlorophyll, polyphenol, and flavonoid content, and ultimately increase crop yield under saline stress (Abdissa et al., 2011; Saleem et al., 2022)

5 Conclusion

Salinity is among the leading factors affecting crop growth and yield. After exposure to salinity stress, defense mechanism is activated to protect plants from the oxidative stress that is causing cell death. Fertilizer application along with proper irrigation management is an assertive strategy to enhance salt tolerance and increase the production of crops. In the present findings, significant decrease in growth of onion plant compared to the control treatments indicated the adverse effects of salinity. Whereas a remarkable increase in growth, yield, antioxidant enzymatic activities and biochemical parameters of onion plants was observed through the application of various concentrations of ammonium nitrate and potassium sulphate fertilizers. Therefore, further study is needed to explore the molecular mechanism that increases the growth parameters and antioxidant enzymatic activities of plants.

Authors Contribution: Conceptualization and supervision, **NT**; **AF:** Experimental work, data analysis and original draft writing. : Assist in Implementation of Research and Analysis Research Plan. **ZA** and **AW**: Software and Statistical Analysis. **SB** and **SH**: writing, Reviewing and Editing. **NT** and **SB:** Conceptualization, Supervision, Acquisition, Correspondence, Writing and Editing. Published manuscript version have read and agreed by all authors.

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The authors declare that any artificial intelligence (AI)-assisted technologies used during the preparation of this manuscript were employed solely to improve language, grammar, readability, or formatting. All scientific content, including the study design, data collection, analysis, interpretation of results, and conclusions, was conceived, verified, and approved by the authors. The authors accept full responsibility and accountability for the accuracy, originality, and integrity of the manuscript.

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