



# Article Effect of Compost and Vermicompost Amendments on Biochemical and Physiological Responses of Lady's Finger (Abelmoschus esculentus L.) Grown under Different Salinity Gradients

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Abstract: In the coming decades, the pressure to use saline water will increase as most of the natural resources with good water quality are being depleted. In order to avoid more stress on the soil plant system, a better understanding of the type of amendments and their integration with the irrigational water quality of any location-specific region is essential. Utilizing salt-affected lands in the best way possible will facilitate food security for the growing human population. An experiment was conducted with the Abelmoschus esculentus L. plant, irrigated with saline water having different NaCl gradients (0, 50, 100 and 150 mM), to evaluate the biochemical and physiological responses under different salinity gradients. Additionally, the effect of compost and vermicompost amendments in soil on plant responses to the changing salinity of irrigated water was observed. The results suggested that the addition of compost and vermicompost in soil not only suppressed the adverse impact of salinity in plants but also increased soil nutrients (TKN, OC, avail. P, avail. K and avail. Ca contents). Moreover, some biochemical parameters and plant growth parameters showed better traits in such manure-amended setups. The enhancement of proline, phenol, ascorbic acid and lipid peroxidation contents in the leaves of Abelmoschus esculentus L. under high salinity levels suggests some secondary metabolite-mediated response possibly due to stress caused by soil salt accumulations. In summary, crop production could be efficiently maintained in saline water-irrigated areas after amending the soils with appropriate organic manure.

Keywords: saline water; NaCl; irrigation; compost; vermicompost; Abelmoschus esculentus L.

# 1. Introduction

Worldwide, there has been a drastic degradation in the quality of natural water resources, primarily because of over-exploitation and improper practices of water treatment and wastewater disposal [1]. Due to the limited availability of good-quality water for crop irrigation, there is pressure to utilize saline water for crop irrigation practices [2]. Irrigation with saline water requires a proper understanding in order to avoid more stress on the soil-plant system. Utilizing salt-affected lands in the best way possible will facilitate food security for the growing human population. Soil and water salinity stress influence



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). physiological, biochemical, and molecular alterations in plants, and that causes a reduction in growth and crop productivity [3,4]. Studies show that plants grown with salinity stress show alterations in physio-biochemical characteristics such as the level of proteins, proline, lipid peroxidation, glutathione reductase, catalase, superoxide dismutase, carotenoid and chlorophyll content as well as soil microbial abundance and their diversity [4–7].

Soil salinity induces two types of effects in plants, i.e., the primary effect (osmotic stress), which disturbs cellular ion balance and causes a secondary effect (specific ion toxicity) [8]. Salts like Na<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Mg<sup>2+</sup>, etc. are essential for different crops or a plants' growth and proliferation, but their elevated root zone concentration causes plant toxicity [9]. When plants are exposed to salt stress, reactive oxygen species (ROS) are generated in response to such conditions [4]. ROS, severe toxic oxygen derivatives such as  $O^{2-}$ ,  ${}^1O_2$ ,  $H_2O_2$ , and  ${}^{\bullet}OH$ , are produced when  $CO_2$  increases due to stomatal closure (because of ionic imbalance inside the plant system) [3]. Different plant species exposed to various saline regimes in different soil types show various adaptations to thrive on salt-affected soils, and the adaptation capabilities vary from species to species [4,5]. Diverse plant species have specific techniques to combat the stressful conditions caused by soil salinity. Plants function in coordination with the lower and higher complex mechanisms working in the plant system to facilitate proper plant defense mechanisms [4]. The lower complex mechanisms include antioxidant stimulation, plant hormone induction, regulation of ion uptake, compartmentalization via  $Na^+/H^+$  antiporters, osmolyte biosynthesis, and modulation in the photosynthetic pathway, whereas higher complex mechanisms include chromosomal aberrations, membrane modification, water utilization efficiency, photosynthesis, and cellular respiration maintenance [10]. In a study by [11], various activity patterns of antioxidant enzymes in response to 100 and 300 mM NaCl indicated that leaves and roots reacted differently to salt stress.

The addition of compost and vermicompost can enhance the soil quality and antioxidants by providing organic matter, micro- and macro-nutrients, humic substances, and beneficial microorganisms [12]. Organic fertilizers (in most experiments, using field practice) have a positive effect on plant yields. At the same time, it is known that their operation depends on the starting substrate; therefore, the use of this on a larger scale involves certain limitations. A study conducted by Beykkhormizi et al. [13] on the impact of a vermicompost amendment on bean plants under salinity stress revealed a reduction in plant height, leaf area, and chlorophyll with increasing salinity. However, it was noted that the vermicompost amendments significantly helped the plants combat salinity stress.

Abelmoschus esculentus L., commonly known as bhindi or lady's finger, which belongs to the family Malvaceae was used in our study. This vegetable is widely used in Indian kitchens as it can be cultivated year-round with India being the world's top producer contributing around 70% of total production [14]. As it is a short-duration crop, it is widely grown for nutrient management practices, and its seeds contain many useful mono-unsaturated fatty acids and crude proteins.

This study investigated the changes in the biochemical and physiological responses of lady finger plants grown in pots irrigated with a solution of different salinity gradients and amended with different ratios of compost and vermicompost (Tables 1 and 2). Overall, this study aims to provide a broader perspective on the impact of salinity stress and the interaction between plants and their growth medium as well as to observe the changes due to the addition of compost and vermicompost along with different concentrations of saline solution used for irrigation. Thus, the focus of this study was on studying the interaction between salt stress and plant growth to identify the diverse responses and patterns in the biochemical and physiochemical changes during the experiment.

NaCl Solution (mM)	pН	EC (dS/m)
0	6.65	0.3
50	6.82	5.2
100	6.74	10.4
150	6.64	14.8

Table 1. Table showing pH and EC of saline solution used for irrigation.

**Table 2.** Table showing the type of amendments along with the saline solution irrigated during the pot experiment.

Treatments	Mixing Ratio	Irrigated with Saline Solution (mM)						
	For compost							
T1	8 kg soil	0						
T2	8 kg soil	50						
Τ3	6 kg soil + 2 kg compost	50						
Τ4	4 kg soil + 4 kg compost	50						
T5	2 kg soil + 6 kg compost	50						
Т6	8 kg soil	100						
Τ7	6 kg soil + 2 kg compost	100						
Τ8	4 kg soil + 4 kg compost	100						
Т9	2 kg soil + 6 kg compost	100						
T10	8 kg soil	150						
T11	6 kg soil + 2 kg compost	150						
T12	4 kg soil + 4 kg compost	150						
T13	2 kg soil + 6 kg compost	150						
For vermicompost								
T14	8 kg soil	0						
T15	8 kg soil	50						
T16	6 kg soil + 2 kg vermicompost	50						
T17	4 kg soil + 4 kg vermicompost	50						
T18	2 kg soil + 6 kg vermicompost	50						
T19	8 kg soil	100						
T20	6 kg soil + 2 kg vermicompost	100						
T21	4 kg soil + 4 kg vermicompost	100						
T22	2 kg soil + 6 kg vermicompost	100						
T23	8 kg soil	150						
T24	6 kg soil + 2 kg vermicompost	150						
T25	4 kg soil + 4 kg vermicompost	150						
T26	2 kg soil + 6 kg vermicompost	150						

## 2. Material and Methods

# 2.1. Experimental Design, Treatments and Plant Material

The pot experiments were conducted at the experimental field of the Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi, India. This study employed lady finger plants (*Abelmoschus esculentus* L.) to assess their response to varying salinity gradients (NaCl salt) concerning the plant morphology and physiology. The pot experiment was carried out in triplicate for each amendment. Earthen pots 30 cm in diameter and 30 m in depth with a carrying capacity of approximately 8 kg were used in the experiment. Mature compost and vermicompost were added in different proportions and treated with different saline solutions (Tables 1 and 2). The compost was made of an organic fraction of municipal solid waste, and it was collected from the solid waste management (SWM) plant at Karsada, Varanasi. The vermicompost was made of an organic fraction of municipal solid waste in the experimental field of I.E.S.D. The initial physiochemical properties of the compost and vermicompost are given in Table 3.

	Compost	Vermicompost
pH	$7.21\pm0.02$	$7.15\pm0.12$
EC (dS/m)	$0.365\pm0.01$	$0.451\pm0.1$
TKN (%)	$1.5\pm0.15$	$1.37\pm0.12$
OC (%)	$31\pm0.52$	$32\pm0.25$
Avail. Na (mg kg $^{-1}$ )	$1561\pm0.7$	$1477 \pm 1.5$
Avail. P (mg kg <sup><math>-1</math></sup> )	$186\pm0.8$	$150\pm2.1$
Avail. K (mg kg $^{-1}$ )	$2035\pm0.25$	$1178\pm2.3$
Avail. Ca (mg kg $^{-1}$ )	$214\pm0.18$	$170\pm1.1$
C/N Ratio	$20.5\pm0.5$	$24.3\pm0.7$

**Table 3.** Initial physiochemical properties of compost and vermicompost (mean  $\pm$  SE, n = 3).

#### 2.2. Soil Physiochemical Analysis

The pH of samples was measured in the suspension of 1:5 (w/v) with a pH meter (Systronics 802, India) and electrical conductivity (EC) using a conductivity meter (Systronics 371, India). The total nitrogen (TKN) contents in the samples were determined by an automatic nitrogen analyzer instrument (Tulin KDIGB 20M, KjelFTRP & KjelDIST, India). The available P in the samples was quantified using the NaHCO<sub>3</sub> extraction method [15,16]. Available Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> in the soil were extracted, and then, concentrations were determined using a flame photometer.

## 2.3. Biochemical Parameters of Plants Grown

Biochemical analyses were conducted using fresh leaves, plugged manually at 45 and 65 days after germination, and samples were stored in a deep freezer until further analyses. Chlorophyll and carotenoid contents were determined using the standard methods [17,18]. Thiol and phenol contents were assessed by following the methods of Bray and Thorpe and Fahey et al. [19,20] respectively. Proline and ascorbic acid contents were determined by the methods of Bates et al. and Keller and Schwager [21,22], respectively. Lipid peroxidation was assessed by MDA (malondialdehyde) levels [23]. Protein content in the leaves was analyzed by following the method of Lowry et al. [24].

#### 2.4. Plant Growth Variables

Plants were randomly selected and sampled in triplicate from each treatment plot at 65 days after sowing, and root and shoot lengths, the number of leaves, and leaf area were measured [25,26]. Leaf area was computed with the help of a portable leaf area meter (Systronics 211, India). For biomass, plants were washed to remove soil particles and oven-dried at 80 °C until a constant weight was achieved. The plants were then weighed separately for biomass measurement as g plant<sup>-1</sup>. For growth indices, such as leaf area ratio (LAR), specific leaf area (SLA), leaf weight ratio (LWR), root shoot ratio (RSR), and specific leaf weight (SLW), formulae by Hunt [27] were used as shown:

LAR (cm<sup>2</sup> g<sup>-1</sup>) = Leaf area/Total biomass

LWR (g  $g^{-1}$ ) = Leaf dry weight/Total biomass

SLA ( $cm^2 g^{-1}$ ) = Leaf area/Leaf biomass

RSR (g  $g^{-1}$ ) = Root dry weight/Shoot biomass

SLW (g cm<sup>-2</sup>) = Leaf dry weight/Leaf area

#### 2.5. Scanning Electron Microscopy (SEM) Analysis

SEM of leaf samples was conducted using a Scanning Electron Microscope (Nova NanoSEM 450, Brno, Czech Republic). The leaf material was fixed in glutaraldehyde solution and washed with a series of ethanol solutions [28,29]. Samples were mounted on the specimen holder.

#### 2.6. Statistical Analysis

The data were analyzed using one-way ANOVA (analysis of variance) with the help of SPSS (version 16, Chicago, IL, USA) software. ANOVA and DMRT (Duncan's multiplerange test) were executed post hoc to test the significance of the difference between the treatments. Sigma Plot software (version 12) was used for plotting graphs. PCA (principal component analysis) was used for dimensional reduction and was carried out using ORIGIN version 2023 to evaluate physiochemical, biochemical, and growth responses concerning different treatments.

#### 3. Results

### 3.1. Soil Analysis at 0 and 45 Days

Under saline treatment, there were changes in the salt and mineral uptake, such as N, Na, P, K, and Ca, resulting in biochemical disturbances and an inhibition of plant growth. Soil parameters were analyzed at 0 days and 45 days for parameters like pH, EC, TKN, OC, and available Na, P, K, and Ca (Tables 4 and 5).

The pH values varied among different treatments at 0 and 45 days, with the lowest pH observed in T6 at both points, and the highest pH observed in T5 and T13 at 0 days and in T13 at 45 days. The addition of compost along with saline solution resulted in a significant reduction in pH in most treatments (T1, T2, T3, T4, T5, T6, T7, T8, T9, T0, T11, T12, and T13) after 45 days. Compared to 0 days, at 45 days, the EC was significantly decreased in T1 and T9, while it increased in T2, T3, T4, T5, T6, T10, T11, T12, and T13. The total nitrogen (TKN) in soil samples showed a significant increase in N contents upon the addition of compost in T3, T4, T5, T7, T8, T9, T11, T12, and T13 at 0 days when compared to the control sample, i.e., T1. However, at 45 days, there was a significant reduction in N for each treatment when compared to 0-day treatments. At 0 days, there was a significant increase in OC content in T3, T4, T5, T7, T8, T9, T11, T12 and T13 when compared to the control sample, i.e., T1. After 45 days, each of the treatments (T1 to T13) showed a significant reduction in carbon content when compared to 0 days. Adding NaCl solution resulted in a significant increase in available Na in treatments T2, T6 and T10 compared to the control (T1), but all treatments showed a significant reduction in Na content at 45 days compared to 0 days. For available P, K and Ca, some treatments (T3, T4, T5, T7, T8, T9, T11, T12, and T13) showed a significant increase at 0 days compared to the control (T1), but all treatments showed a significant reduction in these parameters at 45 days compared to their respective 0-day samples.

The lowest pH was observed in T19, whereas the highest was in T14 at 0 and 45 days. Upon comparing the treatments at 45 days with those at 0 days, T14 showed no significant change in pH, while T15, T16, T17, T18, T20, T21, and T22 showed a significant increase in pH, and T19, T23, T24, T25 and T26 showed a significant decrease. T14 had the lowest EC at both 0 and 45 days. However, when comparing the treatments 45 days with those at 0 days, all treatments at 45 days showed a significant increase in EC. At 0 days, T16, T17, T18, T20, T21, T22, T24, T25 and T26 had a significant increase in TKN and OC, whereas T15, T19 and T23 were similar to T14. However, at 45 days, all the treatments showed a significant reduction in TKN and OC compared to 0 days. At 0 days, T15, T19 and T23 had a significant increase in available Na compared to T14, while T16, T17, T18, T20, T21, T22, T24, T25 and T26 had a taginificant at 45 days compared to 0 days. At 0 days T15, T19, and T23 samples were similar to T14 in terms of available P, K, and Ca, whereas T16, T17, T18, T20, T21, T22, T24, T25, and T26 showed a significant increase. But at 45 days, all treatments showed a significant reduction in available P, K, and Ca compared to those at 0 days.

Treatments	pН	EC (dS/m)	TKN (%)	OC (%)	Avail. Na (mg kg <sup>-1</sup> )	Avail. P (mg kg <sup>-1</sup> )	Avail. K (mg kg <sup>-1</sup> )	Avail. Ca (mg kg <sup>-1</sup> )	
At 0 day									
T1	$7.19\pm0.4~^{ m bc}$	$0.543\pm0.0~^{\rm e}$	$0.66\pm0.0$ $^{ m e}$	$19\pm0.1~^{\mathrm{e}}$	$224\pm1.8\ ^{\rm m}$	$55\pm1.5$ <sup>h</sup>	$146\pm2.2$ <sup>m</sup>	$52\pm1.7~^{ m i}$	
T2	$7.44\pm0.0~^{ m ab}$	$0.896\pm0.0$ <sup>a</sup>	$0.79\pm0.0$ <sup>d</sup>	$19\pm0.1~^{ m e}$	$659 \pm 1.17^{1}$	$59\pm1.3$ <sup>h</sup>	$411\pm1.7~^{\rm i}$	$64\pm2.3$ <sup>h</sup>	
Т3	$7.73\pm0.0$ a	$0.750 \pm 0.0 \ ^{ m bc}$	$0.98\pm0.0~^{ m c}$	$26\pm0.1$ <sup>d</sup>	$784\pm1.0~^{ m j}$	$129\pm7.1$ <sup>d</sup>	$794\pm0.8$ f	$119\pm2.9~^{ m e}$	
<b>T4</b>	$7.78\pm0.0$ $^{\rm a}$	$0.420\pm0.0~^{\rm f}$	$1.09\pm0.0$ <sup>b</sup>	$27\pm0.0~^{ m bc}$	1031 $\pm$ 2.8 $^{ m f}$	$101\pm0.7~^{ m e}$	$1171\pm4.0$ <sup>d</sup>	$168\pm3.6~^{ m c}$	
T5	$7.82\pm0.1$ <sup>a</sup>	$0.263\pm0.0$ g	$1.21\pm0.0$ <sup>a</sup>	$29\pm0.0~^{a}$	$1430\pm6.6~^{\rm b}$	$180\pm0.7~^{\mathrm{a}}$	$2047\pm5.7$ <sup>a</sup>	$218\pm3.0~^{a}$	
<b>T6</b>	$6.18\pm0.0$ <sup>d</sup>	$0.800\pm0.0$ <sup>b</sup>	$0.67\pm0.0~{ m e}$	$19\pm0.1~^{ m e}$	$889\pm3.2~^{ m i}$	$75\pm0.7~{ m g}$	$182\pm0.5$ k	$55\pm0.2~^{ m i}$	
<b>T7</b>	$6.21\pm0.0$ d	$0.699\pm0.0~^{ m cd}$	$0.79\pm0.0$ <sup>d</sup>	$27\pm0.0$ c	$928\pm4.9$ h	$135\pm0.7~^{ m cd}$	$286\pm0.8$ <sup>j</sup>	$86\pm1.5~^{ m f}$	
<b>T8</b>	$6.47\pm0.0$ <sup>d</sup>	$0.638\pm0.0$ <sup>de</sup>	$0.98\pm0.0~^{ m c}$	$27\pm0.0$ <sup>b</sup>	$994\pm10.8~{ m g}$	$101\pm0.7~{ m e}$	$534\pm0.8$ <sup>h</sup>	$75\pm2.2~{ m g}$	
Т9	$6.88\pm0.0\ ^{ m c}$	$0.572\pm0.0$ $^{\mathrm{e}}$	$1.13\pm0.0~^{ m ab}$	$29\pm0.0~^{a}$	$1361\pm0.8~^{ m c}$	$136\pm3.6~^{\mathrm{cd}}$	$824\pm2.9~e^{e}$	$112\pm1.9~{ m e}$	
T10	$7.55\pm0.0$ $^{ m ab}$	$0.832\pm0.0$ $^{ m ab}$	$0.60\pm0.0~{ m e}$	$19\pm0.1~^{ m e}$	$1103\pm2.5~^{\rm e}$	$87\pm0.7~^{ m f}$	$161 \pm 0.1^{11}$	$49\pm1.0~^{ m i}$	
T11	$7.73\pm0.0$ <sup>a</sup>	$0.615\pm0.0~^{ m de}$	$0.81\pm0.0$ <sup>d</sup>	$26\pm0.1$ <sup>d</sup>	$735\pm0.4$ $^{ m k}$	$139\pm3.6~^{ m c}$	$738\pm1.4~{ m g}$	$75\pm1.8~{ m g}$	
T12	$7.73\pm0.0$ $^{\rm a}$	$0.574\pm0.0~^{\rm e}$	$0.95\pm0.0$ $^{\rm c}$	$27\pm0.1~^{ m bc}$	$1251\pm3.0$ <sup>d</sup>	$131\pm0.7~^{ m cd}$	$1192\pm3.9~^{\rm c}$	$130\pm1.4$ <sup>d</sup>	
T13	$7.82\pm0.0$ $^{\rm a}$	$0.548\pm0.0\ ^{\rm e}$	$1.19\pm0.0~^{a}$	$29\pm0.0~^{a}$	$1534\pm1.7$ a	$161\pm0.7~^{ m b}$	$1667\pm0.8$ <sup>b</sup>	$191\pm2.8$ <sup>b</sup>	
				At 4	5 days				
T1	$7.07\pm0.0$ bc	$0.381 \pm 0.0^{1}$	$0.54\pm0.0~^{\rm e}$	$19\pm0.1~^{c}$	$176\pm0.3~{ m f}$	$34\pm0.7^{~j}$	$55\pm0.2$ k	$51\pm0.2$ h	
T2	$6.76\pm0.3$ <sup>c</sup>	$0.908\pm0.0~{\rm g}$	$0.72\pm0.0$ <sup>d</sup>	$19\pm0.3~^{ m c}$	$458\pm4.6~^{ m de}$	$41\pm0.7~^{ m i}$	$282\pm0.1$ h	$74\pm0.1~{ m g}$	
T3	$6.78\pm0.0\ ^{ m c}$	$0.783\pm0.0~^{\mathrm{i}}$	$0.89\pm0.0~^{ m c}$	$24\pm2.2$ <sup>b</sup>	$384\pm0.6~{ m e}$	$85\pm1.5$ f	$364\pm0.1~{ m g}$	$81\pm0.3~^{ m fg}$	
<b>T</b> 4	$6.99\pm0.0~\mathrm{^{bc}}$	$0.694 \pm 0.0~^{ m j}$	$1.00\pm0.0$ <sup>b</sup>	$27\pm0.0$ a	$414\pm5.7~{ m e}$	$84\pm0.7~^{ m f}$	$776\pm0.1$ <sup>d</sup>	$101\pm0.1~{ m e}$	
T5	$7.12\pm0.0~^{ m abc}$	$0.566\pm0.0$ $^{ m k}$	$1.16\pm0.0$ a	$28\pm0.0$ <sup>a</sup>	$595\pm1.4$ <sup>b</sup>	$107\pm5.5$ d	$1145\pm1.2$ a	$141\pm1.2~^{ m bc}$	
<b>T6</b>	$6.11\pm0.0$ <sup>d</sup>	$2.171\pm0.0$ $^{\rm a}$	$0.60\pm0.0~{ m e}$	$20\pm0.2$ c	$622\pm2.8~^{\mathrm{b}}$	$63\pm0.7$ g	$104\pm1.6~^{ m j}$	$58\pm1.2$ <sup>h</sup>	
<b>T7</b>	$6.26\pm0.0$ <sup>d</sup>	$1.163\pm0.0$ $^{\rm c}$	$0.71\pm0.0$ <sup>d</sup>	$26\pm0.0$ <sup>a</sup>	$528\pm0.8~^{ m bcd}$	$97\pm0.7~^{ m e}$	$204\pm3.7~^{ m i}$	$86\pm2.8~^{ m f}$	
<b>T8</b>	$6.75\pm0.0$ <sup>c</sup>	$1.001\pm0.0$ <sup>d</sup>	$0.88\pm0.0~^{ m c}$	$27\pm0.0$ <sup>a</sup>	$561\pm1.3~^{ m bc}$	$88\pm1.3~^{ m f}$	$374\pm1.0~{ m g}$	$145\pm3.0~^{ m b}$	
Т9	$6.82\pm0.0$ <sup>c</sup>	$0.373 \pm 0.0^{1}$	$0.98\pm0.0$ <sup>b</sup>	$28\pm0.0$ $^{a}$	$790\pm114.2~^{\rm a}$	$113\pm2.1~^{ m c}$	$726\pm3.2~^{\rm e}$	$165\pm1.4$ <sup>a</sup>	
T10	$7.05\pm0.0~\mathrm{^{bc}}$	$1.32\pm0.0$ <sup>b</sup>	$0.64\pm0.0~^{ m e}$	$19\pm0.0~^{ m c}$	$828\pm2.2$ a	$55\pm2.3$ h	$116\pm0.5$ $^{ m j}$	$84\pm3.9~^{ m f}$	
T11	$7.26\pm0.0$ $^{\mathrm{ab}}$	$0.957\pm0.0~^{\rm e}$	$0.72\pm0.0$ <sup>d</sup>	$27\pm0.2$ a	$468\pm3.5~^{ m cde}$	$133\pm1.5$ <sup>b</sup>	$544\pm2.1~^{ m f}$	$122\pm1.7$ d	
T12	$7.35\pm0.0$ $^{\mathrm{ab}}$	$0.938\pm0.0~^{\rm f}$	$0.89\pm0.0$ <sup>c</sup>	$27\pm0.0~^{a}$	$592\pm0.3$ <sup>b</sup>	$85\pm1.5~{ m f}$	$950\pm1.6$ <sup>b</sup>	$136\pm5.4~^{ m c}$	
T13	$7.45\pm0.0$ a	$0.851\pm0.0$ h	$1.00\pm0.0$ <sup>b</sup>	$28\pm0.0~^{a}$	$766\pm2.0$ <sup>a</sup>	$155\pm1.3$ a	$857\pm1.0~^{ m c}$	$138\pm3.1~^{ m bc}$	

**Table 4.** Soil parameters were analyzed at 0 days and 45 days for each treatment. Letters in each group show a significant difference at p < 0.05 (mean  $\pm$  SE, n = 3).

Treatments	pН	EC (dS/m)	TKN (%)	OC (%)	Avail. Na (mg kg $^{-1}$ )	Avail. P (mg kg $^{-1}$ )	Avail. K (mg kg <sup>-1</sup> )	Avail. Ca (mg kg <sup>-1</sup> )
				At	) days			
T14	$7.54\pm0.1$ a	$0.289\pm0.0$ <sup>j</sup>	$0.66\pm0.0$ $^{ m e}$	$19\pm0.1~^{ m g}$	$221\pm0.8^{ ext{ l}}$	$38.3\pm1.3$ <sup>h</sup>	$107\pm0.5~^{ m i}$	$99\pm2.6$ g
T15	$6.81\pm0.0~^{ m cde}$	$1.591\pm0.0$ $^{\rm a}$	$0.66\pm0.0~{ m e}$	$20\pm0.1~^{ m e}$	$596\pm2.2$ $^{ m k}$	$55\pm2.8$ $^{ m g}$	$377\pm0.8$ f	$102\pm 6.3~^{\mathrm{fg}}$
T16	$6.95\pm0.0~^{ m bcd}$	$1.163\pm0.0$ <sup>d</sup>	$0.87\pm0.0$ <sup>d</sup>	$28\pm0.3~^{ m cd}$	$982\pm0.6~{ m g}$	$118\pm0.7$ <sup>d</sup>	$551\pm1.1~^{ m e}$	$129\pm0.6$ <sup>d</sup>
T17	$7.11\pm0.0~^{ m bc}$	$0.776\pm0.0~{ m f}$	$0.96\pm0.0~^{ m c}$	$28\pm0.0~^{c}$	$1236\pm2.0$ d	$107\pm0.7~^{ m e}$	$1000\pm0.8$ d	$151\pm2.3$ <sup>b</sup>
T18	$7.13\pm0.0~^{ m bc}$	$0.446\pm0.0$ <sup>h</sup>	$1.33\pm0.0$ a	$29\pm0.0$ a	$1461\pm1.3$ <sup>b</sup>	$127\pm1.5~^{ m c}$	$1163\pm2.9~^{ m c}$	$169\pm3.3$ a
T19	$6.57\pm0.2~{ m e}$	$1.318\pm0.0$ <sup>b</sup>	$0.62\pm0.0~{ m e}$	$19\pm0.1~^{ m f}$	$792\pm2.8$ <sup>j</sup>	$67\pm0~{ m f}$	$127\pm0.5$ h	$109\pm2.4~{ m ef}$
T20	$6.61\pm0.1~^{ m de}$	$0.601\pm0.0~{\rm g}$	$0.81\pm0.0$ <sup>d</sup>	$28\pm0.0$ <sup>d</sup>	$875\pm1.9$ h	$140\pm2.8~^{ m b}$	$227\pm1.1~{ m g}$	$117\pm3.2~^{ m e}$
T21	$6.63\pm0.1$ <sup>de</sup>	$0.411\pm0.0$ <sup>h</sup>	$1.02\pm0.0~^{ m c}$	$28\pm0.0$ <sup>b</sup>	$1044\pm2.0~^{ m f}$	$115\pm1.5$ <sup>d</sup>	$377\pm0.8~{ m f}$	$118\pm3.1~^{ m e}$
T22	$7.17\pm0.1$ <sup>b</sup>	$0.364\pm0.0~^{ m i}$	$1.35\pm0.0$ <sup>a</sup>	$29\pm0.0~^{a}$	$1312\pm2.8~^{\rm c}$	$149\pm2.1$ a	$551\pm1.1~^{ m e}$	$138\pm2.9~^{ m c}$
T23	$6.77\pm0.1~^{ m cde}$	$1.556\pm0.0$ $^{\rm a}$	$0.62\pm0.0~^{ m e}$	$19\pm0.1~^{ m f}$	$841\pm1.1~^{\rm i}$	$55\pm1.3$ $^{ m g}$	$1000\pm0.8$ <sup>d</sup>	$110\pm1.7~{ m ef}$
T24	$6.83\pm0.0~^{ m bcde}$	$1.243\pm0.0~^{ m c}$	$0.85\pm0.0$ <sup>d</sup>	$28\pm0.0~^{ m cd}$	$983\pm2.9$ g	$138\pm1.5$ <sup>b</sup>	$1163\pm2.9~^{ m c}$	$141\pm3.4~^{ m c}$
T25	$7.03\pm0.0~\mathrm{^{bc}}$	$0.968\pm0.0~^{\rm e}$	$1.15\pm0.0$ <sup>b</sup>	$28\pm0.0$ <sup>b</sup>	$1128\pm3.7~^{\rm e}$	$120\pm1.5$ <sup>d</sup>	$1267\pm3.1$ <sup>a</sup>	$143\pm1.2~^{ m bc}$
T26	$7.04\pm0.0~^{ m bc}$	$0.566\pm0.0~^{\rm g}$	$1.32\pm0.0$ $^{\rm a}$	$29\pm0.0~^{a}$	$1535\pm4.0$ a	$135\pm3.4$ <sup>b</sup>	$1188\pm7.6$ $^{\rm b}$	$140\pm1.6~^{\rm c}$
				At 4	5 days			
T14	$7.54\pm0.0$ <sup>a</sup>	$0.425\pm0.0~^{\rm hi}$	$0.60\pm0.0~{ m g}$	$19\pm0.1~^{ m g}$	$221\pm0.7^{1}$	$41\pm2.1~^{ m i}$	$108\pm0.2$ k	$94\pm2.8~^{ m ef}$
T15	$7.1\pm0.1~^{ m cde}$	$1.803\pm0.0$ a	$0.65\pm0.0$ h	$19\pm0.1~^{ m f}$	$318\pm0.5$ k	$43\pm1.5$ hi	$228\pm0.1~^{ m i}$	$83\pm3.4~{ m g}$
T16	$7.14\pm0.0~\mathrm{cd}$	$1.346\pm0.0$ <sup>c</sup>	$0.79\pm0.0$ ef	$28\pm0.0~^{ m de}$	$984\pm3.4$ d	$107\pm2.7~^{ m c}$	$365\pm0.2~{ m g}$	$110\pm4.2~^{ m c}$
T17	$7.21\pm0.0~^{ m bc}$	$1.008\pm0.0~^{\rm e}$	$0.90\pm0.0~{ m def}$	$28\pm0.0~^{ m cd}$	$882\pm0.2~{ m e}$	$90\pm1.5$ d	$794\pm1.4$ <sup>d</sup>	$99\pm1.4~^{ m e}$
T18	$7.33\pm0.0^{\text{ b}}$	$0.561\pm0.0~{\rm g}$	$1.28\pm0.0~^{ m bc}$	$29\pm0.0$ a	$1017\pm3.8~^{\rm c}$	$200\pm3.1~^{\mathrm{a}}$	$1046\pm1.2~^{a}$	$132\pm0.4$ a
T19	$6.44\pm0.0$ <sup>h</sup>	$1.213\pm0.0$ <sup>d</sup>	$0.54\pm0.0$ <sup>h</sup>	$19\pm0.0~{ m g}$	$746\pm4.5~^{ m i}$	$43\pm2.3$ <sup>hi</sup>	$111\pm0.2$ $^{ m k}$	$54\pm0.9$ h
T20	$6.85\pm0.0~^{\rm f}$	$0.852\pm0.0$ f	$0.67\pm0.0$ <sup>ef</sup>	$28\pm0.0~^{ m de}$	$760\pm5.7$ $^{ m h}$	$73\pm0.7~^{ m e}$	$181\pm1.8~^{ m j}$	$60\pm1.9$ h
T21	$6.93\pm0.0$ <sup>ef</sup>	$0.979\pm0.0~^{\rm e}$	$0.95\pm0.0~^{ m cd}$	$28\pm0.0$ <sup>b</sup>	$818\pm2.4$ g	$57\pm2.7~{ m g}$	$310\pm2.9$ h	$90\pm2.7~^{\mathrm{fg}}$
T22	$7.33\pm0.0^{\text{ b}}$	$0.380\pm0.0~^{\rm k}$	$1.20\pm0.0~^{ m ab}$	$29\pm0.0~^{a}$	$1077\pm1.8$ <sup>b</sup>	$65\pm3.9~{ m f}$	$533\pm4.4$ f	$124\pm1.1$ <sup>b</sup>
T23	$6.37\pm0.0$ <sup>h</sup>	$0.932\pm0.0$ $^{ m ef}$	$0.57\pm0.0$ <sup>h</sup>	$19\pm$ 0.0 $^{ m f}$	$686 \pm 5.5^{ j}$	$50\pm2.3~{ m gh}$	$643\pm2.8~^{\mathrm{e}}$	$88\pm1.3~^{ m fg}$
T24	$6.63\pm0.0$ g	$1.158\pm0.0$ <sup>d</sup>	$0.78\pm0.0$ $^{ m f}$	$28\pm0.0~^{\rm e}$	$854\pm1.4~{ m f}$	$111\pm3.4~^{ m c}$	$883\pm2.1~^{ m c}$	$93\pm1.3~{ m ef}$
T25	$6.87\pm0.0~{ m f}$	$1.621\pm0.0$ <sup>b</sup>	$0.99\pm0.0~^{ m cde}$	$28\pm0.0~^{ m bc}$	$981\pm1.1$ d	$87\pm2.1$ d	$928\pm14.8$ <sup>b</sup>	$104\pm4.7~\mathrm{cd}$
T26	$6.97\pm0.0~\mathrm{def}$	$0.507\pm0.0~\mathrm{gh}$	$1.20\pm0.0$ a	$29\pm0.0$ a	$1284\pm1.1$ a	$122\pm1.3$ <sup>b</sup>	$1055\pm10.2$ a	$133\pm1.8$ a

**Table 5.** Soil parameters were analyzed at 0 and 45 days for each treatment. Letters in each group show a significant difference at p < 0.05 (mean  $\pm$  SE, n = 3).

#### 3.2. Biochemical Evaluation of Plants

Biochemical parameters were analyzed at 45 and 65 days of the plant growth (Figure 1). The total chlorophyll content was significantly reduced at 45 and 65 days in T2 (50% at 45 days, 15% at 65 days), T6 (46% at 45 days, 12% at 65 days), T10 (47.7% at 45 days, 9% at 65 days) and T11 (48% at 45 days, 17% at 65 days) when compared to the control (T1); however, T3, T4 T5, T7, T8, T9, T12, and T13 showed higher chlorophyll content than T2, T6, T10, and T11. T15 (27% at 45 days, 9% at 65 days) and T19 (18% at 45 days, 6% at 65 days) had a significant reduction in chlorophyll content compared to the control (T14), while T22 (15%), and T26 (19.6%) showed a significant increase at 45 days but decreased at 65 days. Carotenoid content was highest in the control (T1) and significantly reduced in T2 (58%), T6 (47%), T10 (50%) and T11 (51%), at 45 days, and there was a reduction in carotenoid content in all treatments at 65 days. The carotenoid content of T14 was the highest at 45 days, while it was reduced in T15 (61% at 45 days, 42% at 65 days), T16 (45% at 45 days), T19 (56% at 45 days, 42% at 65 days) and T24 (57% at 45 days, 43% at 65 days) compared to the control (T14). The phenol content was lowest in T1, but it increased significantly in T10 (80% at 45 days, 77% at 65 days) and T23 (254% at 45 days, 354% at 65 days) compared to T1 and T14. The proline content was lowest in T1 and highest in T10 (567% at 45 days, 563% at 65 days) and T23 (438% at 45 days, 470% at 65 days) compared to T1 and T14. The ascorbic content was lowest in T1 and highest in T10 (58% at 45 days, 52% at 65 days) and T23 (51.8% at 45 days and 51.6% at 65 days) compared to T1 and T14. The protein content was highest in T1 and T4 and lowest in T10 (32% at 45 days, 29% at 65 days) for both 45 and 65 days. T18 had the highest protein content (13% increase at 45 days, 10% increase at 65 days), while T23 had the lowest (24% at 45 days and 65 days). Lipid peroxidation was lowest in T4 (5% reduction) at 45 days and T5 (3% reduction) at 65 days and highest in T10 (increase of 106% at 45 days, 84% at 65 days) when compared to T1. T16 showed a significant reduction in lipid peroxidation (36%) compared T14 at 45 days, and T23 had the highest lipid peroxidation (47%) at 65 days. The thiol content was lowest in T10 (18%)at 45 and 65 days) and T13 (18% at 45, 15% at 65 days) and highest in T8 (21% at 45, 22% at 65 days) compared to T1. T21 had the highest thiol content (16% at 45 days and 10% at 65 days), while T24 had the lowest (17% at 45 days, 20% at 65 days) compared to T14.

#### 3.3. Plant Growth, SEM and PCA Analysis

Plant growth parameters such as leaf area ratio (LAR), specific leaf area (SLA), leaf weight ratio (LWR), root shoot ratio (RSR), and specific leaf weight (SLW) were assessed at 65 days under salt stress (Table 6). The results showed that LAR was significantly decreased at T2, T6 and T10 compared to the control group (T1). LWR was similar in T1, T3 and T4 but significantly decreased in T2, T6, T8, T9, T10 and T12. Moreover, SLA was significantly increased in T8 and T11 treatments compared to the control. RSR was found to be significantly reduced in decreased at T2, T6 and T10 treatments compared to the control, while SLW was decreased in T8 and T11 treatments.

Compared to control T14, LAR was found to decrease significantly in T15 and T19, which was due to a decrease in leaf area. LWR was found to increase significantly in T16, T17 and T20 treatments. SLA was found to decrease significantly in T15, T17, T20 and T22 treatments. RSR was found to decrease significantly in T15, T19, T21, and T23 treatments compared to control T14. Furthermore, SLW was found to decrease in T21, T23 and T24 treatments.

To observe the effects of salt stress on the stomata on the abaxial side of leaves of the lady finger plants, SEM analysis was conducted on different samples including T1 (0 mM), T2 (50 mM), T5 (100 mM), and T9 (150 mM) (Figure 2A–D). The results indicated that T1 had bean-shaped stomata which were open and unaffected, whereas T2 showed damaged and shrunken stomata due to salt stress. T3 and T4 were highly affected by salt stress and showed salt exclusion through stomata. In a study, the negative effect of salinity on stomata was observed in *Dianthus caryophyllus* [30].









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**Figure 1.** Biochemical parameters were analyzed at 45 and 65 days (mean  $\pm$  SE, n = 3). Bars with different letters in each group show a significant difference at *p* < 0.05.



**Figure 2.** SEM images showing abaxial side of lady finger leaves at different salinity gradients for the treatments T1, T2, T5, and T9: (**A**) control; (**B**) 50 mM; (**C**) 100 mM; (**D**) 150 mM).

Also, PCA analysis was conducted for treatments T1 to T13 (Figure 3), which revealed that PC1 (variance = 41.04%, eigenvalue = 8.61) had dominant variables including pH, TKN, OC, available P, K, and Ca, carotenoid, chlorophyll, protein, thiol, LAR, LWR, RSR, and SLW. Similarly, PC2 (variance = 20.31%, eigenvalue = 4.26) had dominant variables such as pH, EC, TKN, OC, available Na, P, K, and Ca, phenol, ascorbic, lipid peroxidase, proline, SLA, and RSR. Another PCA analysis was performed for T14 to T26 (Figure 4), which showed that PC1 (variance = 45.07%, eigenvalue = 9.46) had dominant variables including pH, TKN, OC, available P, K, and Ca, carotenoid, chlorophyll, protein, thiol, LAR, LWR, RSR, and SLW. Similarly, PC2 (variance = 19.89%, eigen value = 4.17) had dominant variables including TKN, OC, available Na, P, K, and Ca, phenol, ascorbic, lipid peroxidase, proline, LAR, SLA, and RSR.

Treatments	LAR (cm <sup>2</sup> g <sup><math>-1</math></sup> )	LWR (g $g^{-1}$ )	SLA (cm <sup>2</sup> g <sup><math>-1</math></sup> )	RSR (g $g^{-1}$ )	SLW (g cm $^{-2}$ )
T1	$6.62\pm0.17~^{\rm a}$	$0.044\pm0.002~^{\mathrm{ab}}$	$151.76\pm3.70$ $^{\rm a}$	$0.248 \pm 0.019~^{\rm c}$	$0.0065 \pm 0.0001$
T2	$4.73\pm0.06$ <sup>d</sup>	$0.037 \pm 0.000$ <sup>bcd</sup>	$128.90\pm4.19\ ^{\mathrm{ab}}$	$0.166 \pm 0.019$ <sup>d</sup>	$0.0077 \pm 0.0002$ <sup>b</sup>
<b>T3</b>	$6.32\pm0.36$ $^{\mathrm{ab}}$	$0.044\pm0.007~^{ m ab}$	$151.53\pm19.86~^{\rm a}$	$0.319\pm0.027$ $^{\rm a}$	$0.0068 \pm 0.0008$ <sup>b</sup>
<b>T4</b>	$4.55\pm0.21$ <sup>d</sup>	$0.044\pm0.002~^{ m ab}$	$104.32 \pm 8.32$ <sup>b</sup>	$0.286\pm0.001~^{\mathrm{abc}}$	$0.0097 \pm 0.0008$ <sup>b</sup>
T5	$5.90\pm0.79~^{ m abc}$	$0.041\pm0.000$ $^{\mathrm{ab}}$	$145.07\pm21.30~^{\mathrm{a}}$	$0.298\pm0.028~^{\mathrm{abc}}$	$0.0071 \pm 0.0009 \ ^{\rm a}$
<b>T6</b>	$4.33\pm0.03$ <sup>d</sup>	$0.03\pm0.000$ d	$142.85\pm3.64~^{\rm a}$	$0.180 \pm 0.006$ <sup>d</sup>	$0.0070 \pm 0.0000$ <sup>b</sup>
<b>T7</b>	$6.06\pm0.18~^{ m ab}$	$0.046\pm0.001~^{\rm a}$	$131.99\pm4.51~^{\mathrm{ab}}$	$0.315\pm0.007~^{ m ab}$	$0.0075 \pm 0.0002$ <sup>b</sup>
<b>T8</b>	$6.28\pm0.23$ $^{ m ab}$	$0.039\pm0.001~^{\mathrm{abc}}$	$162.23\pm8.77$ $^{\rm a}$	$0.270 \pm 0.015~^{ m abc}$	$0.0062 \pm 0.0003 \ ^{\mathrm{b}}$
Т9	$5.10\pm0.23~^{ m cd}$	$0.036 \pm 0.001 \ ^{ m bcd}$	$140.58\pm8.96$ $^{\rm a}$	$0.260 \pm 0.003 \ { m bc}$	$0.0071 \pm 0.0004$ <sup>b</sup>
T10	$4.73\pm0.15$ <sup>d</sup>	$0.032 \pm 0.000$ <sup>cd</sup>	$145.87\pm4.17$ a	$0.185 \pm 0.004$ <sup>d</sup>	$0.0068 \pm 0.0001$ <sup>b</sup>
T11	$6.37\pm0.08$ $^{ m ab}$	$0.04\pm0.002~^{ m ab}$	158.41 $\pm$ 7.84 $^{\rm a}$	$0.302 \pm 0.021~^{ m abc}$	$0.0063 \pm 0.0003$ <sup>b</sup>
T12	$5.75\pm0.14~^{ m abc}$	$0.039\pm0.002~^{ m abc}$	$148.30\pm7.93$ $^{\rm a}$	$0.294\pm0.013~^{ m abc}$	$0.0067 \pm 0.0003$ <sup>b</sup>
T13	$5.63 \pm 0.12 \ ^{ m bc}$	$0.043\pm0.002~^{ m ab}$	$130.84 \pm 7.65 \ ^{ab}$	$0.282\pm0.015~^{ m abc}$	$0.0076 \pm 0.0004$ <sup>b</sup>
T14	$6.29\pm0.07$ $^{ m ab}$	$0.043 \pm 0.000 \ ^{ m bc}$	146.79 $\pm$ 1.83 $^{\rm a}$	$0.277 \pm 0.007~^{ m c}$	$0.0068 \pm 0.0000 \ ^{\rm a}$
T15	$4.81\pm0.05~^{ m cd}$	$0.036 \pm 0.000$ <sup>c</sup>	133.19 $\pm$ 0.35 $^{\rm a}$	$0.149 \pm 0.005 \ ^{\rm e}$	$0.0075 \pm 0.0000 \ ^{\rm a}$
T16	$6.85\pm0.05$ $^{\rm a}$	$0.048\pm0.002~^{\rm a}$	143.79 $\pm$ 5.37 $^{\mathrm{a}}$	$0.325 \pm 0.024$ <sup>b</sup>	$0.0069 \pm 0.0002~^{\rm a}$
T17	$5.82\pm0.25~^{ m abc}$	$0.043 \pm 0.000 \ ^{ m bc}$	$136.45\pm4.99$ $^{\rm a}$	$0.282\pm0.006~^{\rm c}$	$0.0073 \pm 0.0002~^{\rm a}$
T18	$5.86\pm1.10~^{ m abc}$	$0.04\pm0.000$ <sup>cde</sup>	$146.15\pm28.43$ $^{\rm a}$	$0.288 \pm 0.022 \ ^{\rm e}$	$0.0073 \pm 0.0014~^{\rm a}$
T19	$4.40\pm0.15$ <sup>d</sup>	$0.031 \pm 0.001 ~{ m f}$	$144.32\pm3.88$ a	$0.207 \pm 0.000$ <sup>d</sup>	$0.0069 \pm 0.0001 \ ^{\rm a}$
T20	$6.14\pm0.14~^{ m abc}$	$0.045\pm0.001$ $^{ m ab}$	$136.00\pm4.97~^{\rm a}$	$0.335 \pm 0.015$ <sup>b</sup>	$0.0073 \pm 0.0002~^{\rm a}$
T21	$6.25\pm0.27~^{ m abc}$	$0.042 \pm 0.001 \ ^{ m bcd}$	$149.95\pm3.46$ $^{\rm a}$	$0.276 \pm 0.001 \ ^{\rm c}$	$0.0066 \pm 0.0001 \ ^{\rm a}$
T22	$5.23\pm0.22$ <sup>bcd</sup>	$0.038 \pm 0.000$ <sup>de</sup>	$138.52\pm4.45$ $^{\rm a}$	$0.284 \pm 0.004~^{ m c}$	$0.0072 \pm 0.0002 \ ^{\rm a}$
T23	$5.08\pm0.84~^{ m bcd}$	$0.031 \pm 0.002 ~^{ m f}$	159.52 $\pm$ 16.18 $^{\mathrm{a}}$	$0.206 \pm 0.003$ <sup>d</sup>	$0.0064 \pm 0.0007~^{\rm a}$
T24	$6.07\pm0.41~^{ m abc}$	$0.04\pm0.002~^{ m cde}$	154.67 $\pm$ 13.61 $^{\rm a}$	$0.323 \pm 0.000$ <sup>b</sup>	$0.0065 \pm 0.0005 \ ^{\rm a}$
T25	$5.77\pm0.11~^{ m abcd}$	$0.039 \pm 0.000$ <sup>cde</sup>	$146.85\pm3.98$ $^{\rm a}$	$0.284\pm0.003~^{\rm c}$	$0.0068 \pm 0.0001 \ ^{\rm a}$
T26	$6.17\pm0.03~\mathrm{abc}$	$0.043 \pm 0.000$ <sup>bc</sup>	143.60 $\pm$ 3.31 $^{\rm a}$	$0.387\pm0.010$ $^{\rm a}$	$0.0069 \pm 0.0001 \ ^{\rm a}$

**Table 6.** Growth parameters were analyzed at 65 days. Letters in each group show a significant difference at p < 0.05 (mean  $\pm$  SE, n = 3).



Figure 3. PCA analysis for T1 to T13 amendments at 65 days.



Figure 4. PCA analysis for T14 to T26 amendments at 65 days.

## 4. Discussions

In low to moderate salinity conditions, several plants which are highly sensitive to salt tend to accumulate more  $K^+$  than  $Na^+$  in their vacuoles [13]. According to a study by Hasegawa et al. [31], K<sup>+</sup> and Na<sup>+</sup> compete with each other in the cell for transport since they pass through a common carrier. During water-deficient conditions, when the water content in soil declines, the movement of K<sup>+</sup> decreases, and its availability to the roots is reduced. Studies have revealed that an increase in the Na<sup>+</sup>/Ca<sup>2+</sup> ratio outside the cell leads to an increase in the  $Na^+$  influx [32,33]. Many studies have reported that salinity stress causes an osmotic effect, which is the primary stress that leads to secondary stress in the form of cellular ion imbalance [4]. This has a major effect on plant growth and development, with reduced root length, shoot length, leaf area, and biomass in most plants with increasing salinity levels [5,34]. High variations in the available P, Na, K and Ca indicate an ion imbalance due to ion toxicity, which may affect different cations and anions in the soil. This may later induce many other stresses in the soil-plant system metabolism, including cellular ion imbalance. As soon as plants experience salinity stress, many other stresses inside plants start to emerge. Few studies showed similar experimental designs, using the NaCl solution of different concentrations for irrigation and to check the effect on the plant's growth (Table 7).

The study showed a significant change in pH and EC in T2, T6 and T10 because of the addition of a saline solution of 50, 100 and 150 mM NaCl solution, respectively, compared to the control T1 (no addition of saline solution and compost). Meanwhile, T2, T6 and T10 showed no significant change in the amounts of TKN, OC, avail. P, avail. K and avail. Ca at 0 days because no compost was added in any proportion. However, the amount of avail Na was significantly increased in T2, T6 and T10 due to the addition of NaCl solution. Treatments like T3, T4, T5, T7, T8, T9, T11, T12, and T13 showed a significant variation in pH, EC, TKN, OC, avail. Na, avail. P, avail. K and avail. Ca contents. The addition of compost in different proportions significantly increased the TKN, OC, avail. P, avail. K, and avail. Ca contents in T3, T4, T5, T7, T8, T9, T11, T12, and T13, as compost is known to have a positive effect on mineral nutrient uptake under salinity conditions [35,36]. Studies have

proven that compost application in salt-affected soils improves the physical conditions of the soil, besides adding mineral nutrients for better crop growth [37].

Treatments like T15, T19 and T23 showed a significant variation in pH, EC and avail. Na contents due to the addition of 50, 100 and 150 mM NaCl solution, respectively, compared to the control T14 (no addition of saline solution and vermicompost). However, the addition of vermicompost in T16, T17, T18, T20, T21, T22, T24, T25 and T26 significantly increased TKN, OC, avail. P, avail. K and avail. Ca contents. Vermicompost has been proven to enhance the mineral uptake and plant growth in salt-affected soils [38]. In some recent studies, it has been [39,40] reported that different vermicompost ratios under salinity conditions significantly decreased salt stress and enhanced soil quality.

Salt (Na), when taken up by plants, does not directly affect their growth, but it can lead to a decrease in turgidity and photosynthetic mechanisms [33]. Salinity stress can cause growth retardation in wheat plants [41], and it also decreases the thiol content, which triggers ROS and affects major routes of redox regulation, i.e., oxidation and reduction [42,43]. The thiol-disulfide cycle is suggested to play a role in the biochemical mechanisms involved in desiccation tolerance and osmoprotectants, particularly proline accumulation at higher amounts in most plants under salinity stress [44]. Thus, the salinity stress shows a negative effect on the protein content in the leaves of many plants such as Lens culinaris, Eruca sativa, and Carthamus tinctorius L., [45–47]. Proline in plants acts as a signaling molecule and antioxidant during abiotic and oxidative stress conditions and also plays a vital role as a metal chelator [48]. Accumulating osmoprotectants' in higher amounts, particularly proline, is a common characteristic of most plants under salinity stress [5]. In our study, similar results were found, which showed that adding the saline solution to certain treatments resulted in a significant decrease in the total chlorophyll, carotenoids, protein, and thiol contents. Excess ROS can block the electron transport system, cause protein degradation and affect the repair process of PSII [49,50].

Phenols are antioxidants that help in the defense mechanism of plants under abiotic and oxidative stresses [51]. In our study, the phenol content was lowest in T1, but it increased significantly in T10 and T23, which shows that at T1, there was no major stress, but in T10 and T23, the stress level was high (150 mm saline solution irrigation), which accounted for the increase in phenol content. Attia et al. [52] reported that in plants of Solanumolanum lycopersicum, exposure to 100 mM NaCl increased their total phenols contents by 60%. Phenols are known to shield the cells from probable oxidative damage and increase cell membrane stability [53]. Phenols can act as a chelating agent and react with free radicals [54], alter peroxidation, and decrease membrane fluidity to prevent free radical diffusion [55]. ROS cause membrane injury due to oxidative stress, which leads to an increase in the level of the peroxidation content in plants [48,51,56]. Ascorbic acid is a known powerful antioxidant that works as a redox buffer (scavenging free oxy-radicals) and a cofactor for enzymes in [57]. In our study, the addition of compost and vermicompost in different proportions enhanced the levels of proline, phenol, ascorbic acid, and lipid peroxidation contents. The enhancement of these contents in the leaves of Abelmoschus esculentus L. under high salinity levels (150 mm saline solution irrigation) like in T10 and T23 suggests a secondary metabolite-mediated response, which could be due to extreme stress.

It has been reported that salt accumulation due to salinity has a negative impact on several aspects of plant growth, such as leaves, branches, and plant height, and it increases cell damage while also inhibiting different metabolic processes [24,33,58]. Salinity stress leads to primary osmotic stress, which then triggers secondary stress in the form of cellular ion imbalance [4]. This can have a major effect on plant growth and development, with many studies reporting a reduction in root length, shoot length, leaf area, and biomass in response to increasing salinity levels [5,34]. In our study, similar results were observed, with a significant decrease in various growth parameters such as leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), root shoot ratio (RSR), and specific leaf weight (SLW) in treatments such as T2, T6, T10, T15, T19 and T23. However, the addition of compost in T3, T4, T5, T7, T8, T9, T11, T12, and T13 and vermicompost in T16, T17,

T18, T20, T21, T22, T24, T25 and T26 appeared to mitigate the damage to plant growth parameters [40,59]. The increase in the growth parameters shows that plants survived with good growth and the organic amendments helped to attain plant growth. In contrast, a decrease in the growth parameters shows that salt stress conditions inhibited the plant from attaining proper growth [60].

There have been different studies that have shown in their experimental results that salinity significantly decreases plant biomass during higher salinity levels, whereas lower to moderate salinity causes adaptive changes such as a reduction in leaves, leaf area, elongation of stems or roots [60–64]. However, providing the proper amount of nutrient supply through compost or vermicompost helps to balance the plant metabolism and reduces the plant growth inhibition process [40,59,65].

**Table 7.** Studies showing effect of irrigation with different gradients of saline solution on plants' growth.

Plants Examined	Irrigated with Saline Solution	Experiment Type	Country	Observations	References
Populus euphratica	50, 100, 150, and 200 mM NaCl	Pot experiments	China	The POD activity increased with the increase in the severity of NaCl stress, but SOD activity was varied at different levels of salt. Results indicated that salt treatment reduced stomatal aperture and leaf photosynthetic capacity	[66]
Phaseolus vulgaris L.	50, 100, and 150 mM NaCl	Tray experiment	Egypt	The treatment of bean plant with 100 mM of salt combined with different concentration of nano chitosan (0.1%, 0.2% and 0.3%) indicated that nano chitosan in all concentrations significantly promoted seed germination and radical length under salt stress.	[67]
Solanum lycopersicum L.	0, 25, 50, 75, 100, 150 mM sodium chloride (NaCl)	Pot experiments	Southern France	The NaCl treatments of 75, 100 and 150 mM salt resulted in shorter plants, decreased stem width, a lower plant dry weight, fewer flowers, and smaller leaf area, while yield was reduced by treatment with concentrations of 50 mM NaCl and above.	[68]
Lavandula species	0, 25, 50, 100 and 200 mM NaCl	Pot experiment	Greece	All lavender species showed low stomatal conductance values, suggesting the presence of a drought defense strategy. L. dentata var. candicans showed the lowest stomatal conducatance value, similar to those of L. dentata var. dentata and followed in ascending order by L. stoechas and L. angustifolia, with the greatest stomatal conductance.	[69]

Plants Examined	Irrigated with Saline Solution	Experiment Type	Country	Observations	References
Salvia officinalis L.	0, 50, 100, 150, and 200 mM with different salt compounds (NaCl, KCl, MgSO <sub>4</sub> , MgCl <sub>2</sub> , Na <sub>2</sub> SO <sub>4</sub> , and CaCl <sub>2</sub> )	Pot experiment	Turkey	The study showed, $\alpha$ -pinene and camphor percentage increased under all salt stress. The percentage of camphene was also augmented under all stress types except CaCl <sub>2</sub> treatment whereas $\beta$ -thujone percentage increased except MgCl <sub>2</sub> treatment. Moreover, NaCl and KCl treatments decreased the percentage of $\alpha$ -thujone while other treatments caused an increase in the percentage.	[70]
Corchorus olitorius L.	50, 100, and 150 mM NaCl	Pot experiment	Bangladesh	Biochar and chitosan supplementation increased oxidative stress tolerance and improved the growth and physiology of salt-affected jute plants, while also significantly reducing Na+ accumulation and ionic toxicity and decreasing the Na <sup>+</sup> /K <sup>+</sup> ratio.	[71]
Abelmoschus esculentus	0, 25, 50 and 75 mM NaCl	Tray experiment	Pakistan	The results clearly indicated that seeds of all varieties can tolerate the lower concentration of salt (25 mM) and higher (50 mM) greatly reduced the seeds germination while at highest concentration (75 mM) no germination was recorded.	[72]

# Table 7. Cont.

# 5. Conclusions

The impact of different salinity levels on the *Abelmoschus esculentus* L. was examined in the present study, and significant alterations were noted in various physiological and biochemical characteristics. The increase in salinity (50 mM, 100 mM, and 150 mM) had a significant effect on the levels of phenol, proline, lipid peroxidation, and ascorbic acid content, whereas a decline was observed in the contents of thiol, protein, carotenoid, and chlorophyll in plants. The growth indices were also negatively influenced by the salinity gradients.

To avoid more stress on the soil-plant system, a better understanding is required of the integration of organic amendments with the type of irrigational water quality (level of salinity in water). The present experiment facilitates a viewpoint to understand the relationship between irrigational water quality and organic amendments. These findings provide valuable insights into the plant's response to varying salinity levels and can help develop a better understanding of this relationship. Also, from an economic point of view, the proper use of amendments will help to save a lot of money, as it can help utilize organic amendments in the best way possible, avoiding the use of chemical fertilizers. Author Contributions: Conceptualization, Writing an original draft, Investigation, Data Analysis, Data curation, Validation, Writing—review and editing, I.S.; Writing—review and editing, V.S.; Writing—review and editing, M.M.; Writing—review and editing, S.S.; Writing—review and editing, V.K.G. Resources, Supervision, Conceptualization, Funding acquisition, Writing—review and editing, R.P.S. All authors have read and agreed to the published version of the manuscript.

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