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# COMPARISON AND SYNERGISTIC IMPACT OF *ALOE VERA* LEAF EXTRACT AND SALICYLIC ACID ON THE PHYSIOLOGICAL AND BIOCHEMICAL ADAPTATION OF CACAO CULTIVARS TO SALINE STRESS

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**Abstract** *Salinity is one of the significant abiotic stress factors that accentuate the growth of Theobroma cacao L., severely limiting its growth, physiological performance, and yield potential. The current paper examined the role of Aloe vera leaf extract (AVE) and salicylic acid (SA) used individually and in combinations in the physiological and biochemical responses of two cacao plants (CCN-51 and ICS-95), under 100 mM NaCl-induced salinity. Salinity had a huge impact on reducing the growth of the plants, the ratio of water to the material (RWC), chlorophyll pigmentation, and the concentration of potassium ($K^+$), and elevated electrolyte leakage (EL), sodium ($Na^+$) accumulation, and the indicators of oxidative stress. Foliar treatment of SA and AVE as a single or combined agent had significant alleviating effects against salt stress, which included the maintenance of membrane stability, osmotic adjustment, antioxidant defense system strengthening, and ionic homeostasis. The SA+AVE treatment had the strongest effect on the increase in chlorophyll content, antioxidant enzyme activities (superoxide dismutase, peroxidase, and catalase), and $K^+/Na^+$ ratio, and a significant decrease in the electrolyte leakage and $Na^+$. CCN-51 was found to have a relatively high salt tolerance among the two cultivars, and this is related to an effective antioxidant mechanism and high ability to lose ions. On the whole, the combination of Aloe vera extract and salicylic acid is a potentially dependable and environmentally friendly approach that allows to increase salt tolerance and cacao yield in a salty environment.*

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**Keywords:** *Multi drug resistant bacteria; antibiotics; biopolymers; drug delivery; skin regeneration*

## Introduction

Salinity stress is regarded as one of the most adverse abiotic factors that restricts agricultural productivity of the world and is a significant threat to the food security of the globe(Amrutha et al., 2023). The causes of soil salinization have been climate change, inappropriate irrigation practices, and high rates of evapotranspiration, which have caused soil salinization at such a rate that the availability of arable land has been gradually decreasing. The buildup of soluble salts, especially sodium chloride (NaCl), destabilizes the soil structure and disturbs the relations between the plants and water, which eventually worsen crops growth and yield(Antolovich et al., 2002). Salinity has already covered a significant share of the irrigated agricultural lands, and the number keeps on the rise year after year. As the world population is estimated to reach over 9 billion in 2050, increasing the salinity resilience of crops has now been an important aspect of sustainable agriculture(Armstrong, 2019; Chaabene et al., 2017).

The salinity stress is harmful to the plants as it causes osmotic imbalance, ion toxicity, and oxidative stress. Sodium ($Na^+$) and chloride ($Cl^-$) overload interfere with nutrient absorption and disrupt cellular ion homeostasis, especially that of potassium ($K^+$). An increase in $Na^+$ causes instabilities in cell membranes, inhibition of enzymatic activity, and decreases the efficiency of photosynthesis. More so, salt stress increases the generation of reactive oxygen species (ROS) that leads to oxidative damage of lipids, proteins, and nucleic acids. Even though the salinity triggers different kinds of defense pathways and mechanisms, such as antioxidant enzyme systems and ion regulation pathways, among others, when subjected to prolonged salinity levels, the defensive mechanisms become overwhelmed, resulting in massive losses in growth and productivity(Dahliani et al., 2023). Cacao (*Theobroma cacao L.*) is a perennial tropical crop of great economic dimension since it mainly provides cocoa and chocolate products. Although cacao prefers humid tropical conditions, soil salinity is becoming a challenge to its cultivation in some parts. Salinity has been linked to depressed growth in vegetation, compromised water relations, degraded chlorophylls, ionic, and oxidative stress on cacao vegetation. Hence, it is necessary to come up with greener, sustainable efforts to expect salinity tolerance in cacao to continue with productivity and cultivating in marginal areas (Demidchik et al., 2014). The exogenous use of plant growth regulators and natural bio-stimulants has received significant interest among other methods of mitigation. Salicylic acid (SA) is a relevant signaling molecule in the process of controlling plant responses to abiotic stressors, such as salinity. SA modulates the activity of antioxidant enzymes and membrane stability, enhances the osmotic adjustment of cells, as well as enhancement of ion homeostasis under stress. Likewise, *Aloe vera* leaf extract (AVE), a bioactive compound, has been found to have antiaging properties, including phenolics, vitamins, amino acids, and natural antioxidants, as a favorable bio stimulant. The works of the past demonstrated that *Aloe vera* extracts could promote the growth of plants and their chlorophyll levels, improve antioxidant defense mechanisms, and reduce the effects of stress(Dixon and Massey Jr, 1951). This is because even though the effects of salicylic acid and *Aloe vera* extract have been reported on several crops, there is hardly any data available on comparative effects and synergistic effects between these two extracts on cacao when under saline conditions. Thus, the current experiment was designed to measure the personal and the synergistic effects of salicylic acid and *Aloe vera* leaf extract on physiological and biochemical activities of cacao plants under salinity stress. Through the analysis of parameters concerning ion regulation and antioxidant defense, membrane stability, and water status, this study aims to find effective measures that can be adopted to increase salt tolerance and allow production of cacao in environments with high salt levels, and allow sustainable production of cacao(El Sabagh et al., 2020; Fagarasanu and Kumar, 2002).

## Materials and Methods

### Experimental Site and Plant Material

The experiment was carried out in a greenhouse with controlled conditions at the Department of Plant Breeding and Genetics, University of the Punjab, Lahore, Pakistan. The two cacao (*Theobroma cacao L.*) cultivars, CCN-51 and ICS-95, were the materials for the investigation. Cacao plants that were one year old, grafted, and well-established were taken from a certified nursery and transplanted in big plastic containers (60L capacity) filled with a mixture of loam soil, sand, and well-decomposed organic compost in a 2:1:1 (v/v/v) ratio. Before transplanting, the soil's physicochemical properties were determined to confirm that the growth conditions were uniform. The experiment was a completely randomized design (CRD) with three biological replicates per treatment, and each plant was considered as an experimental unit. During the day, the temperature in the greenhouse was kept at 26 ± 2 ˚C and at 20 ± 2 ˚C at night, with relative humidity between 70 and 80% under natural photoperiod conditions. The plants were regularly watered to keep the soil moisture at an optimal level during the vegetative and reproductive growth phases.

### Salinity and Exogenous Treatments

Salicylic acid (SA) at 0.5 mM concentration was made and used for one, time foliar application by a hand sprayer till the leaves were uniformly wetted to the point of runoff. *Aloe vera* leaf extract (AVE) was prepared from mature, healthy leaves. Before usage, it was diluted with distilled water at a ratio of 1:25 (v/v). Plant foliar treatments started after their establishment, and 7-day interval applications were continued during both vegetative and reproductive growth stages.

The treatments were as follows:

| Treatments | |
|---|---|
| **T0** | Control (no NaCl, no SA, no AVE) |
| **T1** | 100 mM NaCl |
| **T2** | 0.5 mM SA |
| **T3** | *Aloe vera* extract (1:25 dilution) |
| **T4** | 0.5 mM SA + *Aloe vera* extract |
| **T5** | 100 mM NaCl + 0.5 mM SA |
| **T6** | 100 mM NaCl + *Aloe vera* extract |
| **T7** | 100 mM NaCl + 0.5 mM SA + *Aloe vera* extract |

### Preparation of *Aloe vera* Leaf Extract (ALE)

The *Aloe vera* was thoroughly washed with distilled water, and mature and healthy leaves were collected and harvested. The outer rind was also removed carefully to get the inner gel. Blender was used to homogenize the gel and filtered on muslin cloth. After 15 minutes of centrifugation at a speed of 8,000 rpm, the filtrate was separated to have a clear supernatant. A 1:25 (v/v) dilution of the supernatant was made in distilled water, and foliar treatment was done(Fardsadegh and Jafarizadeh-Malmiri, 2019).

**Data Collection and Measurements**

The data were taken at the active vegetative stage and at the reproductive stage when salinity stress occurred. Morphological, physiological, biochemical, and yield-related parameters were measured at the right stages of growth. Physiological and biochemical investigations were carried out using fully developed and healthy leaves. Measurements were done in triple groupings of biological replicates of each treatment(Hameed et al., 2012).

**Morphological and Biomass Assessment**

The measuring scale was used to measure the height of the plants and the length of the roots (cm). The count of the leaves per plant has been done manually(Isayenkov and Maathuis, 2019). To determine biomass, the plants were comprehensively rooted after completion of the experimental period, and the fresh mass of shoot and root (g) was determined with the help of an analytical scale. Shoot and root dry weights (g) were determined by subjecting the samples to $650^0$C drying at 72 hours under the oven until they had reached dry weight(Inamullah et al., 2011).

**Photosynthetic Pigments**

The determination of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents was done by adhering to the technique of Arnon (1949) with minor adjustments. The leaf samples (0.5 g) were put into a cause of homogenization with 80% acetone and centrifuged at 10000 rpm for a period of 10 minutes. A UV-visible spectrophotometer at 663 nm, 645 nm, and 480 nm was used to measure the absorbance of the supernatant. The concentrations of the pigments were calculated by means of common equations and expressed as mg/g minus fresh weight (FW)(Kuczynska et al., 2015).

**Electrolyte Leakage (%)**

The electrolyte leakage (EL) was calculated to evaluate the stability of the membrane (Liu et al., 2017). In each of the treatments, fully developed and healthy leaves were gathered together. On leaf samples, uniformly sized samples (about 1 cm 2) were cut to a uniform size, and the samples were rinsed thoroughly with deionized water to eliminate electrolytes that were adsorbed on the surface. The samples were placed in test tubes with 10mL of distilled water. The samples were left to incubate at 25 ˚C. Relative EC ($EC_1$) of the solution was then determined after incubation with the use of a digital conductivity meter. Then the samples were heated at $95^0$C and 15 min to break up the tissues and liberate all the electrolytes. When the betatron cooled down to room temperature, the final electrical conductivity ($EC_2$) was measured.

$$EL\% = \left(\frac{EC1}{EC2}\right) X\ 100$$

**Relative Water Content (RWC)**

Relative water content (RWC) was calculated to assess the water of cacao (*Theobroma cacao L.*) plants under salinity stress. The second fully expanded leaf encountered on the apex was sampled in relation to each treatment(Maathuis et al., 1996). The status of fresh weight (FW) was noted instantly. The samples of the leaves were immersed in distilled water for 24 hours at room temperature to get the turgid weight (TW). Next, the samples were dried in an oven at 65 o C until they attained a constant weight to obtain dry weight (DW).

$$RWC\% = \left[\frac{(FW - DW)}{(TW - DW)}\right] X\ 100$$

Where: FW= fresh weight, DW = dry weight, TW= turgid weight.

**Determination of $K^+$ and $Na^+$ (mg $g^{-1}$ DW)**

Leaf tissues were first oven, dried at 65 °C for 72 hours until the weight was constant, and then they were ground into a fine powder with the help of a laboratory mill. A precisely weighed portion of the dried material was digested with a mixture of concentrated nitric acid (HNO) and hydrogen peroxide (HO) in a hot, block digester until the solution became clear(Majeed and Muhammad, 2019). The levels of sodium (Na) and potassium (K) were established by means of a flame photometer (Jenway PFP, 7, UK). The measurement was done through the comparison with the standard calibration curves prepared from the graded standard solutions within the range of 20100 mg L. The values obtained were presented as mg g dry weight (DW).

**Antioxidant Activity**

Antioxidant enzyme activities were determined by taking all the fully expanded leaves of each treatment. The nitroblue tetrazolium (NBT) reduction method was used to measure the activity of Superoxide dismutase (SOD). The guaiacol oxidation assay was used to determine the peroxidase (POD) activity, whereas the rate was used to determine the catalase

(CAT) activity through spectrophotometry using the breakdown of hydrogen peroxide ($H_2O_2$)(Mullan and Pietragalla, 2012). The estimation of proline substrate was performed by the acid ninhydrin method, and malondialdehyde (MDA), a lipid peroxidative product, was measured by use of the thiobarbituric acid (TBA) assay. All the enzymatic procedures were determined on a protein basis (U-mg $^{-1}$ protein) and triplicated.

**Yield attributes**

The characteristics related to growth and yield were measured during physiological maturity. The height of the plants, the diameter of the stem, and overall biomass were noted at harvest. The parameters of yield, such as the number of pods per plant, the length of the pod (cm), and the number of seeds in the pod, were measured in the fruiting period. The average values of three biological replicates of one treatment were used as the mean values(Munns et al., 2010).

**Statistical Analysis**

Data were analyzed using the two-way ANOVA to compare the effects of salinity, *Aloe vera* extract, and salicylic acid(Myśliwa-Kurdziel and Strzałka, 2002). Comparison of means of treatment was done at p 0.05 by use of Tukey's HSD test. Statistical tests were conducted with the help of SPSS (Version 20), and the graphical analysis was created with the help of Microsoft Excel (2021).

**Results and discussions**

**Morphological attributes**

Salinity stress at 100 mM NaCl significantly hampered the morphological characteristics of the different cacao (*Theobroma cacao L.)* cultivars investigated, such as plant height, shoot length, root length, stem diameter, number of leaves, and total biomass accumulation (Table 1). The largest decreases were found under the NaCl stress alone (T1), which also substantiates that cacao plants are salt-sensitive. By enhancing the nutrition of the leaves with salicylic acid (SA) and *Aloe vera* extract (AVE), the negative impacts of salinity were greatly lessened. Out of all the different types of treatments, the two biostimulants applied together, SA+AVE (T4 non, saline, and T7 saline) had the largest positive influence on the growth parameters. Plant height, shoot length, and root length were significantly different from those of NaCl, stressed plants. Similarly, the fresh and dry weight of the shoot and roots was greatly increased by the two biostimulants, with AVE generally having a slightly stronger effect than SA alone. Two cultivars were used in this experiment: CCN-51 and ICS-95. CCN-51 outperformed ICS-95 in terms of growth throughout the whole experimental period under both conditions. Even with salt stress, CCN-51 produced a higher biomass, more leaves, and better root development than the other cultivar; therefore, the salt tolerance of CCN-51 was relatively higher(Nawaz et al., 2010).

**Table 1. Comparative and synergistic effects of *Aloe vera* leaf extract and salicylic acid on morphological attributes of cacao (Theobroma cacao L.) cultivars under salt stress (mean ± SD; Tukey's HSD, p ≤ 0.05**

| Treatment | PH (cm) | RFW (g) | SDW (g) | RDW (g) | SL (cm) | RL (cm) | NOL (per plant) |
|---|---|---|---|---|---|---|---|
| | | | | **CCN-51** | | | |
| **T0** | 28.17 ± 1.26 $^{g}$ | 2.59 ± 0.11 $^{fg}$ | 3.47 ± 0.29 $^{e}$ | 2.05 ± 0.10 $^{c}$ | 27.17 ± 1.26 $^{g}$ | 19.00 ± 1.00 $^{d}$ | 18.67 ± 1.62 $^{f}$ |
| **T1** | 22.67 ± 1.18 $^{h}$ | 2.13 ± 0.13 $^{h}$ | 2.17 ± 0.19 $^{g}$ | 1.50 ± 0.10 $^{e}$ | 23.67 ± 1.53 $^{h}$ | 15.67 ± 1.53 $^{e}$ | 12.33 ± 1.53 $^{g}$ |
| **T2** | 34.27 ± 1.21 $^{c}$ | 3.15 ± 0.23 $^{c}$ | 4.40 ± 0.20 $^{c}$ | 2.30 ± 0.10 $^{b}$ | 34.00 ± 2.65 $^{c}$ | 24.23 ± 1.03 $^{c}$ | 25.33 ± 1.53 $^{c}$ |
| **T3** | 37.65 ± 1.55 $^{b}$ | 3.35 ± 0.24 $^{b}$ | 5.33 ± 0.29 $^{b}$ | 2.63 ± 0.15 $^{ab}$ | 37.33 ± 1.53 $^{b}$ | 28.70 ± 0.52 $^{b}$ | 28.33 ± 1.93 $^{b}$ |
| **T4** | 42.67 ± 1.93 $^{a}$ | 3.40 ± 0.23 $^{a}$ | 6.33 ± 0.15 $^{a}$ | 2.77 ± 0.15 $^{a}$ | 42.67 ± 2.52 $^{a}$ | 31.83 ± 1.53 $^{a}$ | 31.67 ± 1.53 $^{a}$ |
| **T5** | 29.17 ± 1.29 $^{ef}$ | 2.67 ± 0.22 $^{f}$ | 2.80 ± 0.10 $^{ef}$ | 1.80 ± 0.10 $^{d}$ | 29.67 ± 1.15 $^{fg}$ | 18.83 ± 1.04 $^{d}$ | 21.67 ± 1.53 $^{ef}$ |
| **T6** | 31.42 ± 1.03 $^{e}$ | 2.84 ± 0.12 $^{e}$ | 3.57 ± 0.12 $^{e}$ | 2.00 ± 0.30 $^{c}$ | 31.00 ± 1.04 $^{ef}$ | 20.29 ± 0.25 $^{dc}$ | 23.67 ± 1.53 $^{d}$ |
| **T7** | 33.30 ± 1.26 $^{cd}$ | 3.02 ± 0.21 $^{cd}$ | 4.10 ± 0.10 $^{d}$ | 2.07 ± 0.06 $^{c}$ | 33.03 ± 1.41 $^{de}$ | 23.33 ± 0.29 $^{c}$ | 26.00 ± 1.65 $^{c}$ |
| | | | | **ICS-95** | | | |
| **T0** | 24.21 ± 1.87 $^{ef}$ | 1.87 ± 0.13 $^{g}$ | 3.33 ± 0.29 $^{ef}$ | 2.01 ± 0.31 $^{c}$ | 22.23 ± 1.08 $^{d}$ | 17.33 ± 1.53 $^{d}$ | 15.33 ± 0.58 $^{d}$ |
| **T1** | 19.83 ± 1.76 $^{h}$ | 1.23 ± 0.13 $^{gh}$ | 2.78 ± 0.19 $^{g}$ | 1.10 ± 0.00 $^{f}$ | 19.13 ± 0.81 $^{e}$ | 13.23 ± 0.21 $^{f}$ | 9.67 ± 2.08 $^{g}$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **T2** | 30.17 ± 1.29 $^{c}$ | 2.95 ± 0.11 $^{bc}$ | 4.37 ± 0.15 $^{c}$ | 2.12 ± 0.01 $^{bc}$ | 30.67 ± 3.06 $^{b}$ | 22.00 ± 1.00 $^{c}$ | 17.67 ± 1.51 $^{c}$ |
| **T3** | 32.17 ± 1.29 $^{b}$ | 3.04 ± 0.20 $^{b}$ | 4.73 ± 0.46 $^{b}$ | 2.20 ± 0.07 $^{b}$ | 32.10 ± 1.15 $^{ab}$ | 25.27 ± 0.68 $^{b}$ | 21.67 ± 1.53 $^{ab}$ |
| **T4** | 36.17 ± 1.29 $^{a}$ | 3.33 ± 0.12 $^{a}$ | 5.17 ± 0.58 $^{a}$ | 2.27 ± 0.01 $^{a}$ | 36.20 ± 1.39 $^{a}$ | 28.53 ± 0.95 $^{a}$ | 23.67 ± 2.08 $^{a}$ |
| **T5** | 21.33 ± 1.15 $^{g}$ | 1.50 ± 0.10 $^{f}$ | 3.20 ± 0.10 $^{f}$ | 1.40 ± 0.20 $^{e}$ | 21.27 ± 0.25 $^{d}$ | 14.57 ± 0.31 $^{ef}$ | 11.33 ± 2.52 $^{f}$ |
| **T6** | 23.37 ± 1.15 $^{e}$ | 1.65 ± 0.12 $^{e}$ | 3.40 ± 0.20 $^{e}$ | 1.47 ± 0.06 $^{e}$ | 22.40 ± 0.53 $^{cd}$ | 15.47 ± 0.40 $^{e}$ | 13.33 ± 1.53 $^{e}$ |
| **T7** | 27.13 ± 1.23 $^{cd}$ | 1.78 ± 0.12 $^{d}$ | 3.80 ± 0.10 $^{de}$ | 1.70 ± 0.10 $^{d}$ | 26.07 ± 2.00 $^{c}$ | 18.00 ± 1.00 $^{d}$ | 16.00 ± 2.00 $^{d}$ |

*Height of the plants (PH), root fresh weight (RFW), Shoot dry weight (SDW), root dry weight (RDW), Shoot length (SL), root length (RL), Number of leaves (NOL).
*Control T0, 100 mM NaCl T1, 0.5 mM SA T3, *Aloe vera* extract (1:25 dilution) T4, 0.5 mM SA + *Aloe vera* extract T5, 100 mM NaCl + 0.5 mM SA T6, 100 mM NaCl + *Aloe vera* extract T7, 100 mM NaCl + 0.5 mM SA + *Aloe vera* extract.
*Various letters also show that there are critical differences between treatments based on the HSD test of Tukey at p 5.

**Photosynthetic pigments**

Salinity stress severely lowered the amounts of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the two cacao cultivars (Table 2). The NaCl treatment (T1) had the lowest levels of pigments, thus showing impaired photosynthetic performance under saline stress. Besides that, spray of SA and AVE on the leaf surface alleviated the negative effects of salinity stress on pigments, especially the combined treatment (SA+AVE), which resulted in the highest concentration of pigments(Nelson et al., 1998). In CCN-51, chlorophyll a reached 2.84 0.09 mg $g^{-1}$ FW under combined treatment, while ICS-95 got 2.63 0.12 mg $g^{-1}$ FW. A similar pattern appeared for chlorophyll b, total chlorophyll, and carotenoids. In general, CCN-51 showed a better pigment retention under salinity stress as compared to ICS-95, thus indicating improved photosynthetic stability and antioxidant capacity.

**Table 2. The influence of the *Aloe vera* extract and salicylic acid on chlorophyll and carotenoids in cacao under salt stress (mean ± SD, HSD of Tukey's HSD, p ≤ 0.05)**

| Treatment | Chlorophyll a (mg $g^{-1}$ FW) | Chlorophyll b (mg $g^{-1}$ FW) | Total chlorophyll (mg $g^{-1}$ FW) | Carotenoids (mg $g^{-1}$ FW) |
|---|---|---|---|---|
| | | **CCN-51** | | |
| **T0** | 1.84 ± 0.04$^{e}$ | 1.21 ± 0.05$^{e}$ | 3.05 ± 0.06$^{f}$ | 0.92 ± 0.03$^{e}$ |
| **T1** | 1.42 ± 0.06$^{f}$ | 0.98 ± 0.04$^{f}$ | 2.40 ± 0.07$^{g}$ | 0.74 ± 0.04$^{f}$ |
| **T2** | 2.46 ± 0.08$^{c}$ | 1.71 ± 0.06$^{c}$ | 4.17 ± 0.09$^{c}$ | 1.52 ± 0.05$^{c}$ |
| **T3** | 2.68 ± 0.07$^{b}$ | 1.88 ± 0.05$^{b}$ | 4.56 ± 0.10$^{b}$ | 1.64 ± 0.04$^{b}$ |
| **T4** | 2.84 ± 0.09$^{a}$ | 1.96 ± 0.07$^{a}$ | 4.80 ± 0.11$^{a}$ | 1.78 ± 0.06$^{a}$ |
| **T5** | 1.98 ± 0.05$^{d}$ | 1.32 ± 0.04$^{d}$ | 3.30 ± 0.08$^{e}$ | 1.06 ± 0.03$^{d}$ |
| **T6** | 2.12 ± 0.07$^{d}$ | 1.44 ± 0.05$^{d}$ | 3.56 ± 0.09$^{d}$ | 1.18 ± 0.04$^{d}$ |
| **T7** | 2.33 ± 0.08$^{c}$ | 1.60 ± 0.06$^{c}$ | 3.93 ± 0.10$^{c}$ | 1.39 ± 0.05$^{c}$ |
| | | **ICS-95** | | |
| **T0** | 1.62 ± 0.03$^{f}$ | 1.05 ± 0.05$^{e}$ | 2.67 ± 0.07$^{g}$ | 0.83 ± 0.04$^{e}$ |
| **T1** | 1.28 ± 0.04$^{g}$ | 0.86 ± 0.03$^{f}$ | 2.14 ± 0.06$^{h}$ | 0.62 ± 0.03$^{f}$ |
| **T2** | 2.19 ± 0.05$^{c}$ | 1.49 ± 0.06$^{c}$ | 3.68 ± 0.09$^{c}$ | 1.29 ± 0.04$^{c}$ |
| **T3** | 2.36 ± 0.06$^{b}$ | 1.63 ± 0.05$^{b}$ | 3.99 ± 0.08$^{b}$ | 1.41 ± 0.03$^{b}$ |
| **T4** | 2.63 ± 0.12$^{a}$ | 1.74 ± 0.08$^{a}$ | 4.37 ± 0.10$^{a}$ | 1.56 ± 0.05$^{a}$ |
| **T5** | 1.74 ± 0.05$^{e}$ | 1.18 ± 0.04$^{d}$ | 2.92 ± 0.07$^{f}$ | 0.96 ± 0.03$^{d}$ |
| **T6** | 1.88 ± 0.06$^{d}$ | 1.29 ± 0.05$^{d}$ | 3.17 ± 0.08$^{e}$ | 1.08 ± 0.04$^{d}$ |
| **T7** | 2.05 ± 0.07$^{cd}$ | 1.42 ± 0.06$^{c}$ | 3.47 ± 0.09$^{d}$ | 1.21 ± 0.05$^{c}$ |

*T0: control, T1: 100mMNaCl, T2: 0.5mMSA, T3: *Aloe vera* (1:25 dilution) extract, T4: 0.5mMSA+*Aloe vera* extract, T5: 100mMNaCl+0.5 mM SA, T6: 100mMNaCl+ *Aloe vera* extract, T7: 100mMNaCl+ 0.5 mM SA + *Aloe vera* extract.
*The difference among treatments in relation to the HSD test of Tukey at p ≤ 0.05 shows that there are major differences between different letters.

**Physiological attributes**

Electrolyte leakage (EL) went up a lot due to salt stress, which means that the membranes got damaged (Figure 1a). NaCl treatment alone caused a 17.4% rise in EL when compared to the control plants. On the other hand, spraying leaves with SA and AVE noticeably lessened the leakage of the membrane, and the combined treatment showed the greatest decrease, especially in CCN-51. Relative water content (RWC) fell due to the salinity condition (Figure 1b), yet both SA and AVE use helped to retain water. The highest RWC values were those of the combined treatment, thus showing that osmotic adjustment was improved. Under stress, CCN-51 was still able to keep a higher level of RWC as compared to ICS-95 (Pandey et al., 2002).

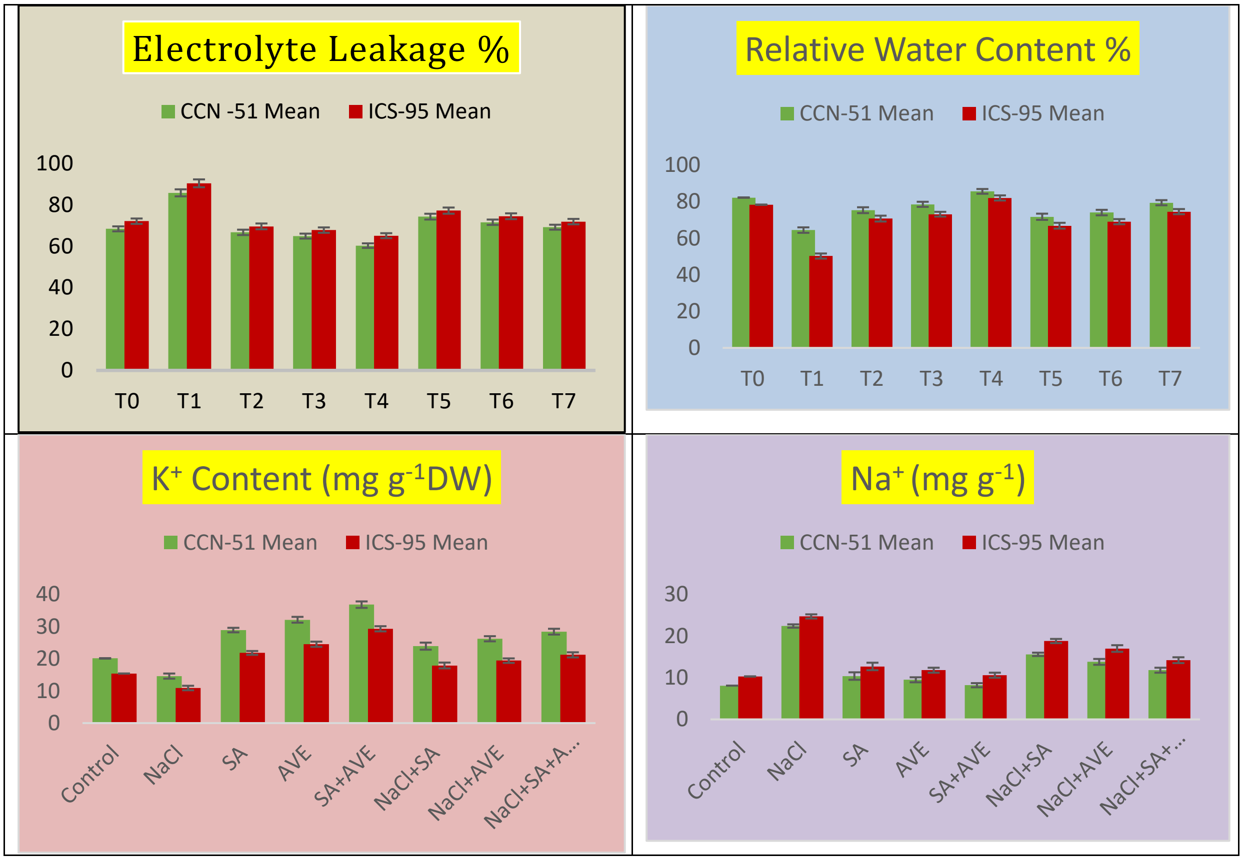


***Figure 1 Effects of Aloe vera extract and salicylic acid on (a) electrolyte leakage, (b) relative water content, (c) $K^+$ content, and (d) $Na^+$ content in cacao cultivars (CCN-51 and ICS-95) under 100 mM NaCl stress. Values represent mean ± SD (n = 3). Different letters indicate significant differences at $p \leq 0.05$ (Tukey's HSD)***

**$K^+$ and $Na^+$ Content (mg $g^{-1}$ DW)**

Salinity stress caused a drastic reduction in K content and a drastic increase in Na accumulation in both cultivars. K levels dropped more substantially when only NaCl was applied. Foliar spraying of AVE and SA raised K concentration, while the combined application gave the greatest K amounts (a 58.7% increase over salt stress alone). Na levels were drastically raised by salinity stress, while SA and AVE were able to significantly reduce Na uptake. SA had a greater inhibitory effect on Na accumulation than AVE. CCN-51 was able to keep a higher K/Na ratio than ICS-95, thus showing a better ionic regulation and salt tolerance(Parihar et al., 2015).

**Antioxidant activity**

The salinity stress caused a drastic increase in the antioxidant enzyme activities as a response to the stress. Enzymatic activities such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were boosted even more by SA and AVE treatments (Figure 2). The joint SA + AVE treatment led to the peak enzyme activities, especially in CCN-51(Quamruzzaman et al., 2021). These findings imply the triggered synergistic cooperation of the antioxidant defense system that helped to increase stress tolerance.

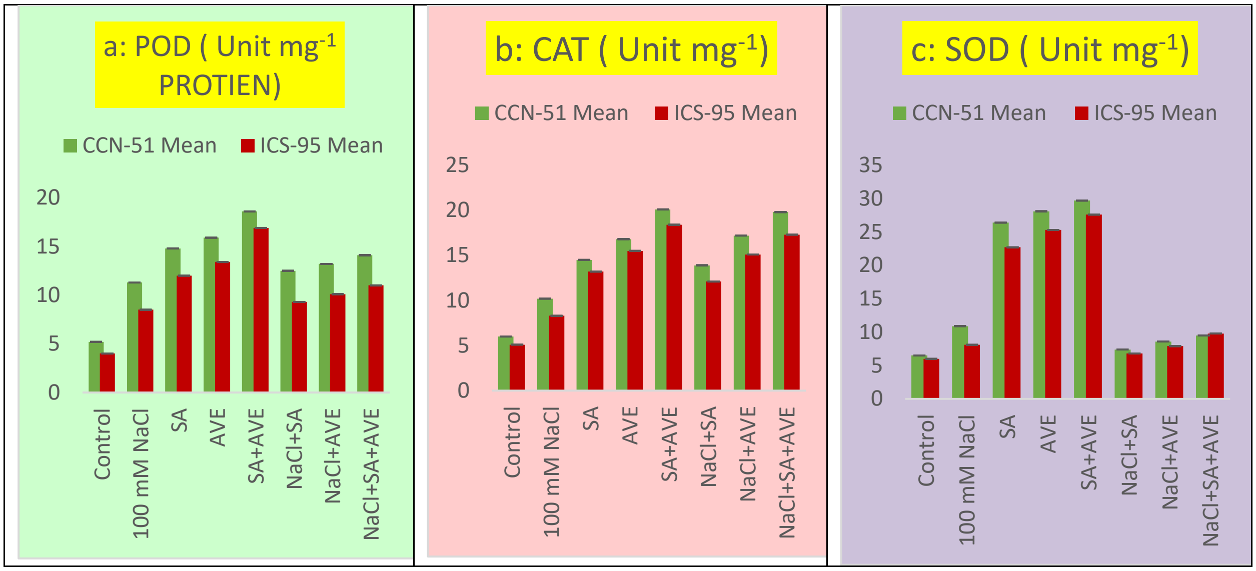


***Figure 2 Effects of Aloe vera leaf extract and salicylic acid on the activities of antioxidant enzymes: (a) peroxidase (POD), (b) catalase (CAT), and (c) superoxide dismutase (SOD) in cacao (Theobroma cacao L.) cultivars (CCN-51 and ICS-95) under salinity stress. Values represent mean SD (n = 3). Different letters refer to significant differences as per Tukeys HSD test at p 0. 05***

**Yield attributes**

Salinity had grave impacts on the pod number, pod length, and the number of seeds per pod (Figure 3). But, yield parameters under saline conditions were enhanced by both treatments (SA and AVE). The overall treatment yielded the greatest number of pods and seeds in the two cultivars(Rademacher, 2015). CCN-51 has always shown better performance on reproductive fitness compared to the ICS-95, not reducing its reproductive success (higher production of pods and seed set in the face of salinity).

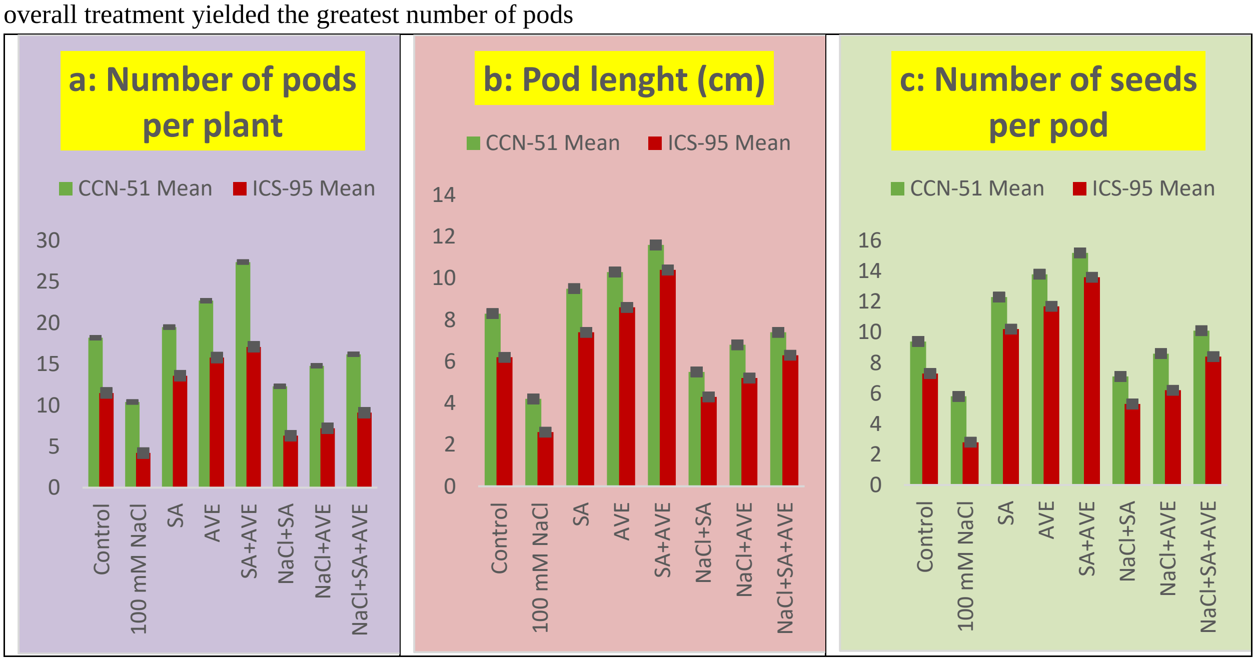


***Figure 2 Comparison of the influences of Aloe vera leaf extract and salicylic acid either alone or in combination on (a) the number of pods per plant, (b) pod length (cm), and (c) the number of seeds per pod in two cultivars of cacao (Theobroma cacao L.) (CCN-51 and ICS-95) under salinity stress (100 mM NaCl). Bars represent the mean± SD (n = 3). Different letters show significant differences among the treatments according to Tukey's HSD test at p 0. 05***

**Pearson Correlation**

Pearson correlation analysis has indicated that there was a strong negative relationship between the concentration of Na plus and growth, physiological, and yield parameters (Figure 4). On the other hand, the positive relationships between $K^+$ content and biomass, photosynthetic pigments, antioxidant enzyme activities, and yield characteristics were high. Most parameters of growth and physiology were negatively correlated with electrolyte leakage, which was positively related to $Na^+$ accumulation. These results show the relevance of ionic balance and antioxidant defense systems in increasing salt tolerance in cacao cultivars(Shah et al., 2021).

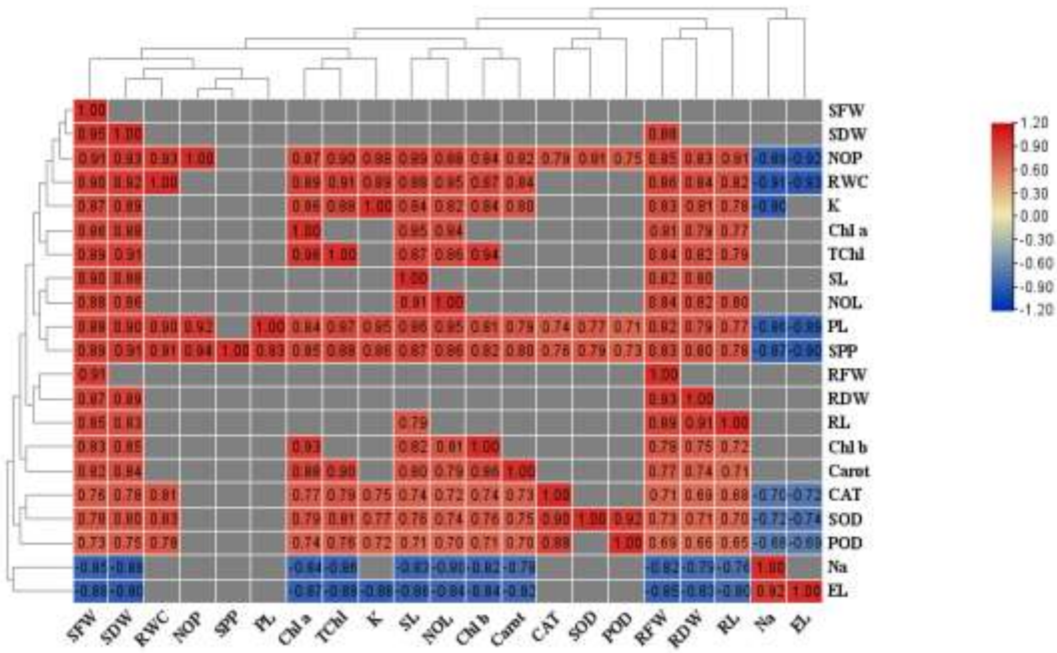


***Figure 3 Pearson correlation analysis of morphological, physiological, antioxidant, ionic, and yield attributes was performed on two different cacao cultivars (CCN-51 and ICS-95) exposed to salinity stress. The color intensity shows the degree of correlation (red = positive; blue = negative). SFW = shoot fresh weight; RFW = root fresh weight; SDW = shoot dry weight; RDW = root dry weight; SL = shoot length; RL = root length; NOL = number of leaves; Chl a = chlorophyll a; Chl b = chlorophyll b; TChl = total chlorophyll; Carot = carotenoids; Na = sodium; K = potassium; EL = electrolyte leakage; RWC = relative water content; CAT = catalase; POD = peroxidase; SOD = superoxide dismutase; NOP = number of pods per plant; PL = pod length; SPP = seeds per pod***.

Salinity stress had severe effects on cacao plants in terms of growth, photosynthetic performance, and ionic balance. High levels of $Na^+$ influx interfered with cellular homeostasis, impaired the availability of $K^+$, and caused oxidative stress that caused cell membrane damage and decreased productivity. These effects were reduced by the exogenous use of SA and AVE significantly. AVE contains antioxidants and bioactive compounds that are highly likely to increase the stability of the membrane as well as nutrient uptake. SA, being a "signaling molecule, triggered stress-receptive signaling, enhancing osmotic and antioxidant responses. More significant increments in the phase of pigment retention, enzyme activity, ionic balance, and yield performance were observed with the combined treatment of SA+AVE, and this indicates a synergistic relationship. The tolerance of CCN-51 was always higher than that of ICS-95, which could probably be explained by the level of more effective ion regulation and antioxidant activity(Singh, 2022; Tarang et al., 2013).

**Conclusion**

The current experiment has shown that salinity stress can harm the growth, physiological stability, ionic homeostasis, and yield performance of cacao cultivars. Salicylic acid and *Aloe vera* extract sprayed onto the foliage were shown to reduce the damage from salt greatly. The combined treatment was best in the improvement of photosynthetic pigments, antioxidant enzyme activities, $K^+/Na^+$ balance, and reproductive performance. CCN-51 was more salt-tolerant than ICS-95 of the two cultivars. The combination of *Aloe vera* extract and salicylic acid is a prospective and sustainable approach to enhancing the productivity of cacao plants in a saline environment. The future studies need to be aimed at dose optimization and clarification of molecular mechanisms regulating increased salt tolerance.

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**Statements and Declarations**

**Data Availability statement**

All relevant data are within the manuscript file.

**Author's Contribution Statement**

QH, MAR, MR, ZA, and MFS conceived the study, collected and analyzed data, wrote the manuscript. MTA, MA supervised, provided resources. MMJ, SHHS, MA, MR and QR critically reviewed, edited, and provided resources. All authors have read the final manuscript and approve its submission.

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**Ethical Statement**

Not applicable

**Conflict of interest**

No conflict of interest.