



Foliar Application of Kaolin Enhances Physiological and Biochemical Resilience of Guar (*Cyamopsis tetragonoloba* L.) Under Salt Stress

Muhammad Ameen¹, Javaid Akhtar², Muhammad Anwar-Ul-Haq³, Kashif Mustafa¹, Mueen Alam Khan⁴ and Zaffar Malik¹

¹Department of Soil Science, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur 63100, Punjab, Pakistan

²Vice-Chancellor Secretariat, University of Sahiwal 57000, Punjab, Pakistan

³Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad 38040, Punjab, Pakistan

⁴Department of Plant Breeding and Genetics, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, 63100, Punjab, Pakistan

Article Info

Keywords:

Kaolin, Soluble sugars, Photosynthesis, Transpiration, Enzymatic Antioxidants

Date:

Received: 02 March 2025

Accepted: 13 October 2025

Published: 15 December 2025

Volume: 6(02), 2025



Copyright: © 2025 by the authors. This article is distributed under the Creative Commons Attribution (CC BY) license, allowing for open access and sharing under specified terms and conditions.

<https://creativecommons.org/licenses/by/4.0/>.

Abstract

Salt stress is a major abiotic constraint limiting crop productivity in arid and semi-arid regions. Guar (*Cyamopsis tetragonoloba* L.), an important industrial and forage crop, is highly sensitive to salinity, which adversely affects its physiological and biochemical functions. The present study was conducted to evaluate the effectiveness of foliar-applied kaolin in mitigating salt stress in guar plants. A pot experiment was performed using clay loam soil selected based on its physicochemical properties. Salinity stress was imposed by adding a calculated amount of NaCl to the soil, while kaolin was applied foliarly at concentrations of 2% and 4%. Physiological attributes including photosynthetic rate, stomatal conductance, substomatal CO₂ concentration, transpiration rate, and chlorophyll content were measured. Osmolyte accumulation (proline and soluble sugars) and antioxidant enzyme activities (SOD, CAT, and POD) were also assessed. The results indicated that salinity significantly reduced plant physiological performance; however, foliar application of kaolin markedly improved photosynthetic rate (1.83-fold), stomatal conductance (24%), substomatal CO₂ concentration (11%), and chlorophyll content, while reducing transpiration rate by 54% compared to untreated plants under saline conditions. Kaolin treatment enhanced osmolyte accumulation, with proline and soluble sugars increasing by (2.56-fold) and (1.37-fold), respectively, contributing to improved osmotic adjustment. Additionally, antioxidant enzyme activities were significantly elevated in kaolin-treated plants, facilitating reactive oxygen species (ROS) detoxification and reducing oxidative damage under salt stress. These findings suggest that foliar application of kaolin is an effective and sustainable strategy to improve guar growth, physiological efficiency, and biochemical resilience under saline environments.

1. Introduction

Salt stress is one of the most pervasive abiotic constraints limiting crop productivity in arid and semi-arid regions, where low rainfall, high evapotranspiration, and inadequate drainage promote the accumulation of soluble salts in the soil

(Hussain et al., 2025). Excessive soil salinity imposes osmotic stress, reducing water uptake, followed by ionic toxicity, primarily due to sodium (Na⁺) and chloride (Cl⁻), which disrupt cellular homeostasis, compromise membrane integrity, and interfere with essential metabolic processes

(Singh et al., 2022). These physiological disturbances reduce stomatal conductance and CO₂ assimilation (Zhang et al., 2022), impair chlorophyll biosynthesis, compromise enzymatic activity, and enhance the production of reactive oxygen species (ROS) (Yin et al., 2024). In regions such as South Asia, where agriculture is frequently exposed to high salinity, sustaining crop productivity is essential not only for agronomic viability but also for ensuring food security and supporting rural livelihoods (Acharya et al., 2025).

Guar (*Cyamopsis tetragonoloba* L.), commonly known as cluster bean, is a legume of significant economic and agronomic importance. It is cultivated primarily for its endosperm, which produces guar gum—a galactomannan polysaccharide widely utilized in food processing, pharmaceuticals, textiles, and oil and gas industries (Alkizwini, 2021). Guar gum possesses unique thickening, stabilizing, and emulsifying properties, making it a critical raw material in both traditional applications and emerging fields such as bioprinting and biodegradable materials. Beyond its industrial value, guar plays an important role in sustainable agriculture owing to its inherent drought tolerance and capacity for biological nitrogen fixation. These traits reduce dependence on synthetic fertilizers and allow cultivation in low-input farming systems in water-limited environments (Koirala, 2024).

Despite its drought tolerance, guar is highly sensitive to salinity. Salt stress adversely affects seed germination, root development, and nodule formation, while compromising photosynthetic performance through chlorophyll degradation and disrupted electron transport. High salinity also impairs leaf water relations by inducing stomatal closure and reducing turgor, which limits CO₂ assimilation. Biochemically, salt-stressed plants accumulate compatible solutes such as proline and soluble sugars, while activating antioxidant enzymes to mitigate ROS-induced oxidative damage (Alalwani et al., 2025). The efficiency of these adaptive responses depends on the severity of salinity and the plant's capacity to maintain oxidative and ionic homeostasis. Failure to regulate these physiological and biochemical processes often results in reduced yield, compromised seed quality, and diminished industrial performance of guar gum, underscoring the importance of effective management strategies in saline environments.

In recent years, foliar-applied particle films, particularly kaolin, have emerged as a practical and cost-effective approach to mitigating abiotic stresses in crops, including salinity. Kaolin, a naturally occurring hydrous aluminum silicate (Al₂O₃·2SiO₂·2H₂O), forms a reflective, porous film on leaf surfaces that reduces leaf temperature, moderates radiation load, and stabilizes leaf energy balance without significantly restricting gas exchange (Ahmad et al., 2024).

These physical effects enhance water use efficiency, reduce excessive transpiration, and maintain photosynthesis under environmental stress (El-Ansary, 2025; Azuara et al., 2023). Under saline conditions, kaolin can preserve stomatal function, maintain internal CO₂ concentration, improve cellular hydration, limit ROS accumulation, and enhance antioxidant enzyme activity and osmotic adjustment (Abdallah et al., 2019). Its immobility, affordability, and environmental safety make kaolin an attractive component of integrated salinity management programs that combine genotypic selection, optimized irrigation, and soil amendments such as gypsum.

The physiological processes by which kaolin reduce stress are complex. Kaolin decreases the extreme stomatal closing by reflecting solar radiation and decreasing leaf temperature, which helps to sustain a desirable balance between CO₂ assimilation and water loss. This assists in maintaining photosynthetic activity and averts metabolic impairments that are usually related to salt stress. Moreover, enhanced leaf water status facilitates ionic homeostasis, restricts the production of ROS, and decreases lipid peroxidation, which allows antioxidant enzymes to work more effectively and osmotic regulation to be performed at a reduced energetic cost. All these cumulative effects underscore the potential of kaolin as a viable, sustainable and cost-effective agent to aid crop performance under unfavorable environmental conditions.

In spite of these promising qualities, the influence of kaolin on the physiological and biochemical performance of salt-stressed guar has not been fully studied. In particular, its effect on chlorophyll retention, photosystem stability, stomatal behavior, internal CO₂ dynamics, and transpiration in salty conditions need to be systematically assessed. Moreover, the possible interplay between decreased transpiration and nutrient movement is not fully understood, and it is not known whether kaolin can potentially inhibit the uptake of key cations (including potassium (K⁺) and calcium (Ca²⁺) or inhibit the toxic accumulation of sodium (Na⁺). It is important to address these uncertainties to come up with evidence-based agronomic strategies that would guarantee the survival of plants and maintenance of industrial-quality guar gum in saline environments.

Consequently, the current study aimed to investigate the effect of foliar-applied kaolin to enhance growth, physiological performance, and biochemical resilience of guar under salt stress in a systematic manner. The research particularly examines the effects of kaolin on stomatal control, photosynthetic performance, osmotic adjustment, and antioxidant defense systems, which give mechanistic understanding of how kaolin improves crop performance in saline soils. The study will explain these physiological and

biochemical processes to guide specific approaches to sustainable production of guar in agroecosystems that are influenced by salt, as well as to maintain the stability of the yield and quality of the product.

2. Materials and Methods

2.1. Experimental Site and Plant Material

The experiment was carried out in a wire house in the Department of Soil Science, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan, in the 2025 growing season. Average day/night temperatures were $32 \pm 3^\circ\text{C}$ / $24 \pm 2^\circ\text{C}$. Genetic uniformity was achieved by using certified seeds of guar (*Cyamopsis tetragonoloba* L.) that were acquired in the Guar Section, Regional Agricultural Research Institute, Bahawalpur. Seeds were surface sterilized in 0.1% sodium hypochlorite for 2 minutes, followed by three thorough rinses with distilled water to remove any residual disinfectant or microbial contaminants. The seeds were then dried at room temperature before sowing. Plastic pots were filled with 10 kg of air-dried, sieved (2 mm) soil. Each pot had five seeds which were sown and thinned to three similar plants per pot after emerging.

2.2. Experimental Design

The experiment was arranged in a completely randomized design (CRD) with three replications. Nine treatment combinations were established, comprising three salinity levels (0, 5, and 10 dS m^{-1} NaCl), two foliar kaolin concentrations (2% and 4% w/v), and their interactions. Salinity was imposed by thoroughly mixing the required amount of NaCl into the soil, while kaolin was applied as a foliar spray following standard procedures for arid-region crops. A 2% kaolin application is known to reduce leaf temperature, whereas a 4% concentration forms a more reflective film that alleviates physiological dehydration under high salinity (Faghihi et al., 2025). Soil moisture was maintained at approximately 70% of field capacity throughout the experiment, and all plants received uniform basal fertilization with nitrogen, phosphorus, and potassium at 60–40–30 kg ha^{-1} , applied as urea, single superphosphate, and sulfate of potash, respectively.

2.3. Salinity and Kaolin Application

The salinity was calculated by a standard formula and the necessary amount of NaCl was mixed well into the soil and then the pots were filled. The physicochemical properties of the soil were examined before the experiment. The initial soil properties were as follows: electrical conductivity (EC) of 1.3 dS m^{-1} , which is non-saline to slightly saline; pH 7.8, which is moderately alkaline reaction; sodium adsorption ratio (SAR) of 3.2 (mmol L^{-1})^{1/2}, which is low sodicity hazard; calcium carbonate (CaCO_3) content of 17.5%, which is

calcareous soil; total nitrogen 367 mg kg^{-1} ; available phosphorus The interpretation of the experimental results took into consideration these properties. The soil was mixed well and left to stabilize after two days before sowing. Each treatment was prepared as kaolin suspensions by adding kaolin powder to distilled water and a little sticking agent to maintain uniform adhesion. Foliar application was done by spraying with a hand sprayer until the leaves were completely covered without any runoff. Sprays were applied at the 15-day intervals throughout the vegetative and flowering phases with the appropriate kaolin concentrations not being surpassed. Distilled water containing the sticking agent was applied to control plants to ensure even comparison.

2.4. Physiological Measurements

The photosynthetic rate, stomatal conductance and transpiration rate were recorded at 9:00 to 11:00 a.m. on fully expanded leaves using a portable infrared gas analyzer (IRGA). Infrared thermometer was used to measure the temperature of the leaves. Relative water content (RWC) was determined using a slightly modified procedure of Weatherley (1950). A SPAD chlorophyll meter was used to determine the content of chlorophyll.

2.5. Biochemical Analysis

Leaf samples were collected 60 days after sowing for biochemical assays. Proline content was determined using the acid-ninhydrin method described by Bates et al. (1973), while soluble sugars were estimated according to the method of Yemm and Willis (1954). Activities of enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), were measured following standard protocols by Beauchamp and Fridovich (1971), Bergmeyer (1973), and Omran (1980).

2.6. Statistical Analysis

Analysis of variance (ANOVA) was used to analyze all data in the statistics 8.1 software. The Tukey Honest Significant Difference (HSD) test was used to compare treatment means at a probability level of 5%. OriginPro 2021 software was used to conduct correlation analysis between physiological and biochemical parameters to assess the relationship between measured traits.

3. Results

3.1. Growth Attributes

The data reveals a significant variation in plant growth among treatments (Table 1). Shoot length ranged from 10.8 cm to 51.5 cm, while root length varied between 13.1 cm and 29.2 cm. The maximum shoot length (51.5 cm) and root length (29.2 cm) were recorded under T₅, followed closely by T₄. These treatments also produced the highest shoot dry weight

Table 1. Effect of salinity and kaolin on the growth performance of Guar under salt stress

Treatment	Shoot length (cm)	Root Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)
T ₁	47.4±0.53b	25.2±1.16bc	5.74±0.07b	1.89±0.05ab
T ₂	34.3±0.46e	18.9±0.78ef	4.35±0.05e	1.31±0.02cde
T ₃	15.5±0.44g	13.1±0.98h	3.09±0.04h	0.92±0.08f
T ₄	50.2±0.44a	27.5±0.17ab	6.05±0.05a	2.02±0.14ab
T ₅	51.5±0.51a	29.2±0.20a	6.26±0.05a	2.12±0.02a
T ₆	38.4±0.51d	21.5±0.20de	4.88±0.04d	1.49±0.07cd
T ₇	43.5±0.40c	23.1±0.82cd	5.18±0.04c	1.65±0.07bc
T ₈	10.8±0.23h	15.5±0.20gh	3.58±0.04g	1.05±0.08ef
T ₉	19.2±0.38f	17.2±0.20fg	3.88±0.02f	1.18±0.10def
HSD Value	2.1918	3.2049	0.2229	0.3851

(6.26 g) and root dry weight (2.12 g). Compared with the control, T₅ showed a substantial increase in vegetative growth and biomass accumulation. In contrast, T₈ and T₃ exhibited markedly reduced growth. The minimum shoot length (10.8 cm) was observed in T₈, representing nearly an 80% reduction compared with T₅. Root length was lowest in T₃ (13.1 cm), indicating strong growth suppression under stress conditions. Overall, growth parameters showed a declining trend under salinity stress, whereas kaolin-treated plants maintained comparatively higher values.

3.2. Physiological Responses

Physiological attributes varied significantly across treatments (Table 2). SPAD chlorophyll value ranged from

25.1 to 46.2 SPAD units. The highest SPAD chlorophyll value was recorded in T₅ (46.2 SPAD), which correlates directly with its superior biomass accumulation. Conversely, T₃ exhibited the lowest chlorophyll content (25.1), suggesting a breakdown of photosynthetic pigments under that specific treatment. High-performing treatments (T₄ and T₅) showed maximum relative water content (RWC) 84–85% and the highest membrane stability index (MSI) 80–82%, respectively. This indicates that these treatments helped the plants to maintain cellular turgor and structural integrity. An inverse relationship was observed between growth and proline accumulation. The highest proline contents were found in T₉ (7.42) and T₈ (7.02), while the lowest levels were found in T₁ (2.08) and T₄ (2.42) respectively. This represents more than a threefold increase under stress conditions.

Table 2. Effect of salinity and kaolin on the physiology of Guar under salt stress

Treatment	SPAD Chlorophyll Value	Relative Water Contents (%)	Membrane Stability Index (%)	Proline Contents (μmol g ⁻¹ FW)
T ₁	42.2±1.59ab	82±0.61b	77±0.64b	2.08±0.02i
T ₂	33.9±1.25de	70±0.51e	64±0.68e	4.85±0.04f
T ₃	25.1±0.68g	57±0.64h	51±0.51h	6.48±0.04c
T ₄	43.6±0.37ab	84±0.26a	80±0.40a	2.42±0.02h
T ₅	46.2±0.78a	85±0.23a	82±0.17a	2.62±0.02g
T ₆	37.5±0.69cd	74±0.38d	68±0.46d	5.42±0.04e
T ₇	39.2±0.78bc	77±0.23c	71±0.25c	5.82±0.04d
T ₈	28.2±0.71fg	63±0.40g	56±0.44g	7.02±0.04b
T ₉	30.9±0.69ef	65±0.35f	58±0.25f	7.42±0.04a
HSD Value	4.4830	2.1215	2.2551	0.1645

3.3. Reactive Oxygen Species

The data (Figure 1A-C) illustrates the levels of three Reactive oxygen species (ROS), Hydrogen Peroxide, Superoxide Anion, and Hydroxyl Radical across nine different treatments. One of the major indicators of oxidative stress in plants is ROS accumulation, especially when plants are under salinity stress. The observed differences between treatments are due to differences in the intensity of stress and the efficiency of treatments applied to reduce oxidative damage.

lipid peroxidation and disturbance in cellular redox homeostasis under stress conditions. However foliar application of kaolin at higher level of NaCl stress T₈ (12.35) and T₉ (10.67) showed significantly more accumulation of H₂O₂ compared to the control. This suggests that under severe salinity, the protective effect of kaolin might be partially limited due to excessive ionic toxicity and osmotic imbalance. Its minimum value was recorded in T₅ (4.6) and T₄ (4.97) respectively, suggesting these treatments may have an inhibitory or antioxidant effect on H₂O₂ production. The

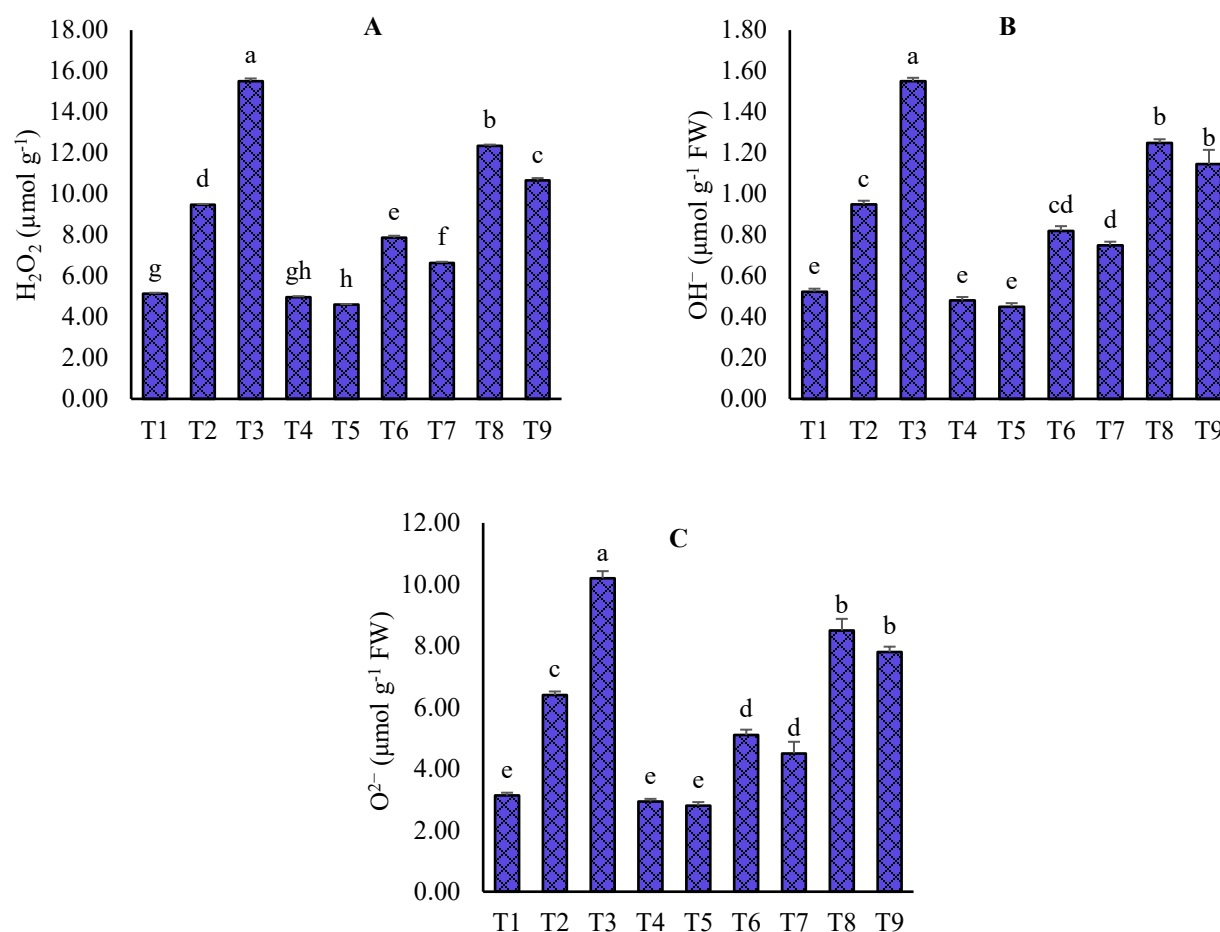


Figure 1. Effect of salinity and kaolin on reactive oxygen species in Guar cultivation under salt stress, Data are presented as mean $\pm$ SE. Different lowercase letters above the bars indicate significant differences between treatments at $p < 0.05$ according to HSD Tukey Multiple Range Test.

3.3.1. Hydrogen Peroxide ($\mu\text{mol g}^{-1}$)

Treatment T₃ showed maximum accumulation (15.51) of H₂O₂, indicating maximum oxidative stress or a specific triggering mechanism in this treatment. Elevated hydrogen peroxide levels are commonly associated with membrane

reduced accumulation in these treatments indicates improved antioxidant defense and better physiological stability.

3.3.2. Superoxide Anion ($\mu\text{mol g}^{-1}$ FW)

The maximum superoxide anion ($10.2 \mu\text{mol g}^{-1}$ FW) was observed in T₃, followed by T₈ ($8.5 \mu\text{mol g}^{-1}$ FW) and T₉ (7.8

$\mu\text{mol g}^{-1}\text{FW}$). A similar trend to hydrogen peroxide indicates consistent oxidative pressure under these treatments. The values are generally lower in magnitude than H_2O_2 , which is expected, as O_2^- is rapidly dismutated into H_2O_2 by enzymes such as SOD. This enzymatic conversion plays a crucial role in regulating superoxide toxicity but may contribute to increased hydrogen peroxide levels. Treatment T_5 ($2.8 \mu\text{mol g}^{-1}\text{FW}$) and T_4 ($2.93 \mu\text{mol g}^{-1}\text{FW}$) showed the least superoxide radical accumulation. Lower superoxide content in these treatments reflects reduced electron leakage and enhanced ROS scavenging efficiency, indicating improved stress tolerance.

3.3.3. Hydroxyl Radical ($\text{nmol g}^{-1}\text{FW}$)

The concentration of hydroxyl radicals is significantly lower (ranging from 0.45 to $1.55 \mu\text{mol g}^{-1}\text{FW}$) compared to the other two parameters, reflecting the highly reactive and short-lived nature of hydroxyl radicals. T_3 exhibited the highest radical damage potential ($1.55 \mu\text{mol g}^{-1}\text{FW}$), suggesting a high rate of the Fenton reaction occurring in this treatment. T_1 , T_4 , and T_5 showed minimum levels ($0.45\text{--}0.52 \mu\text{mol g}^{-1}\text{FW}$), indicating minimal oxidative damage under these conditions.

3.4. Gaseous Exchange Attributes

There was a high level of differences in the gas exchange parameters across all the treatments (Figure 2A-D). Salt stress causes damage to the photosynthetic machinery as is directly indicated by the photosynthetic rate. Photosynthetic rate with T_5 indicated the highest value, which was far better than any other treatment. T_4 was the next, best treatment. T_3 showed the lowest photosynthetic rate, so this treatment was extremely stressful or limiting in the case of the plant. T_5 recorded the highest rate of transpiration at 5.67 and T_3 the lowest rate of transpiration at 2.37, which was very low when compared to the other groups. T_4 (5.33) and T_5 (5.67) were the only ones that had an increase in transpiration than T_1 (5.0). All of the treatments T_2 , T_3 , T_6 , T_7 , T_8 , and T_9 showed a reduction in the transpiration rate relative to T_1 . These results are similar to the visual patterns of the bar graphs, which proved that T_5 was the treatment with the highest physiological activity in the exchange of water vapor, and T_3 was the most constrained. In line with the data on transpiration, T_5 recorded the greatest stomatal conductance of 0.41. T_8 (0.15) and T_3 (0.16) had the lowest values, which implied that these treatments produced the highest level of stomatal closure. Only the T_4 (0.35) and T_5 (0.41) had improved conductance with respect to T_1 (0.33). There was a significant variation in the intercellular CO_2 level (Ci) among the nine experimental treatment groups. These values had a minimum of 172 in treatment T_3 and a maximum of 269 in treatment T_5 , which showed that gas exchange parameters are largely affected by treatment conditions. The maximum Ci

was observed in treatment T_5 (269), then there was T_4 (255) and T_1 (242). Such treatments were the highest data of the dataset. The minimum Ci was recorded in T_3 (172) and T_8 (192). T_3 Ci was about 36% of the highest value in T_5 . T_2 , T_6 , T_7 , and T_9 had moderate levels of treatment with the values varying between 201 and 235. In particular, T_6 (222) and T_7 (235) retained values close to the overall value of the study sample.

3.5. Antioxidant Activity

The activities of key enzymatic antioxidants, including catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), were significantly influenced by the various treatments (Figure 3A–C). Overall, all three enzymes followed a similar trend across the treatment groups, reaching their maximum values in treatment T_7 . CAT activity showed significant variation among the treatments (Figure 3A). The lowest activity was recorded in T_1 (9.3). Activity increased progressively through T_2 and T_3 before declining in T_4 and T_5 . A substantial and significant increase was observed starting from T_6 , with T_7 exhibiting the highest CAT activity (29.1), which was significantly higher ($p < 0.05$) than all other treatments. Activity levels in T_8 and T_9 remained elevated compared to the control but were significantly lower than the peak observed in T_7 . Similarly, POD activity was significantly enhanced by the treatments (Figure 3B). T_1 showed the minimum POD activity. While moderate increases were noted in T_2 and T_3 , the activity increased markedly in the later treatment groups. T_7 reached the maximum POD activity (33.5). Interestingly, T_6 and T_9 showed comparable levels of POD activity. SOD activity followed a consistent pattern with the other antioxidant enzymes (Figure 3C). The activity in T_1 was approximately 19.5. A significant upward trend was observed as the treatments progressed, with a sharp increase from T_5 to T_6 . The maximum SOD activity was recorded in T_7 (43.8). Treatments T_6 and T_9 showed high levels of activity with no statistically significant difference between them, while T_8 showed a slight but significant decrease compared to T_7 and T_9 .

3.6. Soluble sugar contents

The use of various treatments greatly changed the accretion of soluble sugars (Figure 3D). The results showed that there was a fluctuating yet overall upward trend in the concentration of sugar in the groups with the highest concentration being recorded in the last stage of treatment. The minimum level of soluble sugar was observed in the control group (T_1 , ~13). A sharp and significant rise was observed through T_2 and T_3 , with T_3 attaining a local maximum of about 22.7. There was significant decrease in the soluble sugar concentration in T_4 (15.2) as compared to T_3 , then slight but significant increase in T_5 (16.9). Since T_6 ,

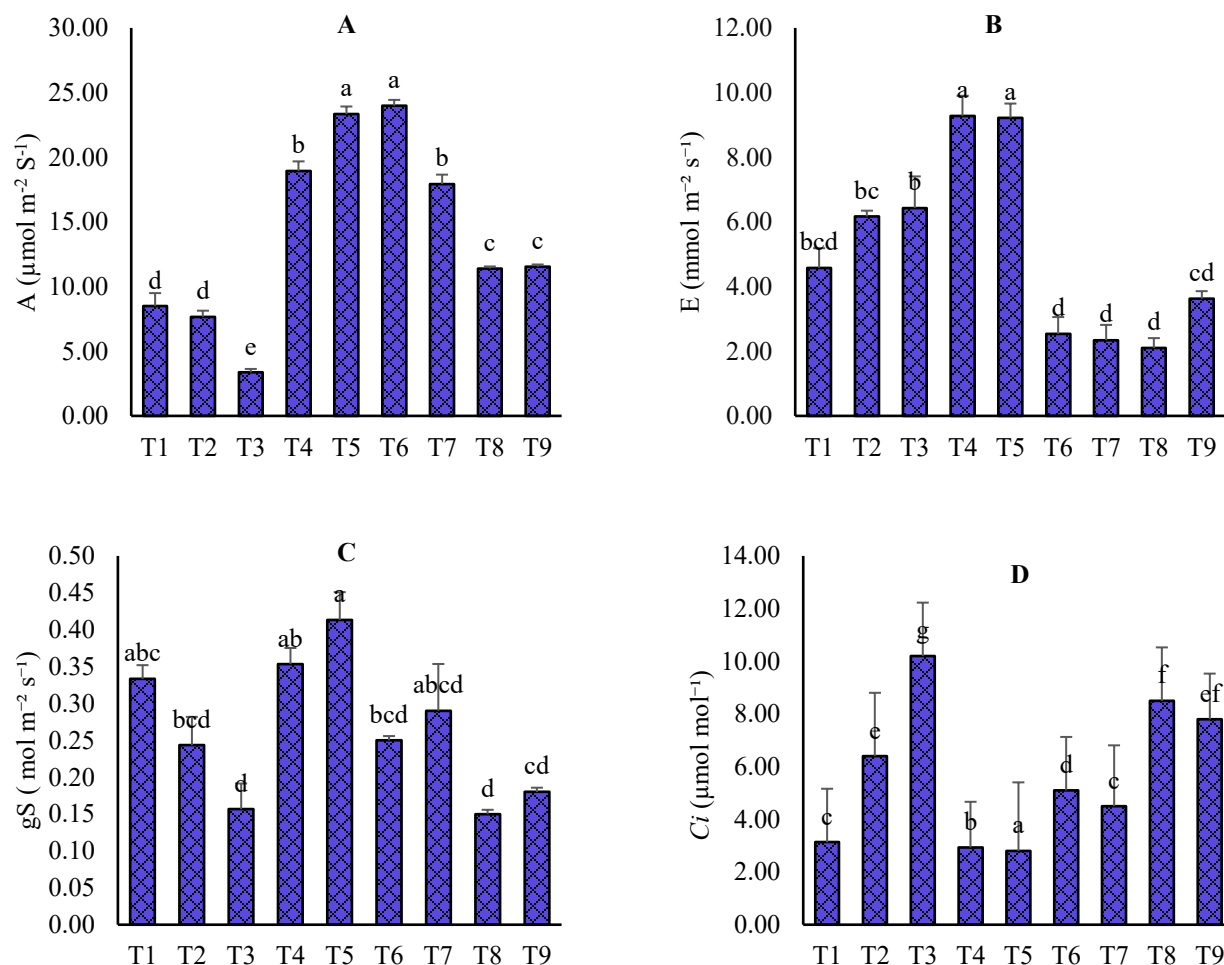


Figure 2: Effect of salinity and kaolin on gas exchange parameters in Guar cultivation under salt stress, Data are presented as mean $\pm$ SE. Different lowercase letters above the bars indicate significant differences between treatments at $p < 0.05$ according to HSD Tukey Multiple Range Test.

the soluble sugar content showed a pronounced and positive upward trend with a value of 20.8 in T₆ and increasing to 24.3 in T₇. Treatment T₉ (30.6) had the highest soluble sugar content, and this was significantly higher ($p < 0.05$) than the other treatment groups.

3.7. Pearson correlation

The Pearson correlation (Figure 4) results between various plant attributes showed that proline contents and reactive oxygen species were negatively correlated to the plant growth, physiological and gas exchange parameters. The chlorophyll contents were positively correlated with leaf stomatal conductance ($r = 0.86$). The rate of transpiration had a negative relationship with proline contents (0.63). The activities of SOD, POD and CAT were positively correlated

with water use efficiency, which showed that there is a relationship between antioxidant defense and efficient water use. Physiology and gas exchange properties were significantly and positively related to total plant biomass, which indicates that the combination of these factors influenced the overall performance of plants in various treatments.

4. Discussion

A significant limiting factor on the growth and productivity of guar is salt stress. In the current research, moderate salinity led to a significant decrease in biomass, whereas high salinity led to a major decrease in plant growth because of chlorosis, necrosis, and slowed metabolic activity. Shoot and root length, and dry matter accumulation were significantly lower

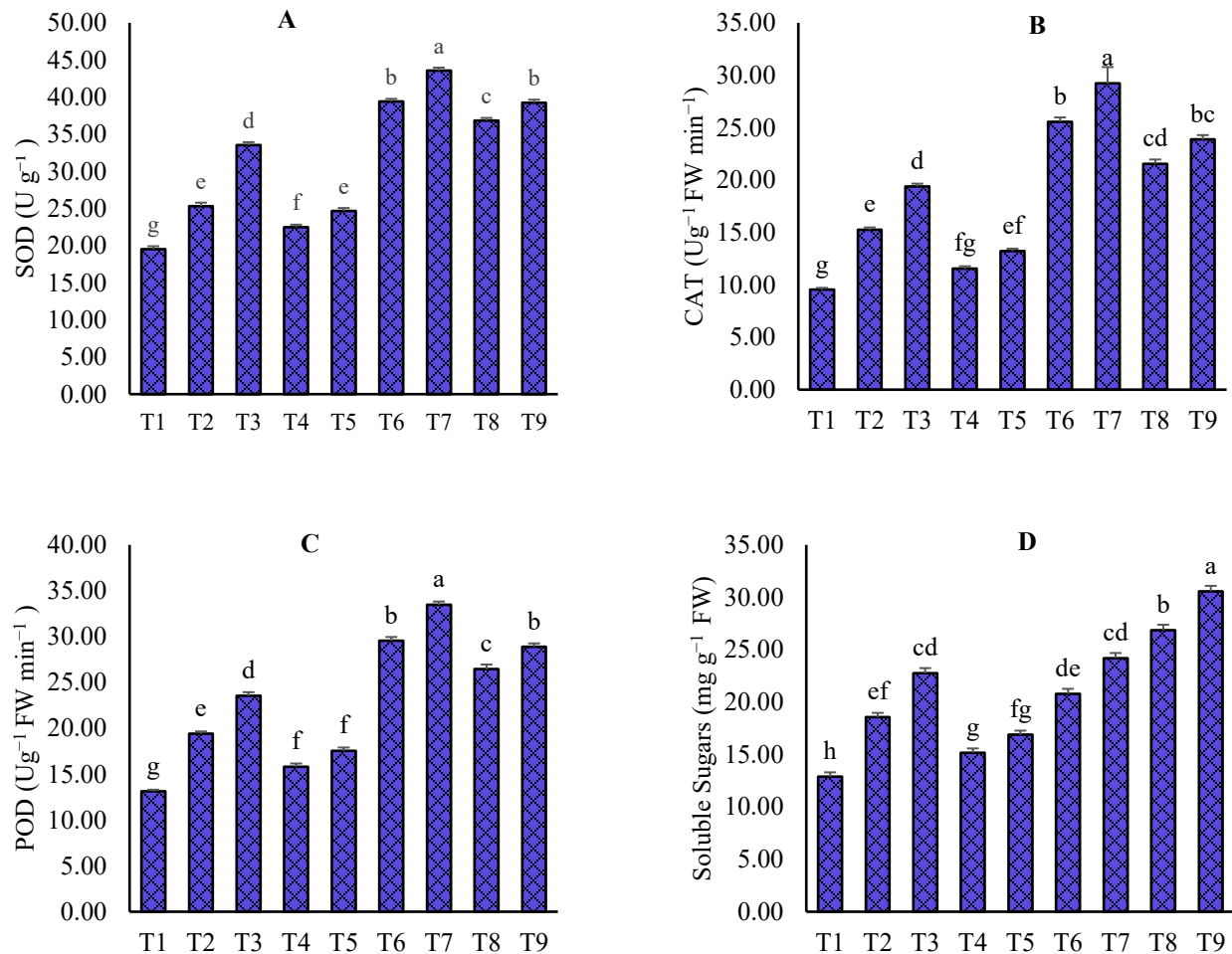


Figure 3. Effect of salinity and kaolin on enzymatic antioxidants and soluble sugar contents in guar cultivation under salt stress. Data are presented as mean $\pm$ SE. Different lowercase letters above the bars indicate significant differences among treatments at $p < 0.05$ according to Tukey's HSD multiple range test.

in T₃ (10 dS m⁻¹) and T₈ (high salinity + kaolin), which means that the excess of Na⁺ and Cl⁻ ions interfere with cellular homeostasis, inhibit water uptake, and limit nutrient allocation. Conversely, the growth of foliar kaolin application (T₄ and T₅) in non-saline conditions enhanced the growth of the shoot and root, which indicates its stimulatory role in plant development in the absence of stress. The results indicate that kaolin does not only protect plants in salty conditions but also improves overall growth performance by means of better leaf microclimate and photoprotection.

Kaolin served as a good antitranspirant, which reduced heat load and alleviated the visual effects of salinity stress. T₄ and T₅ treatments had greater chlorophyll contents, relative water content (RWC) and membrane stability index (MSI) and this

demonstrates its importance in maintaining photosynthetic efficiency and cellular integrity. The higher chlorophyll content with kaolin application promoted light capture, which was converted to high photosynthetic rates and biomass growth. On the other hand, salinity (high) in the absence of kaolin (T₃, T₈) led to low SPAD values, low RWC, and low MSI, which is indicative of oxidative damage and osmotic stress. These findings are aligned with earlier studies that salinity affects the biosynthesis of chlorophyll, interferes with turgor and senescence (Angon et al., 2022).

Osmotic adjustment was observed by the accumulation of proline, which rose with high salinity (T₈, T₉) and was used as a measure of the intensity of stress. The negative correlation that was found between proline concentrations

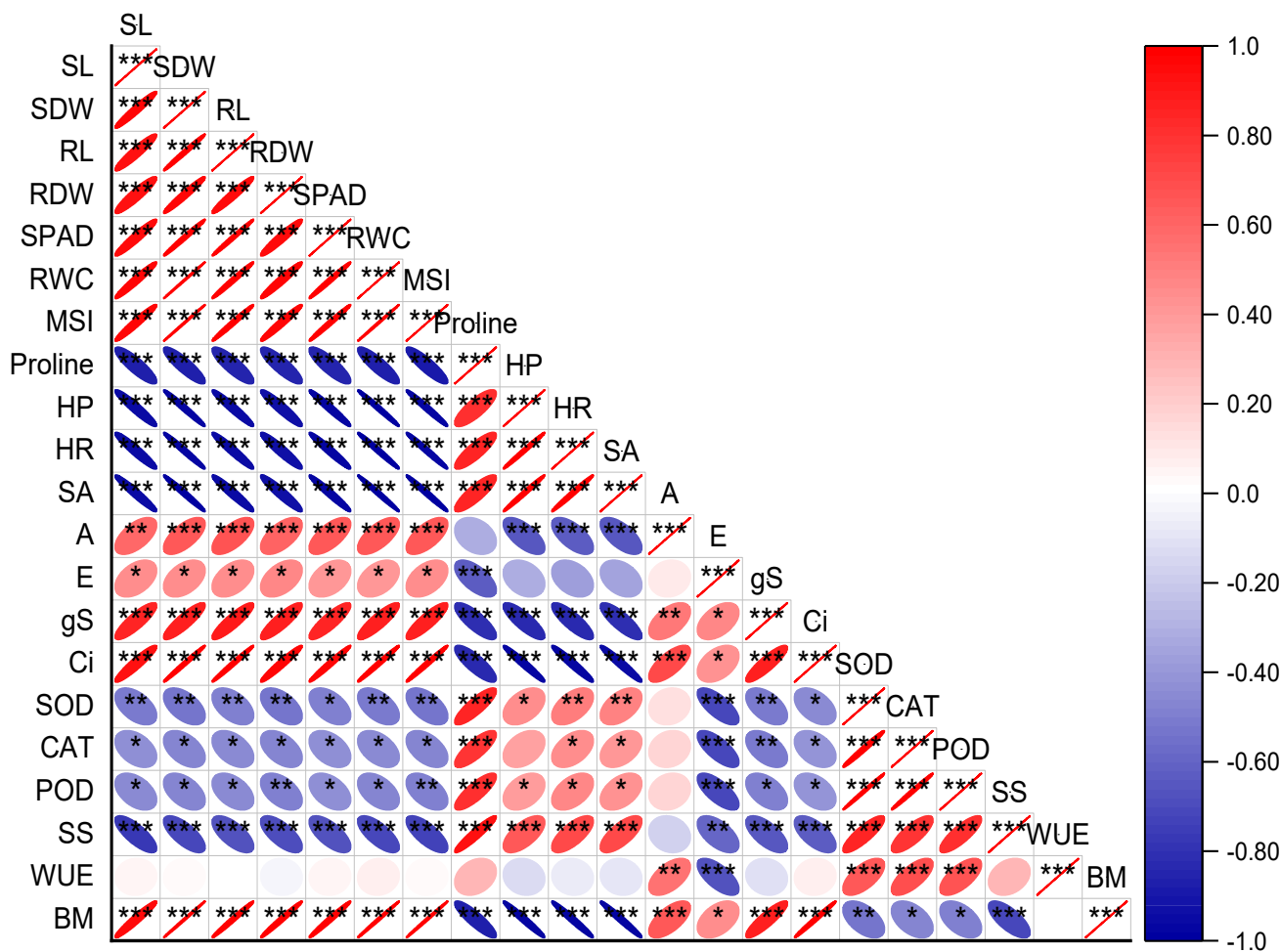


Figure 4. Pearson correlation analysis among biomass (BM), water use efficiency (WUE), superoxide anion (SA), hydroxyl radical (HR), hydrogen peroxide (HP), and soluble sugars (SS). Asterisks indicate levels of significance: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

and growth indicates that plants that are highly stressed use more energy in defense mechanisms instead of synthesizing biomass. Plants treated with kaolin and low salinity (T_4 , T_5) had lower levels of proline, which showed a decrease in the physiological pressure. On the same note, soluble sugars were elevated during stress, as compatible solutes to stabilize cellular osmotic equilibrium. Kaolin treatments showed a moderate increase in sugars, which implies that kaolin suppresses the necessity of a wide osmotic adjustment due to the preservation of water status. These osmoprotective systems play a vital role in protecting metabolic activity and maintaining constant carbon assimilation in salinity. The accumulation of reactive oxygen species (ROS) such as H_2O_2 , superoxide, and hydroxyl radicals was more pronounced under salinity stress, especially in T_3 and T_8 ,

which validates oxidative stress. The plants treated with kaolin (T_4 and T_5) had a much lower ROS level, which is an indirect antioxidant effect due to the preservation of water relations and the alleviation of stomatal stress. The protective effect of kaolin was also facilitated by the further improvement of antioxidant enzyme (SOD, CAT, POD) activity in T_6 and T_7 , which assisted plants to eliminate ROS and reduce lipid peroxidation. These findings indicate that kaolin enhances the resilience of plants both mechanically (reflective film) and physiologically (increased enzyme activity), which is in line with the prior results (Bríto et al., 2019). These trends were reflected in gaseous exchange attributes. The photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 (Ci) were all optimized at T_4 and T_5 , which proved that kaolin inhibits

stomatal closure by reducing the temperature of leaves and vapor pressure deficit. This equilibrium between CO_2 absorption and water conservation enhanced water use efficiency and metabolic performance. Salinity levels in the absence of kaolin, however, caused extreme stomatal limitation and decreased gaseous exchange, which underscores the stress-induced inhibition of carbon assimilation. Photosynthesis is probably maintained during kaolin application by a combination of increased light reflectance and better leaf hydration, which enables more favorable energy balance and photosynthetic efficiency (Bartczak et al., 2025). A mechanistic overview of kaolin-mediated enhancement of physiological and biochemical resilience in guar under salt stress is shown (Figure 5).

C/N ratio remained more stable, facilitating carbon fixation and structural development even under challenging conditions. Shoot and root dry matter became maximal with kaolin application, likely due to increased cell division and elongation, promoting higher structural development. Similarly, kaolin applications improved MSI, indicating reduced electrolyte leakage and enhanced cellular protection under stress. In treatments with higher salinity, MSI declined ($T_3 = 51\%$, $T_8 = 56\%$), whereas lower salinity with kaolin maintained higher MSI ($T_7 = 71\%$), demonstrating the combined effect of kaolin and reduced ionic stress on cell stability. Proline and soluble sugar accumulation under salinity highlighted the biochemical adjustments required for osmotic balance and ROS scavenging. Kaolin reduced

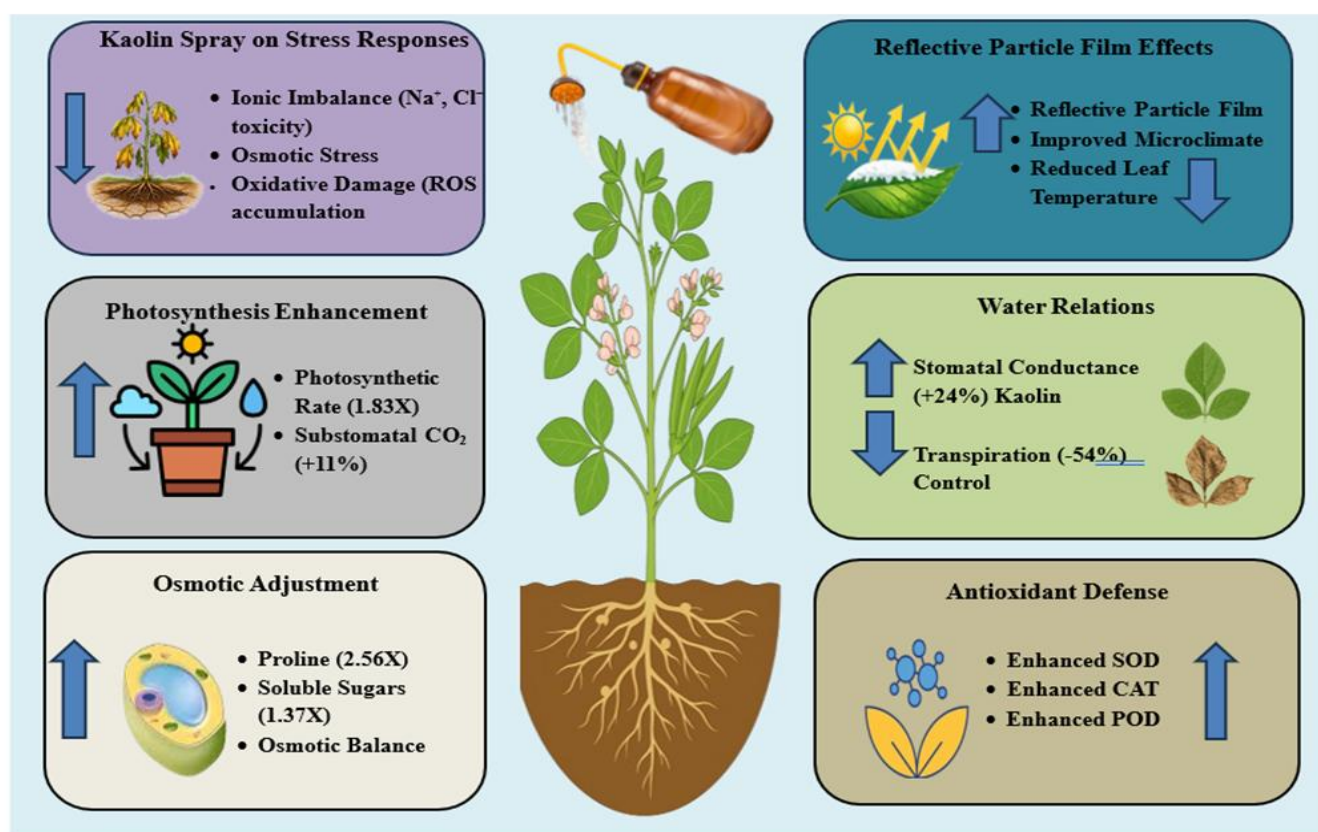


Figure 5. Mechanistic overview of kaolin-mediated resilience in guar under salt stress. Kaolin reduces leaf temperature, maintains stomatal function and water status, enhances chlorophyll and antioxidant activity, regulates osmolytes, and mitigates ROS, supporting growth under salinity.

Kaolin's reflective properties contributed to these benefits by decreasing leaf temperature and excessive radiation, reducing the risk of photoinhibition, and preserving the function of photosystems. Improved energy balance allowed plants to allocate more resources toward growth and osmotic regulation rather than stress defense alone. Consequently, the

excessive proline accumulation under low salinity, suggesting that the plants required less energy for stress mitigation, enabling greater growth. Conversely, T_8 and T_9 displayed higher proline and sugar levels, reflecting the physiological pressure of salinity and the plant's adaptive response to maintain metabolic activity.

Collectively, salt stress severely limits guar growth by inducing osmotic imbalance, ion toxicity, ROS accumulation, and disruption of photosynthetic machinery. Foliar application of kaolin mitigates these effects by: (i) reducing leaf temperature and transpiration, (ii) improving relative water content and membrane stability, (iii) enhancing photosynthetic efficiency, (iv) modulating osmolyte accumulation, and (v) supporting antioxidant defense mechanisms. Treatments T₄ and T₅ consistently showed superior performance, highlighting kaolin as a practical, low-cost, and effective strategy to enhance physiological and biochemical resilience in guar under both saline and non-saline conditions. These findings demonstrate that integrating kaolin into management practices can sustain growth, improve water use efficiency, and maintain plant vigor in arid, salt-affected environments.

5. Conclusion

Salinity has an adverse influence on the growth, physiological performance and biochemical processes of guar. Kaolin applied as foliage increases photosynthesis by eliminating reactive oxygen species (ROS) by improved antioxidant activity and compatible solutes accumulation. Kaolin also enhances the usage of water by regulating the leaf temperature which promotes the ideal stomatal behavior leading to decreased ROS production. Consequently, plants have an increased tolerance to salt stress especially with moderate salinity whereby physiological systems are not resistant. On the practical management side, kaolin is a low cost and simple to apply remedy applicable in both smallholder and commercial cultivation systems. It is most advantageous when used with coinciding applications at the time of high radiation levels as well as the sensitive growth phases, including vegetation growth, flowering, and pod set. The effective dose can be different with the type of crop, but in guar, 4% (w/v) kaolin suspension is the most effective one, but additional optimization of results based on the canopy density and local microclimate can improve the results. In general, the experiment shows that foliar application of 4 percent kaolin is very beneficial to guar in terms of growth, physiological, and biochemical performance during salt stress. It lowers the concentration of ROS, boosts the level of chlorophyll, and lessens water stress, thus improving the general condition and performance of the plant.

6. Funding

No funding was received for this study.

7. Acknowledgment

The authors gratefully acknowledge the support provided by the Saline Agriculture Research Centre, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, for facilitating this study through the provision of

necessary resources and research facilities. The authors also broaden their appreciation to all collaborators and staff members who participated directly or indirectly in the smooth accomplishment of the trial activities.

8. Conflict of Interest

The authors declare that they have no conflicts of interest related to this research or its publication.

9. Authors' Contribution

Muhammad Ameen and Javaid Aktar were involved in the conceptualization of the study, data collection, and initial drafting of the manuscript. Javaid Akhtar supervised the experiment. Muhammad Anwar-Ul-Haq facilitated in lab analysis. Zaffar Malik provided critical intellectual input. Mueen Alam Khan and Kashif Mustafa assisted in data interpretation and manuscript revision. All authors reviewed and approved the final manuscript.

10. SDGs Addressed

This study supports SDG 02 (Zero Hunger) by promoting sustainable and resilient guar production under salt-affected conditions through an eco-friendly foliar kaolin strategy. It also contributes to SDG 13 (Climate Action) by enhancing crop tolerance to salinity stress associated with climate change.

References

- Abdallah, M. M. S., El-Bassiouny, H. M. S., & AbouSeeda, M. A. (2019). Potential role of kaolin or potassium sulfate as anti-transpirant on improving physiological, biochemical aspects and yield of wheat plants under different watering regimes. *Bulletin of the National Research Centre*, 43(1), 134.
- Abdelslam, O. G., & El-khawaga, H. A. (2025). Mitigating salinity stress in two wheat cultivars through Nano-Silicon application: Impact on yield and grain composition Nanosilica particles, *Triticum aestivum*, yield, silica accumulation. *International Journal of Theoretical and Applied Research*, 4(1), 563-576.
- AbuEl-Leil, E. F., AbdelRahman, M. A., & Desoukey, S. F. (2024). Effect of kaolin on productivity, anatomical and biochemical responses to water deficit in *Pelargonium graveolens* grown in sandy soil. *BMC Plant Biology*, 24(1), 1111.
- Acharya, B. R., Sandhu, D., Dueñas, C., Ferreira, J. F., & Grover, K. K. (2022). Deciphering molecular mechanisms involved in salinity tolerance in Guar (*Cyamopsis tetragonoloba* (L.) Taub.) using transcriptome analyses. *Plants*, 11(3), 291.

- Alalwani, A. K., Sekhi, Y. S., Kamis, A., Al-Dulaimy, A. F., Almohammed, O. H., & Al-Taey, D. K. A. (2025). Physiological Activities of Reactive Oxygen Species (ROS) in Plants: A Review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1487, No. 1, p. 012063). IOP Publishing.
- Alkizwini, R. S. (2021). The use of an organo-kaolinite sorbent in a permeable reactive barrier for remediating groundwater contaminated with methylene blue dye: experimental and theoretical investigation. *Environmental Processes*, 8(2), 889-910.
- Angon, P. B., Tahjib-Ul-Arif, M., Samin, S. I., Habiba, U., Hossain, M. A., & Brestic, M. (2022). How do plants respond to combined drought and salinity stress?—A systematic review. *Plants*, 11(21), 2884.
- Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, 55(1), 373-399.
- Atta, K., Mondal, S., Gorai, S., Singh, A. P., Kumari, A., Ghosh, T., ... & Jespersen, D. (2023). Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. *Frontiers in Plant Science*, 14, 1241736.
- Azuara, M., González, M. R., Mangas, R., & Martín, P. (2023). Kaolin foliar-application improves the photosynthetic performance and fruit quality of Verdejo grapevines. In *BIO Web of Conferences* (Vol. 68, p. 01024). EDP Sciences.
- Bartczak, M. H., Croci, M., Tiwari, H., Poni, S., & Frioni, T. (2025). Modeling grapevine leaf reflectance and physiological response to increasing kaolin doses and varying light and water availability. *Plant Physiology and Biochemistry*, 110738.
- Bates, L. S., Waldren, R. P. A., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39(1), 205-207.
- Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1), 276-287.
- Bergmeyer, H. U. (1973). Standardization of the reaction temperature for the determination of enzyme activity. *Clinical Chemistry and Laboratory Medicine*, 11(1), 39-45.
- Boari, F., Donadio, A., Pace, B., Schiattone, M. I., & Cantore, V. (2016). Kaolin improves salinity tolerance, water use efficiency and quality of tomato. *Agricultural Water Management*, 167, 29-37.
- Brito, C., Dinis, L. T., Moutinho-Pereira, J., & Correia, C. (2019). Kaolin, an emerging tool to alleviate the effects of abiotic stresses on crop performance. *Scientia Horticulturae*, 250, 310-316.
- El-Ansary, D. O. (2025). Smart Farming and Orchard Management: Insights and Innovations. *Current Food Science and Technology Reports*, 3(1), 10.
- Faghihi, E., Aghamir, F., Mohammadi, M., & Eghlima, G. (2025). Kaolin application improved growth performance, essential oil percentage, and phenolic compound of *Thymus vulgaris* L. under drought stress. *BMC Plant Biology*, 25(1), 892.
- Firdoos, A., Wang, C., Manzoor, M. A., Ramakrishnan, M., ... & Ding, Y. (2024). Enhancing plant resilience: Nanotech solutions for sustainable agriculture. *Heliyon*, 10(23).
- Flowers, T. J., & Colmer, T. D. (2008). Salinity tolerance in halophytes. *New Phytologist*, 945-963.
- Garcia, A., Grover, K., VanLeeuwen, D., Stringam, B., & Schutte, B. (2023). Growth and performance of guar (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes under various irrigation regimes with and without biogenic silica amendment in Arid Southwest US. *Plants*, 12(13), 2486.
- Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), 909-930.
- Grassi, G., & Magnani, F. (2005). Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell & Environment*, 28(7), 834-849.
- Hailegnaw, N. S., Awoke, G. W., Santos, A. D. C., Schaffer, B., Vargas, A. I., Souza, E. R. D., & Bayabil, H. K. (2025). Assessing salinity-induced impacts on plant transpiration through machine learning: from model development to deployment. *Modeling Earth Systems and Environment*, 11(3), 1-14.
- Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., Siddique, M. J., Zia, R., ... & Imran, A. (2021). Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. *Frontiers in Sustainable Food Systems*, 5, 617157.
- Hissaria, P. (2025). Factors Influencing the Supply & Demand for Guar Gum: A Qualitative Study of

- Stakeholder Perspectives in India and the US. Available at SSRN 5373066.
- Hussain, S. B., Karagiannis, E., Manzoor, M., & Ziogas, V. (2023). From stress to success: Harnessing technological advancements to overcome climate change impacts in citriculture. *Critical Reviews in Plant Sciences*, 42(6), 345-363.
- Jifon, J. L., & Syvertsen, J. P. (2003). Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree Physiology*, 23(2), 119-127.
- Khurizadeh, S., Naderi, R., Meftahizadeh, H., Hazrati, S., & Nicola, S. (2024). Foliar Application of Methyl Jasmonate and Chitosan Improve Growth, Yield, and Quality of Guar (*Cyamopsis tetragonoloba* L.) Under Water-Deficit Stress. *Plants*, 13(21), 3099.
- Koirala, S. (2024). *Genetic variation in black gram and guar for phenology, physiology, growth, and yield under irrigated and rainfed conditions of northern great plains* (Master's thesis, North Dakota State University).
- Lombardini, L., Harris, M.K. & Glenn, D.M. 2005 Effects of particle film application on leaf gas exchange, water relations, nut yield, and insect populations in mature pecan trees. *HortScience* 40, 1376-1380.
- Miller, G. A. D., Suzuki, N., Ciftci-Yilmaz, S. U. L. T. A. N., & Mittler, R. O. N. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment*, 33(4), 453-467.
- Omran, R. G. (1980). Peroxide levels and the activities of catalase, peroxidase, and indoleacetic acid oxidase during and after chilling cucumber seedlings. *Plant Physiology*, 65(2), 407-408.
- Pereira, S., Monteiro, A., Baltazar, M., Maia, C., Pereira, S., Oliveira, M. J., ... & Dinis, L. T. (2025). Modulating grapevine performance and hormonal dynamics under summer stress by the synergistic effects of kaolin and silicon. *Frontiers in Plant Science*, 16, 1639169.
- Sallam, M. E., ElRefaay, E. S., Emara, H. A., & Khidr, Y. A. (2025). Potential of kaolin and fulvic acid antitranspirant substances in suppressing wheat leaf rust and enhancing crop yield under four water irrigation regime levels. *Middle East Journal of Applied Sciences*. 15(03),203-218.
- Sánchez-Suárez, F., Martínez-García, R., de Lerma, N. L., & Peinado, R. A. (2025). Synergistic strategy against the effects of climate change using non-positioned vegetation training systems and the application of kaolin in a vineyard in a semi-arid climate: agronomic and oenological effects. *Agronomy*, 15(12), 2730.
- Sandhu, D., Pallete, A., William, M., Ferreira, J. F., Kaundal, A., & Grover, K. K. (2023). Salinity responses in 24 guar genotypes are linked to multigenic regulation explaining the complexity of tolerance mechanisms in planta. *Crop Science*, 63(2), 585-597.
- Shah, S. H., Houborg, R., & McCabe, M. F. (2017). Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy*, 7(3), 61.
- Shen, C., Yang, W., Kang, Y., Qin, S., Zhang, W., Liu, Y., ... & Han, Y. (2025). Effect of alkaline salt stress on photosynthetic activities of potato plants (*Solanum tuberosum* L.). *Plants*, 14(19), 2979.
- Singh, P., Choudhary, K. K., Chaudhary, N., Gupta, S., Sahu, M., Tejaswini, B., & Sarkar, S. (2022). Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones. *Frontiers in Plant Science*, 13, 1006617.
- Stefanov, M. A., Rashkov, G. D., Borisova, P. B., & Apostolova, E. L. (2024). Changes in photosystem II complex and physiological activities in pea and maize plants in response to salt stress. *Plants*, 13(7), 1025.
- Teolis, I., Liu, W., & Peffley, E. B. (2009). Salinity effects on seed germination and plant growth of guar. *Crop Science*, 49(2), 637-642.
- Vatandoost, S., Davarynejad, G., & Tehranifar, A. (2017). Impact of Kaolin Particle Film on Light Extinction Coefficient and Radiation Use Efficiency of Pistachio (*Pistachia vera*). *AgroLife Scientific Journal*, 6(2), 214-218.
- Weatherley, P.E. (1950) Studies in the Water Relations of the Cotton Plant I. The Field Measurements of Water Deficit in Leaves. *New Phytologist*, 49, 81-97.
- Yin, Y., Cui, D., Chi, Q., Xu, H., Guan, P., Zhang, H., ... & Sun, H. (2024). Reactive oxygen species may be involved in the distinctive biological effects of different doses of 12C⁶⁺ ion beams on Arabidopsis. *Frontiers in Plant Science*, 14, 1337640.
- Zhang, Y., & Liu, X. (2025). Kaolin-induced changes in stomatal conductance and water use efficiency in rice under salt-affected soils. *Plant Science Research*, 47(4), 210-225.
- Zhang, Y., Kaiser, E., Li, T., & Marcelis, L. F. (2022). NaCl affects photosynthetic and stomatal dynamics by

osmotic effects and reduces photosynthetic capacity by ionic effects in tomato. *Journal of Experimental Botany*, 73(11), 3637-3650.

Disclaimer/Publisher's Note: The views, opinions, and data presented in all publications are exclusively those of the respective author(s) and contributor(s) and do not reflect the stance of Pagepal or its editorial team. Pagepal and the editor(s) disclaim any responsibility for harm to individuals or damage to property resulting from the use of any ideas, techniques, instructions, or products mentioned in the content.