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ANTIOXIDANT ENZYME RESPONSES OF RICE (*Oryza sativa* L.*)* PLANTS TO SALINITY STRESS AND SOLID BIOFERTILIZER APPLICATION

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Abstract

This study evaluated the antioxidant enzyme responses of rice (Oryza sativa L.) to salinity stress and solid biofertilizer application under controlled conditions at the Department of Plant Biology and Biotechnology Botanic Garden, University of Benin. Soil samples were collected, bulked, and distributed into 66 bags, Soil salinity was induced using ($NaNO_3$) sodium nitrate at concentrations of 100, 1000, and 10,000 ppm for six weeks to simulate saline conditions The activities of key antioxidant enzymes Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate (Vit. C), and Glutathione Peroxidase (GPx) as well as malondialdehyde (MDA), ascorbate content, and total protein were assessed to determine oxidative stress responses. Results showed that salinity stress altered antioxidant enzyme activities, while solid biofertilizer application modulated these effects. The control recorded an SOD activity of 4.38 u/g protein, whereas treatments such as S1A (6.90 u/g) and S3D (6.76 u/g) showed increased activity, indicating enhanced antioxidant defense. Conversely, some treatments exhibited reduced SOD activity. CAT activity decreased under moderate salinity (S2A–S2E) but increased markedly at higher salinity levels with biofertilizer application (S3A–S3E). GPx activity was significantly enhanced in selected treatments, reaching up to 10.93 u/g. Lipid peroxidation, measured by MDA content, declined in most biofertilizer-treated plants, suggesting reduced oxidative damage. Ascorbate and protein contents also improved, particularly in biofertilizer-amended soils. Overall, the findings demonstrate that solid biofertilizer application can enhance stress tolerance in rice by improving antioxidant defense systems and reducing oxidative damage under saline conditions ($p < 0.05$). Proper biofertilizer management is therefore crucial for optimizing plant resilience in salinity-affected soils.

Keywords: *Salinity stress, Oryza sativa L., Biofertilizers, Antioxidant defense system, Oxidative stress, Soil health, Crop productivity*

Introduction

Rice *(Oryza sativa* L.) forms a significant portion of food consumed in most households in Nigeria. It is an important cereal for human consumption, providing 23% of global human per capital energy and 16% per capital protein. Furthermore, it also boosts national income. The impact of salinity on soil health and crop productivity is a critical concern for agricultural sustainability. Salinity can lead to the accumulation of water-soluble salts in the soil, affecting agricultural production and economic welfare (Rengasamy, 2006). It has been observed that soil salinity surpassing crop-specific thresholds can reduce crop yields, with varying levels of tolerance among different crops (Zörb *et al.,* 2018). Salinity stress has been found to cause a reduction in the growth, yield, and productivity of cereal crops (JAMIL and Ma'arup, 2022). However, oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Jomova *et al.,* 2023). Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA (Baiken *et al.,* 2021). Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, including O_2^- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide). Furthermore, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling (Chandra *et al.,* 2015). The defense system, when presented with increased ROS formation under stress conditions, can be overwhelmed when it is unable to remove the toxic molecular species with increased enzymatic or non-enzymatic antioxidant processes. Oxidative stress from environmental sources and developmental transitions such as seed maturation involves the formation of reactive oxygen species (ROS) in plant cells. The redox-modulated changes that follow are central events in cellular responses. ROS themselves play a role in intracellular redox sensing, activating antioxidant resistance mechanisms, among other adaptive processes (Sies and Jones, 2020). Functional roles of these responses include the protection of redox-sensitive enzymatic processes, the preservation of membrane integrity, and the protection of DNA and proteins.

In rice antioxidants help mitigate oxidative stress caused by environmental stress like salinity, drought and extreme temperature. Antioxidant protect the cell membranes and biomolecules from oxidative damage, it enhances stress tolerance and plant resilience, promote healthy growth, development and yield (Fujita and Hasanuzzaman, 2022.). When plant face stress, they produce ROS, which can damage cells and disrupt normal functioning. Antioxidant help counterbalance ROS, maintaining cellular homeostasis and promote stress tolerance (Nadarajah, 2020). Ascorbic acid and glutathione have each been shown to act as antioxidants in the detoxification of ROS. These compounds have central and interrelated roles, acting both non-enzymatically and as substrates in enzyme-catalyzed detoxification reactions (Potega, 2022). An anti-ROS response

includes the induction of genes that belong to ROS scavenging mechanisms.

Materials and Methods

Preparation of Carrier Molecules

This experiment was carried out in the Botanic Garden at the Department of Plant Biology and Biotechnology in the University of Benin, Benin City Edo State. Organic waste samples were gathered over a three-week period from vendors in a local market in Benin City, Edo State. The collected materials were carefully sorted to eliminate extraneous matter and subsequently categorized into different waste types for proper identification. The separated wastes were shredded into smaller fragments to facilitate efficient drying and grinding. They were then spread under sunlight for several weeks to ensure thorough drying and conversion into chip-like forms suitable for blending. Following complete drying, each waste category was weighed individually to determine the appropriate proportions and mixing ratios prior to milling. The dried food waste was subsequently processed at a local mill and ground into a fine powder. After milling, the powdered material underwent additional drying before being transported to the laboratory for sterilization. Sterilization was achieved by wrapping the organic waste in aluminum foil and subjecting it to autoclaving. Upon completion of sterilization, the microbial inoculant was incorporated into the carrier material.

Introduction of Microbial Inoculant

The inoculum of each soil was introduced in the McFarland solution of 200ml the 7 carrier molecules were weighed with varying weights and dispensed into a conical flask 100ml of this McFarland solution was then introduced into the conical flask and mixed with the molecules and let to sundry on a poly bag for about 5 days. A sample of this biofertilizer was taken to the lab for identification of the microbes present in each.

Determination of Shelf Life

The biofertilizers were tested for viability over a period of two months under laboratory conditions at room temperature.

Experimental Design

A previous experiment was carried out whereby the soil was watered with 500 ml of $NaNO_3$ solution every day for 1 week to ensure that the soil was heavily salinized. The experiment utilized 66 bags of soil, split into two groups of 3. The treatment groups received 100 ml, 1,000 ml, and 10,000 ml of $NaNO_3$ solution three times weekly, whereas the control group received water. To initiate the experiment, 10g of biofertilizer was incorporated into each bag of soil before seeding.

Biomarkers Protocol

Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535nm (Buege and Aust, 1978). The activity of Superoxide Dismutase (SOD) was determined by using the method of Misra and Fridovich (1972). Catalase activity was determined using the method of Claiborne (1985). The activity of Glutathione Peroxidase was measured according to the method of Chance and Maehly (1955).

Data Analysis

Data were analyzed using one-way analysis of variance (ANOVA). Mean separations were performed using Tukey's post-hoc test for multiple comparison at a significance level of $(p < 0.05)$. All experiments were conducted in triplicate, and results are reported as mean ±

standard deviation. Analyses were conducted using Minitab 17 software.

Results and Discussion

To investigate the total protein content in various treatments, Figure 1 reveal significant increases in total protein in the fertilized salinized soil compared to the control. Specifically, the total protein content was 0.03g/dl in the control, whereas it increased to 0.25g/dl in S1A, 0.50g/dl in S1C, 0.88g/dl in S2B, and 1.26g/dl in S2D. These results suggest that the application of solid biofertilizers in salinized soil enhanced the protein content of the plants. This implies that the solid biofertilizers had a more pronounced effect on protein content in the salinized soil, potentially mitigating the negative effects of salinity on plant protein production. Overall, the results indicate that the use of solid biofertilizers can improve protein content in plants grown in salinized soil, which is a desirable outcome for agricultural productivity.

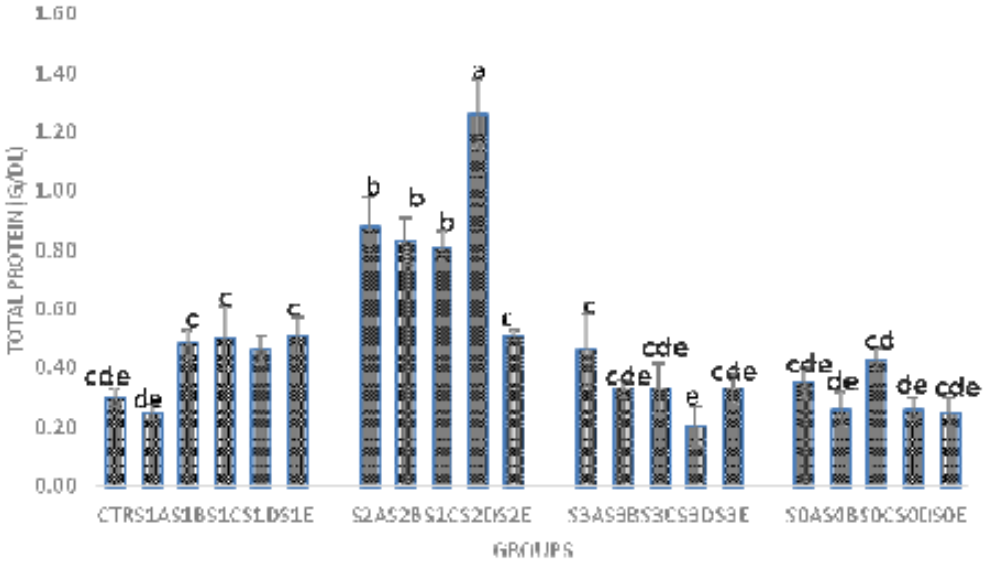

Fig. 1: Total protein activity in rice plants after harvest under different treatment

Values are expressed as mean ±SEM, n = 3, bars with different letters on the chart are significantly different ($p < 0.05$), while those with identical letters are not significantly different. Salinity (S1: 100 ppm, S2: 1,000 ppm, S3: 10,000 ppm). SOA-SOE = Soil with biofertilizer, Biofertilizer combinations. A: Banana + *Klebsiella*, B: Plan + *Bacillus*, C: Pap + Serratia, D: Pap + *Bacillus*, E: Banana + Serratia on plant growth, CTR= control

Figure 2 presents the superoxide dismutase (SOD) activities in the rice plant, showing varying levels of SOD activity across different treatments. The control treatment had an SOD activity of 4.38u/g protein. In contrast, some treatments exhibited increased SOD activity, such as S1A with 6.90 u/g and S3D with 6.76 u/g. However, other treatments showed decreased SOD activity, including S1C with 3.52 u/g and S2C with 2.15 u/g. These results suggest that the application of biofertilizers can modulate SOD activity in rice plants, potentially influencing their antioxidant defense mechanisms.

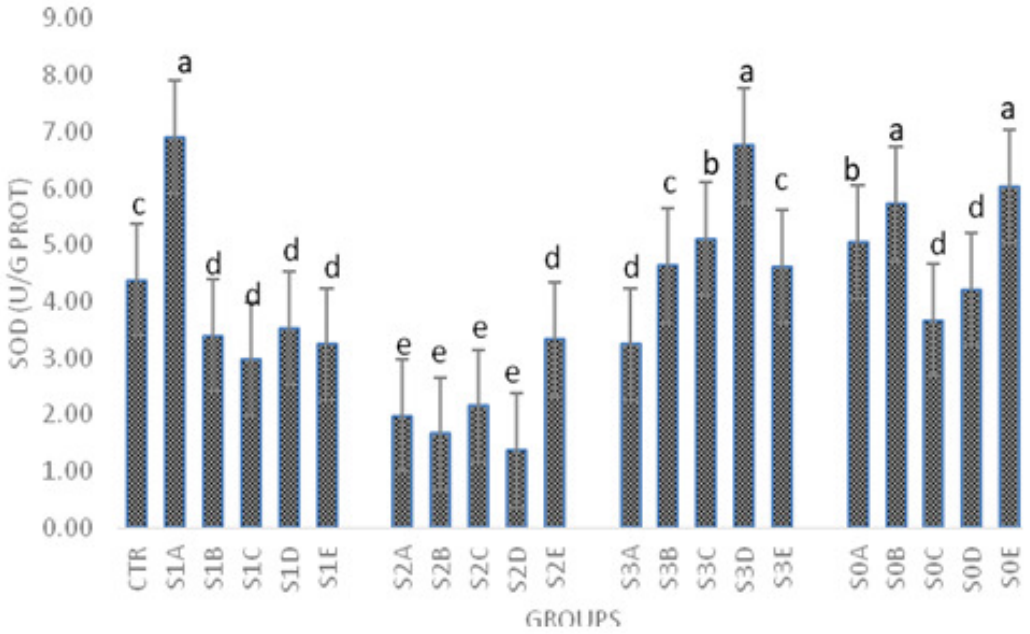

Fig. 2: Superoxide dismutase activity in rice plants after harvest under different treatment
Values are expressed as mean ±SEM, n = 3, bars with different letters on the chart are significantly different ($p < 0.05$), while those with identical letters are not significantly different.
Salinity (S1: 100 ppm, S2: 1,000 ppm, S3: 10,000 ppm). SOA-SOE = Soil with biofertilizer, Biofertilizer combinations. A: Banana + *Klebsiella*, B: Plan + *Bacillus*, C: Pap + Serratia, D: Pap + *Bacillus*, E: Banana + Serratia on plant growth, CTR= control

Catalase (CAT) activity in the rice plant across various experimental treatments as seen in Figure 3 shows that CAT activity was significantly reduced in treatments S2A to S2E, with values ranging from 0.91 to 1.7 u/g, compared to the control value of 2.3 u/g. In contrast, treatments S3A to S3E exhibited significant increases in CAT activity, with values ranging from 2.50 to 5.76 u/g, surpassing the control value. Similarly, in the non-salinized soil, the application of biofertilizers also led to significant increases in CAT activity, with values ranging from 2.3 to 4.67 u/g, compared to the control. These findings suggest that the application of biofertilizers can modulate CAT activity in rice plants, with some treatments showing enhanced antioxidant defense mechanisms, while others exhibit reduced activity. The variations in CAT activity across different treatments may be attributed to the specific biofertilizer used, the level of salinity, or the interactions between these factors.

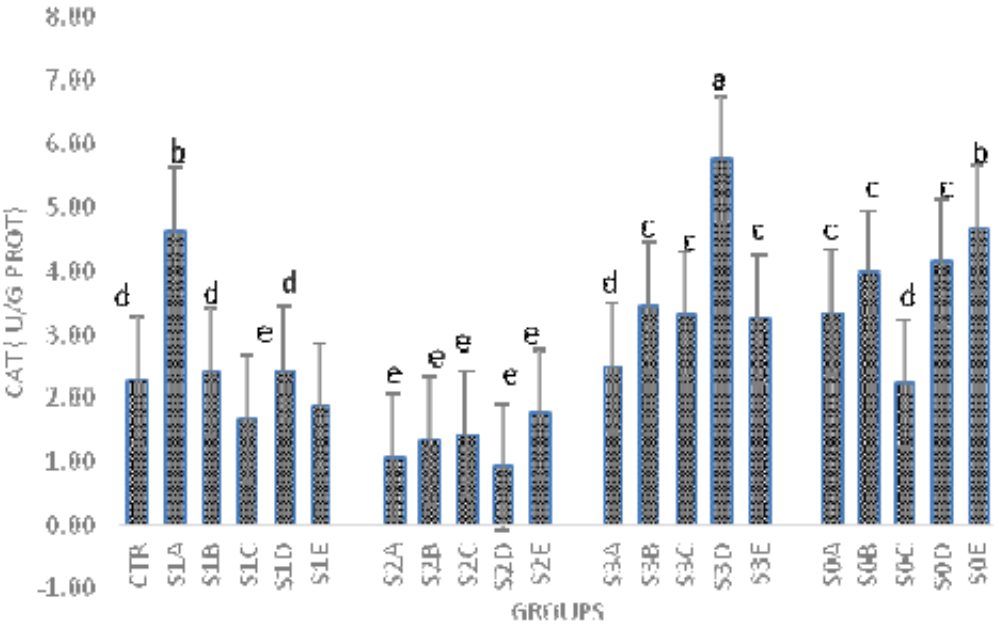

Fig. 3: Catalase (CAT) activity in rice plants after harvest under different treatment.
Values are expressed as mean ±SEM, n = 3, bars with different letters on the chart are significantly different ($p < 0.05$), while those with identical letters are not significantly different.
Salinity (S1: 100 ppm, S2: 1,000 ppm, S3: 10,000 ppm). SOA-SOE = Soil with biofertilizer, Biofertilizer combinations. A: Banana + *Klebsiella*, B: Plan + *Bacillus*, C: Pap + Serratia, D: Pap + *Bacillus*, E: Banana + Serratia on plant growth, CTR= control

Figure 4 presents the glutathione peroxidase (GPx) activities in rice plants under various experimental conditions. The results indicate that GPx activities were significantly enhanced in some treatments, with S1A showing an increase to 10.6 u/g, compared to the control value of 7.54 u/g. Similarly, S3C exhibited a GPx activity of 7.72 u/g. Notably, the application of biofertilizers in samples D and E led to substantial increases in GPx activities, with values reaching 10.16 u/g in SOD and 10.93 u/g in SOE. The enhancement of GPx activities in response to biofertilizer application suggests that these microorganisms can stimulate the plant's antioxidant defense system, leading to improved protection against oxidative stress. GPx is a crucial enzyme that helps to detoxify hydrogen peroxide and other reactive oxygen species, thereby maintaining cellular homeostasis. The increased GPx activities observed in these treatments imply that the biofertilizers can promote plant health and resilience by augmenting the plant's antioxidant defenses. These findings have important implications for agriculture, as they suggest that biofertilizers can be used to enhance plant antioxidant defenses, leading to improved crop yields and reduced oxidative stress. Further research is needed to fully understand the mechanisms underlying the biofertilizer-induced increase in GPx activities and to explore the potential applications of this phenomenon in agricultural settings.

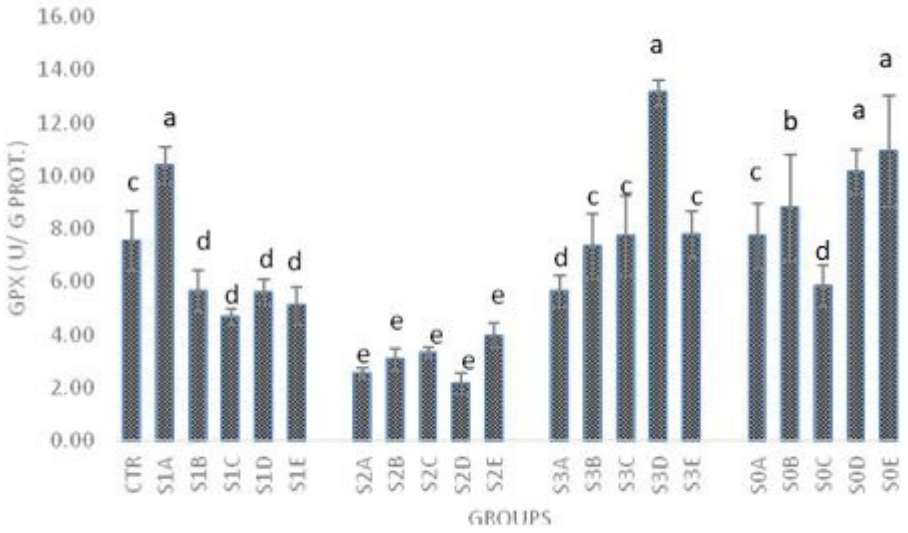

Fig. 4: Glutathione peroxidase (GPx) activity in rice plants after harvest under different treatment

Values are expressed as mean ±SEM, n = 3, bars with different letters on the chart are significantly different ($p < 0.05$), while those with identical letters are not significantly different.

Salinity (S1: 100 ppm, S2: 1,000 ppm, S3: 10,000 ppm). SOA-SOE = Soil with biofertilizer, Biofertilizer combinations. A: Banana + *Klebsiella*, B: Plan + *Bacillus*, C: Pap + Serratia, D: Pap + *Bacillus*, E: Banana + Serratia on plant growth, CTR= control

Figure 5 shows the Malondialdehyde (MDA) concentration, which serve as an indicator of lipid peroxidation and cellular damage in plants. MDA levels are often used to assess the extent of oxidative stress and physiological or metabolic disorders in plants. In this study, the control treatment showed an MDA concentration of 0.56u/g, indicating a baseline level of oxidative stress. However, the results reveal that MDA concentration varied significantly across different treatments. In S1A, MDA concentration increased to 0.87 m/g, suggesting heightened oxidative stress. In contrast, treatments S1B, S1C, S1D, and S1E showed reduced MDA concentration, ranging from 0.51 to 0.66 m/g. Interestingly, treatments S2A to S2E and S3A to S3E exhibited significantly reduced MDA concentration, with values ranging from 0.182 to 0.52 m/g. This suggests that certain biofertilizer treatments may have mitigated oxidative stress and cellular damage in the plants. Notably, the non-salinized soil treatments (SOA to SOE) showed increased MDA concentration, ranging from 0.61 to 0.90m/g, compared to the control. This indicates that even in the absence of salinity, the biofertilizer treatments may have induced some level of oxidative stress or cellular damage. However, the overall trend suggests that microorganisms contained in the biofertilizer can stimulate plants oxidative defense system, which could have positive implications for plant health and resilience.

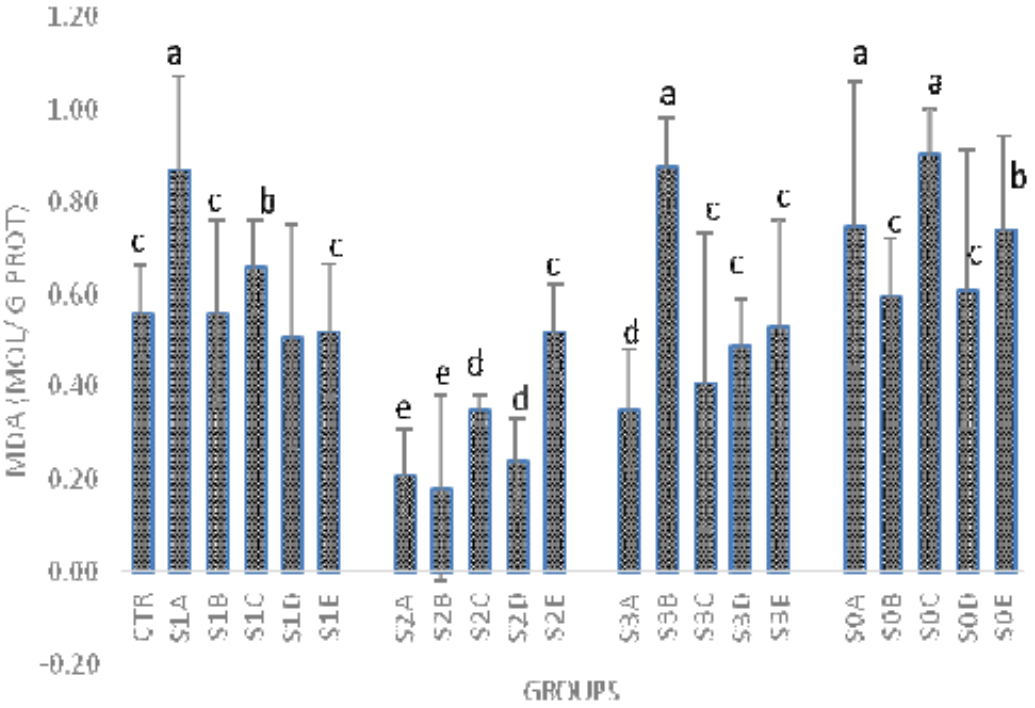

Fig. 5: Malondialdehyde (MDA) concentration in rice plants after harvest under different treatments

Values are expressed as mean ±SEM, n = 3, bars with different letters on the chart are significantly different ($p < 0.05$), while those with identical letters are not significantly different.

Salinity (S1: 100 ppm, S2: 1,000 ppm, S3: 10,000 ppm). SOA-SOE = Soil with biofertilizer, Biofertilizer combinations. A: Banana + *Klebsiella*, B: Plan + *Bacillus*, C: Pap + Serratia, D: Pap + *Bacillus*, E: Banana + Serratia on plant growth, CTR= control

Figure 6 shows the ascorbate content in the experiment, with the control showing a value of 32.99 mg/mL. In contrast, treatments S1A to S1E exhibited similar ascorbate levels, ranging from 35.84 to 41.30 mg/mL, with no significant differences observed. However, a notable increase in ascorbate activities was reported in treatments S2A to S2E, with values ranging from 44.68 to 60.52 mg/mL. Additionally, the non-salinized soil treatments showed varying ascorbate levels, with SOA exhibiting the highest value of 67.01 mg/mL, while SOB, SOC, SOD, and SOE showed values of 34.29, 40.26, 29.09, and 38.70 mg/mL, respectively.

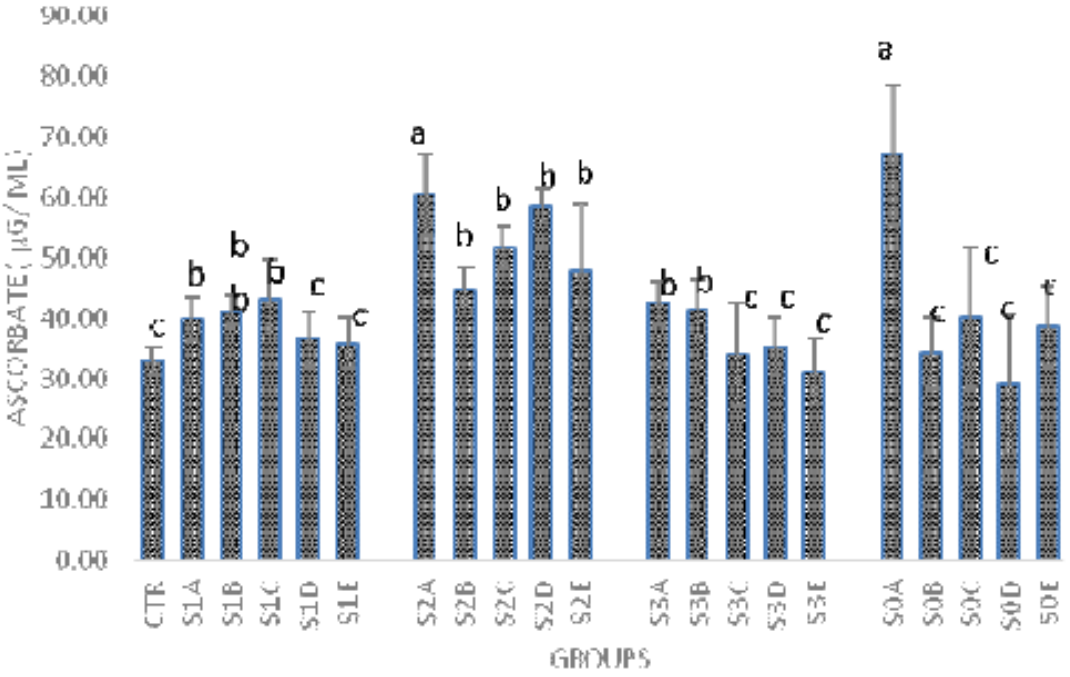

Fig. 6: Ascorbate (Vit. C) activity in rice plants after harvest under different treatments
Values are expressed as mean ±SEM, n = 3, bars with different letters on the chart are significantly different ($p < 0.05$), while those with identical letters are not significantly different.
Salinity (S1: 100 ppm, S2: 1,000 ppm, S3: 10,000 ppm). SOA-SOE = Soil with biofertilizer, Biofertilizer combinations. A: Banana + *Klebsiella*, B: Plan + *Bacillus*, C: Pap + Serratia, D: Pap + *Bacillus*, E: Banana + Serratia on plant growth, CTR= control

Discussion

When plants are exposed to either living stressors (biotic) such as pathogens or non-living stressors (abiotic) like salinity, heat, or drought, they often respond by boosting the activity of certain antioxidant enzymes. These enzymes, such as superoxide dismutase (SOD) and peroxidases, help to counteract damage by lowering lipid peroxidation, stabilizing cell membranes, and removing harmful reactive oxygen species (ROS). This protective mechanism has been observed in numerous studies (Sharma *et al.,* 2012; Meena *et al.,* 2017; Prasad *et al.,* 2019; Singh *et al.,* 2019;). High concentrations of salts particularly chloride ions can upset plant metabolism, leading to physiological disturbances, swelling of cells, and reduced energy production (Prasad *et al.,* 2019; Larcher, 1980). Research suggests that biofertilizers can help plants grown in salty soils by increasing their protein content, an improvement that could boost crop yields. In rice, biofertilizers have been linked to changes in SOD activity, potentially strengthening the plant's antioxidant defence system (Kumar *et al.,* 2018). SOD plays a central role in this system by converting damaging superoxide radicals into harmless oxygen and hydrogen peroxide. Similar benefits have been recorded in pearl millet, where seeds treated with carbon nanoparticle (CNP) solutions showed higher SOD activity than untreated controls (Siddaiah *et al.,* 2018). Beneficial microbes can also

shield plants from environmental stress. For instance, Pseudomonas spp. has been shown to reduce drought stress in maize (Sandhya *et al.,* 2010), with SOD activity differing between treatments, suggesting tailored activation of antioxidant pathways depending on stress intensity. Treated maize plants often exhibited higher sugar, protein, and phenolic levels part of the protective and growth-promoting effects of *Pseudomonas taiwanensis* and nanogypsum. Nanoprimed seeds may accumulate more sugars due to increased α-amylase activity (Mahakham *et al.,* 2017), while treatments with chitosan or nanochitosan have been shown to raise phenolic content in tea leaves by 20–24% (Chandra *et al.,* 2015).

The increase in CAT activity suggests that the biofertilizer triggered a response in the plant to enhance its antioxidant defenses. The exact mechanism behind this increase is unclear, but several factors may contribute to it. Improved nutrient availability, microbial stimulation, induced systemic resistance, and antioxidant properties of the biofertilizer may all play a role. The increase in CAT activity in response to biofertilizer application in non-salinized soil suggests that biofertilizers can have beneficial effects on plant antioxidant defenses, even in the absence of salinity stress. This has important implications for agriculture, as it suggests that biofertilizers can be used to promote plant health and resilience, even in non-stressed conditions. Further research is needed to fully understand the mechanisms behind this effect and to explore its potential applications in agriculture. Catalase (CAT)**,** also responds positively to biofertilizer application. In rice grown in non-saline soils, CAT activity rose significantly with biofertilizer treatment, indicating improved ability to neutralize hydrogen peroxide and other ROS (Sadak and Dawood, 2023). This suggests enhanced resilience against oxidative stress. Similar increases in CAT activity have been documented in wheat varieties with different salt tolerances (Mutlu *et al.,* 2009) and in salt-tolerant cotton (Meloni *et al.,* 2005). The activities of CAT, SOD, and ascorbate peroxidase (APX) can serve as useful markers for evaluating stress severity, while the water plant *Azolla microphylla* has shown promise in protecting against salinity stress (Abraham, 2010).

In the present study, salt-stressed seedlings with higher SOD and CAT activity were better able to cope with salinity, mirroring findings in alfalfa treated with *Pseudomonas aeruginosa* and *Enterobacter aerogenes* (Liu *et al.,* 2019). Here too, the elevated enzyme levels supported plant growth under saline conditions.

Glutathione peroxidase (GPx) activity is enhanced by biofertilizer application, promoting detoxification of hydrogen peroxide and other reactive oxygen species (ROS). This stimulation suggests that microbial inoculants strengthen plant antioxidant defenses even under non-saline conditions. The increase in GPx may result from improved plant–microbe interactions and the inherent antioxidant properties of certain biofertilizers, which directly or indirectly enhance enzymatic activity and overall plant resilience. However, biofertilizer use can also influence malondialdehyde (MDA) levels, a key indicator of lipid peroxidation and membrane damage. While beneficial microbes support growth, they may stimulate ROS production, elevate MDA and indicate oxidative stress. In rice under salinity, MDA assessment helps determine

oxidative injury and the protective potential of biofertilizers. Although slight increases may reflect active defense responses, excessive MDA accumulation can impair plant health, highlighting the importance of proper biofertilizer management. Physiological stress appeared elevated under experimental conditions compared with the control. Malondialdehyde (MDA) increased from 0.56 µg/g in the control to 0.87 µg/g in S1A, while S1B–S1E ranged from 0.51–0.66 µg/g. In contrast, S2A–S2E showed reduced MDA levels (0.182–0.52 µg/g), with similar reductions observed in S3 treatments. In non-saline soils (SOA–SOE), MDA rose from the control value of 0.58 µg/g to 0.61–0.90 µg/g.

Elevated MDA following biofertilizer application may result from microbial-induced oxidative stress, nutrient imbalance, plant defense activation, and microbial plant interactions that enhance reactive oxygen species (ROS) production. Although moderate MDA increases may reflect adaptive defense responses, excessive accumulation indicates membrane lipid peroxidation and cellular damage. Therefore, appropriate biofertilizer management is essential to optimize plant health and minimize oxidative injury.

Conclusion

Plants under biotic and abiotic stress activate antioxidant enzymes (SOD, CAT, Ascorbate (Vit. C), GPx) to detoxify reactive oxygen species and limit cellular damage. Salinity disrupts metabolism, but biofertilizers enhance stress tolerance by boosting antioxidant activity, protein content, and beneficial microbes. Increased SOD and CAT indicate improved resilience, though excessive MDA may signal membrane damage.

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