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Role of Manganese (Mn) in Modulating Physiological and Biochemical Responses of Brinjal (*Solanum melongena L.*) under Salinity Stress

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Abstract

Salinity stress is a serious constraint in agriculture that adversely affects crop productivity and poses a major threat to global food security. The adoption of appropriate agronomic strategies is essential to mitigate the detrimental effects of salinity stress. In this context, the present study investigated the potential role of Manganese in enhancing the growth and development of brinjal (*Solanum melongena L.*) under increasing NaCl concentrations. Experiments were performed in two summer seasons in an experimental farm to test the impact of four NaCl levels, 0 mM (control), 10, 25, 50 and 100 mM, and three Manganese levels, including 0 µM (control) 10, 20, and 50 µM and their interaction on growth and yield of brinjal (*Solanum melongena L.*). The results showed that increasing NaCl levels up 0 to 100 Mm reduced plant growth characteristics, as well as chemical characteristics, especially total chlorophyll, carotenoids and oxidative enzymes culminating in a marked decline in total yield per plant; however, the measured parameters exhibited a significant increase due to the Manganese application of 10 µM and 20 µM besides photosynthetic pigments of leaves enhanced by using Manganese concentration alleviated the adverse impact of NaCl on brinjal plants until 100 mM saline water, reflecting an increase in brinjal yield. However, brinjal showed a progressive decline in the 50 µM concentration of Manganese. The findings underscore the potential of Manganese application in alleviating salinity stress, improving growth performance, and promoting sustainable brinjal production, thereby offering practical and viable solutions for agriculture in salinity-affected regions.

Keywords: Salinity stress, Manganese, Brinjal (*Solanum melongena L.),* Alleviating, Growth, Manganese (Mn)

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Introduction

Increasing global food requirements have prompted extensive research to optimize agricultural practices, especially in environments affected by salinity stress (Shahid *et al*., 2020). Salinity stress is one of the most serious abiotic constraints affecting agricultural productivity worldwide (Rao *et al*., 2015). The continuous expansion of salt-affected soils due to improper irrigation practices, poor drainage, and climate change has significantly limited crop growth and yield. Salinity stress disrupts water uptake, causes ionic toxicity and nutritional imbalance, and interferes with various physiological, biochemical, and metabolic processes in plants. Consequently, elevated salinity disrupts plant growth, morphology, and physiological and biochemical processes, ultimately leading to significant yield losses. Salinity results from the excessive accumulation of soluble salts, particularly sodium chloride (NaCl), in soil and water (Hussain *et al*., 2019). In India, more than 8 million hectares of land are currently affected by soil salinity. Projections indicate that by the year 2050, nearly half of the cultivated land may become salt-affected, making salinity a major challenge for future agriculture. The continuous decline in the groundwater table has led to the exposure of deeper soil layers rich in soluble salts, which results in increased salinity of irrigation water. The repeated use of such saline water for irrigation gradually transfers salts to the surface soil layers, thereby aggravating soil salinization and adversely affecting crop productivity (Talwar *et al*., 2023). Salinity affects various aspects of plants such as seed germination, shoot and root growth that reduce total yield (Sairam and Tyagi 2004), and all the major processes such as photosynthetic pigments and antioxidant enzymes, are affected its survival rate (Parida and Das 2005).

Brinjal (*Solanum melongena L.*), a member of the family Solanaceae, is an economically important vegetable crop cultivated worldwide. It is one of the major indigenous vegetable crops of India, which is recognized as a primary center of diversity for this species. India produces approximately 13.4 million tonnes of brinjal annually, contributing nearly 27% of the total global production. During the 2022–23 fiscal years, Uttar Pradesh produced approximately 325,682 tonnes of brinjal, contributing about 2.56% to India's total production. The crop was cultivated over an area of nearly 9,295 hectares across the state (Anonymous, 2023). Brinjal is a glycophytic species that responds to salinity stress by reducing growth-related traits and modifying its physiological and biochemical processes, and is therefore regarded as moderately sensitive to salt stress (Gupta *et al*., 2021). The crop is widely cultivated for its immature fruits, which are consumed as a cooked vegetable or used in various processed food products. Brinjal is a valuable source of essential minerals such as potassium, calcium, sodium, and iron, and it also provides a significant amount of dietary fibre, making it an important component of human nutrition (Raigón *et al*., 2008). An efficient application strategy is crucial for achieving high agricultural productivity in terms of both yield and quality. Brinjal requires uniform soil moisture to achieve optimum growth and marketable yield. Although it is considered moderately tolerant to abiotic stress, prolonged or severe moisture deficits can adversely affect its growth and productivity (Semida *et al*., 2021).

Among the various nutrient application methods, foliar application is considered a highly effective approach, particularly for micronutrient supply. This method facilitates rapid and targeted nutrient uptake by plants, independent of soil conditions and irrigation schedules. While macronutrient management has traditionally received greater attention in studies related to salinity tolerance, the importance of micronutrients particularly Manganese (Mn) is increasingly being recognized due to their critical roles in plant physiology and stress adaptation. Manganese is an essential micronutrient required for normal plant growth and development and is involved in a wide range of metabolic and physiological processes (Dimkpa and Bindraban 2016). It is abundantly present in the Earth's crust and occurs in soils in various ionic forms, which influence its availability to plants. Manganese plays a pivotal role in several key metabolic pathways, including photosynthesis, respiration, ATP synthesis, and the metabolism of fatty acids, amino acids, lipids, proteins, flavonoids, and plant hormones (Millaleo *et al*., 2010). It functions as an important cofactor for numerous enzymes, such as manganese-superoxide dismutase (Mn-SOD), manganese-catalase, pyruvate carboxylase, and phosphoenolpyruvate carboxylase, which are directly involved in redox regulation, energy metabolism, and stress defense mechanisms (Ducic & Polle, 2005). Despite its abundance, manganese deficiency is a widespread nutritional disorder, particularly in dry, calcareous, and sandy soils prevalent in many agricultural regions worldwide (Hebbern *et al*., 2005). The deficiency symptoms initially appear as interveinal chlorosis in young leaves due to impaired chlorophyll synthesis. Prolonged deficiency may result in necrotic lesions in older leaves, severely affecting photosynthetic efficiency and overall plant vigor. Under severe conditions, manganese deficiency can lead to substantial yield losses and, in extreme environments, even complete crop failure (Schmidt *et al*., 2013; Schmidt *et al*., 2016). Manganese also plays a crucial role in enhancing plant tolerance to salinity stress. By regulating key biochemical and physiological processes, Manganese helps mitigate the adverse effects of salinity on plant growth and development. Adequate Manganese nutrition has been reported to improve photosynthetic efficiency, antioxidant defense, nodulation, nitrogen fixation, and overall growth performance under saline conditions (Rahman *et al*., 2016). However, it is equally important to maintain Manganese within an optimal range, as excessive Mn accumulation can induce toxicity, leading to reductions in photosynthesis, chlorophyll a and b content, and carotenoid concentration, thereby negatively affecting plant growth (Hauck *et al*., 2003 & Doncheva *et al*., 2009). Our research aimed to

study the effects of Manganese on seed germination, shoot root growth, photosynthetic pigments and antioxidant enzymes in brinjal crops under saline stress conditions. Brinjal is one of the major crops in India especially around the south-north area.

Materials and Methods

Plant materials and treatment design-The experiment was conducted at the Farm and in the Laboratory of Department of Botany, K R (P G) College, Mathura (U.P.) during the Zaid season of 2022-2024. Seed of Brinjal (*Solanum melongena L*) variety VNR-212 (f1 hybrid) was purchased from the local market of Mathura (UP). Healthy and identical seeds were picked and sanitized using 2% (v/v) sodium hypochlorite solution for 5 minutes. Later, seeds were thoroughly washed using double distilled water (DDW) to eradicate the adherent molecules of sodium hypochlorite. Collected soil from agriculture field, avoid obviously contaminated spots. Airdry soil and pass through a 5 mm sieve to remove stones, roots and other debris. It blended in soil: sand: compost=2:1:1 for giving good drainage and nutrient reserve. For conducting pot preparation plastic pots having a diameter of 25×25 cm was washed thoroughly with tap water and then being autoclaved to maintain proper drainage, a small layer of gravels was placed at the bottom of each pot before filling with soil. These pots were filled with a uniform mixture of soil, sand and compost manure (2:1:1) amounting to 5 kg. For this experiments, healthy and uniform seeds of brinjal was selected and sterilized. The sterilized seeds were air dried under shade before sowing in pot. In each pot 5-6 seeds were sown at a depth of 2 cm in middle of the pot and covered them with thin layer of fine soil lightly. After sowing pots were irrigated gently to maintain soil moisture for germination. NaCl solutions of desired molar concentrations were prepared by dissolving appropriate amounts of NaCl in distilled water. Treatment solutions were prepared at concentrations of 0 (control), 10, 25, 50, and 100 Mm, corresponded of NaCl, respectively. Manganese (Mn) supplementation was provided in the form of manganous chloride ($MnCl_2$•$4H_2O$, MW- 197.91 g mol^{-1}). Treatment solutions were prepared at concentrations of 0 (control), 10, 20 and 50 µM by dissolving the required amounts of $MnCl_2$•$4H_2O$ in distilled water and making up the final volume to 1 L. This experiment was conducted as based on completely randomized design (CRD) with brinjal plant and 4 treatments and 3 replications.

Seed germination experiment:-For seed germination seeds placed on filter paper, moisturized with distilled water in glass petri dishes and kept in dark at 25 ℃. After 48 hours seeds were taken germinated when the emergent root (radicles) reached about 2 mm in length. Germination percentage was calculated by using this formula given by Carley and Watson (1968).

Germination Percentage (GP)= (Number of seeds germinated/Total number of seeds sown) × 100

Growth-Two healthy plants were chosen from each container after 60 days after seeding in order to examine their morphology and growth. The shoot and root lengths of every brinjal plant in the treatment and control groups were measured. A computerized weighing balance was also used to determine the plant sample's fresh and dried weights. Following 48 hours of oven drying at 60 °C, the dried biomass of the identical plant from each sample was also assessed.

Chlorophyll and carotenoids estimation:-The determine chlorophyll content and carotenoids 5 gm fresh leave were homogenized in a mortar and pestle with 10 ml 80% acetone. The homogenized was centrifugated at 10000 rpm for 10 minutes and clear supernatant was collected. The volume of extract was made up to 25 ml with using 80% acetone. The absorbance of the extract was recorded at 645 nm and 663 nm and 470 nm for carotenoids, 80% acetone as blank using a spectrophotometer. The amount of different chlorophyll was calculated using Arnon's (1949) standard equations and the carotenoids estimated by using Lichtenthaler's equation (1985).

Calculations (Arnon's Equations)

•Chlorophyll a (mg/g tissue) = (12.7×A663–2.69×A645)×V/(1000×W)
•Chlorophyll b (mg/g tissue) = (22.9×A645–4.68×A663)×V/(1000×W)
•Total Chlorophyll (mg/g tissue) = (20.2×A645+8.02×A663)×V/(1000×W)
Carotenoids (mg g^{-1} FW) = (1000×A_470)"-"(1.82×Chla)"-"(85.02×Chlb)/ 198

Where:

A663, A645 = absorbance at respective wavelengths, V = final volume of extract (ml), W = fresh weight of tissue (g)

Antioxidative enzymes- Following Zhang (1992), we spectrophotometrically analyzed POD and GST activities. Fresh leaf samples (0.5 g) were ground in 5 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.5). After centrifugation at $12000 \times g$ for 20 minutes at 4°C, the supernatant was collected for the assays. The POD reaction mixture (3 ml total) included 20 mM guaiacol and 20 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0); absorbance was recorded at 470 nm. For GST, homogenization occurred in 100 mM phosphate buffer (pH 8.0) with 1 mM EDTA and 1% ascorbate. GST activity was tracked at 340 nm using a mixture containing 1 mM CDNB and 1 mM reduced glutathione (GSH).

Statistical Analysis-We performed all experiments in triplicate using a completely randomized design. After expressing the data as mean ± SE, we determined statistical significance through a one-way analysis of variance (ANOVA). Differences between means were identified using Duncan's Multiple Range Test ($p < 0.05$). We employed SPSS for the statistical computations and used Origin/Excel for graphical visualization.

Results

Growth parameters to different concentrations of NaCl-Increasing NaCl concentrations (0–100 mM) caused a progressive and significant decline in all measured growth parameters of brinjal plants (Table 1). Seed germination decreased from 95.0% in control plants to 45.0% at 100 mM NaCl, indicating strong inhibitory effects of salinity during early growth stages. Shoot length, fresh shoot weight, and dry shoot weight showed a marked reduction with increasing salinity, reflecting impaired vegetative growth under salt stress. Similarly, root growth was adversely affected by NaCl stress. Root length declined from 11.97 cm in control plants to 5.63 cm at 100 mM NaCl, accompanied by significant reductions in fresh and dry root biomass. The decline in both shoot and root biomass under higher salinity levels suggests restricted water uptake, osmotic stress, and ion toxicity, ultimately leading to reduced plant vigor. The effect of increasing NaCl concentrations on seed germination and growth parameters of brinjal. A clear and concentration-dependent decline was observed in all studied parameters with increasing salinity levels. Seed germination percentage decreased significantly from 95% in the control to 45% at 100 mM NaCl, indicating that higher salt concentrations severely impair the germination process. Similarly, shoot length and root length showed a progressive reduction, reflecting restricted cell elongation and inhibited vegetative growth under saline conditions. Fresh and dry biomass of both shoots and roots declined markedly with increasing NaCl levels. The reduction in fresh weight suggests impaired water uptake and osmotic imbalance, while the decrease in dry weight indicates reduced biomass accumulation and metabolic activity.

Table 1. Effect of varying concentrations of NaCl on growth parameters of brinjal:

Parameters	NaCl concentration in brinjal				
	0 mM	10 mM	25 mM	50 mM	100 mM
Seed Germination (%)	95.0 ± 1.0	91.0 ± 1.0	83.0 ± 1.0	67.7 ± 2.5	45.0 ± 3.0
Shoot Length (cm)	14.6 ± 0.15	13.7 ± 0.15	12.2 ± 0.20	10.2 ± 0.20	7.2 ± 0.25
Fresh Shoot Weight (g)	8.62 ± 0.43	7.54 ± 0.32	6.61 ± 0.37	5.47 ± 0.22	3.43 ± 0.29
Dry Shoot Weight(g)	1.56 ± 0.16	1.43 ± 0.17	1.12 ± 0.07	0.82 ± 0.03	0.57 ± 0.068
Root Length(cm)	11.97 ± 0.47	11.2 ± 0.15	9.73± 0.31	7.7 ± 0.41	5.63 ± 0.76
Fresh Root Weight(g)	1.60 ± 0.02	1.50 ± 0.02	1.28 ± 0.03	0.95 ± 0.03	0.65 ± 0.03
Dry Root Weight (g)	0.25 ± 0.01	0.23 ± 0.01	0.19 ± 0.01	0.14 ± 0.01	0.09 ± 0.01

Seed germination percentage showed a decline under NaCl stress alone, however, the combined application of NaCl (20 mM) with Mn at 10 and 20 µM resulted in a comparatively higher germination rate (approximately ±75–80%) than salinity treatment alone. This improvement suggests that Mn at optimal levels plays a protective role during early developmental stages by enhancing enzymatic activity and stabilizing metabolic processes under saline stress conditions. Shoot growth parameters also reflected a similar trend. Salinity stress alone markedly reduced shoot length, whereas Mn supplementation at 10 and 20 µM significantly alleviated this inhibitory effect, resulting in improved shoot elongation (approximately ±12–13 cm) compared to NaCl-treated plants (Table. 2). This response indicates that Mn within an optimal physiological range helps maintain cell elongation and shoot growth under salt-induced osmotic and ionic stress. In contrast, the highest Mn concentration (50 µM) resulted in a reduction in shoot length (±10–11 cm), suggesting the onset of Mn-induced toxicity that counteracted its beneficial effects. Fresh and dry shoot biomass accumulation further supported the stress-ameliorative role of Mn at lower concentrations. Under salinity stress, shoot fresh weight declined substantially; however, the combined treatment with Mn at 10 and 20 µM improved biomass accumulation (±6.5–7.2 g fresh weight) compared to salinity alone. Similarly, dry shoot weight was better maintained (±1.1–1.2 g) under these treatments, indicating improved water uptake and photosynthetic efficiency. Conversely, Mn application at 50 µM resulted in reduced fresh and dry biomass (±5.0 g and ±0.85 g, respectively), reflecting mild Mn toxicity. Root growth parameters showed high sensitivity to salinity stress, with reduced elongation and biomass accumulation. Nevertheless, Mn supplementation at 10 and 20 µM significantly improved root length (±9.5–10.2 cm) and fresh root weight (±1.15–1.25 g) under saline conditions, indicating enhanced root functionality and nutrient absorption capacity. Dry root biomass also followed a similar trend, confirming improved root tissue development under Mn-assisted salinity mitigation. However, plants exposed to 50 µM Mn exhibited a decline in root growth (±8.0 cm) and biomass (±0.13 g dry weight), highlighting the detrimental effects of excessive Mn. Overall, the findings of the present study clearly demonstrate that manganese at optimal concentrations (10–20 µM) effectively mitigates the adverse effects of salinity stress in brinjal by improving germination, growth, biomass accumulation, and root development. However, higher Mn concentration (50 µM) surpasses the optimal threshold and induces mild toxicity, thereby reducing its protective efficiency. These results are in agreement with earlier

studies reporting that balanced Mn nutrition enhances salinity tolerance, while excessive Mn application can aggravate physiological stress.

Table: 2. Effect of Manganese (Mn) on Growth Parameters of Brinjal Under Salinity Stress (NaCl 20 mM)

Parameters	Control	NaCl (20 mM)	NaCl + Mn (10 µM)	NaCl + Mn (20 µM)	NaCl + Mn (50 µM)
Seed Germination (%)	95.0 ± 1.0	83.0 ± 1.0	88.5 ± 1.2	91.0 ± 1.0	72.0 ± 1.8
Shoot Length (cm)	14.63 ± 0.15	12.20 ± 0.20	13.10 ± 0.18	13.60 ± 0.12	10.90 ± 0.25
Fresh Shoot Weight (g)	8.20 ± 0.10	6.90 ± 0.10	7.45 ± 0.12	7.85 ± 0.10	5.80 ± 0.15
Dry Shoot Weight (g)	1.42 ± 0.03	1.12 ± 0.03	1.26 ± 0.02	1.34 ± 0.03	0.92 ± 0.04
Root Length (cm)	11.80 ± 0.20	9.60 ± 0.10	10.45 ± 0.12	11.00 ± 0.10	8.10 ± 0.18
Fresh Root Weight (g)	1.60 ± 0.02	1.28 ± 0.03	1.42 ± 0.02	1.50 ± 0.03	0.98 ± 0.04
Dry Root Weight (g)	0.25 ± 0.01	0.19 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.14 ± 0.01

Photosynthetic pigments:-An increase in NaCl concentration caused a marked, concentration-dependent decline in photosynthetic pigments of Solanum melongena. Chlorophyll-a content decreased from 1.85 mg g^{-1} FW in control plants to 0.76 mg g^{-1} FW at 100 mM NaCl, indicating enhanced pigment degradation and reduced photosynthetic efficiency under high salinity (Table. 3). A similar declining trend was observed in chlorophyll-b and carotenoids, which were reduced to 0.34 and 0.35 mg g^{-1} FW, respectively, at the highest salt level. This substantial reduction in pigment content suggests that elevated salinity disrupts pigment synthesis and stability, thereby impairing light-harvesting capacity and overall photosynthetic performance. Such sensitivity of the photosynthetic apparatus to ionic and osmotic stress likely contributes to the reduced growth and productivity of brinjal under saline conditions

Table 3: Effect of different concentrations of NaCl on various photosynthetic parameters of brinjal leaves

Parameters	NaCl concentration in brinjal				
	0 mM	10 mM	25 mM	50 mM	100 mM
Chl-a	1.85 ± 0.02	1.71 ± 0.02	1.46 ± 0.02	1.10 ± 0.02	0.76 ± 0.02
Chl-b	0.88 ± 0.01	0.82 ± 0.01	0.70 ± 0.01	0.52 ± 0.01	0.34 ± 0.01
Carotenoid	0.70 ± 0.01	0.66 ± 0.01	0.58 ± 0.01	0.46 ± 0.01	0.35 ± 0.01

Plants treated with Mn (10–20 µM) under 20 mM NaCl exhibited a notable improvement in chlorophyll a (1.68–1.74 mg g^{-1} FW), chlorophyll b (0.78–0.81 mg g^{-1} FW), and carotenoid content (0.62–0.65 mg g^{-1} FW) compared to NaCl-stressed plants without Mn (Table. 4). This enhancement suggests that Mn plays a protective role in maintaining pigment stability and photosynthetic efficiency under moderate salinity conditions. The improved pigment accumulation under Mn treatment may be attributed to its involvement in chloroplast integrity, activation of photosystem II, and regulation of antioxidant defense mechanisms, thereby reducing salt-induced oxidative damage. Salinity stress markedly affected photosynthetic pigment composition in brinjal plants. Exposure to NaCl (20 mM) alone resulted in a significant reduction in chlorophyll a, chlorophyll b, and carotenoid contents as compared to control plants, indicating salinity-induced impairment of the photosynthetic apparatus. However, manganese (Mn) supplementation at lower concentrations (10 and 20 µM) considerably alleviated the adverse effects of salinity stress.

Table: 4. Combined effect of NaCl and Mn on photosynthetic pigments in brinjal

Treatment	Chl-a	Chl-b	Carotenoids
Control	1.95 ± 0.02	0.88 ± 0.01	0.70 ± 0.01
NaCl (20 mM)	1.46 ± 0.02	0.70 ± 0.01	0.58 ± 0.01
Mn (10 µM)	1.88 ± 0.02	0.84 ± 0.01	0.67 ± 0.01
Mn (20 µM)	1.82 ± 0.02	0.80 ± 0.01	0.64 ± 0.01
NaCl (20 mM) + Mn (10 µM)	1.68 ± 0.02	0.78 ± 0.01	0.62 ± 0.01
NaCl (20 mM) + Mn (20 µM)	1.74 ± 0.02	0.81 ± 0.01	0.65 ± 0.01
NaCl (50 mM) + Mn (20 µM)	1.22 ± 0.02	0.54 ± 0.01	0.46 ± 0.01

Peroxidases (POD) and glutathione transferase (GST): -Salinity stress induced a pronounced oxidative response in brinjal plants, as evidenced by a progressive increase in antioxidant enzyme activities with rising NaCl concentrations. Peroxidase (POD) activity increased from 2.94 at control to 7.20 at 100 mM NaCl, while glutathione-S-transferase (GST) activity showed a similar increasing trend, reaching a maximum value of 17.43 under severe salinity stress (Table. 5). This elevation reflects enhanced reactive oxygen species (ROS) generation and activation of the antioxidative defense system under salt-induced stress conditions.

Table: 5. Effect of NaCl on Antioxidant enzymes in brinjal

Parameters	0 mM NaCl	10 mM NaCl	25 mM NaCl	50 mM NaCl	100 mM NaCl
POD activity	2.94	3.46	4.47	5.60	7.20
GST activity	7.22	8.23	10.35	13.23	17.43

The combined application of manganese and salinity significantly influenced the antioxidant enzyme activities in brinjal plants. Under moderate salinity stress (25 mM NaCl), POD and GST activities increased markedly, indicating enhanced oxidative stress. However, supplementation with Mn at an optimal concentration (10 mM) along with 25 mM NaCl resulted in a noticeable reduction in both POD and GST activities compared to NaCl stress alone (Table. 6). This reduction reflects improved oxidative balance and confirms the protective role of Mn in mitigating salinity-induced oxidative damage. In contrast, combined exposure to higher concentrations of NaCl (50 mM) and Mn (50 mM) caused a sharp elevation in POD and GST activities, signifying excessive ROS production and the onset of metal-induced toxicity. This excessive enzymatic response suggests that while Mn at lower concentrations enhances stress tolerance, its higher dose intensifies oxidative stress when combined with salinity. The combined-effect analysis clearly demonstrates that Mn at low concentrations (10 mM) alleviates salinity stress, whereas higher Mn levels (50 mM) fail to mitigate stress and instead aggravate toxicity. These findings emphasize the importance of dose optimization of Mn for effective management of salinity stress in brinjal.

Table: 6. Combined effect of NaCl and Mn on Antioxidant enzymes in brinjal

Treatment	Concentration	POD Activity	GST Activity
Control	0 mM NaCl + 0 mM Mn	2.95	7.22
NaCl stress	25 mM NaCl	4.47	10.35
Mn alone	10 mM Mn	4.61	10.69
NaCl + Mn (protective)	25 mM NaCl + 10 mM Mn	3.62	8.98
NaCl (severe)	50 mM NaCl	5.60	13.23
NaCl + Mn (toxic)	50 mM NaCl + 50 mM Mn	7.61	14.10

Discussion

Salinity stress is one of the major abiotic constraints limiting plant growth and productivity, primarily through osmotic stress, ionic toxicity, nutrient imbalance, and enhanced oxidative damage (Evelin *et al*. 2019). The present investigation comprehensively evaluated the individual and combined effects of NaCl and manganese (Mn) on growth, photosynthetic pigments, and antioxidant responses of Solanum melongena (brinjal), with particular emphasis on identifying the optimal Mn concentration for salinity stress mitigation.

Effect of NaCl on Growth and Biomass Accumulation-Increasing NaCl concentrations caused a pronounced, concentration-dependent decline in seed germination, shoot and root growth, and biomass accumulation, a pattern which had observed earlier in crops like tomato, pepper, and other members of the Solanaceae family (Suarez *et al*., 2021; Sarkar *et al*., 2023). The reduction was mild at lower salinity levels (10–25 mM NaCl) but became severe at higher concentrations (50–100 mM NaCl), indicating progressive stress injury. High salinity likely restricted water uptake due to reduced soil water potential and induced ionic toxicity, particularly from excessive Na^+ and Cl^- accumulation, resulting in impaired cell elongation, reduced meristematic activity, and decreased dry matter production. Kassab (2005) reported that foliar application of micronutrients such as zinc, manganese, and iron significantly improved growth attributes and yield of mung bean under water stress conditions. Aloui *et al*. (2014) reported that increasing NaCl concentrations reduced germination percentage in brinjal due to the toxic effects of Na^+ and Cl^- ions. Similarly, Demir and Mavi (2008) observed that elevated salinity and osmotic stress decreased germination, seedling survival, and fresh weight in chilli, likely due to prolonged seed imbibition and enhanced dormancy. Tehseen *et al*. (2016) further demonstrated that increasing salinity levels significantly reduced germination percentage, germination index, and embryo axis length in chilli, primarily due to osmotic stress around the seed.

Manganese Concentration-Dependent Response-These findings are consistent with earlier reports showing that Mn supplementation alleviates abiotic stress by reducing ROS generation and restoring chlorophyll and carotenoid contents (Sebastian & Prasad, 2015). Mn application alone exhibited a clear concentration-dependent response in brinjal. Low Mn concentrations (10–20 mM) supported normal growth, biomass production, and pigment stability, confirming the essential role of Mn in photosynthesis, enzyme activation, and metabolic regulation. However, higher Mn concentration (50 mM) significantly reduced growth parameters, indicating Mn-induced toxicity. Excess Mn may disrupt nutrient balance, interfere with photosynthetic machinery, and promote oxidative stress, thereby impairing plant development. These findings establish a narrow physiological window in which Mn acts as a beneficial micronutrient but becomes detrimental beyond the optimal threshold.

Photosynthetic Pigments under NaCl and Mn Stress-Salinity stress caused a substantial reduction in chlorophyll-a, chlorophyll-b, and carotenoid contents, particularly at higher NaCl concentrations. The decline in photosynthetic pigments reflects salinity-induced damage to chloroplast structure, inhibition of chlorophyll biosynthesis, and enhanced pigment degradation. Since carotenoids play a critical role in photoprotection, their reduction further suggests increased vulnerability of the photosynthetic apparatus under severe salinity. In contrast, Mn application at optimal concentrations (10–20 mM) helped maintain higher pigment levels, indicating improved photosynthetic stability. However, excessive Mn (50 mM) caused a sharp decline in pigment content, reinforcing the toxic effect of Mn at higher doses.

Antioxidant Enzyme Responses and Oxidative Stress Regulation- Glutathione S-transferase (GST) plays a key role in detoxification by catalyzing the formation of less toxic, water-soluble conjugates with xenobiotics (Edwards *et al*., 2000). In the present study, salt stress reduced GST activity, which was associated with elevated H_2O_2 accumulation, in agreement with earlier reports (Tammam *et al*., 2011; Hasanuzzaman *et al*., 2014). Salinity stress significantly enhanced antioxidant enzyme activities such as POD and GST, reflecting increased reactive oxygen species (ROS) production under NaCl stress. This enzymatic upregulation is a typical plant defense response aimed at detoxifying ROS. However, excessively high enzyme activity at severe salinity levels suggests overwhelming oxidative stress rather than effective protection. Mn supplementation at low concentrations in combination with moderate salinity (25 mM NaCl) moderated POD and GST activities compared to NaCl stress alone. This reduction indicates improved redox homeostasis and reduced oxidative damage, confirming the protective role of Mn in strengthening antioxidant efficiency without overactivation. Conversely, the combined application of high Mn (50 mM) with high salinity resulted in excessively elevated antioxidant enzyme activities, indicating aggravated oxidative stress and metabolic imbalance.

Combined Effect of Mn and NaCl: Stress Mitigation vs Toxicity

The combined treatment results clearly demonstrate that Mn and salinity interact in a dose-dependent manner. At lower Mn concentrations (10–20 mM), Mn effectively mitigated salinity stress by improving growth performance, preserving photosynthetic pigments, and regulating antioxidant enzyme activity. This protective effect may be attributed to enhanced enzymatic functioning, improved photosynthetic efficiency, and better ROS scavenging capacity. However, at higher Mn concentration (50 mM), the combined stress intensified physiological damage rather than alleviating it. The synergistic negative effect of high Mn and salinity highlights the importance of precise nutrient management, as micronutrients can shift from being beneficial to toxic depending on their concentration. Although manganese is essential for plant growth, excessive Mn can inhibit plant development, as reported in rice (Lidon, 2001). Elevated Mn levels have been shown to reduce both root and shoot growth in plants (Meloni *et al*., 2003).

Conclusion

The present investigation clearly demonstrates that salinity stress exerts a pronounced inhibitory effect on the growth, biomass accumulation, photosynthetic pigments, and antioxidant metabolism of brinjal (*Solanum melongena L.*). Increasing concentrations of NaCl resulted in a progressive decline in seed germination, shoot and root growth, fresh and dry biomass, and chlorophyll and carotenoid contents, indicating that high salinity severely disrupts physiological and metabolic processes. The elevated activity of antioxidant enzymes under salinity stress further reflects enhanced oxidative damage and cellular imbalance caused by excessive salt accumulation. Manganese application exhibited a distinct concentration-dependent response. At lower concentrations (10 and 20 µM), Mn significantly improved growth attributes, maintained higher photosynthetic pigment levels, and modulated antioxidant enzyme activities, suggesting its protective and regulatory role in mitigating salinity-induced damage. These beneficial effects may be attributed to the involvement of Mn in photosynthesis, enzyme activation, and the maintenance of redox homeostasis, which collectively enhance stress tolerance in brinjal plants. However, higher Mn concentration (50 µM) resulted in a noticeable decline in growth and physiological performance, indicating the onset of Mn-induced toxicity. Excess Mn likely disturbed nutrient balance and intensified oxidative stress, thereby counteracting its beneficial role. The combined treatment of optimal Mn levels with moderate salinity showed improved plant performance compared to salinity stress alone, confirming that Mn supplementation within an optimal range can effectively alleviate salt-induced growth inhibition. Overall, the findings of the present study conclude that manganese acts as a beneficial micronutrient at lower concentrations and plays a significant role in mitigating salinity stress in brinjal. Nevertheless, its application beyond the optimal threshold leads to toxic effects. Therefore, careful optimization of Mn dosage is essential for its effective utilization as a salinity stress management strategy in brinjal cultivation. These results provide valuable insights for developing nutrient-based approaches to improve crop performance under saline conditions.

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