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The Impact of Iron Nanoparticles on Growth Parameters and Antioxidant Responses of Chilli Plants Under Salinity Stress

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Abstract

This study investigates the efficacy of Fe2O3 iron nanoparticles applied as a foliar spray on chilli seedlings (*Capsicum annuum L.*) under salt stress conditions. Salinity significantly impairs chilli growth, affecting morphological and physiological parameters, including root and shoot length, biomass, and photosynthetic efficiency. The application of iron nanoparticles was found to enhance growth and mitigate oxidative stress by improving antioxidant enzyme activities and chlorophyll content. Results indicated that foliar application of 50 ppm Fe2O3 nanoparticles significantly improved growth characteristics and physiological responses, thereby increasing resilience to salinity stress. This research highlights the potential of nanotechnology in agriculture, particularly in enhancing chilli productivity and sustainability in the face of increasing salinity in agricultural soils.

Introduction

Chillies (*Capsicum spp.*) are a crucial crop globally, particularly in Pakistan, where they are integral to the economy and nutrition. Worldwide, chilli production exceeds 7 million tons, with India as the leading producer, followed by China and Pakistan, which contributes approximately 300,000 tons annually [33,35]. In Pakistan, chillies are cultivated over an area of about 1.5 million hectares, making them a key cash crop for smallholder farmers [33]. Nutritionally, chillies are rich in vitamins A and C, capsaicin, and various antioxidants, which provide health benefits such as anti-inflammatory and antimicrobial properties [34]. Despite their importance, chilli productivity is significantly limited by various abiotic stresses, with salinity being one of the major factors affecting production both globally and in Pakistan. Salinity stress not only reduces growth and yield but also impacts the nutritional quality of the crop, making it imperative to develop strategies for enhancing chilli resilience to such environmental challenges [16]. Addressing these stresses is essential for improving the sustainability and profitability of chilli cultivation in the face of changing climatic conditions.

Salt stress significantly impacts the morphological, physiological, and biochemical parameters of chilli plants (*Capsicum spp.*), leading to detrimental effects on growth and development. Morphologically, salt stress has been shown to reduce root and shoot length, as well as fresh and dry biomass, indicating stunted growth and overall plant vigor [4,11]. Physiologically, salt stress induces oxidative stress, characterized by the accumulation of Reactive Oxygen Species (ROS), which can disrupt essential cellular functions and lead to increased electrolyte leakage and reduced relative water content [11]. This oxidative stress negatively affects photosynthesis and transpiration rates, further impairing plant health [4,16]. Biochemically, chilli plants respond to salt stress by enhancing the production of osmoprotectants such as proline, which helps mitigate the adverse

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effects of salinity by improving antioxidant enzyme activities, including Superoxide Dismutase (SOD) [9,11]. Additionally, the accumulation of antioxidant compounds, such as flavonoids, has been observed as a response to salt stress, contributing to the plant's defense mechanisms against oxidative damage [16]. Furthermore, the interaction between salt stress and specific genotypes reveals significant variability in tolerance levels, with some cultivars exhibiting better growth and biochemical responses than others under saline conditions (Kpinkoun *et al.*, 2019). Overall, the multifaceted impact of salt stress on chilli plants underscores the importance of understanding these responses for developing more resilient cultivars.

Salinity adversely affects the morphological and yield parameters of chillies (*Capsicum annuum* L.), leading to significant reductions in the number of fruits per plant, fruit length, and fruit width. Recent studies indicate that increased salinity levels correlate with decreased fruit yield, as plants struggle to absorb water and nutrients effectively [32]. Specifically, higher salinity levels have been shown to reduce the average fruit weight and overall yield per plant, ultimately impacting the economic viability of chilli cultivation [32,38]. Furthermore, morphological traits such as fruit size and number are critical indicators of plant health, which are compromised under saline conditions [38]. Addressing salinity stress is essential for enhancing chilli productivity and ensuring sustainable agricultural practices [32].

The application of nanoparticles, particularly iron nanoparticles, via foliar spray has emerged as a promising strategy to mitigate the adverse effects of salt stress on chilli plants (*Capsicum annuum* L.). Recent studies indicate that iron nanoparticles enhance plant growth and physiological parameters under saline conditions by improving iron availability, which is often limited during salt stress [17]. The foliar application of these nanoparticles not only boosts chlorophyll content and photosynthetic efficiency but also enhances antioxidant enzyme activities, thereby reducing oxidative stress [5]. Furthermore, iron nanoparticles have been shown to improve fruit yield and quality by promoting better root development and nutrient uptake, which are crucial under saline conditions [36]. This innovative approach highlights the importance of utilizing nanotechnology in agriculture to enhance crop resilience against abiotic stresses, ensuring sustainable production and food security [43]. Overall, the use of iron nanoparticles represents a significant advancement in agricultural practices, particularly for crops like chillies that are highly sensitive to salinity stress.

This study aims to investigate the efficacy of Fe_2O_3 iron nanoparticles applied as a foliars spray on chilli seedlings under salt stress conditions. Given the increasing salinity of agricultural soils, which adversely affects crop growth and yield, the need for innovative solutions to enhance plant resilience is critical. By employing iron nanoparticles, this research seeks to elucidate their potential in improving physiological and morphological parameters of chilli plants, thereby mitigating the detrimental effects of salinity [28]. The findings of this study are expected to contribute significantly to sustainable agricultural practices, promoting enhanced productivity in chilli cultivation amidst challenging environmental conditions [29]. Ultimately, this research underscores the importance of utilizing nanotechnology to address the pressing challenges posed by salinity stress in crop production.

Material and methods

Plant material and treatments: The present study was conducted at the research farm of the Vegetable Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad. Seeds of two registered chilli lines, "Wiz-21" and "Iso-87," were sown in peat moss-filled seedling trays in November 2022. Once the seedlings reached a height of 12 cm, they were transplanted into plastic pots containing a soil and peat moss mixture in a 1:1 ratio. The experimental design employed was a Complete Randomized Design (CRD) with three replications, incorporating three factors: salinity, genotypes, and iron nanoparticles. Salinity treatments included a control group (0 mM NaCl) and a salt stress group (50 mM NaCl), with salt stress applied fifteen days post-transplantation. Iron nanoparticles (Fe_2O_3) were sourced from the Department of Botany, University of Agriculture, Faisalabad. Three concentrations of iron nanoparticles were utilized: 0, 25, and 50 mg/g. The nanoparticles were applied using a handheld sprayer 24 and 48 hours after the initiation of salt stress. Following a 10-day period post-application, shoot and root lengths and fresh weights were measured. Subsequently, the samples were placed in an oven at 65°C for drying, and the root and shoot dry weights were recorded.

Chlorophyll a, b, and proline content levels were measured using the method of Arnon [7] and Bates et al. [8]. Total pigments and carotenoids were estimated following method described by Lichtenthaler and Buschmann [26] and [37]. Potassium and sodium ions concentration was estimated following Zhao et al. [45] whereas calcium ion content was determined following method described by Fujiwara et al. [15]. H_2O_2 and Malondialdehyde (MDA) were estimated following Heath and Packer [19] whereas total soluble protein concentrations were measured following [10].

Samples for enzymes extraction and estimation were prepared following method outlined by Yin et al. [41]. Superoxide Dismutase (SOD) and Peroxide (POD) enzymes activity was estimated using methods described by Giannopolitis and Ries [18] and Polle et al. [31]. Catalase (CAT) and Ascorbate Peroxidase (APX) enzymes activity was estimated following Jiang and Huang [22].

Statistical analysis

Data collected was subjected to analysis of variance as described by Steel *et al*., 1997. The significance of differences among treatment means was tested using Least Significant Difference test at 5% probability level using Statistix® 8.1 software.

Results

Stress from salinity significantly reduced the development characteristics of chillies. (Table 1). When compared to the control value of NaCl (0 mM), the biomass and root and shoot lengths were dramatically reduced when exposed to strong NaCl (50 mM). Iron nanoparticles greatly reduced the harmful impacts of the salinity and markedly enhanced biomass output and growth characteristics. On the other hand, compared to the other value of Iron NPs (25 ppm) application in both genotypes, respectively, the application of Iron NPs (50 ppm) foliar spray significantly improved the growth features. Foliar spray of Iron NPs (50 ppm) in Wiz-21 appreciably improved the Shoot Length (SL: 5.36%), Root Length (RL: 6.66%), Shoot Fresh Weight (SFW: 4.51 %), Shoot Dry Weight (SDW: 7.22%), Root Fresh Weight (RFW: 6.5%), Root Dry Weight (RDW: 3.06%), and Leaves Per Plant (LPP: 7.14%) as compared to ISO-87 it decreases the value of SDW and no. of leaves.

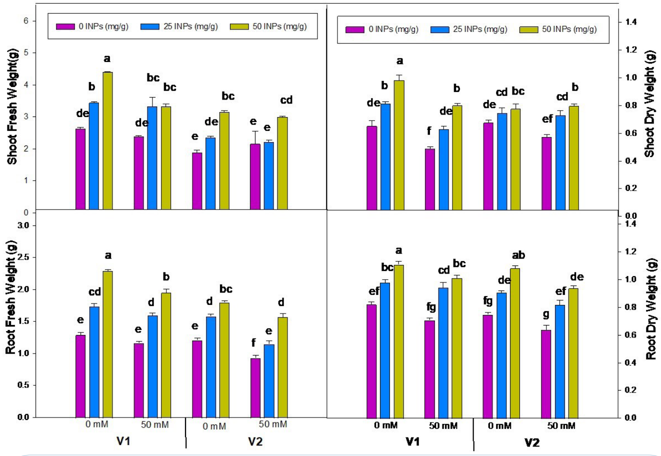

Figure 1: Effect of foliar application with Fe2O3NPs on Growth traits of Chilli (*Capsicum annuum L*).

The application of INPs had a significant impact on shoot length and shoot fresh weight in both genotypes. In growth parameters control values with the addition of Iron NPs increased the growth of root and shoot length (6.66%) and (5.36%) respectively, shoot and root fresh and dry weight (0.45%), (17.47%), (12.5%) and (7.47%) respectively, number of leaves (11.76) and plant height (2.8%) respectively of VIZ and some values were decreased of ISO in root fresh weight and root length.

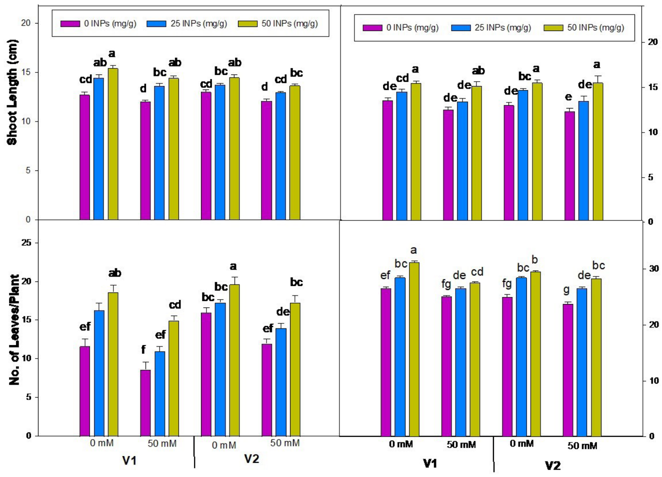

Figure 2: Effect of INPs on **(A)** Shoot length **(B)** Root length **(C)** Number of leaves **(D)** Plant height of chilli plants under salinity.

Mostly significant and some non-significant interaction has also been observed between salinity and INP application in terms of growth attributes. In both genotypes, ISO had some non-significant values. Root dry weight and shoot dry weight show a more significant interaction (Table 1).

Table 1: Mean square from analysis of variance of data for growth traits of chilli treated with foliar spray of INPs under salinity.

Main Effects	Df	SFW	SDW	RFW	RDW	SL	RL	No. of leaves	PH
V	1	5.62***	0.01*	0.80***	0.04***	1.77**	0.01*	56.25***	3.01***
NaCl	1	0.52**	0.09***	0.59***	0.08***	6.25***	4.55**	117.36***	32.11***
NPs	2	4.36***	0.17***	1.70***	0.28***	12.38**	19.54***	93.69**	47.76***
V+NaCl	1	0.48*	0.04***	0.02*	0.01**	6.77ns	0.09*	1.36*	3.36***
V+NPs	2	0.28**	0.02***	0.05***	0.01*	0.91**	0.28*	4.08ns	0.41**
ssNaCl+ NPs	2	0.32*	0.01*	0.01*	0.01**	0.01*	0.76*	1.36*	0.26*
V+NaCl+ NPs	2	0.17*	0.01*	0.03**	6.69***	0.03*	0.04*	1.86**	0.43**
Error	24	0.06	0.01	0.01	0.01	0.14	0.42	1.61	0.05

*, **, *** Significant at 0.05, 0.01, and 0.001 levels respectively; ns=Non-Significant; SL: Shoot Length, RL: Root Length, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, NOL: No. of Leaves, PH: Plant Height.

As demonstrated in Figure 3, various doses of Fe_2O_3NPs considerably enhanced photosynthetic pigments as compared to the untreated control. Compared to other treatments, it is clear that foliar application of Fe_2O_3NPs at a concentration of 50 ppm has the most beneficial effect on photosynthetic pigment content. Both genotypes of chillies experienced a notable decrease in photosynthetic rate (Pn) due to salt stress (Figure 3C). For the saline control, foliar treatment of all levels of INPs dramatically increased the photosynthetic rate of both genotypes in a salty environment without the use of an iron nanoparticle foliar spray. Applying INP was more successful than using iron nanoparticle foliar spray in increasing the photosynthetic rate in both genotypes. However, a higher photosynthetic rate was observed in VIZ-21 (21.7%µmol CO_2 m-2s-1) while the lowest was recorded in ISO-87 (17.20% µmol CO_2 m-2s-1) genotype.

Under salinity, the activities of POD, SOD, and CAT increased noticeably. The strategies for applying Iron NPs greatly enhanced the activity of the antioxidants (Figure 4). But in both genotypes, the combination of INPs with salinity and foliar spray was shown to be the best performer, as it significantly raised the activity of CAT, POD, and SOD in VIZ-21 under heavy NaCl compared to control by 14.28, 5.20, and 51.85%, respectively (Figure 4). INPs decrease the POD activity. Salinity increases the level of POD but iron nanoparticles decrease its activity for managing the concentration in both genotypes. INPs and NaCl increase the activity of SOD, POD, and CAT in ISO-87 by 2.19, 29.03, and 10%. Antioxidants and photosynthetic pigments show significant results which given in (Table 2).

The collected data, which are shown in Table 2, demonstrate that applying foliar treatments containing varying concentrations of Fe_2O_3 nanoparticles significantly increased the amount of proline, malonaldehide, total soluble protein, and H_2O_2 in Capsicum annuum L. Plant cultivated in salinity as opposed to controls. The data also demonstrate that the foliar application of a 50 ppm Fe_2O_3NPs treatment produced the biggest record of the osmolyte under study. There was a substantial ($p<0.05$) difference in MDA activity between all INP levels and the saline control in both genotypes (Figure 5).

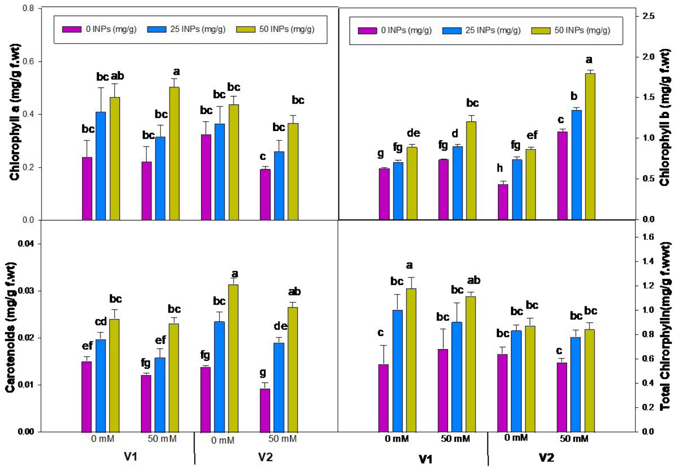

Figure 3: Impact of different concentrations of iron nanoparticles sprayed topically on (A) Chlorophyll a, (B) Chlorophyll B, (C) Carotenoids, and (D) Total Chlorophyll of Chilli plants under saline.

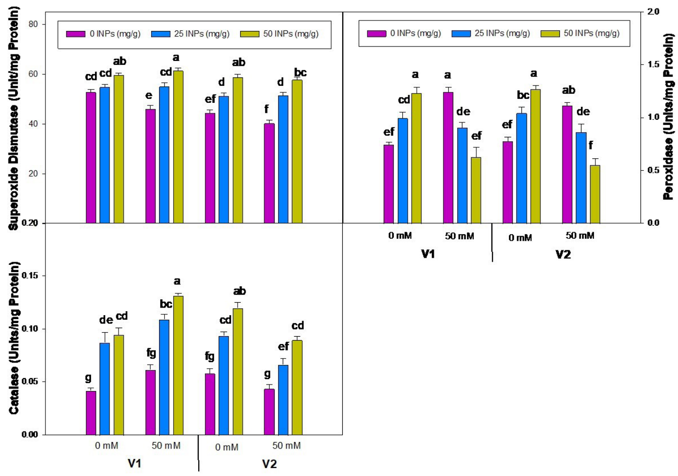

Figure 4: Impact of different concentrations of iron nanoparticles sprayed topically on activities of antioxidants; POD (A), SOD (B), and CAT (C) of Chilli plants under saline.

Table 2: Mean square from analysis of variance of data for Photosynthetic pigments and antioxidants of Chilli treated with foliar spray of INPs under saline.

Main Effects	Df	Chl a	Chl b	Car	Ttl. Chl	SOD	POD	CAT
V	1	0.04*	0.35***	4.60**	0.20*	162.68***	0.10*	7.50**
NaCl	1	0.03*	1.96***	1.18***	0.32**	24.09*	0.14***	1.17ns
NPs	2	0.11***	0.66***	5.63***	0.47***	548.61***	0.20**	0.01***
V+NaCl	1	0.04*	0.61***	9.33ns	0.24*	15.71*	0.33**	0.01***
V+NPs	2	0.03*	0.03***	4.51***	0.35**	18.14*	0.52ns	2.26**
NaCl+ NPs	2	0.03*	0.05***	3.16*	0.30**	32.78**	0.86***	3.52*
V+NaCl+ NPs	2	0.07**	0.07*	2.09*	0.41**	16.91*	4.98ns	2.05**
Error	24	0.01	0.01	4.81	0.02	4.18	0.01	7.31

*, **, *** Significant at 0.05, 0.01 and 0.001 levels respectively; ns=Non-Significant; Chl. *a*: Chlorophyll *a*; Chl. *b*: Chlorophyll *b*; Chl.*a*/*b*: Chlorophyll *a*/*b,* Carotenoid; POD: Peroxidase; SOD: Superoxide Dismutase; CAT: Catalase.

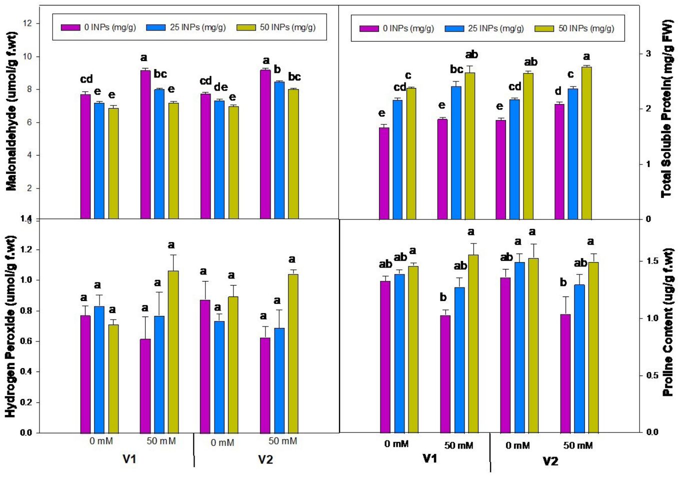

Figure 5: Impact of different concentrations of iron nanoparticles sprayed topically on Maloneldehyde (A), Total Soluble Protein (B), Hydrogen Peroxide (C) and Proline Contents (D) of Chilli plants under saline.

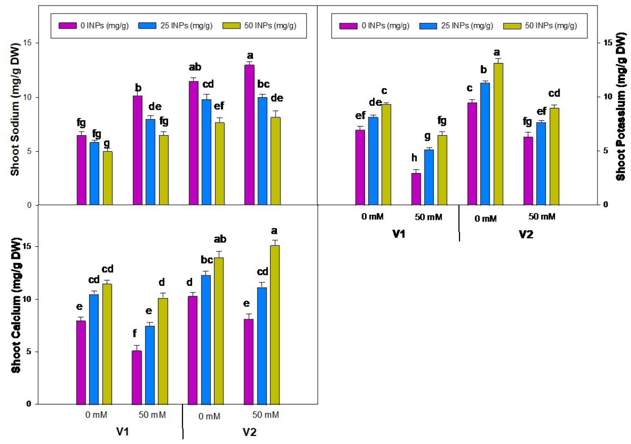

Figure 6: Impact of different concentrations of iron nanoparticles sprayed topically on ionic status of the shoot; Sodium (A), Potassium (B), and Calcium (C) of Chilli plants under saline.

At 50 mM salinity stress of examined chillies, salt stress significantly increased the formation of Cl-ions (10.34 mg/g FW) in the tissues (Table 3). The Wiz-21 and ISO-87 genotypes' leaf Na^+ concentrations were significantly impacted by salt stress. When iron nanoparticles were sprayed foliarly, the content of Na^+ in the leaves decreased but increased in salinized circumstances. Maximum reduction in ISO-87 (5.55 mg g-1 D.W.) in Na^+ concentration was recorded at 50 mM and INPs concentration in leaves of Wiz-21induced (16.66 mg g-1 D.W.) as shown in Figure 6. Salt stress dramatically reduced the potassium (K^+) concentration in leaves. On the other hand, in both genotypes, foliar application of iron nanoparticles under salt stress increased the leaf K^+ content. However, the maximum increment (7.69 mg g-1 D.W.) was recorded in the VIZ-21 genotype (Figure 6).

Table : Mean square from analysis of variance of data for hydrogen and lipid peroxide, TSP, Proline, and ionic contents of Chilli treated with foliar spray of INPs under saline.

Main Effects	Df	MDA	TSP	H_2O_2	Proline	Shoot Na^+	Shoot K^+	Shoot Cl^-
V	1	0.63***	0.13***	0.09*	0.12*	82.50***	79.50***	84.02***
NaCl	1	9.59***	0.40***	1.87ns	0.18**	22.56***	108.50***	21.77***
NPs	2	4.30***	1.79***	0.14**	0.30***	35.96***	27.79***	68.89***
V+NaCl	1	0.29**	0.09*	0.02ns	0.26*	6.67***	0.80*	6.25**
V+NPs	2	0.14*	0.04**	0.22**	1.14**	2.29**	0.09ns	1.09ns
NaCl+ NPs	2	0.42***	0.23**	0.15**	0.08*	2.27**	0.94*	5.00***
V+NaCl+ NPs	2	0.09*	0.06***	0.37*	0.90**	0.29ns	0.92*	2.64ns
Error	24	0.02	0.01	0.02	0.01	0.38	0.23	0.46

Salt stress enhanced MDA contents. The impact of applying varying doses of Fe_2O_3 NPs topically on the H2O2 and MDA levels in chillies. Plants grown under saline conditions are presented in (Figure 5). Data showed that, when Fe_2O_3NP levels increased up to 25 and 50 Fe_2O_3NPs, respectively, the contents of H_2O_2 and MDA fell and remained lower than the control plant as compared to the untreated control plant. TSP and proline content concentrations were greatly increased by INPs, with the greatest increase occurring during intense application.

Discussion

The effects of salinity stress on the growth and development of chillies (*Capsicum annuum* L.) have been extensively documented, revealing that elevated salinity levels can significantly impair various growth parameters. In the present study, it was observed that exposure to high NaCl concentrations (50 mM) resulted in a marked reduction in biomass, root length, and shoot length compared to the control group (0 mM NaCl) [21]. This aligns with previous research indicating that salinity stress adversely affects plant growth by disrupting physiological processes and nutrient uptake [27]. The detrimental impact of salinity is primarily attributed to osmotic stress and ion toxicity, which lead to reduced water availability and increased accumulation of harmful ions such as Na^+ and Cl^- in plant tissues [23].

To mitigate the adverse effects of salinity, the application of iron nanoparticles (Fe_2O_3 NPs) has emerged as a promising strategy. The results of this study demonstrated that foliar application of Fe_2O_3 NPs significantly alleviated the negative impacts of salinity on chilli plants, enhancing biomass and growth characteristics [44]. Specifically, the application of 50 ppm Fe_2O_3 NPs resulted in notable improvements in shoot length, root length, and both fresh and dry weights of shoots and roots. This finding is consistent with previous studies that have reported the beneficial effects of iron nanoparticles on plant growth under stress conditions, attributed to their role in enhancing nutrient availability and promoting antioxidant activity [13,30].

The observed improvements in growth parameters with the application of Fe_2O_3 NPs can be explained by their ability to enhance photosynthetic pigment content, as evidenced by the significant increase in chlorophyll levels in treated plants [23]. Enhanced chlorophyll content is crucial for improving photosynthetic efficiency, which is often compromised under salinity stress [42]. In this study, the foliar application of 50 ppm Fe_2O_3 NPs resulted in the highest chlorophyll content, thereby contributing to improved photosynthetic rates in both chilli genotypes. This is particularly important as salinity stress has been shown to reduce the photosynthetic rate, which directly affects plant growth and productivity [39].

Moreover, the application of Fe_2O_3 NPs was found to enhance the activity of key antioxidant enzymes, including Peroxidase (POD), Superoxide Dismutase (SOD), and Catalase (CAT). These enzymes play a vital role in mitigating oxidative stress induced by salinity by scavenging Reactive Oxygen Species (ROS) and protecting cellular components from damage [27]. The study revealed that the combination of salinity stress and Fe_2O_3 NPs application resulted in a significant increase in the activity of these antioxidant enzymes, particularly in the Wiz-21 genotype, which exhibited the highest increase in CAT, POD, and SOD activities compared to the control. This suggests that the application of iron nanoparticles not only enhances growth but also fortifies the plant's defense mechanisms against oxidative stress.

In addition to growth and antioxidant activity, the study also assessed the impact of Fe_2O_3 NPs on osmotic adjustment in chilli plants under salinity stress. The results indicated that foliar application of $Fe2O_3$ NPs significantly increased the levels of proline, total soluble proteins, and Malondialdehyde (MDA) in the plants [12]. Proline serves as an important osmolyte that helps maintain cellular turgor and protects cellular structures under stress conditions [6]. The increase in total soluble proteins further indicates enhanced metabolic activity and stress tolerance in treated plants [25]. Conversely, the reduction in MDA levels suggests that Fe_2O_3 NPs may help mitigate lipid peroxidation caused by oxidative stress, thereby preserving membrane integrity [40].

The study also highlighted the effects of salinity on ions accumulation in chilli plants. High salinity levels led to increased Na^+ concentrations in the leaves, which can be detrimental to plant health [20]. However, the application of Fe_2O_3 NPs effectively reduced Na^+ accumulation in the leaves, particularly in the ISO-87 genotype, which recorded the lowest Na^+ concentration under salinity stress. This reduction in Na^+ levels is critical for maintaining ion homeostasis and overall plant health, as excessive Na^+ can lead to ion toxicity and disrupt physiological processes [14]. Furthermore, the application of Fe_2O_3 NPs under salinity stress was associated with an increase in potassium (K^+) concentrations in the leaves, which is essential for various physiological functions, including osmoregulation and enzyme activation (Mulyono et al., 2022).

Conclusion

In conclusion, the findings of this study underscore the significant negative impact of salinity stress on the growth and development of chillies, as evidenced by reduced biomass and growth parameters. However, the application of iron nanoparticles, particularly at a concentration of 50 ppm, demonstrated a remarkable ability to alleviate the adverse effects of salinity. This was achieved through enhancements in growth characteristics, photosynthetic pigment content, antioxidant enzyme activity, and osmotic adjustment mechanisms. The results suggest that the use of iron nanoparticles could be a viable strategy for improving the resilience of chilli plants to salinity stress, thereby contributing to sustainable agricultural practices in saline-prone areas.

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