

Foliar application of selenium nanoparticles, multiwalled carbon nanotubes and their hybrids stimulates plant growth and yield characters in rice (*Oryza sativa* L.) under salt stress[☆]

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ABSTRACT

Salt stress (NaCl) is a major abiotic factor that severely affects plant growth and crop yield, with rice (*Oryza sativa* L.) being particularly vulnerable. Excessive soil salinity has been shown to significantly reduce rice productivity, posing a threat to global food security. To mitigate these adverse effects, research has increasingly focused on using nanomaterials to enhance plant tolerance to salt stress. This study investigates the potential of selenium nanoparticles (SeNPs), multiwalled carbon nanotubes (MWCNTs), and their hybrid form (SeNPs+MWCNTs) in alleviating NaCl-induced stress in rice plants exposed to 50 mM and 100 mM NaCl. Nanoparticle synthesis, hybridization, and localization in the foliar parts of the plants were confirmed using Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infrared Spectroscopy (FTIR), and fluorescence microscopy with methylene blue dye, respectively. Among the treatments, SeNPs+MWCNTs hybrids (160 µg/mL) demonstrated the most promising effects, significantly enhancing various growth and yield parameters under saline conditions. Notably, this treatment improved shoot length (17 %), root length (14 %), and key yield traits, including shoot dry weight (32 %), root dry weight (31 %), total dry weight (32 %), number of panicles (31 %), panicle length (19 %), panicle weight (22 %), number of spikes per panicle (28 %), spike length (18 %), spike weight (19 %), number of fertile spikelets (32 %), and 100-grain weight (29 %) compared to the individual SeNPs and MWCNTs treatments. These findings provide valuable insights into the potential application of nanomaterials for improving rice growth under saline stress.

1. Introduction

Salinity (NaCl) stress is a major abiotic factor that significantly impacts crop productivity, posing a severe threat to global food security (Francini and Sebastiani, 2019; Razzaq et al., 2020). Approximately 1.125 billion hectares of land worldwide are affected by NaCl stress (Hossain, 2019), leading to adverse effects on plant growth, physiological functions, and biochemical activities (El Sabagh et al., 2021). Nanotechnology, which utilizes synthetic nanomaterials, has emerged as a promising approach to mitigate abiotic stresses. Nanoparticles have

demonstrated the ability to preserve photosynthetic function, scavenge reactive oxygen species (ROS), and alleviate osmotic and ionic stress. However, excessive use of nanoparticles can lead to environmental toxicity and adverse effects on plants (Singh et al., 2023). Despite these concerns, nanomaterials show promise in combating NaCl stress, with numerous studies highlighting their potential in agriculture (El-Badri et al., 2022; Ganjouei et al., 2023; Raza et al., 2023).

Multi-walled carbon nanotubes (MWCNTs) are known to enhance photosynthesis, electron transmission, and auxin bioprotocols in plants, thereby improving growth and yield under both stressed and non-

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stressed conditions (Joshi et al., 2018a, 2018b; Zhao et al., 2019; Li et al., 2022). MWCNTs alleviate NaCl stress by improving water absorption, stomatal conductance, transpiration, photosynthetic efficiency, and biomass in rice plants (Xu et al., 2023). Similarly, selenium nanoparticles (SeNPs) are recognized for their greater penetrating power, free radical scavenging activity, and low toxicity, making them beneficial for plant growth under NaCl stress (Zohra et al., 2021; Nikam et al., 2023). Several studies have shown that foliarly applied SeNPs positively affect plant growth, yield, and stress mitigation in crops like garlic, date palm, and pineapple mint (El-Saber, 2021; Mahdi et al., 2022; Kiumarzi et al., 2022). Additionally, SeNPs have been demonstrated to improve rice traits, including root characteristics, leaf water content, and panicle yield under salt stress (Badawy et al., 2021).

Despite individual studies on SeNPs and MWCNTs, the combined potential of these nanoparticles has not been explored for rice plants under NaCl stress. This study investigates the synergistic effects of SeNPs and MWCNTs (SeNPs+MWCNTs) in alleviating NaCl stress in rice, aiming to fill this gap in research. Given the detrimental impact of NaCl stress on rice growth and productivity (Islam et al., 2019; Thu et al., 2017), the use of SeNPs+MWCNTs hybrids offers a novel approach to mitigating these effects. Rice, a crucial crop that feeds nearly half of the global population, was chosen for this study due to its high sensitivity to NaCl stress (Rezvi et al., 2023).

To evaluate the efficacy of the nanoparticles, a field trial was conducted on rice plants subjected to 50 mM and 100 mM NaCl treatments. Nanoparticles were applied foliarly, and a comprehensive analysis of various growth and yield parameters was performed, including plant height, dry biomass, panicle number, panicle dimensions (length and weight), spike characteristics (number, length, and weight), number of fertile spikelets, and 100-grain weight. The synthesis and hybridization of the nanoparticles were confirmed using Field Emission Scanning Electron Microscopy (FESEM) and Fourier Transform Infrared Spectroscopy (FTIR). FESEM analysis provided detailed insights into the surface morphology, size, and agglomeration behavior of the nanoparticles, while FTIR spectra identified the functional groups involved in nanoparticle stabilization. Fluorescence microscopy further validated the uptake of the nanoparticles by foliar tissues. This study seeks to investigate the synergistic effects of SeNPs and MWCNTs, offering valuable insights that can contribute to improving crop productivity and facilitating the sustainable management of NaCl-affected agricultural systems.

2. Material and methods

2.1. Functionalization of MWCNTs

MWCNTs (multi-walled carbon nanotubes) were sourced from Sigma-Aldrich, with outer and inner diameters of 10 ± 1 nm and 4.5 ± 0.5 nm, respectively. Prior to foliar application on rice plants, the MWCNTs underwent functionalization using nitric acid (HNO_3). The functionalization process involved treating the MWCNTs with concentrated nitric acid at $0-4^\circ\text{C}$ for 24 hours (Tripathi et al., 2011; Joshi et al., 2018b). This treatment facilitated the incorporation of carboxylic and hydroxyl groups onto the MWCNT surface, enhancing their ability to bond effectively with plant tissues. Following functionalization, the MWCNTs were washed five times with deionized water and subjected to ultra-centrifugation at 15,000 rpm for 10 minutes per cycle to remove any excess nitric acid (Kaur et al., 2013). After centrifugation, the MWCNTs were dried overnight in an oven at 80°C before being prepared for foliar application to the rice plants.

2.2. Synthesis of SeNPs and SeNPs+MWCNTs

Selenium nanoparticles (SeNPs) were synthesized using the sol-gel method. To initiate the process, 1.2 g of sodium selenite (Na_2SeO_3) and 50 g of D-glucose were dissolved together. Following this, 50 mL of

ethylene glycol was added with continuous stirring, and 30 mL of deionized water was introduced. The resulting mixture was subjected to ultrasonic treatment using an ultrasonicator (LABMAN: LMUC-2) for 30 minutes at room temperature to achieve a uniform suspension. The transparent suspension was then heated in an oven at 85°C for 90 minutes, maintaining a pH between 6 and 8. After 30 minutes of heating, the sample was removed and stirred for an additional 10 minutes using a magnetic stirrer (REMI: 2-MLH). The formation of SeNPs was indicated by the appearance of a brick-red color. The sample was collected in a centrifuge tube and washed 4–5 times with deionized water. The supernatant was discarded, and the pellet was isolated. The pellet, consisting of SeNPs, was then dried to yield the final powdered form of SeNPs.

For the preparation of SeNPs+MWCNTs hybrids, 20 mg of multi-walled carbon nanotubes (MWCNTs) were added to the reaction mixture described above. This resulted in the direct attachment of SeNPs to the defect sites on the MWCNTs, thereby forming the SeNPs+MWCNTs composite.

2.3. Experimental area and material

The field experiment was conducted at Akal University, located in Talwandi Sabo, Bathinda District, Punjab, India. The experimental site is situated at geographical coordinates 29.9882680°N , 75.0786780°E , with an elevation of 213 m above sea level. The rice seeds (DSR 122) employed in this study were procured from Punjab Agricultural University, Ludhiana, Punjab, India. Multiwalled carbon nanotubes (MWCNTs) used in the experiment were obtained from Sigma-Aldrich.

2.4. Characterization of nanoparticles

2.4.1. Fourier-transformed infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR; Bruker, Model-Tensor 27) was employed to analyze the synthesized selenium nanoparticles (SeNPs), as well as to identify the functional groups present in multiwalled carbon nanotubes (MWCNTs) and the SeNPs+MWCNTs hybrid, facilitating a detailed understanding of their chemical structures and interactions.

2.4.2. Field emission scanning electron microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM; TESCAN, USA, Model-MIRA 3LMH, 2012) was utilized to examine the surface morphology of freshly synthesized selenium nanoparticles (SeNPs), multiwalled carbon nanotubes (MWCNTs), and their hybrid (SeNPs+MWCNTs), providing detailed insights into their structural characteristics and particle interactions at the nanoscale level.

2.4.3. Fluorescence microscopy

The localization of SeNPs, MWCNTs, and SeNPs+MWCNTs in rice leaf tissues was confirmed using fluorescence microscopy (Olympus FSX-100, Japan) with methylene blue as a fluorescent marker. A solution containing 100 mL of methylene blue and 100 mL of MWCNTs was prepared in a 250 mL conical flask and wrapped in aluminium foil to prevent photobleaching. The mixture was agitated for 72 hours at 150 rpm on an orbital shaker, followed by centrifugation at 5000 rpm for 30 minutes. The supernatant was carefully decanted and used for foliar application on rice plants. Leaf tissue samples were rinsed with deionized water and manually sectioned using fresh razor blades. The sections were placed in petri dishes filled with water for rinsing and mounted on glass slides using glycerin. Cross-sectional images of control, SeNPs, MWCNTs, and SeNPs+MWCNTs-treated leaf tissues were observed under fluorescence microscopy.

2.5. Experimental procedure

To initiate the experiments, rice seeds were sown in polythene bags

with a capacity of 7 kg and dimensions of 15 in. in length and breadth. The soil mixture in the bags was composed of soil, sand, and farmyard manure in a 3:1:1 ratio, respectively. Prior to sowing, the rice seeds were surface sterilized for 1–2 minutes using a 10 % sodium hypochlorite solution. The seeds were then thoroughly rinsed 2–3 times with distilled water to remove any residual sterilizing solution. The experimental setup included 12 treatment groups, consisting of control plants and plants subjected to different concentrations of NaCl, SeNPs, MWCNTs, and SeNPs+MWCNTs. For NaCl-induced stress, the polythene bags were saturated with 50 mM and 100 mM NaCl solutions and allowed to dry for one day. Control plants were irrigated with distilled water. Six seeds were sown per bag, and after 15 days, the plants were thinned to four per bag to ensure optimal growth. Throughout the experiment, plants were regularly irrigated to prevent water stress.

During the booting phase, rice plants were treated with foliar sprays of nanoparticle solutions (SeNPs, MWCNTs, and their hybrid SeNPs+MWCNTs) at a concentration of 160 µg/mL. Upon maturation, the rice plants were harvested for subsequent analysis, enabling the evaluation of the effects of SeNPs, MWCNTs, and SeNPs+MWCNTs on various growth and yield parameters. This experimental framework provided an opportunity to explore the potential benefits of these nanoparticles in enhancing growth and stress resilience in rice plants, thereby offering valuable insights for agricultural applications.

2.6. Growth and yield traits

Following the uprooting of rice plants, precise measurements of shoot and root lengths were taken. The plants were subsequently oven-dried at 50°C to determine shoot and root dry weights, which are critical indicators of plant growth. For yield assessment, the total dry weight of the plants was recorded alongside the number of panicles per plant. Additionally, the length and weight of the panicles were meticulously measured, along with the number of spikes per panicle, to further evaluate the plant's reproductive output. The number, weight, and length of fertile spikelets per panicle were also quantified, providing valuable insights into the plants' reproductive potential. Lastly, to evaluate grain yield, the weight of 100 grains was determined. These growth and yield-related parameters were measured under both normal and NaCl-stressed conditions, providing a comprehensive understanding of the impact of SeNPs, MWCNTs, and their hybrid (SeNPs+MWCNTs) on rice plant development and productivity. The data gathered from these measurements will serve as a robust foundation for assessing the efficacy of these nanoparticles in promoting plant growth, alleviating stress, and enhancing yield potential. The findings will significantly contribute to the ongoing exploration of nanoparticle applications in agriculture, particularly in mitigating abiotic stresses such as salinity, thus offering promising solutions for sustainable agricultural practices.

2.7. Statistical analysis

The experiments were performed with a minimum of three biological replicates for each treatment. Data are presented as the mean values \pm standard deviation (SD) or standard error. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with Tukey's post hoc test to determine the significance of differences among treatment groups. The analysis was performed using Microsoft Excel 2019. A p -value ≤ 0.05 was considered statistically significant, and p -values ≤ 0.01 were considered highly significant.

3. Results and discussion

3.1. Preliminary results

Our study was conducted under controlled laboratory conditions, employing foliar application as the mode of nanoparticle (NP) administration to rice plants. Foliar application offers a dual advantage: it

minimizes environmental impact by reducing nutrient leaching and facilitates direct absorption of NPs through the leaf surface. Based on preliminary trials, we selected NaCl concentrations of 50 mM and 100 mM from an initial gradient of 25, 50, 75, 100, 125, and 150 mM for further experimentation (Fig. 1a). Lower concentrations failed to elicit significant physiological responses, while higher concentrations resulted in diminished plant performance. Excessive NaCl accumulation in the soil impairs plant growth and development by disrupting water uptake and inducing ionic imbalances within plant cells. This leads to phenotypic manifestations such as leaf necrosis, wilting, and stunted growth. Additionally, NaCl stress triggers oxidative damage at the cellular level, adversely affecting photosynthetic efficiency and nutrient acquisition, ultimately resulting in osmotic, ionic, and oxidative stress. The 50 mM and 100 mM concentrations were deemed optimal, as they were sufficiently robust to induce discernible stress responses while avoiding excessive toxicity.

Similarly, based on prior research on foliar NP applications in rice, we selected concentrations of 80, 120, 160, and 200 µg/mL for selenium nanoparticles (SeNPs) and multi-walled carbon nanotubes (MWCNTs) (Fig. 1b and c). These concentrations were chosen based on their observed effects on shoot elongation in preliminary trials; lower concentrations failed to significantly enhance shoot length, whereas higher concentrations resulted in growth inhibition. Among these, 160 µg/mL exhibited the most pronounced positive impact on plant growth parameters. Therefore, this concentration was selected to evaluate the ameliorative potential of NPs under NaCl-induced stress conditions.

Our investigation systematically examined the role of SeNPs, MWCNTs, and their combined application (SeNPs+MWCNTs) in mitigating the adverse effects of NaCl stress on rice growth and yield. Key agronomic traits assessed included plant height, dry biomass, panicle count, panicle length and weight, spike number, spike length and weight, number of fertile spikelets, and 100-grain weight. The findings provide crucial insights into the efficacy of nanoparticle-mediated stress mitigation strategies, underscoring their potential for enhancing rice productivity under saline conditions.

3.2. Characterization of nanoparticles

Field Emission Scanning Electron Microscopy (FESEM) analysis was performed to examine the surface morphology of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their composite (SeNPs+MWCNTs). The FESEM images confirmed that SeNPs exhibited a well-defined spherical morphology, with particle sizes ranging from 30 to 200 nm (Fig. 2a). In contrast, MWCNTs displayed characteristic thread-like tubular structures, with an outer diameter of 10 ± 1 nm and an inner diameter of 4.5 ± 0.5 nm (Fig. 2b). Notably, the SeNPs+MWCNTs composite demonstrated a uniform distribution of SeNPs along the MWCNT surface without any observable aggregation (Fig. 2c, encircled regions). Importantly, no structural distortions or agglomerations were detected in the individual SeNPs or MWCNTs, reinforcing the stability of the synthesized nanomaterials (Fig. 2a & b). These morphological characteristics align with previous reports on SeNPs and MWCNTs (Joshi et al., 2020; Radzi et al., 2024), further validating the synthesis approach.

Fourier-transform infrared (FTIR) spectroscopy was conducted within the spectral range of 4000–500 cm^{-1} to identify the functional groups associated with the synthesized nanoparticles (Fig. 2d–f). The FTIR spectra revealed distinct absorption peaks corresponding to various functional groups, confirming the successful synthesis and structural integrity of SeNPs, MWCNTs, and the SeNPs+MWCNTs composite. The observed peaks were indicative of characteristic vibrational modes associated with O-H, C-H, C-O, C-C, C=C, C=N, and C-CH₃ functional groups (Table 1). These spectral signatures corroborate findings from previous studies that have employed FTIR analysis to characterize nanoparticle composition and functionality (Joshi et al., 2018b; Radzi et al., 2024). Collectively, the FESEM and FTIR analyses

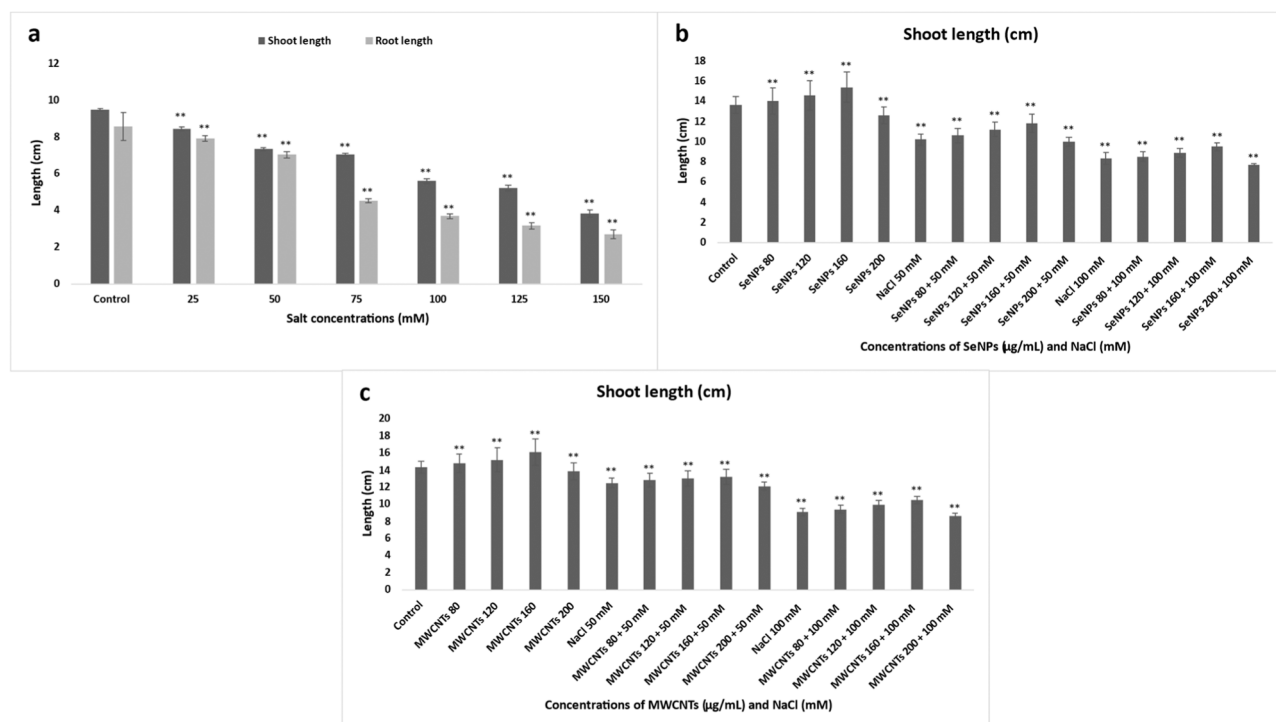


Fig. 1. (a) Effect of varying concentrations (25, 50, 75, 100, 125, and 150 mM) of NaCl on shoot and root length (b) effect of foliar applied SeNPs at varying concentrations (80, 120, 160, and 200 µg/mL) and NaCl (50 and 100 mM) on shoot length (c) effect of foliar applied MWCNTs at varying concentrations (80, 120, 160, and 200 µg/mL) and NaCl (50 and 100 mM) on shoot length of rice seedlings in laboratory conditions during optimization. Statistical analysis via one-way ANOVA revealed significant differences between treatments, denoted by LSD values * $p < 0.05$; ** $p < 0.001$). Error bars represent mean ± Standard error.

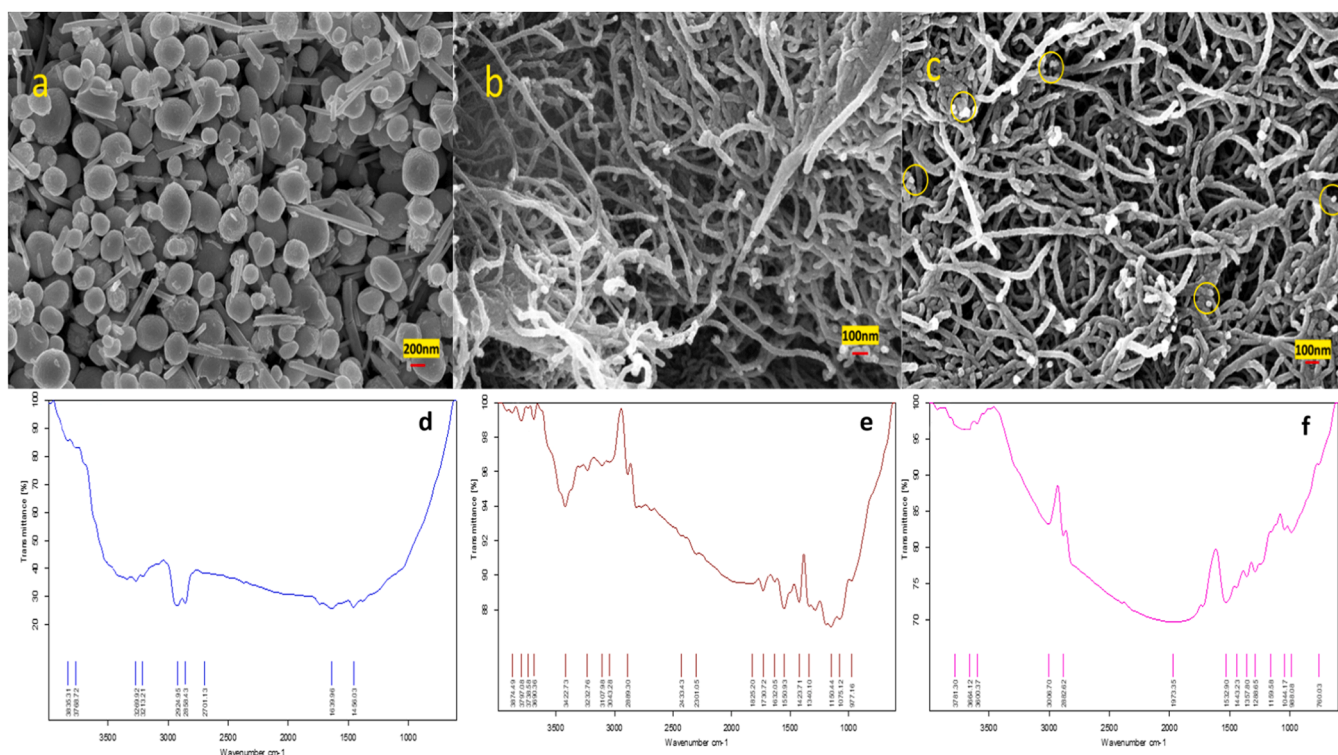


Fig. 2. FESEM images of (a) SeNPs, (b) MWCNTs, and (c) SeNPs+MWCNTs; FTIR spectra of (d) SeNPs, (e) MWCNTs, and (f) SeNPs+MWCNTs.

Table 1
FTIR spectra peak table.

Sr. No.	Peaks (cm ⁻¹)	Assignment
1.	988	O-H bending
2.	1044	C-CH ₃ stretching
3.	1150	C-O
4.	1456	C-C stretching
5.	1532	C=N, C=C stretching
6.	1550	C=C
7.	1632, 1639	β-sheet structure of amide I
8.	2858	Stretching of C-H of methoxy group
9.	2882	C-H
10.	2924	Stretching of C-H of methyl group
11.	3213, 3269, 3422	O-H stretching

provide strong evidence of the structural and chemical stability of the synthesized nanomaterials, highlighting their potential for further applications in plant stress mitigation

3.3. Effect of nanoparticles on growth parameters on rice plants under NaCl stress

3.3.1. Shoot length

Nanoparticles (NPs) offer excellent bioavailability and low toxicity, making them promising agents for improving crop productivity under abiotic stress conditions. In this study, selenium nanoparticles (SeNPs) and multi-walled carbon nanotubes (MWCNTs) were utilized to mitigate the adverse effects of NaCl stress on rice plants. The findings provide valuable insights into the role of these nanoparticles in enhancing plant growth under both normal and saline conditions.

Foliar application of SeNPs, MWCNTs, and their hybrid formulation (SeNPs+MWCNTs) at an optimized concentration of 160 µg/mL significantly promoted shoot elongation, a key indicator of plant development. Compared to the control group, SeNPs, MWCNTs, and SeNPs+MWCNTs increased shoot length by 14 %, 15 %, and 17 %, respectively (Table 2). These results align with previous studies reporting the growth-enhancing effects of MWCNTs in oats, wheat, rice, mung bean, and maize (Joshi et al., 2018a, b; Haq et al., 2019; Joshi et al., 2020; Shekhawat et al., 2021; Luo et al., 2022). Similarly, the beneficial impact of SeNPs on shoot elongation has been documented in wheat (Ikram et al., 2020) and barley (Siddiqui et al., 2021), reinforcing their potential as plant growth modulators.

Under moderate NaCl stress (50 mM), shoot length decreased by 18 %. However, foliar application of SeNPs, MWCNTs, and SeNPs+MWCNTs at 160 µg/mL effectively counteracted this reduction, leading to respective shoot length increases of 11 %, 14 %, and 15 % compared to NaCl-stressed plants. These findings are consistent with previous research demonstrating that MWCNTs at concentrations of 500 and 1000 µg/mL enhanced shoot elongation in barley under drought

and salt stress conditions (Karami and Sepehri, 2018). Likewise, SeNPs have been reported to improve shoot length in salt-stressed common bean (Rady et al., 2021), lemon balm (Ghasemian et al., 2021), and mustard (El-Badri et al., 2022).

In response to severe NaCl stress (100 mM), shoot length was reduced by 30 % compared to unstressed plants (Fig. 4a). However, foliar application of SeNPs+MWCNTs significantly mitigated this reduction, resulting in a 14 % increase in shoot length—outperforming the individual effects of SeNPs (10 %) and MWCNTs (12 %) (Table 2). These findings align with previous reports demonstrating that SeNPs and MWCNTs alleviate NaCl-induced growth inhibition in barley (Habibi and Aleyasin, 2020), mustard (Sarkar and Kalita, 2022), and peppermint (Fallah et al., 2023). Notably, SeNPs+MWCNTs exhibited the most pronounced enhancement in shoot length under normal conditions, suggesting a potential synergistic effect between these nanoparticles.

The improved shoot elongation observed across all NP treatments may be attributed to the complementary mechanisms of SeNPs and MWCNTs. SeNPs exhibit potent antioxidant properties, protecting cellular structures from oxidative damage, while MWCNTs enhance water uptake, improving plant hydration under salt stress. These findings underscore the potential of nanotechnology-based approaches for enhancing crop resilience to salinity stress, offering a promising strategy for sustainable agriculture.

3.3.2. Root length

Foliar treatment with SeNPs, MWCNTs, and SeNPs+MWCNTs at a concentration of 160 µg/mL resulted in significant increases in root length by 13%, 15%, and 19%, respectively (Fig. 4 a). Previous studies have also demonstrated that treatment with MWCNTs significantly increased root length in oats (Joshi et al., 2018a), wheat (Joshi et al., 2018b), maize (Haq et al., 2019; Hu et al., 2021), and rice (Joshi et al., 2020). However, when exposed to NaCl concentration of 50 mM, the plants exhibited a 19% reduction in root length compared to the control. The application of SeNPs, MWCNTs, and SeNPs+MWCNTs mitigated this reduction, resulting in increase of 9%, 11%, and 14%, respectively, under NaCl stress conditions (50 mM) (Table 2). These findings are consistent with those of Karami and Sepehri (2017), who observed enhanced root length in barley plants under NaCl stress conditions due to MWCNTs. The application of CNTs likely facilitate nutrient absorption by enhancing root traits, which is attributed to the improved water retention capacity provided by MWCNTs (Tiwari et al., 2014; Yuan et al., 2017). Additionally, CNTs have been shown to up-regulate the expression of water uptake channels, such as aquaporins, in tobacco cells (Khodakovskaya et al., 2012). Lahiani et al. (2013) also reported that CNTs induce genes related to aquaporins. It is hypothesized that CNTs may create new pores for water permeation and enhance the capillary action of water, thus improving overall water uptake. Similarly, El-Badri et al. (2022) reported that SeNPs positively impacted

Table 2

Effect of foliar treatment with SeNPs, MWCNTs, and SeNPs+MWCNTs at 160 µg/mL and soil-treated NaCl (50 and 100 mM) on shoot length, root length, shoot dry weight, root dry weight, and total dry weight of rice plants after harvest. Values are mean ± Standard error, with significance determined by one-way ANOVA ($p < 0.05$).

Treatments	Shoot length	Root length	Shoot dry weight	Root dry weight	Total dry weight
Control	70.4 ± 1.51c	22.8 ± 0.83d	11.6 ± 0.48d	3.2 ± 0.26d	14.8 ± 0.32d
SeNPs	80.4 ± 1.14b	25.8 ± 1.30c	13 ± 0.85c	3.6 ± 0.46c	16.7 ± 0.69c
MWCNTs	81 ± 1b	26.2 ± 1.48b	13.8 ± 0.40b	3.9 ± 0.43b	17.8 ± 0.66b
SeNPs+MWCNTs	82.3 ± 1.92a	27.2 ± 0.83a	15.3 ± 0.60a	4.2 ± 0.13a	19.6 ± 0.63a
NaCl 50 mM	57.6 ± 1.51 f	18.4 ± 0.54 h	7.2 ± 0.75 h	2.2 ± 0.54 h	9.4 ± 0.66 h
SeNPs+ 50 mM	64 ± 0.70e	20 ± 0.70 g	8 ± 0.57 g	2.4 ± 0.39 g	10.5 ± 0.36 g
MWCNTs+ 50 mM	65.4 ± 0.89d	20.4 ± 0.54 f	8.5 ± 0.54 f	2.5 ± 0.43 f	11.1 ± 0.57 f
SeNPs+MWCNTs+ 50 mM	66.2 ± 1.30d	21 ± 0.70e	9.4 ± 0.45e	2.6 ± 0.54e	12 ± 0.66e
NaCl 100 mM	49.2 ± 1.78i	15.4 ± 1.14 l	4.7 ± 0.76 l	1.3 ± 0.33 l	6.1 ± 1.04 l
SeNPs+ 100 mM	54.2 ± 0.83 h	17 ± 1k	5.2 ± 0.56k	1.4 ± 0.21k	6.7 ± 0.44k
MWCNTs+ 100 mM	55.2 ± 0.83 g	17.4 ± 0.54j	5.4 ± 0.91j	1.5 ± 0.22j	6.9 ± 1j
SeNPs+MWCNTs+ 100 mM	56 ± 1 g	18 ± 1i	6 ± 0.45i	1.6 ± 0.24i	7.7 ± 0.64i

mustard plant root length under 150 mM NaCl stress. Additionally, Morales-Espinoza et al. (2019) noted that plants can easily uptake and transport nanoparticles, suggesting that SeNPs might interact with plants at cellular and subcellular levels, promoting changes in morpho-physiochemical attributes (Khan et al., 2019; Sotoodehnia-Korani et al., 2020). Increasing the NaCl concentration to 100 mM further reduced root length compared to control. This may be due to excessive NaCl that hinders physiological and biochemical activities (Shahid et al., 2020). However, the application of SeNPs, MWCNTs, and SeNPs+MWCNTs resulted in respective increase in root length of 10%, 13%, and 17% under 100 mM NaCl level. This aligns with findings from previous studies conducted on rice, mustard, and grape plants, where the individual effects of SeNPs and MWCNTs were also observed under NaCl stress conditions, leading to enhanced root length (Badawy et al., 2021; Sarkar and Kalita, 2022; Li et al., 2022). Here our research highlights that SeNPs+MWCNTs results in a more significant boost in root length compared to applying SeNPs or MWCNTs individually. This finding suggests the potential of SeNPs+MWCNTs hybrid treatment in promoting nutrient uptake and overall plant health (Fig. 4 a).

3.3.3. Shoot dry weight

In the present study, foliar application of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their hybrid formulation (SeNPs+MWCNTs) significantly increased shoot dry weight per plant compared to the control, with respective enhancements of 12 %, 19 %, and 32 % (Table 2). These findings align with previous studies that reported improved shoot dry weight upon the application of SeNPs and MWCNTs in wheat (SeNPs; Ikram et al., 2020), oats (MWCNTs; Joshi et al., 2018a), rice (MWCNTs; Joshi et al., 2020), and maize (MWCNTs; Hu et al., 2021).

However, exposure to 50 mM NaCl stress led to a significant reduction in shoot dry weight, with a 38 % decrease compared to control plants. Notably, foliar application of SeNPs, MWCNTs, and SeNPs+MWCNTs effectively mitigated this reduction, leading to respective shoot dry weight increases of 11 %, 18 %, and 30 % under 50 mM NaCl stress (Fig. 4b). These results are consistent with previous reports indicating that SeNPs and MWCNTs enhance shoot biomass accumulation in various crops, including strawberry (Zahedi et al., 2019), common bean (Rady et al., 2021), wheat (Soliman et al., 2023), and peppermint (Fallah et al., 2023).

Under severe NaCl stress (100 mM), plants exhibited the most substantial reduction in shoot dry weight, with a significant 59 % decline compared to unstressed plants. Nevertheless, foliar application of SeNPs and MWCNTs at 160 µg/mL alleviated this stress-induced reduction, increasing shoot dry weight by 10 % and 14 %, respectively, compared to untreated plants under 100 mM NaCl stress (Table 2). Remarkably, SeNPs+MWCNTs demonstrated the highest mitigation effect, leading to a 27 % increase in shoot dry weight under 100 mM NaCl stress conditions. These findings align with previous studies highlighting the beneficial role of SeNPs in enhancing shoot biomass in barley (Habibi and Aleyasin, 2020), foxtail millet (Nasibi et al., 2022), peppermint (Ghasemian et al., 2021), lemon balm (Ghanbari et al., 2023), and wheat (Soliman et al., 2023).

Overall, these results demonstrate that the hybrid formulation of SeNPs+MWCNTs exerted the most pronounced effect on shoot dry weight under both normal and NaCl-stressed conditions, outperforming the individual applications of SeNPs and MWCNTs. This superior efficacy may be attributed to their complementary mechanisms of action, where SeNPs enhance plant physiological functions and stress tolerance, while MWCNTs facilitate nutrient uptake and water retention (Zohra et al., 2021; Joshi et al., 2020). These findings underscore the potential of SeNPs+MWCNTs as an effective nanotechnological intervention for enhancing crop resilience under salinity stress, thereby contributing to sustainable agricultural practices.

3.3.4. Root dry weight

This study demonstrated a significant increase in root dry weight following the foliar application of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their hybrid formulation (SeNPs+MWCNTs). Specifically, SeNPs increased root dry weight by 14 %, while MWCNTs led to a 23 % increase. Notably, the SeNPs+MWCNTs hybrid exhibited the most pronounced improvement, enhancing root dry weight by 31 % compared to the control (Table 2). These findings align with previous studies reporting that SeNPs and MWCNTs significantly improve root biomass in wheat, oats, and maize (Ikram et al., 2020; Joshi et al., 2018a, b; Hu et al., 2021; Hao et al., 2023).

Under 50 mM NaCl stress, root dry weight was reduced by 32 % compared to unstressed plants. However, foliar application of SeNPs, MWCNTs, and their hybrid formulation at a concentration of 160 µg/mL mitigated this reduction. SeNPs improved root dry weight by 12 %, MWCNTs by 15 %, and the hybrid SeNPs+MWCNTs demonstrated the highest enhancement, increasing root dry weight by 20 % under 50 mM NaCl stress (Fig. 4b).

The most severe reduction in root dry weight (59 %) was observed under 100 mM NaCl stress. Despite this, foliar application of SeNPs and MWCNTs alleviated the adverse effects of high salinity, increasing root dry weight by 11 % and 14 %, respectively. Remarkably, the hybrid formulation of SeNPs+MWCNTs exhibited a superior response, leading to a 23 % increase in root dry weight under 100 mM NaCl stress (Table 2). These results corroborate previous studies demonstrating that SeNPs enhance root biomass in barley (Habibi and Aleyasin, 2020), lemon balm (Ghasemian et al., 2021), and wheat (Soliman et al., 2023), while MWCNTs improve root growth in peppermint (Fallah et al., 2023).

The superior effectiveness of SeNPs+MWCNTs under both normal and high-salinity conditions suggests a synergistic interaction between these nanoparticles. Their combined application likely facilitates improved nutrient uptake, enhanced water retention, and greater stress tolerance, ultimately leading to a significant increase in root biomass under saline conditions. These findings highlight the potential of SeNPs+MWCNTs as a promising nanotechnological approach for mitigating salinity-induced stress and improving plant resilience.

3.3.5. Total dry weight

In unstressed rice plants, the foliar application of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their hybrid formulation (SeNPs+MWCNTs) led to significant increases in total plant dry biomass by 13 %, 20 %, and 32 %, respectively, compared to the control (Table 2). These findings are consistent with previous reports demonstrating that MWCNTs significantly enhance total dry biomass in rice and maize (Joshi et al., 2020; Luo et al., 2022).

Exposure to 50 mM NaCl stress resulted in a substantial 36 % reduction in total plant dry weight compared to unstressed plants (Fig. 4b). However, foliar application of SeNPs, MWCNTs, and their hybrid formulation effectively alleviated this decline, increasing total dry biomass by 11 %, 17 %, and 28 %, respectively, under salt stress. Similar improvements in plant biomass following SeNPs application have been observed in pineapple mint and rice grown under saline conditions ranging from 30 to 90 mM NaCl and 7–7.2 dS/m salinity levels (Kiumarzi et al., 2022; Badawy et al., 2021).

At a higher salinity level of 100 mM NaCl, total plant dry weight was reduced by 60 % compared to the control. Despite this severe reduction, foliar application of SeNPs, MWCNTs, and their hybrid formulation mitigated the adverse effects of salt stress, leading to biomass increases of 10 %, 14 %, and 26 %, respectively, under 100 mM NaCl conditions (Table 2). These findings align with previous studies reporting that SeNPs improved plant biomass in mustard (Sarkar and Kalita, 2022) and wheat (Zafar et al., 2024), while MWCNTs enhanced dry weight in salt-stressed grape plants (Li et al., 2022).

Overall, our findings suggest that while individual applications of SeNPs and MWCNTs contribute to improved plant biomass under both

normal and saline conditions, their hybrid formulation (SeNPs+MWCNTs) demonstrates a superior effect. This enhanced efficacy may be attributed to their combined role in mitigating salt stress by improving osmotic balance, maintaining ion homeostasis, and enhancing the plant's antioxidant defense mechanisms (El-Badri et al., 2022; Soliman et al., 2023; Fallah et al., 2023).

3.4. Effect of nanoparticles on yield parameters on rice plants under NaCl stress

3.4.1. Number of panicles per plant

Foliar application of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their hybrid formulation (SeNPs+MWCNTs) significantly increased the total number of panicles per plant in rice by 18 %, 23 %, and 31 %, respectively, compared to unstressed plants (Table 3). These findings highlight the potential of nanoparticles in promoting reproductive development under optimal conditions.

However, exposure to 50 mM NaCl stress resulted in an 18 % reduction in the number of panicles per plant, likely due to the detrimental effects of salinity on reproductive processes such as flower development and fertility. Despite this reduction, foliar application of SeNPs, MWCNTs, and their hybrid formulation effectively mitigated the adverse effects of NaCl stress, enhancing panicle production by 13 %, 16 %, and 19 %, respectively, under this salinity level (Table 3). This improvement may be attributed to the role of nanoparticles in alleviating salt stress by enhancing osmotic adjustment, regulating hormonal balance, and scavenging reactive oxygen species (ROS), thereby maintaining reproductive integrity and ensuring proper floral development (Etesami et al., 2021).

At a higher NaCl concentration of 100 mM, a substantial reduction of 38 % in panicle number was observed compared to unstressed plants, underscoring the severe negative impact of elevated salinity on plant reproductive performance (Fig. 4c). Nevertheless, foliar supplementation with SeNPs, MWCNTs, and their hybrid formulation counteracted these adverse effects, increasing panicle number by 8 %, 13 %, and 21 %, respectively, in rice plants subjected to 100 mM NaCl stress. The observed improvements may be linked to the ability of nanoparticles to preserve key reproductive processes such as pollen viability, pollen tube growth, and fertilization efficiency under saline conditions.

These findings align with previous research demonstrating the beneficial effects of SeNPs on reproductive traits in salt-stressed plants.

Table 3

Effect of foliar treatment with SeNPs, MWCNTs, and SeNPs+MWCNTs at 160 µg/mL and soil-treated NaCl (50 and 100 mM) on the number of panicles, panicle length, and panicle weight of rice plants. Values are mean ± Standard error, with significance determined by one-way ANOVA ($p < 0.05$).

Treatments	Number of panicles	Panicle length	Panicle weight
Control	7.8 ± 1.92d	18 ± 0.70d	2.1 ± 0.10d
SeNPs	9.2 ± 0.83c	20.2 ± 1.30c	2.4 ± 0.11c
MWCNTs	9.6 ± 1.14b	20.6 ± 1.14b	2.5 ± 0.12b
SeNPs+MWCNTs	10.2 ± 0.83a	21.4 ± 0.89a	2.6 ± 0.06a
NaCl 50 mM	6.4 ± 1.14 h	15 ± 1.87 h	1.5 ± 0.06 h
SeNPs+ 50 mM	7.2 ± 0.44 g	16.8 ± 1.30 g	1.6 ± 0.06 g
MWCNTs+ 50 mM	7.4 ± 0.89 f	17.2 ± 1.30 f	1.8 ± 0.14 f
SeNPs+MWCNTs+ 50 mM	7.6 ± 0.54e	17.6 ± 1.14e	2 ± 0.03e
NaCl 100 mM	4.8 ± 0.83 i	10.6 ± 0.89 i	1.1 ± 0.14 i
SeNPs+ 100 mM	5.2 ± 0.44k	11.6 ± 0.54k	1.2 ± 0.06k
MWCNTs+ 100 mM	5.4 ± 0.54j	11.8 ± 0.83j	1.3 ± 0.16j
SeNPs+MWCNTs+ 100 mM	5.8 ± 0.83i	12.4 ± 0.89i	1.4 ± 0.08i

Badawy et al. (2021) reported that seed priming and foliar application of SeNPs (6.25 mg/L) significantly enhanced panicle number in rice under NaCl stress, likely due to the antioxidant properties of selenium, which help mitigate oxidative damage under saline conditions. Similarly, Rady et al. (2021) found that foliar application of selenium dioxide nanoparticles (1 mM) improved pod formation in common bean plants under electrical conductivity (EC) levels of 7.55–7.61 dS/m. Furthermore, Sheikhalipour et al. (2021) demonstrated that foliar application of Cs–Se NPs (10 and 20 mg/L) improved fruit production in bitter melon (*Momordica charantia*) subjected to 50 and 100 mM NaCl stress.

Overall, our results underscore the potential of SeNPs, MWCNTs, and their hybrid formulation in mitigating the deleterious effects of salinity on rice reproductive development, likely through their combined influence on stress tolerance mechanisms, osmotic regulation, and antioxidant defense pathways.

3.4.2. Panicle length

Under optimal growth conditions, foliar application of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their hybrid (SeNPs+MWCNTs) significantly increased panicle length by 12 %, 15 %, and 19 %, respectively, compared to untreated control plants (Table 3). These findings highlight the potential of nanoparticles in enhancing reproductive organ development in rice.

However, exposure to 50 mM NaCl stress resulted in a 17 % reduction in panicle length relative to the control, indicating the detrimental effects of salinity on reproductive growth. Despite this decline, foliar application of SeNPs (160 µg/mL) in combination with 50 mM NaCl stress improved panicle length by 12 %. Similarly, MWCNTs under the same stress condition enhanced panicle length by 15 %, while the SeNPs+MWCNTs hybrid exhibited the highest improvement of 17 %, demonstrating its superior efficacy in mitigating salinity-induced damage.

As NaCl concentration increased to 100 mM, a substantial 41 % reduction in panicle length was observed compared to unstressed plants (Fig. 4c). Nonetheless, foliar supplementation with SeNPs, MWCNTs, and their hybrid effectively alleviated these adverse effects, leading to increases of 9 %, 11 %, and 17 %, respectively, in panicle length under 100 mM NaCl stress (Table 3). This improvement is likely attributable to the ability of nanoparticles to enhance root system architecture, improve nutrient and water uptake efficiency, and strengthen the vascular network for effective resource allocation under salinity stress.

These findings align with previous studies. Joshi et al. (2020) reported that panicle length in rice plants treated with MW70, MW80, and MW90 increased by 19 %, 47 %, and 47 %, respectively, underscoring the positive impact of MWCNTs on reproductive traits. Furthermore, Badawy et al. (2021) demonstrated that seed priming and foliar application significantly improved panicle length in rice plants exposed to NaCl stress.

Overall, our study underscores the promising role of SeNPs, MWCNTs, and their hybrid in enhancing panicle development under both normal and salt-stressed conditions. These benefits may be attributed to their synergistic effects on plant physiological resilience, resource allocation, and stress tolerance mechanisms.

3.4.3. Panicle weight

Foliar application of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their hybrid (SeNPs+MWCNTs) significantly enhanced panicle weight by 12 %, 17 %, and 22 %, respectively, compared to untreated control plants (Table 3). These findings highlight the potential of nanoparticle-based treatments in improving reproductive biomass allocation in rice.

However, exposure to 50 mM NaCl stress resulted in a 29 % reduction in panicle weight relative to the control, underscoring the detrimental effects of salinity on reproductive development. Despite this decline, foliar supplementation with SeNPs increased panicle weight by 10 %, while MWCNTs led to a 21 % improvement. Notably, the

combined application of SeNPs+MWCNTs exhibited the most substantial effect, enhancing panicle weight by 34 % under moderate NaCl stress.

Under severe NaCl stress (100 mM), panicle weight decreased by 47 % compared to non-stressed plants (Fig. 4c). Nevertheless, foliar treatment with SeNPs and MWCNTs mitigated these adverse effects, leading to respective increases of 13 % and 20 %. Notably, SeNPs+MWCNTs treatment at 100 mM NaCl resulted in the highest mitigation, with a significant 27 % improvement in panicle weight.

These findings underscore the efficacy of nanoparticle-based treatments in enhancing reproductive performance under salinity stress. Our results align with previous studies, where Joshi et al. (2020) reported that rice plants treated with MW70, MW80, and MW90 exhibited panicle weight increases of 19 %, 48 %, and 55 %, respectively. Similarly, Badawy et al. (2021) demonstrated that seed priming and foliar application strategies significantly improved panicle weight in rice plants under saline conditions.

Overall, our study highlights the potential of SeNPs, MWCNTs, and their hybrid in mitigating salinity-induced reductions in panicle weight. This improvement is likely attributed to their role in enhancing osmotic balance, nutrient uptake efficiency, and reproductive organ resilience under stress conditions.

3.4.4. Number of spikes per panicle

The number of spikes per panicle is a crucial determinant of rice yield, as a higher spike count directly correlates with increased grain production. In this study, foliar application of selenium nanoparticles (SeNPs), multi-walled carbon nanotubes (MWCNTs), and their hybrid (SeNPs+MWCNTs) at a concentration of 160 µg/mL resulted in significant increases of 13 %, 15 %, and 28 %, respectively, compared to the untreated control plants (Table 4).

However, exposure to 50 mM NaCl stress led to a 19 % reduction in the number of spikes per panicle relative to non-stressed plants. This decline can be attributed to NaCl-induced disruptions in reproductive development, adversely affecting flower initiation, pollen viability, and pollen tube growth. Despite this stress, foliar application of SeNPs, MWCNTs, and SeNPs+MWCNTs mitigated the negative effects, leading to respective increases of 11 %, 18 %, and 21 % in the number of spikes per panicle under 50 mM NaCl stress (Table 4).

Under severe salinity stress (100 mM NaCl), the number of spikes per panicle decreased by 49 % compared to unstressed conditions (Fig. 4d). However, foliar supplementation with SeNPs, MWCNTs, and their hybrid improved spike formation under these challenging conditions. SeNPs increased the number of spikes per panicle by 13 %, MWCNTs by 21 %, while the combined treatment of SeNPs+MWCNTs exhibited the most substantial improvement of 25 %. The superior efficacy of SeNPs+MWCNTs in mitigating salinity stress may be attributed to their role in preserving essential reproductive processes, such as flower initiation, pollen viability, and pollen tube growth, which are crucial for spikelet formation.

Table 4

Effect of foliar treatment with SeNPs, MWCNTs, and SeNPs+MWCNTs at 160 µg/mL and soil-treated NaCl (50 and 100 mM) on the number of spikes, spike length, spike weight, fertile spikelets, and 100-grain weight of rice plants. Values are mean ± Standard error, with significance determined by one-way ANOVA ($p < 0.05$).

Treatments	Number of spikes	Spike length	Spike weight	Fertile spikelets	100-grain weight
Control	9.4 ± 0.89d	4.4 ± 0.35d	0.126 ± 0.02d	33.4 ± 3.2d	1.89 ± 0.005d
SeNPs	10.6 ± 0.54c	4.9 ± 0.43c	0.14 ± 0.02c	38.8 ± 0.83c	2.12 ± 0.001c
MWCNTs	10.8 ± 0.83b	5 ± 0.19b	0.142 ± 0.02b	40.6 ± 1.67b	2.17 ± 0.017b
SeNPs+MWCNTs	12 ± 0.70a	5.2 ± 0.32a	0.15 ± 0.01a	44.2 ± 1.30a	2.44 ± 0.13a
NaCl 50 mM	7.6 ± 0.54 h	3.9 ± 0.53 h	0.07 ± 0.03 h	24.2 ± 1.30 h	1.50 ± 0.06 h
SeNPs+ 50 mM	8.4 ± 1.14 g	4.2 ± 0.56 g	0.09 ± 0.007 g	27.2 ± 1.48 g	1.72 ± 0.05 g
MWCNTs+ 50 mM	9 ± 0.70 f	4.3 ± 0.21 f	0.094 ± 0.015 f	28 ± 1.87 f	1.73 ± 0.07 f
SeNPs+MWCNTs+ 50 mM	9.2 ± 0.83e	4.3 ± 0.33e	0.096 ± 0.013e	29.6 ± 1.51e	1.86 ± 0.02e
NaCl 100 mM	4.8 ± 0.44 l	3.1 ± 0.74 l	0.056 ± 0.016 l	16.8 ± 0.83 l	1.27 ± 0.015 l
SeNPs+ 100 mM	5.4 ± 0.54k	3.5 ± 0.81k	0.064 ± 0.016k	18.4 ± 2.30k	1.41 ± 0.017k
MWCNTs+ 100 mM	5.8 ± 0.44j	3.6 ± 0.83j	0.068 ± 0.02j	20 ± 1j	1.44 ± 0.01j
SeNPs+MWCNTs+ 100 mM	6 ± 0.70i	3.7 ± 0.13i	0.07 ± 0.02i	22.8 ± 1.09i	1.47 ± 0.005i

These findings align with previous research by Joshi et al. (2020), who demonstrated that seed priming with MWCNTs at concentrations of 70, 80, and 90 µg/mL enhanced the number of spikelets in rice by 29.8 %, 50.8 %, and 41.5 %, respectively. The consistency between our results and prior studies further underscores the potential of MWCNTs to enhance reproductive performance in rice under both normal and salt-stressed conditions.

3.4.5. Spike length

Spike length is a critical determinant of grain yield, as longer spikes generally result in a higher number of grains per panicle. In this study, foliar application of selenium nanoparticles (SeNPs) increased spike length by 12 %, while multi-walled carbon nanotubes (MWCNTs) led to a 13 % improvement. Notably, the combined treatment of SeNPs+MWCNTs exhibited the most substantial enhancement, with an 18 % increase compared to the untreated control (Table 4).

Under 50 mM NaCl stress, spike length decreased by 12 % compared to non-stressed plants, likely due to salinity-induced disruptions in cellular expansion and reproductive development. However, foliar application of SeNPs, MWCNTs, and their hybrid (SeNPs+MWCNTs) alleviated this reduction, resulting in respective improvements of 10 %, 11 %, and 12 % in spike length per panicle under 50 mM NaCl stress.

Severe salt stress (100 mM NaCl) further exacerbated this decline, leading to a significant reduction in spike length compared to control plants (Fig. 4d). However, foliar supplementation with SeNPs, MWCNTs, and SeNPs+MWCNTs effectively counteracted these detrimental effects, increasing spike length by 13 %, 18 %, and 20 %, respectively, under 100 mM NaCl stress (Table 4).

These findings align with previous studies highlighting the role of nanoparticles in mitigating salt-induced reproductive stress. Soliman et al. (2023) reported that SeNPs enhanced spike length in wheat plants under NaCl stress. Similarly, Joshi et al. (2018a) observed that spike length in oat plants treated with MW70, MW80, and MW90 increased by 28 %, 27 %, and 30 %, respectively. Joshi et al. (2018b) further demonstrated that MWCNT application improved spike length in wheat plants by 16 %, 21 %, and 21 % at the same concentrations. In rice, Joshi et al. (2020) reported that MWCNT treatment led to increases of 25 %, 48 %, and 27 % in spike length for MW70, MW80, and MW90, respectively.

Overall, these results underscore the efficacy of nanoparticle-based foliar treatments in mitigating the adverse effects of salinity, thereby enhancing rice reproductive performance and improving crop resilience under salt stress conditions.

3.4.6. Spike weight

The application of SeNPs, MWCNTs, and their hybrid (SeNPs+MWCNTs) significantly improved spike weight, increasing it by 11 %, 13 %, and 19 %, respectively, compared to untreated control plants (Table 4). This indicates the potential of nanoparticle-based treatments in enhancing rice reproductive performance by promoting

spike development and grain formation.

However, exposure to 50 mM NaCl stress resulted in a marked 41 % reduction in spike weight compared to non-stressed plants, likely due to the detrimental effects of salinity on reproductive tissue development and assimilate partitioning. Despite this decline, foliar supplementation with SeNPs, MWCNTs, and SeNPs+MWCNTs effectively alleviated the negative impact of salt stress, leading to respective increases of 22 %, 27 %, and 30 % in spike weight under 50 mM NaCl stress (Table 4).

Under severe salt stress (100 mM NaCl), spike weight was further reduced by 56 % compared to unstressed plants (Fig. 4d). However, the application of nanoparticles significantly mitigated these adverse effects, with SeNPs improving spike weight by 14 %, MWCNTs by 21 %, and the SeNPs+MWCNTs hybrid showing the highest enhancement of 25 % under 100 mM NaCl conditions. The observed improvements in spike weight could be attributed to the role of SeNPs and MWCNTs in counteracting NaCl-induced physiological stress, thereby sustaining reproductive development, promoting nutrient uptake, and enhancing cellular homeostasis.

These findings are consistent with prior studies demonstrating the efficacy of nanoparticle applications in improving plant reproductive traits under stress conditions. Joshi et al. (2018a) reported that spike weight in oat plants treated with MW70, MW80, and MW90 increased by 25 %, 47 %, and 54 %, respectively. Similarly, Joshi et al. (2018b) observed improvements of 20 %, 25 %, and 27 % in wheat plants treated with MW70, MW80, and MW90. Furthermore, Joshi et al. (2020) documented that spike weight in rice plants increased by 23 %, 49 %, and 52 % following treatment with MW70, MW80, and MW90, respectively.

Overall, these findings highlight the effectiveness of nanoparticle-based treatments in mitigating salinity stress and enhancing spike development, thereby contributing to improved rice yield potential under salt-stressed conditions.

3.4.7. Number of fertile spikelets per panicle

The number of fertile spikelets per panicle is a critical determinant of grain yield and overall crop productivity. In this study, foliar application of SeNPs (160 µg/mL) increased the number of fertile spikelets per panicle by 16 %, while MWCNTs resulted in a 22 % improvement. Notably, the combined application of SeNPs+MWCNTs exhibited the highest enhancement, with a 32 % increase compared to the control (Table 4).

Under moderate salinity stress (50 mM NaCl), the number of fertile spikelets per panicle decreased by 28 % relative to non-stressed plants. This decline is likely attributed to salinity-induced disruptions in reproductive processes, including impaired pollen viability and reduced fertilization efficiency. However, foliar supplementation with SeNPs and MWCNTs mitigated these adverse effects, increasing the number of fertile spikelets by 12 % and 16 %, respectively. The highest improvement was observed with the combined SeNPs+MWCNTs treatment, which enhanced the number of fertile spikelets by 22 % under 50 mM NaCl stress.

Under severe salinity stress (100 mM NaCl), there was a drastic 50 % reduction in the number of fertile spikelets per panicle compared to control plants (Fig. 4e). Despite this, foliar application of SeNPs, MWCNTs, and their combination significantly alleviated these negative effects, leading to respective increases of 10 %, 19 %, and 36 % in fertile spikelets per panicle under 100 mM NaCl stress (Table 4). These findings highlight the potential of nanoparticle-mediated stress mitigation in sustaining reproductive success under saline conditions.

Consistent with our results, Soliman et al. (2023) reported that SeNP application enhanced spikelet formation in wheat under NaCl stress. Additionally, Joshi et al. (2018a) demonstrated that MW70, MW80, and MW90 treatments increased spikelet numbers in oat plants by 18 %, 22 %, and 24 %, respectively. Similar trends were observed in wheat, where MW70, MW80, and MW90 application enhanced spikelet numbers by 13 %, 22 %, and 20 % (Joshi et al., 2018b). In rice, Joshi

et al. (2020) found that MW70, MW80, and MW90 treatments resulted in 27 %, 67 %, and 47 % increases in spikelet number, respectively. Furthermore, Badawy et al. (2021) demonstrated that seed priming and foliar application significantly improved the number of filled grains per panicle in rice plants under salinity stress.

These findings underscore the efficacy of nanoparticle-based treatments in enhancing reproductive resilience and maintaining grain yield under salinity stress, highlighting their potential application in sustainable rice production under challenging environmental conditions.

3.4.8. 100-grain weight

The weight of 100 grains is a critical agronomic parameter directly linked to grain size, a fundamental determinant of overall grain yield. Larger grains contribute to higher productivity, making it essential to evaluate factors influencing grain weight. In this study, foliar application of SeNPs and MWCNTs under non-stress conditions significantly enhanced 100-grain weight. Specifically, SeNPs increased grain weight by 12 %, MWCNTs by 15 %, and the combined application of SeNPs+MWCNTs exhibited the most pronounced effect, improving 100-grain weight by 29 % compared to the control (Table 4).

Under moderate salinity stress (50 mM NaCl), 100-grain weight declined by 21 % relative to non-stressed plants, indicating the detrimental impact of salinity on grain development. However, foliar application of SeNPs, MWCNTs, and their combination mitigated this reduction, leading to respective increases of 15 %, 16 %, and 24 % in 100-grain weight under 50 mM NaCl stress.

Exposure to severe salinity stress (100 mM NaCl) resulted in a substantial 33 % reduction in 100-grain weight compared to control conditions (Fig. 4e). Nevertheless, nanoparticle application demonstrated notable improvements under high salinity. SeNPs and MWCNTs enhanced 100-grain weight by 10 % and 13 %, respectively, while SeNPs+MWCNTs exhibited the highest increase of 15 % under 100 mM NaCl stress (Table 4).

These findings are consistent with previous reports. Zafar et al. (2024) observed an increase in 1000-grain weight in wheat plants treated with SeNPs under varying levels of NaCl stress, reinforcing the beneficial role of SeNPs in promoting grain yield. The underlying mechanism may be attributed to the efficient cellular uptake of SeNPs and their antioxidant properties, as selenium is an integral component of glutathione peroxidase, a key enzyme in oxidative stress defense (Zohra et al., 2021).

Moreover, our results align with those of Joshi et al. (2018a, 2018b, 2020), who demonstrated that seed priming with MWCNTs significantly enhanced multiple yield-related parameters, including the number of panicles per plant, panicle length, panicle weight, number of spikes per panicle, spike length, spike weight, number of fertile spikelets per panicle, and 100-grain weight in oats, wheat, and rice. Similarly, Badawy et al. (2021) reported improvements in these traits in rice plants under NaCl stress following seed priming and foliar application of SeNPs, highlighting their potential to enhance grain yield under stress conditions.

These results underscore the potential of SeNPs and MWCNTs in mitigating salinity-induced yield losses and improving grain productivity, demonstrating their viability as an agronomic strategy for sustainable crop production in saline environments.

3.5. Tracking of nanoparticles

Fluorescence microscopy confirmed the uptake and localization of SeNPs, MWCNTs, and their hybrid in rice leaf tissues. Cross-sections of control and treated leaf samples were analyzed, revealing distinct green fluorescence in SeNP-, MWCNT-, and SeNP+MWCNT-treated tissues, whereas no fluorescence was observed in control samples. This fluorescence signal indicates the successful uptake and translocation of SeNPs and MWCNTs through vascular bundles (Fig. 3). The observed green fluorescence likely arises due to the intrinsic photoluminescent

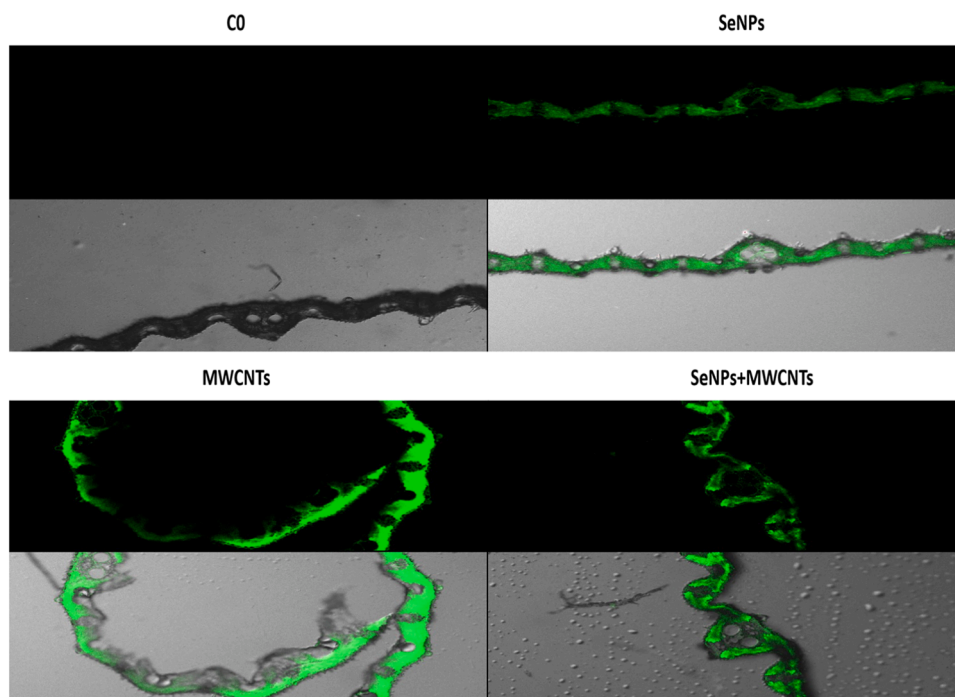


Fig. 3. Fluorescence microscopy images of leaf transverse section of rice plants with the foliar application of using control (no color), SeNPs (green signal), MWCNTs (green signal), and SeNPs+MWCNTs (green signal).

properties of SeNPs, which become visible upon excitation at specific wavelengths under fluorescence microscopy. A similar nanoparticle uptake mechanism through foliar application was previously reported by Joshi et al. (2018) in their study on MWCNTs.

Fluorescence imaging is a powerful tool for understanding how SeNPs are taken up, transported, and stored within plant tissues. Identifying their exact location in specific cell types or organelles is essential to uncover their interactions with plant cells. Additionally, comparing SeNP distribution across different plant species and experimental conditions can help reveal the key factors that influence nanoparticle absorption, movement, and possible effects on plant health and growth.

3.6. Rationale for SeNPs-MWCNTs hybrid application

Salinity stress, primarily driven by NaCl accumulation, poses a major challenge to global crop productivity by inducing oxidative damage and disrupting ion homeostasis in plant cells. Recent advancements in agricultural nanotechnology highlight selenium nanoparticles (SeNPs) as a promising intervention to enhance plant resilience against NaCl-induced stress. Studies on crops such as wheat and barley have demonstrated that SeNPs effectively mitigate oxidative stress by scavenging reactive oxygen species (ROS) (Khan et al., 2023). Our findings align with these reports, indicating that SeNPs not only protect cellular components but also regulate ion balance by modulating sodium (Na⁺) and potassium (K⁺) uptake, thereby preventing toxic Na⁺ accumulation under salinity stress.

Compared to conventional strategies such as soil amendments with inorganic salts or the development of NaCl-tolerant crop varieties, SeNPs offer a more targeted and efficient approach to mitigating salinity stress. While NaCl-tolerant cultivars provide moderate stress tolerance, SeNPs have been shown to significantly enhance antioxidative defense systems, resulting in improved growth and yield stability under saline conditions (Sadegh-Zadeh et al., 2018). Additionally, SeNPs safeguard photosynthetic pigments and enzymatic activity from ROS-induced damage, thereby preserving photosynthetic efficiency, carbon assimilation, and biomass production. This is consistent with findings in rice and tomato, where SeNP application improved photosynthetic

performance under NaCl stress (Ganju et al., 2022).

In parallel, multi-walled carbon nanotubes (MWCNTs) have gained recognition for their role in enhancing nutrient uptake and water retention—both critical factors for plant survival under saline conditions. MWCNTs improve soil water-holding capacity, increase nutrient bioavailability, and modulate hormonal signaling pathways that regulate plant growth and stress responses (Mahmoud and Abdelhameed, 2023). Compared to other nanomaterials, such as ZnO nanoparticles, MWCNTs demonstrate superior efficacy in improving water retention and nutrient acquisition, with studies on maize and wheat reporting significant growth enhancement under NaCl stress (Bhardwaj et al., 2022). However, it is essential to consider potential phytotoxic effects at higher concentrations, as observed in crops like spinach and lettuce, where oxidative stress and membrane damage have been reported (Adhikari et al., 2021).

The combined application of SeNPs and MWCNTs demonstrates a synergistic effect on plant growth and stress tolerance, surpassing the benefits observed with either nanomaterial alone. This co-application not only strengthens the antioxidant defense mechanisms provided by SeNPs but also optimizes nutrient and water uptake facilitated by MWCNTs, leading to enhanced plant resilience and productivity under NaCl stress. These findings are consistent with studies on hybrid nanomaterial applications in rice and canola, where co-treatment with CNTs and SeNPs resulted in superior growth and yield compared to individual applications (Khan and Upadhyaya, 2019; Verma et al., 2024). Notably, the combined use of SeNPs and MWCNTs offers a more effective alternative to traditional salinity management techniques, such as soil conditioning or the application of chemical growth regulators (García-Ovando et al., 2022). This nanotechnological approach presents a promising avenue for improving crop resilience and yield under saline conditions, paving the way for sustainable agricultural practices in salt-affected regions.

The combined application of SeNPs and MWCNTs offers a highly effective strategy to mitigate NaCl stress, surpassing conventional approaches. However, its efficacy depends on nanoparticle concentration, plant species, and environmental conditions, necessitating precise optimization. While foliar-applied nanoparticles enhance stress

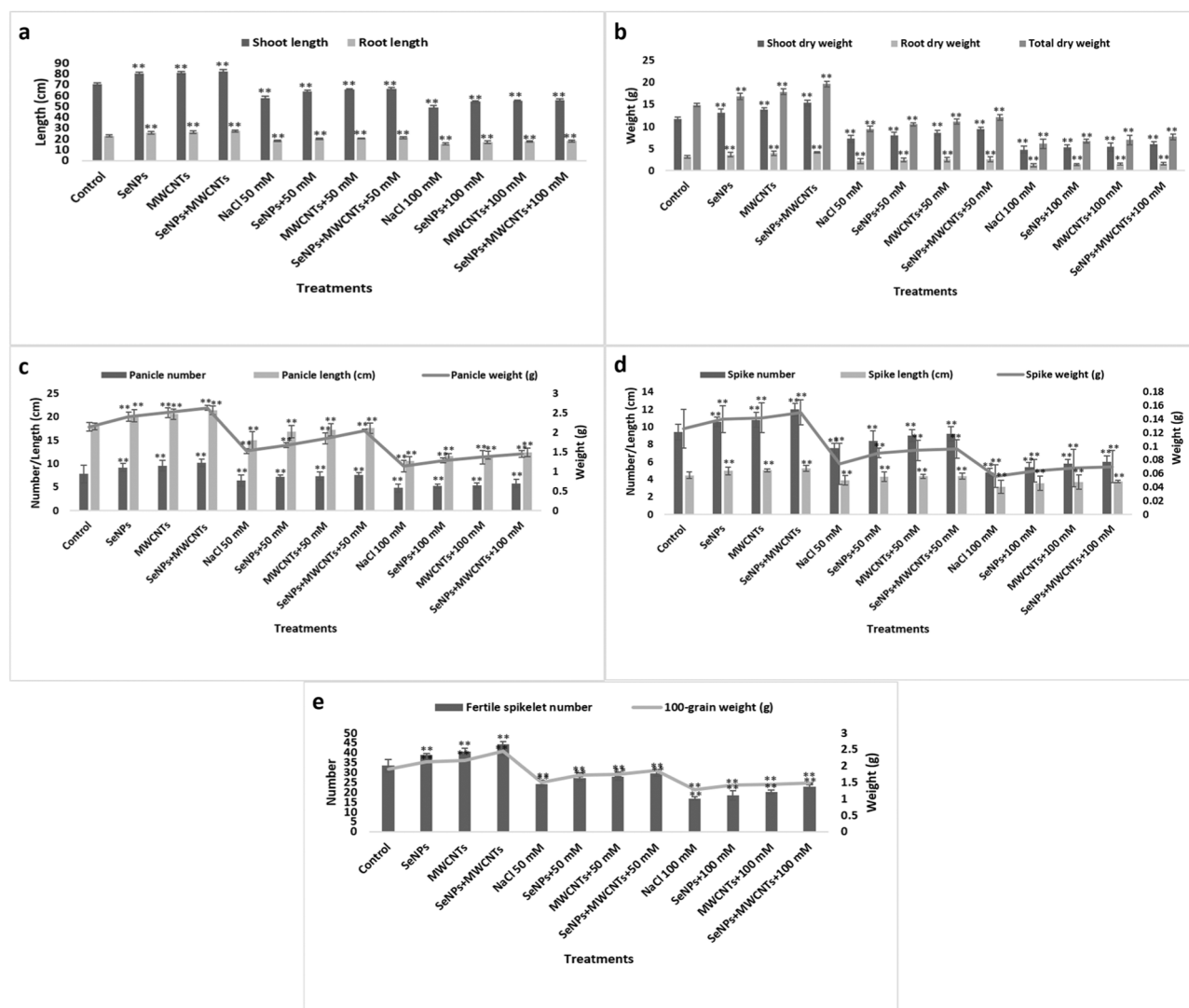


Fig. 4. Effect of foliar treatment with SeNPs, MWCNTs, and SeNPs+MWCNTs at 160 µg/mL and soil-treated NaCl (50 and 100 mM) on the (a) shoot and root length (b) shoot, root, and total dry weight (c) number of panicles, panicle length, and panicle weight (d) number of spikes, spike length, and spike weight (e) number of fertile spikelets and 100-grain weight of rice plants after harvest. Statistical analysis via one-way ANOVA revealed significant differences between treatments, denoted by LSD values * $p < 0.05$; ** $p < 0.001$. Error bars represent mean \pm Standard error.

resilience, they pose ecological risks, including environmental persistence, soil health disruption, and biodiversity loss due to unintended accumulation. Sustainable application strategies must balance efficacy with ecological safety. Future research should focus on precision-targeted delivery, eco-friendly formulations, large-scale field trials, and long-term soil and microbial health assessments to ensure the safe, efficient, and sustainable integration of nanotechnology in agriculture.

4. Conclusion

This study demonstrates the synergistic potential of selenium nanoparticles (SeNPs) and multi-walled carbon nanotubes (MWCNTs) in mitigating NaCl-induced stress in rice. Foliar application of SeNPs, MWCNTs, and their hybrid (SeNPs+MWCNTs) at 160 µg/mL led to significant improvements in shoot and root growth, dry biomass accumulation, and overall plant productivity. Additionally, these nanoparticles enhanced panicle number, panicle length, panicle weight, fertile spikelet count, and 100-grain weight, all of which are crucial for grain yield. Among the treatments, SeNPs+MWCNTs exhibited the most pronounced effects, highlighting a synergistic interaction that enhances stress tolerance and yield stability.

The superior performance of SeNPs+MWCNTs is attributed to their complementary physiological roles. SeNPs play a crucial function in scavenging reactive oxygen species (ROS), enhancing antioxidant enzyme activity, and maintaining ion homeostasis, thereby reducing oxidative stress and preventing Na^+ toxicity. Meanwhile, MWCNTs improve water retention, nutrient uptake, and metabolic efficiency, ensuring sustained plant growth even under saline conditions. This combination not only reinforces plant defense mechanisms but also optimizes physiological processes, resulting in improved resilience and yield sustainability under NaCl stress.

Despite these promising outcomes, the application of nanoparticles must be optimized to minimize potential ecological risks. Concerns such as nanoparticle persistence, unintended interactions with soil microbiota, and environmental bioaccumulation must be addressed through careful formulation and controlled application. Future research should focus on precision-targeted delivery systems, eco-friendly formulations, large-scale field trials, and long-term assessments of soil and microbial health. By integrating nanotechnology into sustainable agriculture, this study offers a scalable and innovative solution for enhancing crop resilience and productivity in salt-affected environments, ultimately contributing to global food security.

CRediT authorship contribution statement

Awasthi Amit: Validation, Software. **Kaur Simranjeet:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Garg Tashima:** Writing – original draft, Visualization, Investigation, Data curation. **Joshi Anjali:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **kumar Avneesh:** Writing – review & editing, Visualization, Validation. **Kumar Vajinder:** Writing – review & editing, Software, Formal analysis, Data curation. **Jindal Neha:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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