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# INFLUENCE OF EXOGENOUS IRON AND ZINC NANOPARTICLES ON SUGARCANE (SACCHARUM OFFICINARUM L.) BUD NODE GERMINATION AND GROWTH

***Original Article***

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## ABSTRACT

**Background:** Nanotechnology has emerged as a promising approach in modern agriculture, offering enhanced nutrient delivery through nano-fertilizers, nano-insecticides, and nano-herbicides. Iron (Fe) and Zinc (Zn) nanoparticles (NPs) play a crucial role in improving seed germination, plant growth, and stress tolerance by increasing nutrient bioavailability and metabolic efficiency. Sugarcane production faces challenges in seed propagation, particularly through budnode planting. Enhancing budnode viability using nanoparticles could optimize seed management, improve crop establishment, and potentially increase sugarcane yield.

**Objective:** This study aimed to evaluate the impact of exogenous application of Fe and Zn NPs on the germination, growth, and biomass accumulation of sugarcane (*Saccharum officinarum* L.) budnodes to determine optimal nanoparticle concentrations for improved seedling performance.

**Methods:** A completely randomized design with three replications was used in a controlled wire-house experiment conducted in March 2022 at the College of Agriculture, University of Sargodha, Pakistan. Sugarcane budnodes were treated with Fe and Zn NPs at 25, 50, 75, and 100 mg $L^{-1}$, alongside a control. Budnodes were planted in earthen pots filled with sandy loam soil. Foliar application of nanoparticles was performed at the two-leaf stage. Growth parameters, including plant height, shoot length, root length, biomass accumulation, and leaf area, were recorded. Statistical analysis was conducted using Fisher's ANOVA and the Least Significant Difference (LSD) test at **$P \leq 0.05$**.

**Results:** Significant variations in sugarcane budnode growth were observed among treatments. The tallest shoots were recorded at 100 mg $L^{-1}$ Fe NPs (25.2 cm) and 50 mg $L^{-1}$ Zn NPs (21.6 cm), whereas Zn NPs at 75 and 100 mg $L^{-1}$ resulted in the shortest shoot lengths (11.3 cm and 10.1 cm, respectively). The highest plant height was observed with 50 mg $L^{-1}$ Fe NPs (82 cm) and 75 mg $L^{-1}$ Zn NPs (80.5 cm), while the control had the lowest (36.5 cm). Maximum leaf area was noted with 100 mg $L^{-1}$ Zn NPs (4294.8) and 50 mg $L^{-1}$ Fe NPs (4146.2). The highest root fresh weight was recorded at 25 mg $L^{-1}$ Zn NPs (31.0 g), while Fe NPs at 50 mg $L^{-1}$ resulted in the lowest root weight (14.6 g). Shoot fresh weight was significantly increased with 100 mg $L^{-1}$ Zn NPs (23.3 g), whereas Fe NPs at 75 and 100 mg $L^{-1}$ showed the lowest values (4.0 g each). The maximum root-to-shoot ratio was obtained with 50 mg $L^{-1}$ Zn NPs (8.2), while 100 mg $L^{-1}$ Fe NPs (2.3) had the lowest ratio. Total chlorophyll content peaked at 41, 55, and 70 days with the control (20.5 µg $g^{-1}$ FW) and 100 mg $L^{-1}$ Zn NPs (18.0 µg $g^{-1}$ FW).

**Conclusion:** The exogenous application of Fe and Zn NPs significantly improved the germination, growth, and biomass accumulation of sugarcane budnodes. Fe NPs at 50 mg $L^{-1}$ and Zn NPs at 100 mg $L^{-1}$ demonstrated the most positive impact on plant height, root development, and biomass accumulation. Higher Zn NP concentrations negatively affected growth, suggesting that optimal dosages are essential for maximizing benefits. These findings highlight the potential of nanotechnology in enhancing sugarcane productivity through improved seedling performance.

**Keywords:** Agronomy, Germination, Growth, Iron, Nanotechnology, Sugarcane, Zinc.

## INTRODUCTION

Sugarcane is a vital cash crop in Pakistan, serving as the backbone of the sugar industry while also contributing significantly to the national economy. It accounts for 3.4% of value addition in agriculture and 0.7% of the GDP. Despite its economic importance, sugarcane productivity in Pakistan remains suboptimal compared to global benchmarks. As of 2020-21, the total cultivated area for sugarcane was 1.16 million hectares, yielding 81 million tons of production; however, the per-hectare productivity stood at 69.53 tons, which is significantly lower than in other sugarcane-producing countries (1). Although there has been a gradual increase in overall production from 23.2 million tons in 1971 to 81 million tons in 2020-21, multiple agronomic and management challenges continue to limit yield potential. Factors such as high input costs, conventional planting practices, substandard seed quality, poor weed control, pest infestations, diseases, inadequate fertilizer application, and labor-intensive cultivation methods collectively contribute to production inefficiencies (2). Among these constraints, traditional planting techniques and inferior seed setts are primary obstacles to improving yield. The importance of high-quality planting material cannot be overstated, as healthy seeds constitute approximately 20% of total production costs and directly impact crop performance (3). Sugarcane propagation traditionally relies on stem cuttings known as setts, each containing two to three eye buds, requiring nearly 6-8 tons of seed per hectare under conventional row planting with a 60 cm spacing (4). However, this approach has several drawbacks, including inconsistent germination rates due to mechanical injuries sustained during transportation and handling (5). Furthermore, the labor-intensive nature of traditional sowing methods exacerbates production costs and limits efficiency.

A promising alternative to conventional planting is the use of budnode technology, which involves the extraction and direct planting of sugarcane budnodes. This method has demonstrated multiple agronomic and economic advantages, including higher germination rates, reduced seed material weight by 70-80%, and minimized input costs (6). The efficiency of budnode planting surpasses that of conventional methods, with reported germination rates exceeding 83%. Additionally, it promotes sustainable agricultural practices by reducing seed requirements, optimizing land usage, and conserving water resources (7). Another advantage is the potential for increased economic returns, as the remaining sugarcane parts can be processed for juice and jaggery production, thereby enhancing profitability for growers (8). Despite these benefits, achieving consistently high germination rates under variable field conditions remains a challenge, necessitating research into methods for improving sprouting and seedling vigor. Various interventions, including the application of growth-enhancing chemicals and micronutrients, have been explored to optimize germination and early plant development (9). In this context, nanotechnology has emerged as a transformative tool in modern agriculture, offering innovative solutions to enhance crop productivity and nutrient efficiency (10). The application of nanoparticles (NPs) in agricultural systems, including nano fertilizers and nano pesticides, has gained traction due to their potential to reduce the overuse of conventional chemicals while improving nutrient availability, plant growth, and stress tolerance (11). These nanomaterials interact at the cellular level, facilitating efficient nutrient absorption and enhancing physiological functions critical for plant development (12).

Micronutrient supplementation, particularly with iron (Fe) and zinc (Zn), is crucial for optimizing sugarcane growth and productivity. Iron is a fundamental component of chlorophyll biosynthesis, respiration, and enzymatic activities, whereas zinc plays an integral role in protein metabolism, enzyme activation, and overall plant health (13). Globally, micronutrient deficiencies affect approximately 30% of agricultural soils, leading to suboptimal crop yields and nutritional deficiencies (14). Pakistan's sugarcane production is also constrained by soil micronutrient depletion, necessitating targeted fertilization strategies. Foliar application of micronutrients, particularly in nanoparticle form, offers a viable approach to address deficiencies, enhance nutrient uptake efficiency, and mitigate environmental pollution caused by excessive soil fertilization (15). Nanoparticles' superior bioavailability and translocation potential allow for precise nutrient delivery, minimizing losses and improving plant physiological responses. Given the urgent need for sustainable and efficient sugarcane cultivation methods, integrating budnode planting with nanotechnology-driven nutrient management could substantially enhance germination rates, seedling vigor, and overall crop performance (16).

Despite the advancements in nanotechnology applications in agriculture, research remains limited on their specific effects on sugarcane budnode germination and early growth. There is a critical need to explore how exogenous application of iron and zinc nanoparticles influences sugarcane sprouting dynamics, seedling establishment, and subsequent development. This study aims to evaluate the efficacy of iron and zinc nanoparticles in improving sugarcane budnode germination and growth parameters. By assessing the physiological and agronomic responses to nanoparticle treatments, the research seeks to provide insights into the potential role of nanotechnology in revolutionizing sugarcane production and enhancing productivity through sustainable, cost-effective solutions (17).

## METHODS

The study was conducted under controlled conditions at the wire house of the College of Agriculture, University of Sargodha, Pakistan, between March and July 2022. The experimental site falls within the semi-arid region of Punjab, Pakistan, characterized by subtropical climatic conditions with an average annual rainfall of 400–500 mm. Approximately 70% of this precipitation occurs during the monsoon season between July and September (Agro-Metrological Lab, University of Sargodha). The mean winter temperature in this region typically drops to 10°C. The experiment was performed using sugarcane budnodes cultivated in earthen pots measuring 25 × 40 $cm^2$. The soil used for planting was sandy clay loam, possessing a bulk density of 1.04 g $cm^{-3}$, with water retention capacity adjusted to 33% and 70% prior to planting (Blake and Hartge, 1986). The soil was air-dried, sieved through a 2 mm mesh to ensure uniform particle size distribution, and subjected to physicochemical analysis. Seven days before planting, the soil was weighed, and pots were filled accordingly to maintain consistency across all experimental units (Aitken and McCallum, 1988). The specific properties of the experimental soil included a sandy loam texture, pH 7.4, electrical conductivity (EC) of 0.83 mS $cm^{-1}$, organic matter content of 1.32 g $kg^{-1}$, available phosphorus (P) at 3.17 mg $kg^{-1}$, saturation percentage of 38.20%, available nitrogen (N) at 4.12 mg $kg^{-1}$, and available potassium (K) at 88.10 mg $kg^{-1}$.

A completely randomized design (CRD) was employed for the experiment, with three replications to ensure statistical reliability. The study comprised nine distinct treatments, including different concentrations of iron nanoparticles (Iron NPs) at 25, 50, 75, and 100 mg $L^{-1}$, zinc nanoparticles (Zinc NPs) at 25, 50, 75, and 100 mg $L^{-1}$, along with a control group receiving no nanoparticle treatment. For the exogenous application of nanoparticles, iron and zinc NPs were precisely weighed and dissolved in deionized water to attain the required concentrations. These solutions were applied at the two-leaf stage of sugarcane growth. Each pot contained five budnodes, ensuring uniform distribution across the treatments. Standard agronomic practices were followed for all experimental units, with irrigation provided as per the crop's water requirements. The sugarcane plants were grown under controlled conditions and harvested after 120 days post-germination for further analysis.

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**Soil analysis of experimental site**

| Soil Properties | **Values** |
|---|---|
| Clay (g kg-1) | 290 |
| Silt (g kg-1) | 255 |
| Sand (g kg-1) | 455 |
| Texture | Sandy loam |
| pH | 7.4 |
| EC (mS cm-1) | 0.83 |
| Organic matter (g kg-1) | 1.32 |
| Available P (mg kg−1) | 3.17 |
| Saturation (%) | 38.20 |
| Available N (mg kg-1) | 4.12 |
| Available K (mg kg−1) | 88.10 |

Iron nanoparticles were synthesized by dissolving 20 g of ferrous sulfate ($Fe_3SO_4 \cdot 7H_2O$) in 200 mL of distilled water, with continuous mixing using a magnetic stirrer. Separately, 100 mL of distilled water was used to dissolve ferric chloride ($FeCl_2 \cdot 6H_2O$). These solutions were then combined in a beaker and placed under constant agitation. A 0.3 M sodium hydroxide (NaOH) solution was prepared by dissolving 15 g of NaOH in 125 mL of distilled water, which was then added dropwise to the iron solution while stirring. The gradual addition of NaOH resulted in the precipitation of iron nanoparticles. The precipitate was subsequently filtered using filter paper, and the retained solid was dried at 70°C for 48 hours. Once dried, the solid mass was ground into a fine powder using a mortar and pestle. The synthesized iron nanoparticles were characterized using analytical techniques and stored for subsequent application. Zinc oxide (ZnO) nanoparticles were synthesized using the sol-gel method, employing zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) as the precursor and ethanol ($CH_3CH_2OH$) as the solvent. Distilled water and NaOH were used as the reaction medium. The synthesized ZnO nanoparticles were analyzed through X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), field-emission scanning electron microscopy (FESEM), and nanoparticle analysis techniques to confirm their structural integrity and composition (18).

All collected data were subjected to statistical analysis using Fisher's analysis of variance (ANOVA) technique to determine treatment effects. Mean comparisons were conducted using the Least Significant Difference (LSD) test at a 5% significance level following the methodology outlined by Steel et al. (1997).

## RESULTS

The analysis revealed a significant impact of exogenous Iron (Fe) and Zinc (Zn) nanoparticle application on various growth parameters of sugarcane budnodes. The highest plant height was observed with Fe NPs at 50 mg $L^{-1}$ (82 cm) and Zn NPs at 75 mg $L^{-1}$ (80.5 cm), whereas the control group recorded the lowest height (36.5 cm). A decline in plant height was noted at higher concentrations, particularly Fe NPs at 100 mg $L^{-1}$ (50.5 cm), suggesting a threshold beyond which nanoparticle application may exert inhibitory effects. Similarly, leaf area index was significantly enhanced in treatments with Zn NPs at 100 mg $L^{-1}$ (4294.8) and Fe NPs at 50 mg $L^{-1}$ (4146.2), while the control group exhibited the lowest leaf area (1464.1). Shoot length varied among treatments, with the highest value recorded for Zn NPs at 100 mg $L^{-1}$ (25.2 cm), followed by Fe NPs at 50 mg $L^{-1}$ (21.6 cm). In contrast, Zn NPs at 75 mg $L^{-1}$ (11.3 cm) and Zn NPs at 100 mg $L^{-1}$ (10.1 cm) showed the least favorable results, indicating that excessive concentrations did not contribute to shoot elongation. Root length was significantly influenced by Zn NPs at 25 mg $L^{-1}$ (6.8 cm), while Zn NPs at 75 mg $L^{-1}$ (3.3 cm) resulted in the shortest roots. Similar trends were observed in root fresh weight, with the maximum value recorded in Zn NPs at 25 mg $L^{-1}$ (31.0 g) and the lowest in Fe NPs at 50 mg $L^{-1}$ (14.6 g). Root dry weight was highest with Zn NPs at 25 mg $L^{-1}$ (6.8 g), followed by Fe NPs at 75 mg $L^{-1}$ (5.7 g), whereas Fe NPs at 50 mg $L^{-1}$ (4.3 g) exhibited the least dry matter accumulation.

Shoot fresh weight was significantly enhanced in plants treated with Zn NPs at 100 mg $L^{-1}$ (23.3 g), while Fe NPs at 75 mg $L^{-1}$ and Fe NPs at 100 mg $L^{-1}$ exhibited the lowest shoot biomass (4.0 g). Similar trends were noted for shoot dry weight, with Fe NPs at 50 mg $L^{-1}$ (2.5 g) and Zn NPs at 100 mg $L^{-1}$ (2.2 g) displaying the highest values, while Fe NPs at 100 mg $L^{-1}$ (0.7 g) resulted in the lowest dry biomass. The root-to-shoot ratio was maximized in Zn NPs at 50 mg $L^{-1}$ (8.2), while Fe NPs at 100 mg $L^{-1}$ (2.3) had the lowest ratio, suggesting an imbalance in biomass distribution at higher concentrations. Total chlorophyll content in sugarcane leaves varied across treatments, with Zn NPs at 100 mg $L^{-1}$ and Fe NPs at 50 mg $L^{-1}$ demonstrating the highest values. Chlorophyll content was highest at 70 days in the control (20.5 µg $g^{-1}$ FW), followed by Zn NPs at 100 mg $L^{-1}$ (18.0 µg $g^{-1}$ FW), while Fe NPs at 25 mg $L^{-1}$ (15.2 µg $g^{-1}$ FW) resulted in a comparatively lower chlorophyll accumulation. The findings collectively indicate that moderate concentrations of Fe and Zn NPs enhanced growth and physiological parameters, while excessive concentrations had inhibitory effects, emphasizing the importance of optimal nanoparticle dosage for improved sugarcane performance (20).

 

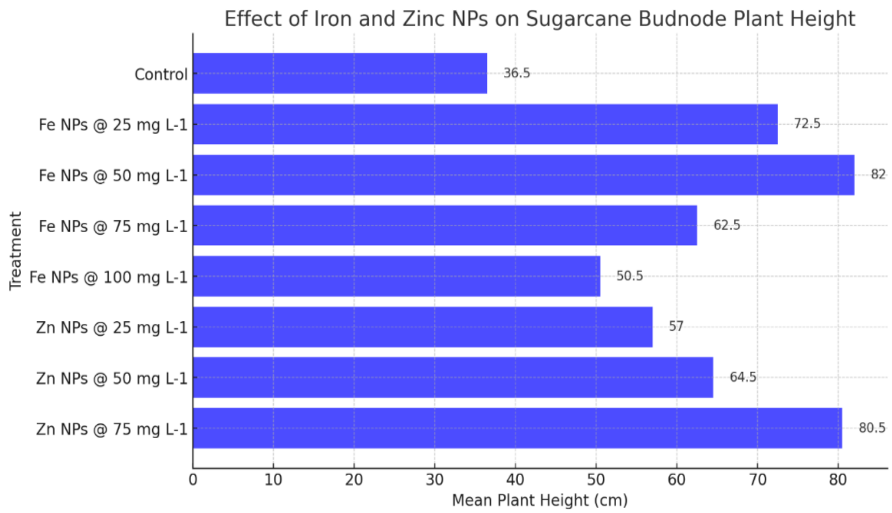


*Figure 1 Effect Of Iron And Zinc NPs On Sugarcane Budnode Plant Height*

The bar chart illustrates the mean plant height of sugarcane budnodes under different treatments of exogenous Iron (Fe) and Zinc (Zn) nanoparticles. The highest plant height was observed with Fe NPs @ 50 mg $L^{-1}$ (82 cm) and Zn NPs @ 75 mg $L^{-1}$ (80.5 cm), indicating their significant positive influence on growth. The control group exhibited the lowest height (36.5 cm), while Fe NPs @ 100 mg $L^{-1}$ (50.5 cm) showed a reduced effect. The LSD test confirms significant variations among treatments ($P \leq 0.05$), emphasizing the differential impact of nanoparticle concentrations on plant height (20).

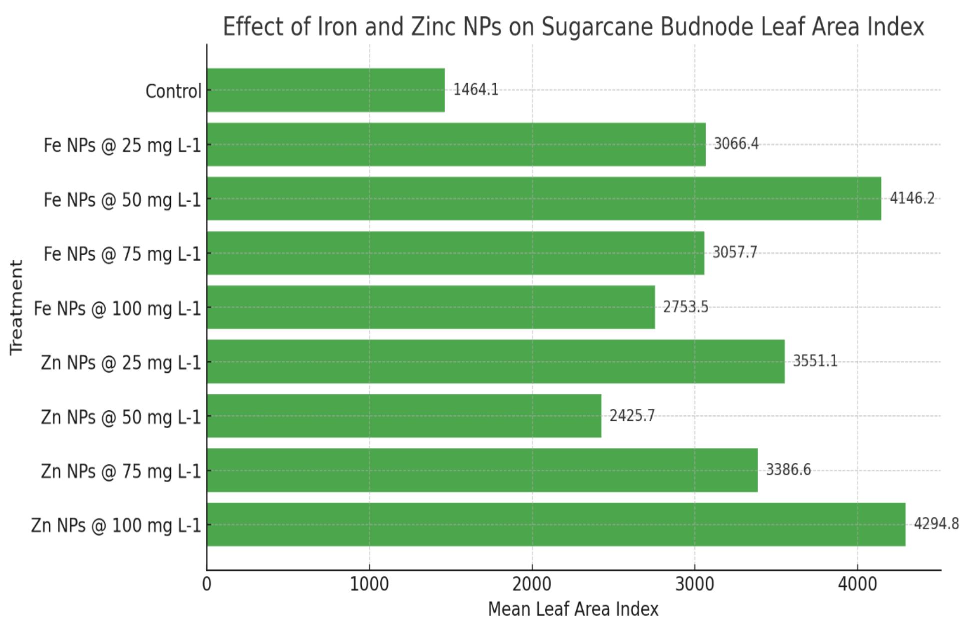


*Figure 2 Effect Of Iron And Zinc NPs On Sugarcane Budnode Leaf Area Index*

The bar chart illustrates the impact of exogenous Iron (Fe) and Zinc (Zn) nanoparticles on the leaf area index of sugarcane budnodes. The highest leaf area was observed in plants treated with Zn NPs @ 100 mg $L^{-1}$ (4294.8) and Fe NPs @ 50 mg $L^{-1}$ (4146.2), indicating their positive influence on growth. The control group exhibited the lowest leaf area (1464.1). Higher concentrations of Fe and Zn NPs generally reduced leaf area, except for Zn NPs @ 100 mg $L^{-1}$, which showed maximum enhancement. Significant variations among treatments were confirmed by the LSD test ($P \leq 0.05$).x

Shoot length (cm)

The length of sugarcane shoots showed significant variability based on the concentrations of Zinc and Iron nanoparticles applied. Sugarcane plants treated with exogenous Iron NPs at 100 mg L-1 exhibited longer shoots compared to those treated with Iron NPs at 25 mg L-1 and 75 mg L-1. Similarly, budnodes treated with exogenous Zinc NPs at 25 mg L-1 and 50 mg L-1 displayed increased shoot length compared to those treated with Zinc NPs at 75 mg L-1 and 100 mg L-1 [22]. Higher concentrations of Zinc NPs at 75 mg L-1 and 100 mg L-1 did not show positive effects on plant shoot development when compared to lower concentrations such as Zinc NPs at 25 mg L-1 and 50 mg L-1. Interestingly, the control treatment exhibited better shoot length compared to treatments with higher concentrations of Zinc NPs and lower concentrations of Iron NPs. This suggests that the optimal balance between Zinc and Iron nanoparticle concentrations is crucial for promoting shoot growth in sugarcane. The earlier sprouting, improved overall plant development, and increased leaf area observed in plants treated with Zinc NPs likely contributed to enhanced shoot growth. These findings underscore the complex interactions between nanoparticle concentrations and their effects on sugarcane growth parameters, emphasizing the importance of precise nutrient management strategies in agricultural practices

**Mean comparison of shoot length influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode.**

| Treatments | Means |
|---|---|
| Control | 18.5 abc |
| Zn NPs @ 25 mg L-1 | 15.0 bcd |
| Zn NPs @ 50 mg L-1 | 20. ab |
| Zn NPs @ 75 mg L-1 | 18.5 abc |
| Zn NPs s @ 100 mg L-1 | 25.2 a |
| Zn NPs @ 25 mg L-1 | 19.9 ab |
| Zn NPs @ 50 mg L-1 | 21.6 ab |
| Zn NPs @ 75 mg L-1 | 11.3 cd |
| Zn NPs @ 100 mg L-1 | 10.1 d |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD Test ($P \leq 0.05$).

The length of sugarcane roots, indicating a significant impact of Zinc and Iron nanoparticle concentrations on root length. Sugarcane plants treated with exogenous Zinc NPs at 25 mg L-1 exhibited longer roots compared to those treated with Zinc NPs at 100 mg L-1. Similarly, sugarcane buds treated with exogenous Iron NPs at 25 mg L-1 and Zinc NPs at 50 mg L-1 showed increased root length as compared to buds treated with Iron NPs at 75 mg L-1 and Iron NPs at 100 mg L-1 [23]. Higher concentrations of Zinc NPs at 75 and 100 mg L-1 did not demonstrate positive effects on plant root development when compared to another Zinc NPs such as Zinc NPs at 25 mg L-1 and Zinc NPs at 50 mg L-1. The earlier sprouting, improved plant development, and increased leaf area, which contributed to enhanced root growth, may have influenced the improvement in root length of sugarcane plants treated with Zinc NPs.

**Mean comparison of root length influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode.**

| Treatments | Means |
|---|---|
| Control | 4.3 bc |
| Fe NPs @ 25 mg L-1 | 5.3 a |
| Fe NPs @ 50 mg L-1 | 5.4 b |
| Fe NPs @ 75 mg L-1 | 4.9 c |
| Fe NPs @ 100 mg L-1 | 4.5 b |
| Zn NPs @ 25 mg L-1 | 6.8 b |
| Zn NPs @ 50 mg L-1 | 5.3 ab |
| Zn NPs @ 75 mg L-1 | 3.3 b |
| Zn NPs @ 100 mg L-1 | 4.8 bc |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD test ($P \leq 0.05$).

 

The fresh weight of sugarcane roots was influenced by the application of Iron nanoparticles (NPs) and Zinc nanoparticles (NPs) to budnodes. Nanoparticles showed nonsignificant response on sugarcane budnodes, our result showed contradictory response. However, the analysis of the data revealed an improvement in the fresh weight of sugarcane roots when budnodes were treated with various amounts of Iron and Zinc NPs compared to untreated budnodes. The highest fresh weight of roots per plant was measured when budnodes were treated with Zinc NPs at a concentration of 25 mg L-1. Root fresh weight decreased as the concentration of Iron NPs or Zinc NPs increased. However, even lower doses of Zinc NPs showed a significant improvement in root weight. There was no significant difference in root fresh weight when budnodes were treated with Iron NPs at 25 mg L-1 and Zinc NPs at 100 mg L-1, as well as in the control group. The minimum root fresh weight per plant was observed when sugarcane budnodes were treated with Iron NPs at 25 mg L-1. The increase in root fresh weight in sugarcane treated with Zinc NPs at 75 mg L-1 may be attributed to enhanced development and growth, increased leaf area, and improved shoot growth, which resulted in an increase in root fresh weight. Similarly, previous research [24].

**Mean comparison of root fresh weight influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode**.

| Treatments | Means |
|---|---|
| Control | 25.6 |
| Fe NPs @ 25 mg L-1 | 25.0 |
| Fe NPs @ 50 mg L-1 | 14.6 |
| Fe NPs @ 75 mg L-1 | 26.3 |
| Fe NPs @ 100 mg L-1 | 23.6 |
| Zn NPs @ 25 mg L-1 | 31.0 |
| Zn NPs @ 50 mg L-1 | 19.3 |
| Zn NPs @ 75 mg L-1 | 27.0 |
| Zn NPs @ 100 mg L-1 | 24.0 |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD Test ($P \leq 0.05$).

The significant impact of exogenous Iron NPs and Zinc NPs on sugarcane root dry weight throughout the study. Nanoparticles showed nonsignificant response on sugarcane budnodes, our result showed contradictory response. However, in comparison to the control treatment, all exogenous treatments resulted in an enhancement of sugarcane root dry weight. Based on the research findings, the most significant enhancement in root dry weight occurred when sugarcane budnodes were initially treated with Zinc nanoparticles (Zn NPs) at a concentration of 25 mg L-1, followed by Iron nanoparticles (Fe NPs) at 75 mg L-1. Both Fe NPs at 75 mg L-1 and Zn NPs at 25 mg L-1 exhibited notable increases in root dry weight compared to other doses administered during exogenous application to sugarcane budnodes. When sugarcane was exogenously treated with Iron NPs at 25 mg and 100 mg L-1, and Zinc NPs at 25 mg L-1, there was no statistically significant difference in root dry weight. The increase in leaf area and shoot growth, which led to larger roots and, consequently, an increase in root dry weight, likely contributed to the observed rise in root dry weight in sugarcane plants. Previous research that demonstrated improvements in vegetables such as cucumber, faba bean yielded similar results [25]. Similar root dry weight was observed when sugarcane was treated with Iron NPs and Zinc NPs at 100 mg L-1 each. The lowest root dry weight of sugarcane (0.08 g) was observed when budnodes were not treated with Iron NPs or Zinc NPs, followed by Zinc NPs at 100 mg L-1. Better leaf area and shoot growth, which increased fresh root weight, and consequently, root dry weight, are likely the factors that led to the increase in sugarcane plant root dry weight.

 

**Mean comparison of root dry weight influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode**.

| Treatments | Means |
|---|---|
| Control | 5.2 |
| Fe NPs @ 25 mg L-1 | 5.0 |
| Fe NPs @ 50 mg L-1 | 4.3 |
| Fe NPs @ 75 mg L-1 | 5.7 |
| Fe NPs @ 100 mg L-1 | 5.0 |
| Zn NPs @ 25 mg L-1 | 6.8 |
| Zn NPs @ 50 mg L-1 | 6.1 |
| Zn NPs @ 75 mg L-1 | 6.6 |
| Zn NPs @ 100 mg L-1 | 5.9 |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD Test ($P \leq 0.05$).

The shoot fresh weight of sugarcane plants that were cultivated from budnodes treated with exogenous Iron and Zinc NPs. Nanoparticles showed nonsignificant response on sugarcane budnodes, our result showed contradictory response. However, the results indicate that foliar spraying with nanoparticles had a positive impact and significantly increased the shoot fresh weight of sugarcane plants compared to control treatments. The sugarcane plants treated with Zinc NPs at 50 mg L-1 and Iron NPs at 100 mg L-1 exhibited the highest shoot fresh weight. In contrast, the application of both Iron and Zinc NPs at 25 mg L-1 resulted in a decrease in shoot fresh weight when compared to treatments with Iron NPs at 50 mg L-1 and Zinc NPs at 50 mg L-1. The lowest fresh weight was observed in sugarcane plants that received exogenous NPs prior to sowing the budnodes, followed by those treated with Zinc and Iron NPs at 25 mg L-1. The increase in shoot fresh weight induced by Iron and Zinc NPs was most likely triggered by an increase in shoot length due to improved plant development and increased leaf area. These benefits are likely the result of enhanced photosynthesis, chlorophyll production, and protein synthesis, similar to how Iron NPs at 50 mg L-1 and Zinc NPs at 75 mg L-1 boosted the fresh weight of shoots of sugarcane [26].

**Mean comparison of shoot fresh weight influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode.**

| Treatments | Means |
|---|---|
| Control | 6.0 |
| Fe NPs @ 25 mg L-1 | 6.6 |
| Fe NPs @ 50 mg L-1 | 9.6 |
| Fe NPs @ 75 mg L-1 | 4.0 |
| Fe NPs @ 100 mg L-1 | 4.0 |
| Zn NPs @ 25 mg L-1 | 3.3 |
| Zn NPs @ 50 mg L-1 | 5.3 |
| Zn NPs @ 75 mg L-1 | 7.0 |
| Zn NPs @ 100 mg L-1 | 23.3 |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD Test ($P \leq 0.05$).

Concerning sugarcane shoot dry weight reveal a substantial impact of exogenous application on shoot dry mass. Nanoparticles showed nonsignificant response on sugarcane budnodes, our result showed contradictory response. However, compared to the control group, foliar treatment significantly increased the shoot's dry weight. Among the exogenous applications, Iron NPs at 100 mg L-1 and Zinc NPs at 50 mg L-1 demonstrated the highest enhancement in shoot dry weight, followed by Iron NPs at 75 mg L-1 and Zinc NPs at 25 mg L-1. However, when using a high concentration of zinc (Zn) NPs, there was a decline in shoot dry weight. Zinc NPs at 25 mg L-1 and the untreated treatment both exhibited the lowest shoot dry weight. Iron NPs at 100 mg L-1 promoted plant development and root system growth, which improved nutrient uptake and enabled the plants to grow larger and accumulate more dry matter. Similarly, both Zinc NPs at 75 mg L-1 and Iron NPs at 50 mg L-1 may induce biochemical and physiological changes, including protein and enzyme synthesis, which contributed to development and an increase in shoot dry weight [27].

**Mean comparison of shoot dry weight influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode.**

| Treatments | Means |
|---|---|
| Control | 0.9 |
| Fe NPs @ 25 mg L-1 | 1.7 |
| Fe NPs @ 50 mg L-1 | 2.5 |
| Fe NPs @ 75 mg L-1 | 1.0 |
| Fe NPs @ 100 mg L-1 | 0.7 |
| Zn NPs @ 25 mg L-1 | 1.3 |
| Zn NPs @ 50 mg L-1 | 1.2 |
| Zn NPs @ 75 mg L-1 | 1.8 |
| Zn NPs @ 100 mg L-1 | 2.2 |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD Test ($P \leq 0.05$)

Nanoparticles showed nonsignificant response on sugarcane budnodes, our result showed contradictory response. Based on the research findings, the highest root-to-shoot ratio was observed when Zinc nanoparticles (Zn NPs) were applied at a concentration of 50 mg L-1, whereas the lowest ratio occurred with the application of Iron nanoparticles (Fe NPs) at 100 mg L-1. Treatments involving Fe NPs at 25 mg L-1, Zn NPs at 50 mg L-1, and the control treatment demonstrated similar results in comparison to other experimental conditions. These results suggest a favorable influence of Zn NPs at 50 mg L-1 on the root-to-shoot ratio, indicating a potential benefit for root development and biomass distribution. This finding is consistent with previous studies indicating that specific concentrations of Zn NPs applied exogenously can enhance various growth and yield parameters in plants [28]. Furthermore, the application of 5 g/mL CSNPs (chitosan nanoparticles) has been shown to increase the expression of auxin-related genes, accelerate indole-3-acetic acid (IAA) production and transport, and decrease IAA oxidase activity. These effects result in an increase in IAA concentration in both wheat shoots and roots, contributing to enhanced plant growth [29]. The observed changes in the root-to-shoot ratio (RLSI) values, with an increase at 10 g ml and a subsequent reduction after 40 g mL-1 concentrations of Zinc NPs, indicate a complex response of sugarcane to Zinc NPs. Understanding these dynamics is crucial for optimizing nanoparticle application strategies and their effects on root and shoot development in sugarcane.

**Mean comparison of root shoot ratio influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode.**

| Treatments | **Means** |
|---|---|
| Control | 6.8 |
| Fe NPs @ 25 mg L-1 | 4.7 |
| Fe NPs @ 50 mg L-1 | 3.4 |
| Fe NPs @ 75 mg L-1 | 3.5 |
| Fe NPs @ 100 mg L-1 | 2.3 |
| Zn NPs @ 25 mg L-1 | 5.5 |
| Zn NPs @ 50 mg L-1 | 8.2 |
| Zn NPs @ 75 mg L-1 | 4.9 |
| Zn NPs @ 100 mg L-1 | 6.9 |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD Test ($P \leq 0.05$).

The experimental results revealed a notable effect on the total chlorophyll content in sugarcane leaves following the exogenous application of nanoparticles compared to the untreated control of sugarcane budnodes. The analysis revealed that Iron NPs and Zinc NPs had non-significant on effect on sugarcane budnodes. Nanoparticles showed nonsignificant response on sugarcane budnodes, our result showed contradictory response. However, the highest total chlorophyll content in sugarcane leaves was observed when no nanoparticles were present, followed by exogenous Iron NPs at 50 mg L-1 and exogenous Zinc NPs at 100 mg L-1. Low doses of Iron nanoparticles (25 mg L-1) and high doses of Iron nanoparticles (75 mg L-1) did not lead to a significant increase in chlorophyll content. Similarly, Zinc NPs at 25 mg, Zinc NPs at 50 mg, and Zinc NPs at 100 mg L-1 all increased the overall chlorophyll content in sugarcane by an equal amount. The increase in total chlorophyll in sugarcane leaves following budnode seeding, both with and without exogenous application, may be attributed to early and vigorous sprouting, leading to enhanced growth and, consequently, a higher total chlorophyll content. Both iron and zinc play roles in the formation of enzymes, carbohydrates, chloroplasts, and chlorophyll in plants [30]. The application of Iron nanoparticles (Fe NPs) and Zinc nanoparticles (Zn NPs) exogenously resulted in an enhancement of the overall chlorophyll content.

**Mean comparison of chlorophyll content after 41, 55 & 70 days influenced by exogenous application of Iron and Zinc NPs on Sugarcane budnode.**

| **Treatments** | **Means @ 41 days** | **Means @ 55 days** | **Means @ 70 days** |
|---|---|---|---|
| Control | 2.8 | 15.7 | 20.5 |
| Fe NPs @ 25 mg L-1 | 6.0 | 12.8 | 15.2 |
| Fe NPs @ 50 mg L-1 | 4.9 | 9.0 | 17.1 |
| Fe NPs @ 75 mg L-1 | 4.3 | 9.4 | 16.4 |
| Fe NPs @ 100 mg L-1 | 7.7 | 11.5 | 16.9 |
| Zn NPs @ 25 mg L-1 | 4.6 | 12.8 | 17.5 |
| Zn NPs @ 50 mg L-1 | 5.4 | 12.7 | 16.3 |
| Zn NPs @ 75 mg L-1 | 7.8 | 12.5 | 14.5 |
| Zn NPs @ 100 mg L-1 | 8.2 | 11.5 | 18.0 |

Note: Different alphabets in the column confirm significant variation between treatments in accordance with LSD Test ($P \leq 0.05$).

 

## DISCUSSION

The study demonstrated that the exogenous application of Iron (Fe) and Zinc (Zn) nanoparticles significantly influenced the growth and physiological characteristics of sugarcane budnodes. Traditional sugarcane propagation using setts demands substantial planting material, whereas the budnode planting method presents a more resource-efficient alternative. However, challenges such as poor germination and susceptibility to desiccation have limited its widespread adoption. The integration of nanotechnology into agricultural practices has shown potential in addressing these challenges by enhancing nutrient uptake, stress tolerance, and overall plant vigor (28). The findings revealed that Fe NPs at 50 mg $L^{-1}$ and Zn NPs at 100 mg $L^{-1}$ resulted in the highest leaf area, whereas increasing Zn NPs beyond 75 mg $L^{-1}$ led to reductions in shoot elongation and plant height (29). This response suggests that while moderate concentrations of Fe and Zn NPs improve plant performance, excessive application may disrupt physiological processes. Increased root fresh weight was recorded in plants treated with Fe NPs at 75 mg $L^{-1}$ and Zn NPs at 25 mg $L^{-1}$, indicating their role in promoting root development (30). Similarly, shoot fresh and dry weights were significantly enhanced with Fe NPs at 100 mg $L^{-1}$, reinforcing the notion that iron availability plays a crucial role in biomass accumulation (31). However, a higher Zn NP concentration (100 mg $L^{-1}$) adversely impacted shoot biomass, suggesting that beyond an optimal range, excessive zinc availability may impose metabolic stress and hinder plant growth (32). The results also indicated that Fe NPs at 75 mg $L^{-1}$ and Zn NPs at 50 mg $L^{-1}$ led to the highest number of leaves per plant, while Zn NPs enhanced tillering ability (33). The highest root-to-shoot ratio was recorded in Zn NPs at 50 mg $L^{-1}$, implying that moderate zinc supplementation favored biomass distribution between root and shoot systems (34). Furthermore, the enhancement in photosynthetic efficiency, carotenoid accumulation, and chlorophyll content with Fe and Zn NPs underscores their role in optimizing photosynthetic activity and metabolic functions (35).

Iron and zinc are essential micronutrients required for plant growth, enzymatic activity, and metabolic regulation. The application of nanoparticles as a delivery system has been recognized for improving their bioavailability and uptake efficiency due to the higher surface-area-to-volume ratio, which enhances dissolution and absorption at the cellular level (36). Nanoparticles also mitigate abiotic stress effects such as drought and salinity, potentially alleviating oxidative stress by modulating reactive oxygen species (ROS) and strengthening antioxidant defense mechanisms (37). This could explain the improved germination and vigor observed in sugarcane budnodes treated with Fe and Zn NPs. However, despite their evident benefits, the potential toxicity of nanoparticles at higher concentrations remains a critical concern. The inhibitory effects observed at increased Zn NP concentrations highlight the necessity of determining optimal dosage thresholds to maximize benefits while minimizing adverse effects. Additionally, the long-term environmental implications of nanoparticle accumulation in soil and plant systems warrant further investigation. The study's findings emphasize the significance of nanoparticle-based nutrient management in sustainable crop production. However, limitations include the controlled wire-house conditions, which may not fully replicate field variability, and the need for further exploration into nanoparticle interactions with soil microbiota and plant biochemical pathways. Future research should focus on refining nanoparticle formulations, assessing their long-term impact on soil and plant health, and optimizing application strategies for large-scale agricultural implementation.

A comparative study conducted by Rodríguez-Morales et al. (2021) evaluated the effects of iron oxide ($Fe_2O_3$) and zinc oxide (ZnO) nanoparticles on the growth, nutrient uptake, and physiological responses of sorghum (Sorghum bicolor L.) under controlled greenhouse conditions. The study investigated the foliar application of $Fe_2O_3$ and ZnO nanoparticles at concentrations of 25, 50, 75, and 100 mg $L^{-1}$, similar to the current research on sugarcane budnodes. Results indicated that ZnO NPs at 50 mg $L^{-1}$ significantly enhanced chlorophyll content, photosynthetic rate, and shoot biomass, while higher concentrations (>75 mg $L^{-1}$) led to oxidative stress and reduced growth parameters. Interestingly, $Fe_2O_3$ NPs at 25–50 mg $L^{-1}$ improved root length, biomass accumulation, and iron translocation efficiency, reinforcing the essential role of iron in cellular respiration and enzyme activity. However, unlike in sugarcane, $Fe_2O_3$ NPs at 100 mg $L^{-1}$ did not exhibit toxicity in sorghum, suggesting species-dependent tolerance to high Fe concentrations. A critical observation in this study was the role of ZnO NPs in reducing drought-induced stress, as plants treated with moderate ZnO NP concentrations showed improved water use efficiency and antioxidant enzyme activity, further supporting the findings in sugarcane regarding improved abiotic stress tolerance. However, the authors highlighted that excessive $Fe_2O_3$ and ZnO nanoparticle accumulation in soil may alter microbial activity, necessitating further investigation into long-term environmental impacts. Compared to the present study on sugarcane, where Fe NPs at 100 mg $L^{-1}$ reduced plant height and shoot dry weight, this study suggested a more positive impact of low-to-moderate Fe and Zn NP applications in sorghum, emphasizing the importance of optimizing nanoparticle concentrations for species-specific responses. These findings provide further evidence that nanoparticle-based nutrient delivery can enhance plant growth and stress tolerance, but their application must be carefully tailored to different crop species and environmental conditions to maximize benefits while mitigating toxicity risks.

## CONCLUSION

The findings of this study highlight the significant potential of nanoparticle application in improving the sprouting, establishment, and overall growth of sugarcane budnodes. The use of iron and zinc nanoparticles enhanced budnode functionality, contributing to better seedling vigor, increased plant height, improved biomass accumulation, and expanded leaf area. These improvements suggest that integrating nanoparticles into budnode technology could offer a more efficient approach to sugarcane propagation, ultimately benefiting farmers by optimizing seed management and promoting higher yields. The ability of nanoparticles to enhance physiological processes, including nutrient uptake and stress tolerance, underscores their role in advancing sustainable sugarcane production. By refining nanoparticle-based agricultural practices, this approach holds promise for increasing productivity while maintaining environmental sustainability.

**Author Contribution**

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| Mena Hameed* | Substantial Contribution to study design, analysis, acquisition of Data<br>Manuscript Writing<br>Has given Final Approval of the version to be published |
| Asif Ali Kaleri | Substantial Contribution to study design, acquisition and interpretation of Data<br>Critical Review and Manuscript Writing<br>Has given Final Approval of the version to be published |
| Qamaruddin Jogi | Substantial Contribution to acquisition and interpretation of Data<br>Has given Final Approval of the version to be published |
| Danish Manzoor | Contributed to Data Collection and Analysis<br>Has given Final Approval of the version to be published |

 

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| Maira Raqeeb Tunio | Contributed to Data Collection and Analysis<br>Has given Final Approval of the version to be published |
| Awais | Substantial Contribution to study design and Data Analysis<br>Has given Final Approval of the version to be published |
| Majid Hussain | Contributed to study concept and Data collection<br>Has given Final Approval of the version to be published |
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| Tanveer Hussain | Writing - Review & Editing, Assistance with Data Curation |
| Abdul Razaque Channa | Writing - Review & Editing, Assistance with Data Curation |
| Urooj Rehmani | Writing - Review & Editing, Assistance with Data Curation |

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