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Effect of Iron Oxide and Zinc Oxide Nanoparticles on *Lactuca Sativa* Growth In Hydroponics Setup

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ABSTRACT
This study aims to explore innovative methods to enhance the nutritional value of leafy vegetables in the face of climate change challenges. The focus is on employing hydroponics, a soilless cultivation technique, and nanoparticles to increase the iron and zinc content in the *Lactuca sativa*. The research is driven by the necessity to mitigate nutritional deficiencies exacerbated by climate change impacts on traditional agricultural practices.The research involves a comprehensive approach comprising laboratory experiments and controlled hydroponic setups. *Lactuca sativa* is cultivated using hydroponic systems infused with iron and zinc nanoparticles. The nanoparticles' efficacy in promoting mineral uptake and their impact on plant growth and nutrient absorption are thoroughly investigated. Comparative analysis between conventionally grown vegetables and those cultivated through hydroponics with nanoparticles are conducted to assess the nutrient enhancement.The research demonstrates the feasibility of using hydroponics coupled with nanoparticles to enhance the iron and zinc content in leafy vegetable (*Lettuce*). Results indicate that this novel method significantly increases the nutritional value of these vegetable compared to conventional cultivation methods. Implementation of such strategies holds promise in addressing nutritional deficiencies, particularly in regions vulnerable to climate change impacts. Further scaling and optimization of this approach could contribute significantly to sustainable agriculture practices, ensuring food security and nutrition resilience in a changing climate scenario.

Highlights
1. Both iron oxide and zinc oxide nanoparticles significantly promote growth in *Lactuca sativa* in hydroponic systems.
2. Iron oxide nanoparticles improve iron assimilation, while zinc oxide nanoparticles boost zinc uptake, enhancing overall plant health.
3. Zinc oxide nanoparticles increase antioxidant enzyme activity, aiding stress resistance in Lactuca sativa.
4. Application of iron oxide nanoparticles leads to higher chlorophyll content, enhancing photosynthetic efficiency.
5. Zinc oxide nanoparticles promote root growth at 50 mg/l on day thirty as compared to iron oxide nanoparticles.

1. INTRODUCTION:
The ever-increasing human population and resultant increasing food supply, demands improvement in agriculture production science. To do so, use of fertilizers is widely practised to increase food production thereby we can reach a global consumption of 201.84 million tons (FAO, 2019). Now the soil is receiving an excessive amount of applied mineral fertilizers which is

demeriting the soil quality and giving many negative impacts on the ecosystems and human health (Nisar pahalvi *et al.,* 2021). Nature is experiencing negative impact by a real runoff, excessive soil erosion and reduction of soil organic matter, metal accumulation, leading to soil fertility imbalance (Penuelas *et al.*, 2023). Taking into consideration, the need for innovation and sustainable technology has been focused for developing environmentally sustainable productivity via agriculture for example by including organic amendments, plant breeding, microbial inoculants (Bonanomi *et al.*, 2018; Koskey *et al.*, 2021). Unfortunately, the given avenues are still not fulfilling their objectives and adequate effect to mitigate the current obstacles in agriculture. In this context, use of hydroponics and supplementation of nanoparticles like iron and zinc oxide proposed to be used in agriculture as an alternative and reported as future of agriculture (Monisha *et al.*, 2023; Sonawane 2018; Sathyanarayana *et al.*, 2022). The integration of hydroponics and nanotechnology along with sensors, IOT is driving us towards precision farming. The need of precision farming generated as soil quality is decreasing, use of soil and water has been diverted and its scarcity in the upcoming concern of agriculture (Chadwick et al, 2023).

The certain applications of nanotechnology in the hydroponics have been related by improving nutrient uptake by plants in presence of nanoparticles, use of nutrients nanoparticles for plant growth, incorporation nanoparticles to improve of substrate culture (Maluin *et al.*, 2021). The real utility of improvement in many vegetables and crop has already been evident mainly in rice (Wang *et al.*, 2021), lettuce (Jacquez *et al.*, 2023), tomato (Haghighi and Daneshmand, 2013), spinach (Smital and Mansee, 2018) and others. Studies encouraged the use of engineered nanoparticles such as calcium phosphate nanoparticles, p-nano fertilizers, N-nano fertilizers for the applications in soil, foliar, fertigation and in hydroponics (Carmona *et al.,* 2022). The nanoparticles showcased specific physical and chemical features that make them capable of crossing cellular barriers by importing novel actions on using organisms. In agriculture nanoparticles showcased their utilities as nano pesticides, nano fungicides, nano herbicides, and nano fertilizer (Singh *et al.*, 2021). The nanoparticles can be given to the various parts of plants such as seeds, roots, pollen- isolated cells and protoplasts (Singh *et al.,* 2021). Nanotechnology and hydroponics integration with IOT is non promoted as sustainable agriculture to work in nature as well as committed to satisfy increasing food demand. Nanoparticles also impart many positive effects in seed germination, root or shoot growth as well as increasing biomass and overall grain yield (Zhao *et al.*,2020). In the present study we have evaluated the effect of iron and zinc oxide nanoparticles on overall growth of *Lactuca sativa* growing under hydroponic conditions and treatment given to the root submerged in setup.

2. METHOD:

2.1. Preparation of seeds and plantlets production:

The commercially available seeds of *Lactuca sativa* (Lettuce) produced from company "All That Grows" India and readily sown in the moist cocopeat. The seeds were moistened intermittently till it germinated since kept in dark. Upon five to six days of incubation, seedlings found to be germinated which were maintained in cocopeat under greenhouse for indirect sunlight exposure. Upon 20 days the plantlet of four leaves is readily used for transfer in sets of experiments.

2.2. Preparation of hydroponic solution:

The standard hydroponic nutrients prepared as suggested in Johnson's formulation available in Siddiqui *et al.*, (1989) article This formulation included both macronutrients and micronutrients in specific concentrations, optimized for plant growth under controlled environments.Macronutrients provided were nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). These were supplied using salts such as $NH_4H_2PO_4$, NH_4NO_3, KH_2PO_4, KNO_3, $Ca(NO_3)_2·4H_2O$, and $MgSO_4·7H_2O$. These elements support key functions like chlorophyll synthesis, root development, and enzymatic activity. Micronutrients included iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo), sourced respectively from Fe-EDTA, $MnCl_2·4H_2O$, $ZnSO_4·7H_2O$, $CuSO_4·5H_2O$, H_3BO_3, and $Na_2MoO_4·2H_2O$. These trace elements play critical roles in photosynthesis, enzyme function, and reproductive processes. The total concentration of nutrient medium wsa maintained in the range of 200 to 800 ppm throughout the study where reverse osmosis water had an initial total dissolved solid level as 50. During study, shift in pH was regularly maintained at 5.5 to 6.5 using acidic solution of dilute HCl(0.1-1N) .

2.3. Preparation of Iron and Zinc oxide nanoparticles:

The readily available iron oxide nanoparticles with chemical abstract service number (CAS No. 1317-16-9) as brown powder with chemical formula Fe_2O_3 having molecular weight 231-553g/mol with purity 99.50% and traces of Sulphur < 0.01%, Nickel < 0.02%, Magnesium <0.04% and Aluminum < 0.02 used in the present study was obtained from Platonic nanotech Pvt. Ltd, Mahagma, India. Similarly, readily available zinc oxide nanoparticles with chemical abstract service number (CAS No. 1314-13-2) used in the present study were obtained from Platonic Nanotech Pvt. Ltd., Mahagma, India. These nanoparticles were provided in the form of a fine white powder with the chemical formula ZnO, having a molecular weight of 81.38 g/mol and a purity of 99.90%. Trace elemental analysis indicated the presence of Lead (Pb) < 0.005%, Cadmium (Cd) < 0.002%, Copper (Cu) < 0.01%, and Iron (Fe) < 0.02%

2.4. Experimental Setup:

Twenty healthy lettuce plants grown up to 20 days in cocopeat were maintained in net pot and transferred to Johnson's nutrient of hydroponics made in deep water culture with ample aeration in each set. In an experimental set up control set made with continuous nutrient supply without nanoparticles treatment.

In an experimental setups three concentrations were allowed to be exposed via supplementing in hydroponics solution as 10 mg/liter, 30 mg/liter and 50 mg/liter of iron oxide and 30 mg/liter, 90 mg/liter and 150 mg/liter zinc oxide nanoparticles.The variation in iron and zinc nanoparticle concentrations is scientifically justified. Zinc oxide is more soluble and bioavailable than iron oxide, requiring higher doses to achieve comparable uptake (Dimkpa et al., 2012; Wang et al., 2016). Additionally, ZnO has a lower phytotoxicity threshold, potentially causing oxidative stress at lower concentrations, whereas iron is generally safer at higher levels (Raliya et al., 2015; Nair et al., 2011). Lastly, the plant's nutritional demand differs—iron is needed in smaller amounts for chlorophyll synthesis, while zinc supports broader metabolic functions and is required in higher quantities (Broadley et al., 2007; Alloway, 2008). Accordingly, inoculation of nanoparticles at a given concentration loaded upon 24^{th} days of plant germination i.e. on 4^{th} day after plants transferred to hydroponic set up.

2.5. Statistical analysis:

The overall growth response of lettuce towards iron oxide and zinc oxide nanoparticles at available concentration with respect to control (plant without nanoparticles in the hydroponic medium in natural condition)is statistically analyzed for significant change with $P<0.05$ and statistically evaluated by Newman-Keuls ANOVA test using Graph Pad Prism. The statistically analyzed data of root length, shoot length, leaves length, leaves number, and plant weight recorded to analyze its effect.

3. RESULT:

In the present study overall growth performance of lettuce towards variable concentrations of iron oxide nanoparticles and similarly of zinc oxide nanoparticles have been reported as per days of growth progression for a number of parameters as follows:

3.1. Iron oxide treatment has been studied for 7^{th} day, 15^{th} ,30^{th} and 45^{th} day in hydroponic setup with respect to root length,shoot length, leave length and leave number and it is tabulated.

3.1.1 Root length:

At the 7-day mark, all treatment groups had root lengths like the control group. Lettuce root lengths exposed to iron oxide nanoparticles at different concentrations (10 mg/l, 30 mg/l, and 50 mg/l) were assessed at four time intervals: 7, 15, 30, and 45 days after post-treatment. The control group did show a typical increase for root length during that time. The treatment groups showed varying responses.

A statistically significant increase in root length was observed on the 45th day at the 30 mg/L concentration of iron oxide nanoparticles, where the root length reached16±0.81 cm. This value was significantly higher compared to the control group (15 ± 2.0 cm) with $P < 0.05$. This suggests that 30 mg/L concentration had a positive effect on root development at later stages of growth.Root length observation is shown in figure 1 and 9 for more clarification.

3.1.2 Shoot length:

The shoot lengths of lettuce varied in response to treatments with iron oxide nanoparticles at various time intervals. Statistical analysis of lettuce shoot length revealed a significant increase in shoot length at Day 45 when treated with iron oxide nanoparticles at concentrations of 30 mg/L with a mean value of(25 ± 1.6 cm) with $P < 0.01$, followed by 50 mg/L which also shows significant increase (25±1.1 cm) with $P < 0.01$.These findings suggest that both 30 mg/l and 50 mg/l concentrations of iron oxide nanoparticles effectively promoted shoot elongation at later stages of growth. Shoot length observation is elucidated in figure 2 and 10 for more clarification.

3.1.3 Leaves length:

Significant increase in leaf length were observed at 30 mg/L and 50 mg/L treatments throughout the study. On day 7, the 30 mg/L treatment (6.3 ± 1.4 cm) was significantly longer than the control (5.0 ± 1.7 cm). By day 15, both 30 mg/L (5.0 ± 1.7 cm) and 50 mg/L (5.3 ± 1.0 cm) treatments showed significant growth compared to

Journal of Molecular Science
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DoI-10.004687/1000-9035.2025.185

the control (3.0 ± 1.8 cm). This trend continued at days 30 and 45, with the 50 mg/L treatment consistently producing the longest leaves.Leaves length observation is elucidated in figure 3 and 11 for more clarification. Observations regarding leaf length are elucidated in Figure 3 and 11.

3.1.4 Leaves number

A marked enhancement in leaf development was evident by day 45 following treatment with iron oxide nanoparticles. The 30 mg/l concentration resulted in the greatest increase in leaf number (25 ± 3.1), followed by 50 mg/l (21 ± 2.6) and 10 mg/l (20 ± 5.3), all significantly surpassing the control group (13 ± 2.3) ($P < 0.05$). These findings highlight a cumulative and dose-responsive stimulatory effect of iron oxide nanoparticles on plant growth, with 30 mg/l demonstrating optimal efficacy. Observations regarding leaves number are elucidated in Figure 4 and 12 for more clarification.

3.1.3.1. Total plant weight

Upon 45th day of treatment with iron oxide nanoparticles significant improvement in plant weight has been recorded in lettuce once the load of 10 mg/lit and 30mg/lit. has been introduced in hydroponics water with mean weight per plant reached as 76 ± 3.2 gm and 79 ± 5.0 gm, respectively while in the control set it was recorded as 57 ± 2.6 gm. The variation in weight is found to be statistically significant ($P < 0.0001$). In contrast, as the dose of iron oxide nanoparticles increased up to 50 mg/lit. It has been observed that total plant weight reduced to 34 ± 5.3gms and reduced significantly as compared to control with 23 gm per plant weight difference .Observations regarding total plant weight is elucidated in figure 17.

3.2 Zinc oxide treatment has been studied for 7th day, 15th ,30th and 45th day in hydroponic setup with respect to root length, shoot length, leave length and leave number and it is tabulated.

3.2.1.1 Root length

Zinc oxide nanoparticles promoted increased root length during the later phases, especially at concentrations of 30 and 50 mg/l on 30th and 45th day of the treatment.A statistically significant increase in root length was observed on the 45th day at the 50 mg/l concentration of iron oxide nanoparticles, where the root length reached 6.1 ± 0.25 cm. This value was significantly higher as compared to the control group (5.2 ± 0.25 cm) with $P < 0.05$. This suggests that 50 mg/l concentration had a positive effect on root development at later stages of growth.The significant root length was observed also observed on on the 30 th day at 30 mg/l reaching5.9±0.12 cm with $P<0.05$ and 50 mg/l, reaching 6.1±0.12 cm with $P<0.05$. As an essential micronutrient that plays a role in auxin metabolism and enzyme activation, zinc may account for the enhanced root elongation (Rameshraddy et al., 2017). In hydroponic systems, nanoparticles improve the solubility of nutrients and their absorption efficiency (Servin et al., 2015), which aids in root growth over time. The noticeable yet gradual response is consistent with previous research indicating that both a specific concentration threshold and time are required for physiological adjustment and advantages (Dimkpa et al., 2012). Observations regarding root length are elucidated in Figure 5 and 13 for more clarification.

3.2.1.2 Shoot length

Shoot length of lettuce exhibited a marked increase at 15 days with 10 mg/l (11 ± 0.58 cm) and 50 mg/l (12 ± 0.58 cm), both statistically significant ($P < 0.0001$) compared to control (4.0 ± 0.49 cm). A similar significant increase was observed with 30 mg/l (12 ± 1.2 cm, $P < 0.0001$). No significant differences were observed at 7, 30, or 45 days among any treatments and control. Observations regarding leaves number are elucidated in Figure 6 and 14 for more clarification.

3.2.1.3 Leaves length

At day 7, 10 mg/l significantly reduced leaf length (1.0 ± 0.75 cm, $P < 0.05$) compared to control (1.6 ± 0.37 cm). On day 15, 50 mg/l showed a highly significant increase (5.0 ± 1.5 cm, $P < 0.0001$) versus control (3.1 ± 0.73 cm). No significant changes were observed at days 30 or 45 among treatments. Observations regarding leaves length are elucidated in Figure 7 and 15 for more clarification.

3.2.1.4 Leaves number:

At day 7, no significant changes in leaf number were observed across ZnO NP treatments compared to control, indicating limited early response.At day 15, all treated groups showed significant increases in leaf number compared to control (8.3 ± 0.58), with 30 mg/l having the highest (21 ± 1.7, $P < 0.05$). At days 30 and 45, leaf numbers remained significantly higher across all treatments, particularly at 50 mg/l (17 ± 0.58 at day 45, $P < 0.0001$), showing a strong dose-dependent effect over time. Observations regarding leaves number are elucidated in Figure 8 and 16 for more clarification.

4. Total plant weight:

After 45 days of treatment, lettuce plants that received iron oxide nanoparticles at concentrations of 10 mg/L and 30 mg/L exhibited a notable increase in overall plant weight, achieving 76 ± 3.2 g and 79 ± 5.0 g respectively, in

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1404-1413
DoI-10.004687/1000-9035.2025.185

comparison to the control group (57 ± 2.6 g). This suggests that moderate iron supplementation positively influences biomass accumulation. However, at the highest concentration (50 mg/L), the weight of the plants significantly decreased to 34 ± 5.3 g, indicating phytotoxic effects from higher doses. Conversely, zinc oxide nanoparticles at concentrations of 10 mg/L (61 ± 1.0 g) and 30 mg/L (64 ± 3.2 g) did not produce substantial changes, whereas the application of 50 mg/L resulted in a significant increase in biomass to 78 ± 6.0 g, exceeding the control (57 ± 2.9 g). This implies that a higher concentration of ZnO nanoparticles is necessary to provoke a meaningful growth response**,** in contrast to iron oxide nanoparticles, which were effective at moderate levels. Observations regarding total plant weight are elucidated in Figure 18 and 19 for more clarification.

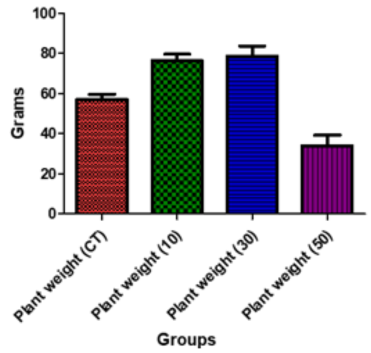


Figure 18 : Overall change in weight of lettuce treated with Iron oxide nanoparticle

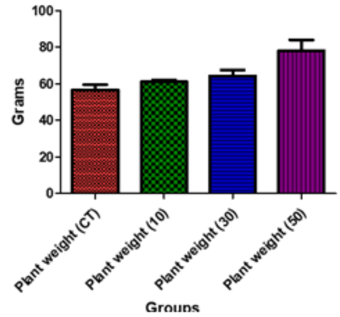


Figure 19 : Overall change in weight of lettuce treated with Zinc oxide nanoparticle

5. DISCUSSION:

The present study highlights the concentration- and time-dependent effects of iron oxide (Fe_2O_3) and zinc oxide (ZnO) nanoparticles (NPs) on the growth and physiological traits of hydroponically grown lettuce (*Lactuca sativa*). The observed variation in root length, shoot biomass, and leaf development suggests that nanoparticle-mediated plant responses follow a nonlinear pattern, consistent with the concept of hormesis—a biphasic response characterized by growth inhibition at lower concentrations and stimulation at moderate to high concentrations under specific exposure durations (Rico et al., 2011; Tripathi et al., 2017).

5.1 Effects of Iron Oxide Nanoparticles:

Root elongation was initially suppressed at 10 mg/L, likely because of early phytotoxic responses or oxidative stress induced by nanoparticle exposure, a phenomenon previously reported in *Arabidopsis thaliana* and lettuce under metal nanoparticle stress (Dimkpa et al., 2012). However, concentrations of 30 mg/L and 50 mg/L showed significant improvements in root and leaf growth after 30–45 days, indicating a delayed stimulatory effect. This may be attributed to the enhanced nutrient uptake, iron-mediated activation of enzymatic processes, and improved oxidative stress management (Tripathi et al., 2017; Sabaghnia & Janmohammadi, 2015).

The improved shoot growth observed in the later stages is indicative of time-sensitive metabolic activation. Iron, an essential micronutrient, plays a vital role in chlorophyll biosynthesis, respiratory chain function, and cellular metabolism (Rico et al., 2011). The significant increase in leaf number, particularly at 30 mg/L (25 ± 3.1, $P < 0.05$), supports the notion that moderate iron nanoparticle concentrations enhance vegetative development in hydroponic systems by facilitating efficient micronutrient delivery and utilization (Sabaghnia & Janmohammadi, 2015).

Interestingly, the plant biomass measurements revealed a bell-shaped response to Fe_2O_3 NPs. While 10 mg/L and 30 mg/L treatments increased plant weight to 76 ± 3.2 g and 79 ± 5.0 g respectively, the 50 mg/L treatment resulted in a marked decline to 34 ± 5.3 g. This decline suggests possible phytotoxicity or nutrient imbalance at elevated nanoparticle concentrations, corroborating earlier findings that excessive micronutrient application can impair plant metabolic functions (Servin et al., 2015).

5.2 Effects of Zinc Oxide Nanoparticles:

ZnO nanoparticles exhibited a distinct pattern of influence on plant morphology and biomass. Root elongation and leaf development were significantly enhanced at concentrations of 30 and 50 mg/L, particularly after 30 days. Zinc's known role in auxin metabolism, enzyme activation, and protein synthesis likely underlies this growth stimulation

(Rameshraddy et al., 2017; Rossi et al., 2019). The initial inhibition observed at 10 mg/L might reflect early oxidative stress or suboptimal micronutrient availability, as noted in previous studies (Dimkpa et al., 2012).

Leaf length exhibited a significant and early response to zinc oxide nanoparticless, with improvements evident from day 7 onward at concentrations of ≥30 mg/L (*P* < 0.0001). This suggests a more immediate bioavailability and systemic integration of zinc into lettuce physiology under hydroponic conditions (Rico et al., 2011). Consistent increases in leaf number at 50 mg/L further affirmed the involvement of zinc in promoting cellular division and organogenesis through enhanced metabolic efficiency (Siddiqi & Husen, 2017).

In contrast to iron, zinc nanoparticles require higher concentrations to elicit a notable effect on biomass accumulation. Plants treated with 50 mg/L ZnO achieved a significant biomass of 78 ± 6.0 g, outperforming both the control (57 ± 2.9 g) and lower treatments (10 mg/L: 61 ± 1.0 g; 30 mg/L: 64 ± 3.2 g). This aligns with previous findings that the physiological benefits of zinc are dose-dependent, with suboptimal concentrations failing to activate key metabolic pathways (Rossi et al., 2019; Tripathi et al., 2017).

5.3 Comparative Insights and Implications

Comparative analysis of Fe_2O_3 and ZnO NPs revealed that while iron was effective at moderate concentrations (10–30 mg/L), zinc required higher levels (50 mg/L) to achieve comparable growth promotion. These results underscore the element-specific dynamics of nanoparticle uptake and utilization in hydroponic systems. Furthermore, the delayed yet significant responses to nanoparticle treatment reinforce the importance of temporal factors in assessing nanomaterial efficacy.

Overall, these findings support the hypothesis that nanoparticles, when applied at optimal concentrations and durations, can serve as effective nanofertilizers, improving plant performance by enhancing nutrient uptake, photosynthesis, and stress resilience (Dimkpa et al., 2012; Servin et al., 2015). However, the observed toxicity at higher Fe_2O_3 concentrations highlights the need for careful dose calibration to avoid detrimental effects.

5.4 Conclusion

This study has put forward that use of iron and zinc oxide nanoparticles is an effective system to implement the overall growth of *Lactuca sativa* once supplemented in hydroponically grown conditions. It has been noted that both nanoparticles are able to increase overall leaves number by fifteen significantly in plant *Lactuca sativa* and hence are able to increase overall weight of plant. The result evidenced the nanoparticles' ability to increase plant weight and improves plant leaves number once treatment given as 10 mg/lit and 50 mg/lit for iron and zinc oxide nanoparticles, respectively.

Control | 10 mg/lit of iron oxide nanoparticle | 30mg/lit of iron oxide nanoparticle | 50mg/lit of iron oxide nanoparticle

Fig. 1 : Lettuce growth response upon seven days of treatment with iron oxide nanoparticles

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1404-1413
DoI-10.004687/1000-9035.2025.185


Control


10 mg/lit of iron oxide nanoparticle


30 mg/lit of iron oxide nanoparticle


50 mg/lit of iron oxide nanoparticle

Fig. 2 : Lettuce growth response upon fifteen days of treatment with iron oxide nanoparticles


Control.


10 mg/l of iron oxide nanoparticles .


30 mg/L of iron oxide nanoparticles.


50 mg/L of iron oxide nanoparticles.

Fig. 4 : Lettuce growth response upon forty five days of treatment with iron oxide nanoparticles Top to bottom (Control, 10, 30 and 50 mg/lit).


Control


10 mg/lit. of zinc oxide nanoparticle

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1404-1413
DoI-10.004687/1000-9035.2025.185

30 mg/lit. of zinc oxide nanoparticle

50 mg/lit. of zinc oxide nanoparticle

Fig. 5: Lettuce growth response upon seventh days of treatment with zinc oxide nanoparticles

Control

10mg/lit of zinc oxide nanoparticle

30mg/lit. of zinc oxide nanoparticle

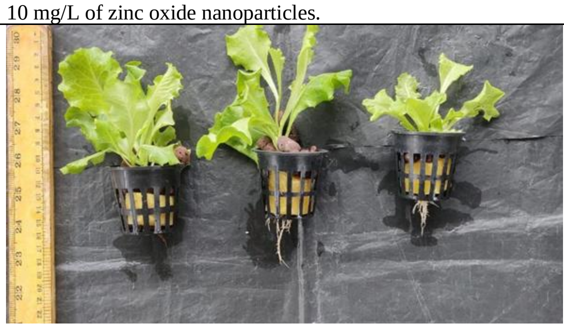
50mg/lit. of zinc oxide nanoparticle

Fig. 6: Lettuce growth response upon fifteen days of treatment with zinc oxide nanoparticles.

Contro

10 mg/L of zinc oxide nanoparticles.

30 mg/L of zinc oxide nanoparticles



50 mg/L of zinc oxide nanoparticles.

Fig. 7: Lettuce growth response upon thirty days of treatment with zinc oxide nanoparticles.

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1404-1413
DoI-10.004687/1000-9035.2025.185

Control

10 mg/lit of zinc oxide nanoparticle

30 mg/lit of zinc oxide nanoparticle

50 mg/lit of zinc oxide nanoparticle

Fig. 8: Lettuce growth response upon forty fifth days of treatment with zinc oxide nanoparticles.

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