



Effects of Different Levels of Iron Nanoparticles on the Growth and Biomass Production of Garden Cress (*Lepidium sativum* L)

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Abstract

Iron (Fe) is an essential micronutrient involved in several physiological processes in plants, including photosynthesis, respiration, chlorophyll formation, and electron transport. However, the low availability of Fe in many soils can restrict plant growth and productivity. Nanotechnology has emerged as a potential approach for improving nutrient delivery because iron nanoparticles may provide a highly reactive and potentially more readily available source of Fe. The present study investigated the effect of different levels of iron nanoparticles on the growth of garden cress (*Lepidium sativum* L.). Seeds were germinated and cultivated in 9-L pots, and plants were exposed to four levels of commercially obtained iron nanoparticles (0.5, 1.0, 1.5, and 2.0 g), in addition to an untreated control. Each treatment consisted of 15 replicates, and plants were evaluated after 21 days. Shoot and root dry weights were used as indicators of plant biomass response. The results showed a clear increase in shoot biomass with increasing nano-iron level, with mean shoot dry weights of approximately 1.28, 3.24, 3.85, and 5.31 g at 0.5, 1.0, 1.5, and 2.0 g nano-iron, respectively. Root dry weight also increased progressively, reaching approximately 0.067, 0.102, 0.165, and 0.205 g at the corresponding treatment levels. Thus, the highest tested nano-iron level produced the greatest shoot and root biomass among the evaluated treatments. These findings suggest that iron nanoparticles may promote early vegetative growth of garden cress under the experimental conditions used. However, because nanoparticle composition, particle size, application concentration in mass per volume, and detailed statistical procedures were not documented in the available experimental record, further controlled experiments are required to establish the optimum dose and evaluate the physiological and environmental implications of nano-iron application.

Keywords: Iron nanoparticles; Nano-fertilizer; Plant growth; Shoot biomass; Root biomass

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1. Introduction

Iron (Fe) is an essential micronutrient required for normal plant growth and development. Although Fe is abundant in the Earth's crust, its availability to plants is frequently limited because it occurs predominantly in poorly soluble forms, particularly under neutral to alkaline soil conditions. Plants have therefore evolved specialized mechanisms for Fe acquisition, transport, chelation, and intracellular homeostasis. (Zhang et al., 2019). The importance of Fe extends beyond its role as a mineral nutrient. Iron participates in electron-transfer reactions and is required for chlorophyll biosynthesis, photosynthetic electron transport, respiration, and the activity of several enzymes. Consequently, inadequate Fe availability can impair photosynthesis and produce chlorosis, reduced metabolic activity, and restricted plant growth (Therby-Vale et al., 2022). Iron nutrition is therefore closely associated with both plant productivity and the nutritional quality of harvested plant material (Briat et al., 2015). Conventional Fe fertilizers can be limited by poor utilization efficiency and by the tendency of Fe to become unavailable following application, particularly in soils with unfavorable chemical conditions. These limitations have stimulated interest in alternative approaches for improving Fe delivery to plants. Iron-based nanomaterials have attracted considerable attention because their small particle size and high surface-area-to-volume ratio can modify their interaction with plant tissues and the surrounding environment. Recent reviews indicate that Fe-based nanomaterials can influence plant biomass, nutrient uptake, photosynthesis, and

antioxidant responses, although their effects depend strongly on nanoparticle type, particle size, concentration, plant species, exposure duration, and application method (Cui et al., 2023).

Experimental evidence supports the potential of nano-iron as a plant-growth-promoting input. For example, Fe₃O₄ nanoparticles have been reported to improve growth, photosynthetic performance, respiration, and mineral accumulation in wheat (Feng et al., 2022). Similarly, foliar application of iron oxide nanomaterials enhanced biomass and physiological performance in soybean, with responses depending on both nanoparticle size and concentration (Cao et al., 2022). Other studies have demonstrated positive effects of Fe nanoparticles on crop biomass and yield, although the optimum concentration is not universal (Dola et al., 2022).

Importantly, garden cress (*Lepidium sativum* L.) is itself a relevant model for investigating plant responses to iron nanomaterials. A previous study specifically examining iron oxide nanoparticle uptake in garden cress demonstrated that plants can interact with and accumulate iron oxide nanoparticles and reported substantial biomass and chlorophyll responses relative to a conventional chelated Fe treatment (Ju et al., 2019). This provides a useful scientific basis for examining the response of *L. sativum* to different nano-iron levels. Nevertheless, the biological response to nanoparticles is not necessarily linear. Beneficial effects at an appropriate dose may be replaced by toxicity at excessive concentrations. A recent review of iron oxide nanoparticles concluded that low concentrations can stimulate germination, biomass production, and photosynthetic pigments, whereas excessive exposure may generate oxidative stress and damage plant tissue (Bhatia et al., 2025). Similarly, a meta-analysis of nanoscale zero-valent iron demonstrated that plant responses vary according to dose, particle size, application method, exposure duration, and plant species (Cui et al., 2023).

Therefore, determining the response of individual crop species to different nano-iron levels is necessary before recommending an application rate. The present study was designed to evaluate the effect of four levels of iron nanoparticles on the biomass production of garden cress, with particular emphasis on shoot and root dry weight after 21 days of treatment. The working hypothesis was that nano-iron application would enhance vegetative growth and biomass accumulation relative to untreated plants, with the magnitude of the response varying according to the applied level.

2. Materials and Methods

2.1 Plant Material

Garden cress (*Lepidium sativum* L.) was used as the experimental plant. Seeds were initially soaked in water for approximately 6–12 h before sowing. Following soaking, the seeds were transferred to soil-filled pots with a capacity of 9 L. Five seeds were initially sown in each pot, and seedlings were subsequently thinned to three plants per pot. These experimental details are based on the original study record.

2.2 Iron nanoparticle treatments

A commercially available iron nanoparticle powder obtained from (Sepehr Parmis, Iran), was used. Four quantities of the nanoparticle preparation (0.5, 1.0, 1.5, and 2.0 g) were prepared separately in water for application to the plants. The original manuscript describes the application as spraying the nano-iron preparation onto the cress plants. For clarity, the experimental treatments were designated as follows: T0: untreated control, T1: 0.5 g nano-iron, T2: 1.0 g nano-iron, T3: 1.5 g nano-iron, T4: 2.0 g nano-iron. A critical methodological limitation is that the original record does not specify the final solution volume for each treatment. Consequently, the treatments should be reported as grams of nanoparticle preparation rather than converted to g L⁻¹ or mg L⁻¹. The exact nanoparticle chemical composition, oxidation state, mean particle diameter, morphology, surface coating, and physicochemical characterization were also not reported in the available manuscript and should not be retrospectively assumed.

2.3 Treatment Experimental design

Five treatments were evaluated, comprising four nano-iron levels and an untreated control. Each treatment consisted of 15 replicates. Plants were maintained for 21 days before biomass assessment.

2.4 Determination of plant biomass

At the end of the 21-day experimental period, shoot and root biomass were evaluated using dry weight as the principal growth indicator. The results in the original manuscript are presented graphically as the effect of nano-iron concentration on shoot and root dry weight. The use of dry biomass is appropriate for assessing treatment-associated changes in accumulated plant material because it is less affected by differences in tissue water content than fresh weight.

2.5 Statistical Analysis

Data were analyzed using a one-way analysis of variance (ANOVA) and results were reported as mean $\pm$ standard deviation (SD).

3. Results

3.1 Effect of nano-iron on shoot dry weight

The application of iron nanoparticles was associated with a progressive increase in shoot dry biomass across the tested treatment levels. According to the numerical data represented in the original shoot graph, mean shoot dry weight increased from approximately 1.28 g at 0.5 g nano-iron to 3.24 g at 1.0 g, 3.85 g at 1.5 g, and 5.31 g at 2.0 g nano-iron. Thus, the highest treatment produced the greatest shoot biomass among the evaluated nano-iron concentrations. Relative to the 0.5-g treatment, shoot dry weight was approximately 2.54-, 3.01-, and 4.16-fold greater at 1.0, 1.5, and 2.0 g, respectively. The results showed increase significantly in shoot dry weight (Fig .1).

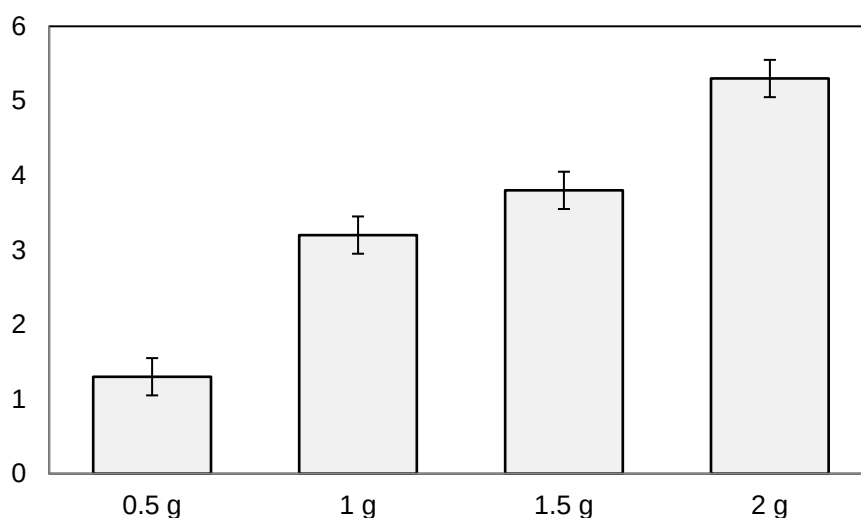


Fig. (1) Effect of different concentrations of nano-iron on the shoots of garden cress plants (21 days after spraying)

3.2 Effect of nano-iron on root weight

A similar trend was observed for root biomass. Mean root dry weight increased from approximately 0.067 g at 0.5 g nano-iron to 0.102 g at 1.0 g, 0.165 g at 1.5 g, and 0.205 g at 2.0 g nano-iron. The increase was therefore progressive over the experimental concentration range, with the highest treatment producing approximately three times the root dry weight recorded at the lowest nano-iron treatment (Fig. 2).

4. Discussion

The present study demonstrated a consistent positive association between increasing nano-iron level and biomass accumulation in garden cress. Both shoot and root dry weights increased progressively across the four tested nano-iron treatments, with the highest biomass recorded at 2.0 g. This pattern suggests that, under the experimental conditions, nano-iron availability was associated with enhanced vegetative growth of *L. sativum*.

The observed response is biologically plausible because Fe is indispensable for several processes directly related to biomass production. Iron is required for chlorophyll formation, photosynthetic electron transport, respiration, and numerous redox reactions. Improving Fe availability can therefore support photosynthetic carbon assimilation and energy metabolism, ultimately increasing the resources available for plant growth (Therby-Vale et al., 2022) .

The strong increase in shoot biomass observed in the present experiment is consistent with previous research demonstrating positive effects of iron-based nanomaterials on plant growth. For example, Fe₃O₄ nanoparticles enhanced growth and photosynthetic performance in wheat and were associated with increased Fe, phosphorus, and potassium concentrations in leaves. Likewise, foliar application of iron oxide nanomaterials has been shown

to stimulate biomass and physiological performance in soybean, although the response depended on nanoparticle size and concentration (Cao et al., 2022).

The response of garden cress is particularly interesting because a previous investigation directly examined iron oxide nanoparticle uptake in *L. sativum*. That study reported enhanced biomass and chlorophyll production following exposure to chelated iron oxide nanoparticles compared with a conventional Fe-EDTA fertilizer, demonstrating that garden cress can respond substantially to nano-enabled Fe treatments (Ju et al., 2019). The present findings therefore add further evidence that *L. sativum* may be a responsive species for evaluating nano-iron effects.

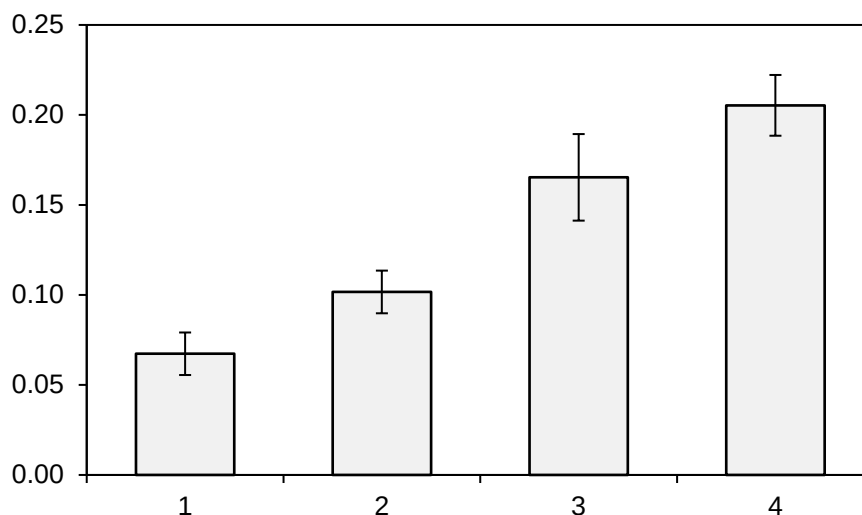


Fig. (2) Effect of different concentrations of nano-iron on the roots of garden cress plants. (21 days after spraying)

The increase in root dry weight is also noteworthy. Root development is fundamental to nutrient and water acquisition, and enhanced Fe nutrition can influence metabolic processes supporting root growth. Previous research indicates that iron-based nanomaterials can increase plant biomass and influence nutrient acquisition, while the magnitude and direction of the response depend on nanoparticle properties and exposure conditions (Cui et al., 2023). The progressive increase in root biomass in the present experiment may therefore reflect improved Fe availability and/or physiological stimulation associated with the nano-iron treatment.

However, the apparent dose-dependent increase observed here should not be interpreted as evidence that 2.0 g represents the universally optimal or agronomically recommended concentration. The study examined only four levels, and the highest dose tested was also the highest-performing dose. Consequently, the response curve beyond 2.0 g remains unknown. A higher concentration might continue to stimulate growth, produce no additional benefit, or induce phytotoxicity.

This point is important because the effects of iron nanoparticles are known to be concentration-dependent. A recent review concluded that iron oxide nanoparticles can stimulate plant biomass and photosynthetic processes at appropriate concentrations but can produce oxidative stress and growth inhibition at excessive concentrations (Bhatia et al., 2025). Similarly, a large meta-analysis found that the effects of iron-based nanomaterials depend on plant species, particle size, exposure duration, dose, and nanoparticle type. (Hou et al., 2024) Therefore, the positive response observed in *L. sativum* should be considered specific to the tested experimental conditions.

The increase in biomass may also involve mechanisms other than simple Fe nutrition. Nanoparticles can interact with plant surfaces and biological membranes and may influence nutrient transport, antioxidant systems, photosynthesis, and cellular signaling. Studies of Fe-based nanomaterials have reported enhanced antioxidant activity and improved nutrient acquisition under particular experimental conditions (Cui et al., 2023). However, the present experiment measured only root and shoot dry weight. It therefore cannot determine whether the observed growth promotion resulted from improved Fe nutrition, enhanced photosynthesis, altered antioxidant activity, changes in nutrient uptake, or a combination of mechanisms.

Another important consideration is the absence of sufficient characterization of the nanoparticle preparation in the original methodology. The manuscript identifies the commercial source but does not specify the chemical

form of the iron nanoparticles, particle diameter, surface characteristics, aggregation state, or Fe oxidation state. These properties can substantially influence nanoparticle uptake and biological activity. Indeed, experimental studies have shown that nanoparticle size and chemical form can alter plant responses (Cao et al., 2022). Future work should therefore characterize the material before application using appropriate techniques such as X-ray diffraction (XRD), transmission or scanning electron microscopy (TEM/SEM), and particle-size analysis, depending on the nanoparticle formulation.

The lack of an explicitly reported application volume is another methodological limitation. Reporting treatments simply as 0.5, 1.0, 1.5, and 2.0 g makes it difficult to compare the present findings with other studies that use mg L⁻¹ or mg/plant. For foliar application, the actual concentration and spray volume are critical because nanoparticle exposure depends on the amount deposited on the leaf surface. Future studies should therefore report the final suspension concentration, application volume per plant, frequency of application, spraying equipment, and environmental conditions.

Despite these limitations, the results provide preliminary evidence that nano-iron can promote biomass accumulation in garden cress. The particularly strong increase in shoot dry weight suggests that the treatment may have stimulated above-ground vegetative development, while the simultaneous increase in root biomass indicates that the response was not restricted to the aerial portion of the plant.

5. Conclusion

Overall, the findings are consistent with the broader literature indicating that appropriately applied Fe-based nanomaterials can improve plant growth and nutrient status (Feng et al., 2022). Nevertheless, the environmental safety and long-term consequences of nanoparticle application should also be considered. Iron nanoparticles may undergo transformations in soil and plant tissues, and their biological effects can change depending on environmental conditions (Zheng et al., 2023). Therefore, future experiments should combine biomass measurements with Fe accumulation, chlorophyll content, photosynthetic activity, antioxidant enzymes, nutrient composition, and nanoparticle fate.

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