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Impact of zinc oxide and titanium dioxide nanoparticles on growth parameters of chickpeas (*Cicer arietinum* L.)

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Abstract

Agricultural productivity remains a fundamental concern for farmers and agricultural scientists. Today, global food security is increasingly threatened by environmental challenges and a rapidly growing population. Environmental stressors, such as salinity, drought, heavy metals, ozone, sulfur oxides, and nitrogen oxides have increased crop yield losses. Various agricultural management practices and techniques are being employed to reduce yield loss and minimize environmental impact on plants. Among these, the application of nanoparticles, such as nanofertilizers, nanoinsecticides, nanofungicides, and nanosensors, has emerged as a promising approach for achieving agricultural sustainability, particularly in pest and soil nutrient management. Therefore, the present study was conducted to assess the effectiveness of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on the chickpeas cultivar. Two sets of experiments were conducted: seed germination (Petri dishes) and a field experiment analyzing various physiological, morphological, and biomass parameters. In the seed germination experiment, TiO_2 nanoparticles were more effective than ZnO nanoparticles, achieving a 100% germination rate at 48 h. Furthermore, in the field experiment, the biomass of the selected cultivar was higher at a 50 parts/million (ppm) nanoparticle concentration compared to 25 ppm. Conclusively, the application of both nanoparticles showed a positive impact on seed germination and plant growth. The nanoparticles hold significant potential for future agricultural applications, offering innovative solutions for agricultural yield and environmental sustainability by enhancing nutrient delivery, soil health, and pest control. Therefore, this study will be helpful for farmers and scientists seeking to harness the potential of nanomaterials for sustainable agricultural production.

Keywords: Zinc oxide; Titanium dioxide; Nanoparticles; *Cicer arietinum*; Seed germination; Plant growth; Biomass of plants

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1. Introduction

Agricultural productivity is one of India's most complex topics, with both positive and negative aspects. The rising global population growth and simultaneous environmental challenges highlight the urgent need for innovative and sustainable solutions in agriculture to meet the growing demand for food worldwide.[1] Agricultural seeds face susceptibility to environmental stressors, leading to compromised seed vigor, hindered crop growth, and reduced yield. While conventional seed treatments by agrochemical-based products improve seed germination, they also pose significant environmental risks.[2] Consequently, there is a pressing need for sustainable technologies, such as nano-based agrochemicals to address these challenges. Nano-agrochemicals offer the potential to mitigate the dose-dependent toxicity associated with seed treatments, thereby enhancing seed viability and ensuring the controlled release of active constituents. However, the excessive use of nano-agrochemicals raises authentic concerns regarding their safety, exposure levels, and impacts on the environment and human health.[3,4] Consequently, there is a critical need for comprehensive assessments and policy regulations to evaluate and manage these risks.

Agricultural production plays a key role in the economies of developing nations, serving as the primary provider of food for a rapidly expanding population globally, which currently exceeds 7.5 billion people.[5,6] Seeds constitute a fundamental input for sustainable agricultural productivity, and approximately 90% of crops are grown from seeds. High-quality seeds are essential for generating vigorous seedlings, thereby contributing to effective agricultural practices.[7] However, agriculture faces multifaceted environmental challenges, such as salinity, drought, and heavy metals in soil, along with the impacts of climate change. These factors can detrimentally affect seed germination, sprout growth, and, ultimately, crop yield. Moreover, seeds are susceptible to damage from seed-borne diseases and pests, leading to irregular seed latency, reduced viability, and impaired water absorption, all of which negatively impact crop and final yield.[8]

In terms of agricultural innovation, nanotechnology is a promising new area that offers long-term solutions to the urgent problems affecting global food security. Through the utilization of nanoparticles' unique features, such as their heightened reactivity and surface area, nano-based agrochemicals and seed priming methods hold the potential to transform seed treatments, augment crop yields, and guarantee the enduring viability of farming methods. However, it is essential to proceed with caution and conduct comprehensive assessments to mitigate potential risks to the environment and human health, ensuring that nanotechnology is harnessed responsibly for the benefit of society as a whole. Nanotechnology emerged as a promising field in agriculture, offering innovative solutions to enhance crop productivity and stress tolerance.[4,9] To guarantee sufficient crop establishment and the effective use of production resources in profitable agriculture, fast and identical seed germination is crucial. Nano-enabled seed treatment has gained considerable attention due to its potential to increase germination and overall plant growth.

The integration of nanotechnology in agriculture has led to a new period of revolution, contributing promising solutions to increase crop yield and sustainability. Among the myriad applications of nanotechnology in agriculture, nano-enabled seed treatments have garnered significant consideration for their potential to revolutionize conventional seed germination practices and seedling vigor indices. This research delves into recent advancements in nano-enabled seed treatments and their profound impact on the germination, seedling vigor, and growth physiology of chickpeas (*Cicer arietinum* L.) cultivars. As a staple crop rich in protein and essential nutrients, chickpeas play an important role in food security worldwide. Therefore, understanding the effects of nanoparticles, specifically titanium dioxide (TiO_2) and zinc oxide (ZnO), on seed germination and seedling growth is key for improving chickpea cultivation practices. This study examines the comparative effectiveness of TiO_2 and ZnO nanoparticles on seed germination rates and vigorous indices, elucidating their respective roles in promoting robust seedling growth and overall plant biomass. By elucidating the biochemical mechanisms underlying the observed effects, including antioxidant enzyme activity, chlorophyll content, and nutrient uptake, this research contributes valuable insights to the burgeoning field of nanotechnology-enabled agriculture. Through comprehensive investigation and analyses, this study aims to inform sustainable agricultural practices and pave the way for informed decision-making in crop management strategies.

To ensure sufficient crop establishment and the effective use of production resources in profitable agriculture, fast and uniform seed germination is crucial. Many crop species have semi-permeable coatings in their seed coats that limit solute leakage while facilitating gas exchange and water absorption. The dense layer of aniline blue staining on the seed coats may have an impact on water permeability and, in turn, seed germination. With varied degrees of effectiveness, treatments, such as scarification, nicking, and removal of the seed coat have been studied to improve the permeability of the seed coat to water and oxygen, hence improving seed germination and vigor of the seedlings. However, triploid seeds still have less seedling vigor than diploid seeds. Thus, new seed priming methods are required to increase seed germination and seedling vigor.

Therefore, the present study was conducted to understand the mechanisms of ZnO and TiO_2 nanoparticles on seed germination and plant growth development. Factors such as nanoparticle concentration, size, and shape play vital roles in determining their bio-effectiveness on plants. Therefore, this study offers a low-cost treatment approach that may enhance seed germination and support sustainable agriculture.

2. Materials and methods

2.1. Experimental design

Two experiments were conducted: (i) seed germination and (ii) a field experiment analyzing plant growth and physiology. Both experiments were conducted in triplicates. The germination experiment was conducted using 150 mm glass Petri dishes (Sidhi Trading Co., India) with a completely randomized design. Each Petri dish contains 30 uniform seeds of chickpeas (*C. arietinum* L.) treated with tap water (T1), 25 parts per million (ppm) TiO_2 (T2), 50 ppm TiO_2 (T3), 25 ppm ZnO (T4), and 50 ppm ZnO (T5), in room temperature. A total of 30 seeds were placed in dishes that were lined with cotton and watered. For the field experiment, the same treatments were applied in 1 m^2 plots.

2.2. Seed and nanoparticle selection

The local variety (Swetha [ICCV2]) of chickpeas (*C. arietinum L.*) was selected for seed treatment using ZnO and TiO_2 nanoparticles.

2.3. Seed germination

The germination experiment was conducted at room temperature. It was continuously monitored until the radicle length touched half of the seed length. Seed germination was assessed. The germination characteristics were measured in triplicate using the following formula in Equation I.[10]

$$GP = (NS \div TNS) \times 100 \qquad (I)$$

Where GP refers to germination percentage, NS is the number of germinated seeds, and TNS is the total number of seeds sown.

2.4. Plant analysis

Plants were harvested 10 and 20 days after germination (DAG) and transferred to the lab for growth measurement and physiological analysis.[11]

2.5. Growth and biomass analysis

Plant lengths (cm/plant) were measured using a meter scale (Jlab, India). The graphical method was used for measuring the leaf area of plants (cm^2). The dry masses of plants were weighed after hot air oven drying at 80°C.[11,12]

2.6. Biochemical analysis

2.6.1. Total chlorophyll and carotenoid content

Photosynthetic pigments were measured using a 100 mg leaf sample mixed with 10 mL of 80% acetone (v/v) solution. The solution's optical density was measured at 663 nm and 645 nm using the formulas described by Maclachlan and Zalik[13] and Yentsh and Duxbury.[14]

2.6.2. Ascorbic acid estimation

The concentration of ascorbic acid was determined based on its ability to reduce 2, 6-dichlorophenolindophenol. A 500 mg fresh leaf sample was homogenized in 20 mL of extraction solution (containing 5 g of oxalic acid and 0.075 g of EDTA in 100 mL of distilled water) and centrifuged for 15 min at 12,000× g. From the resulting supernatant, 1 mL was mixed with 5 mL of 2, 6-dichlorophenolindophenol solution. The absorbance of the resulting pink solution (Es) was measured at 520 nm. Subsequently, in the same solution, one drop of ascorbic acid solution was added, and the absorbance (Et) was measured at the same wavelength. For the blank (Eo), 1 mL of ascorbic acid solution was used in place of the sample.[15] Ascorbic acid concentration was calculated using the following formula in Equation II:

$$\text{Ascorbic acid (mg/g fresh leaf)} = [\{Eo - (Es - Et)\} \times V]/(v \times W \times 1{,}000) \qquad (II)$$

2.7. Statistical analysis

The experiment was conducted with fully randomized block designs. The statistical package for the social sciences (SPSS) (SPSS Inc., version 17.0) software was used for Duncan's Multiple Range Test analysis. The least significant differences were estimated at the $p>0.05$ level among treatments. The mean values of each parameter were given in triplicate of samples and the mean and standard deviation were calculated using Microsoft Excel (2013).

3. Results and discussion

3.1. Nanoparticle effectiveness on seed germination and vigor indices

Germination and quality of seed have a direct impact on agricultural production, with high-quality seeds making a substantial contribution to total crop yields. The Indian seed industry is essential for giving farmers access to high-quality seeds, fostering research and development, and providing a wide range of product options. Therefore, the experiment for seed germination improvements will be beneficial for crop production. In the present study, two types of nanoparticles were used to enhance seed germination. It was found that both TiO_2 and ZnO nanoparticles improved seed germination. The vigorous index is as follows: 50 ppm TiO_2 >50 ppm ZnO >25 ppm TiO_2 >25 ppm ZnO > control (Figure 1). ZnO nanoparticles showed improved germination rates and increased seedling vigor. TiO_2 nanoparticles stimulated seed germination and promoted healthy seedling growth. TiO_2 particularly enhanced root and shoot growth, contributing to overall plant biomass. TiO_2 demonstrated a higher effectiveness in seed germination compared to ZnO nanoparticles, reaching a 100% germination rate at 48 h. Nanoparticle treatment, specifically with ZnO and TiO_2, positively impacted seed germination in chickpeas cultivars compared to untreated seeds. The germination rate – defined as the percentage of seeds that successfully sprouted – increased in nanoparticle-treated seeds.[4,16] Nanoparticle-treated seeds exhibited faster and more uniform germination compared to untreated seeds. The improvement in seed germination indicates that nanoparticle treatment enhanced the physiological processes necessary for seedling emergence and establishment.[17] According to a study, nanoparticles can improve water absorption, nutritional uptake, and stress tolerance, which in turn can improve seed germination and seedling growth. This method is known as "nanopriming." It is a method that involves applying nanoparticles to induce various physiological and biochemical changes that promote faster and more reliable germination.

3.2. Nanoparticle effectiveness on plant growth and development

3.2.1. Root length

Nanoparticles can have both positive and negative effects on plant morphological characteristics depending on the type of nanoparticle, concentration, and plant species. In certain instances, higher concentrations of nanoparticles can cause inhibition and even toxicity, but lower quantities can promote plant growth. Variations in root and shoot length, seed germination, and total biomass are examples of morphological changes that can be induced by nanoparticles.[18] In the present study, nanoparticle-treated plants showed an increase in root length compared to control plants. Both ZnO and TiO_2 nanoparticles likely stimulated root growth, resulting in longer and more

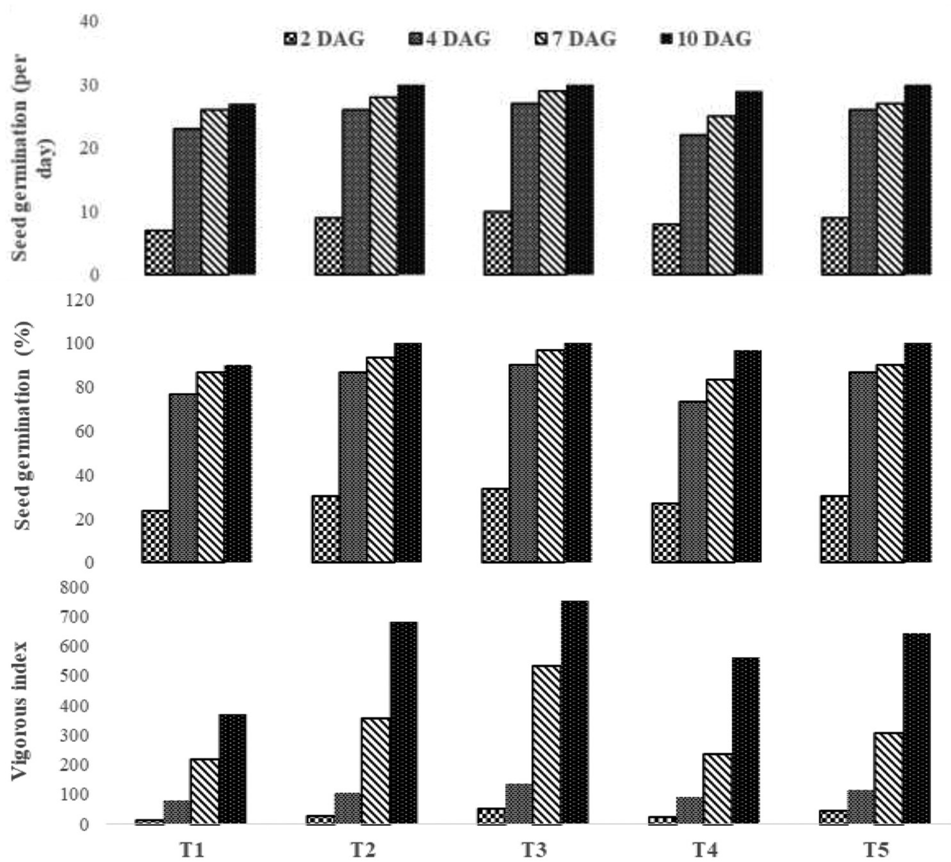

Figure 1. Effectiveness of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on seed germination and vigor index of selected *Cicer arietinum* seeds. T1 is the control, T2 is 25 parts/million (ppm) TiO_2, T3 is 50 ppm TiO_2, T4 is 25 ppm ZnO, and T5 is 50 ppm ZnO.
Abbreviation: DAG: Days after germination.

extensive root systems. Longer root length is beneficial for nutrient uptake and water absorption, ultimately contributing to improved plant health and productivity. The 50 ppm TiO_2 showed the highest growth, followed by 50 ppm ZnO, 25 ppm TiO_2, 25 ppm ZnO, and control (Figure 2).

Shoot length, which refers to the length of the above-ground parts of the plant, also increased in the nanoparticle-treated cultivar. Enhanced shoot length indicates increased vegetative growth and biomass accumulation. The promotion of shoot growth by nanoparticle treatment suggests improved photosynthetic capacity and overall plant health. In this study, the shoot length followed the same trends as the root length of the cultivar. The 50 ppm TiO_2 showed the highest shoot growth, followed by 50 ppm ZnO, 25 ppm TiO_2, 25 ppm ZnO, and control (Figure 2).

Total plant height was determined by summing root and shoot lengths. A significant increase in total height was observed with the exogenous application of ZnO and TiO_2. The increase in total height suggests that nanoparticle treatment promoted overall plant growth and development.[9] Among the treatments, 50 ppm TiO_2 resulted in the greatest plant height, followed by 50 ppm ZnO, 25 ppm TiO_2, 25 ppm ZnO, and control (Figure 2). This result indicates a dose-dependent effect on selected cultivars. Nanotechnology represents a promising green approach for improving agricultural yield and soil fertility by activating nutrient availability and energy metabolism

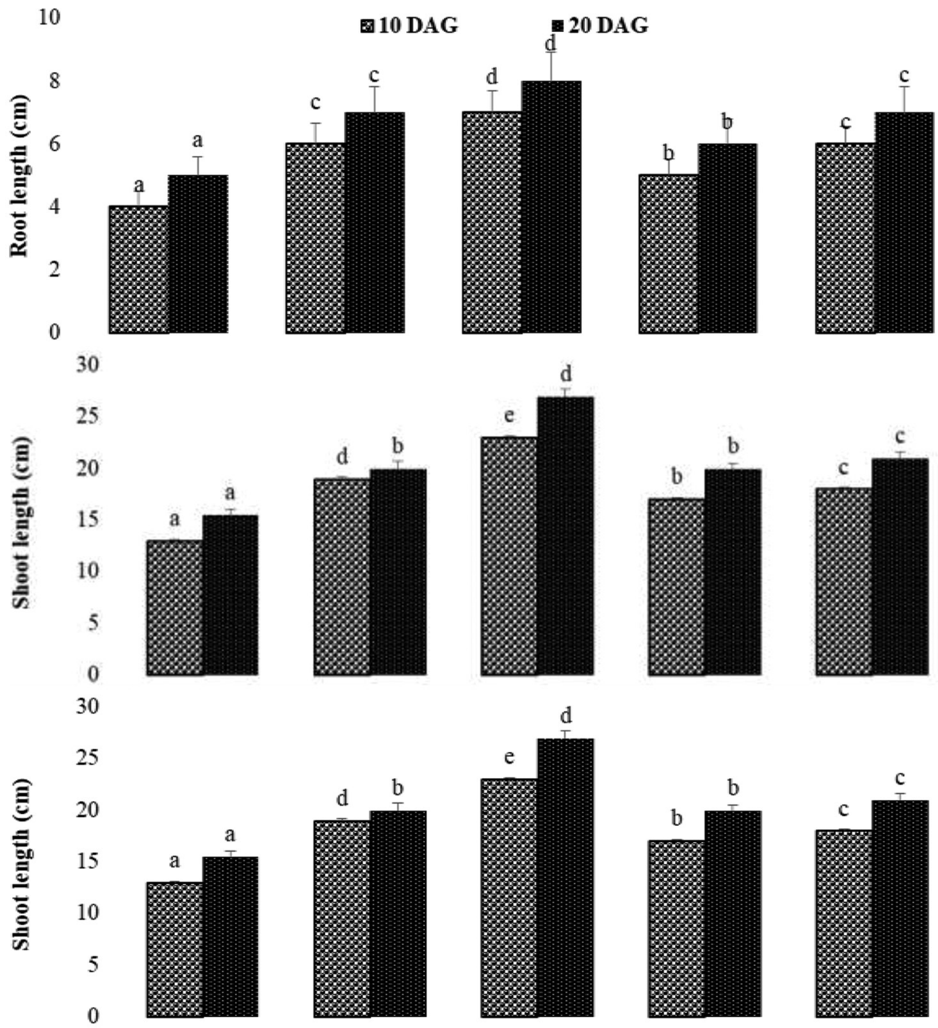

Figure 2. Effectiveness of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on root length, shoot length, and total plant height (cm) of *Cicer arietinum* cultivar. Mean ± standard deviation of three replicates is shown by thin vertical bars. T1 is the control, T2 is 25 parts/million (ppm) TiO_2, T3 is 50 ppm TiO_2, T4 is 25 ppm ZnO, and T5 is 50 ppm ZnO.

Note: Values within same letter are not significantly different $p<0.05$, according to Duncan's multiple range test.

Abbreviation: DAG: Days after germination.

in plant cells. To increase crop yields, nanoparticles are often used as nanofertilizers, nanoinsecticides, and nanofungicides. Various nanoparticles, such as TiO_2, ZnO, silicon oxide, magnesium oxide, gold, and silver, are being used to improve soil fertility, manage nutrients, and boost crop yields.[4]

3.3. Nanoparticle effectiveness on biomass

Nanoparticles can improve plant growth through various processes, such as nutrient delivery, stress tolerance, and hormone regulation promotion. They can improve fruit quality and productivity by scavenging free radicals, enhancing nutrient uptake, and inducing stress response pathways.[4,9] In the present study, biomass accumulation was higher in treatments with nanoparticle application, indicating their effectiveness as enhancers of plant growth pathways. TiO_2 nanoparticles showed significant efficacy in promoting biomass accumulation in chickpeas cultivars. The promotion of root and shoot heights by TiO_2 likely led to increased biomass production. ZnO nanoparticles also had a positive effect on biomass, although lower compared to TiO_2. T3-treated cultivars showed the highest biomass, followed by T2, T5, T4, and T1 (Figure 3). Nanoparticle-treated *C. arietinum L.* cultivars exhibited higher fresh weight compared to untreated plants. The increase in fresh weight indicates greater water content and overall biomass accumulation in nanoparticle-treated plants. Enhanced fresh weight suggests improved growth and physiological activity in response to nanoparticle treatments.

Nanoparticle-treated chickpeas cultivars also showed an increase in dry weight compared to control plants. Dry weight represents the mass of the plant's tissues after removing water content, providing a measure of the plant's physical biomass. The increase in dry weight indicates enhanced accumulation of essential components such as cellulose, lignin, and proteins in nanoparticle-treated plants.[19] The higher dry weight reflects improved biomass production and the potential for increased yield in nanoparticle-treated chickpeas cultivars. These changes indicate enhanced biomass accumulation, growth, and physiological activity in plants. Enhanced root and shoot growth contributed to increased biomass, suggesting the potential for improved plant yield. While both TiO_2 and ZnO nanoparticles showed positive effects on growth, TiO_2 appeared to be more effective in promoting

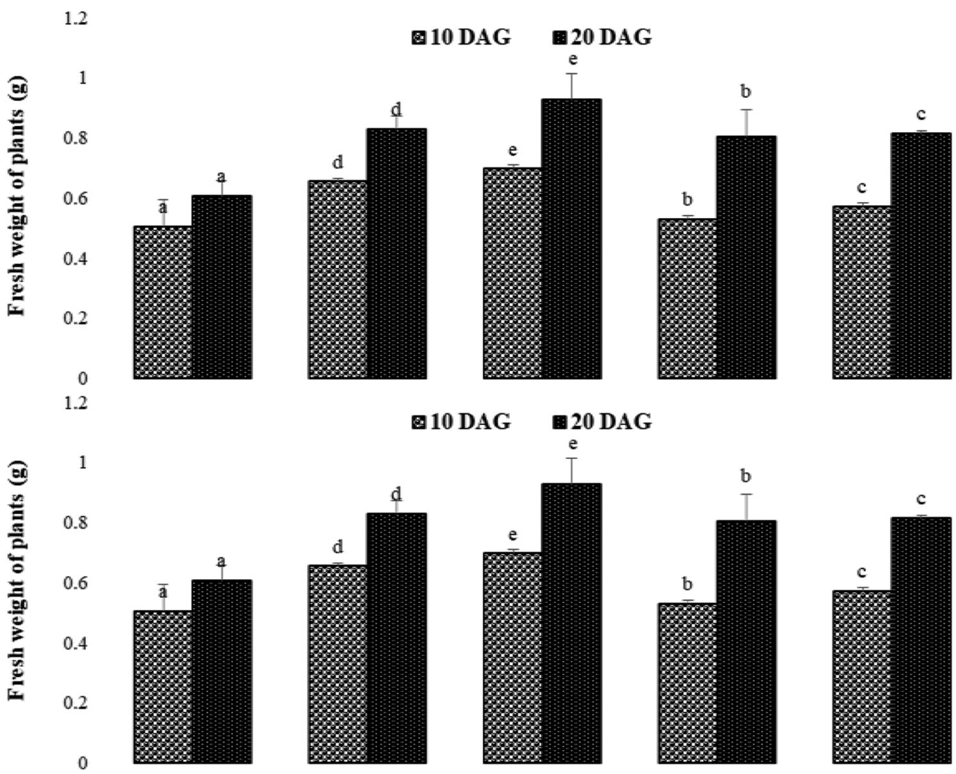

Figure 3. Effectiveness of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on fresh and dry biomass (g) of *Cicer arietinum* cultivar. Mean ± standard deviation of three replicates is shown by thin vertical bars. T1 is the control, T2 is 25 parts/million (ppm) TiO_2, T3 is 50 ppm TiO_2, T4 is 25 ppm ZnO, and T5 is 50 ppm ZnO.
Note: Values within same letter are not significantly different p<0.05, according to Duncan's multiple range test.
Abbreviation: DAG: Days after germination.

biomass accumulation, suggesting its potential as a seed treatment to enhance crop productivity. The application of nanoparticles, especially TiO_2, holds promise for improving the growth and biomass production of chickpeas cultivars, which could have significant implications for agricultural productivity and sustainability.

3.4. Biochemical changes

3.4.1. Chlorophyll a, b, and total chlorophyll

Chlorophyll pigments play an important role in photosynthesis in plants, algae, and cyanobacteria. It is essential for turning light energy into chemical energy, which enables plants to make oxygen and glucose. Photosynthesis and life on Earth would not be possible without chlorophyll. Therefore, analyzing chlorophyll pigments in plants can provide valuable insights into the plant's physiology. In this study, the applied nanoparticles, ZnO and TiO_2, increased the chlorophyll content in chickpeas cultivars (Figure 4). ZnO nanoparticles enhanced chlorophyll synthesis, leading to increased chlorophyll a and b content.[20] Similarly, TiO_2 nanoparticles stimulated chlorophyll biosynthesis pathways, resulting in higher levels of chlorophyll a and b. The total chlorophyll content, representing the sum of chlorophyll a and b, showed a significant increase when plants were treated with nanoparticles. Higher chlorophyll content suggests improved photosynthetic capacity and light absorption, which can positively influence plant growth and biomass accumulation.[4,11]

In the present study, higher chlorophyll contents were seen in the 50 ppm TiO_2-treated cultivars, while ZnO-treated cultivars showed the least increment of chlorophyll content compared to the control. Chlorophyll a, b, and total chlorophyll content increased with plant age, with higher levels observed at 20 DAG than at 10 DAG across all treatments (Figure 4). The application of selected nanoparticles positively impacted chlorophyll pigment levels. The findings suggest that the enhanced chlorophyll pigment in plants, resulting from nanoparticle treatment, improved overall plant growth. Plant chlorophyll may be complexly affected by nanoparticles, increasing or decreasing its content and affecting photosynthesis. By boosting ribulose-1,5-bisphosphate carboxylase/oxygenase activity and photosystem II efficiency, certain nanoparticles, such as TiO_2 and mesoporous silica, can promote photosynthesis and raise chlorophyll content. Other nanoparticles, including superparamagnetic iron nanoparticles, can have a detrimental effect on the amount of chlorophyll and the effectiveness of photosystem II, which could result in less photosynthesis. The type of nanoparticle, its size, concentration, and the type of plant influence the effects of nanoparticles on chlorophyll content.[21]

3.4.2. Carotenoids

Carotenoids play a crucial role in photoprotection and light harvesting during photosynthesis.[12,22] Increased carotenoid content indicates enhanced photoprotection against excess light and oxidative stress, which can contribute to improved plant resilience and productivity. Carotenoids facilitate chlorophyll in absorbing light energy and offer photoprotection by releasing excess energy as heat. In addition, they aid in the scavenging of reactive oxygen species (ROS), protecting the plant from oxidative damage caused by stressors, such as temperature. In the present study, the highest carotenoid content was noted in T3, followed by T5, compared to the control. Carotenoid content increased with plant age, with higher levels observed at 20 DAG compared to 10 DAG across all treatments (Figure 4). Generally, the application of nanoparticles enhances carotenoid production in plant cells and helps improve plant health. However, understanding the role of different nanoparticles and their mechanisms in plant cell environments will be a helpful tool for future research.

3.4.3. Ascorbic acid

Ascorbic acid is a water-soluble antioxidant that scavenges ROS and protects cells from oxidative damage.[11] The present study demonstrates elevated levels of ascorbic acid in nanoparticle-treated chickpeas cultivar, indicating enhanced antioxidant defense mechanisms. Ascorbic acid helps to neutralize ROS generated during stress conditions, such as high light intensity or drought, thereby protecting cellular structures and maintaining physiological functions.[23] The presence of increased levels of ascorbic acid contributes to improved stress tolerance and overall plant health in nanoparticle-treated chickpeas cultivars. It scavenges ROS and protects cellular components from damage, thereby maintaining cell integrity and function. Elevated levels of ascorbic acid indicate improved antioxidant defense mechanisms in the plant, which can enhance stress tolerance and overall plant health. In the present study, ZnO and TiO_2 nanoparticle treatments enhance photosynthetic pigments (chlorophyll a, b, and carotenoids) and antioxidant compounds (ascorbic acid). These changes collectively contribute to improved photosynthetic efficiency, photoprotection, and stress tolerance, ultimately leading to enhanced growth and biomass production in selected cultivars. Consistently, the highest ascorbic acid was noted in 50 ppm TiO_2 (3.95 mg/g fresh leaf) treatment, while 25 ppm ZnO (2.45 mg/g fresh leaf)

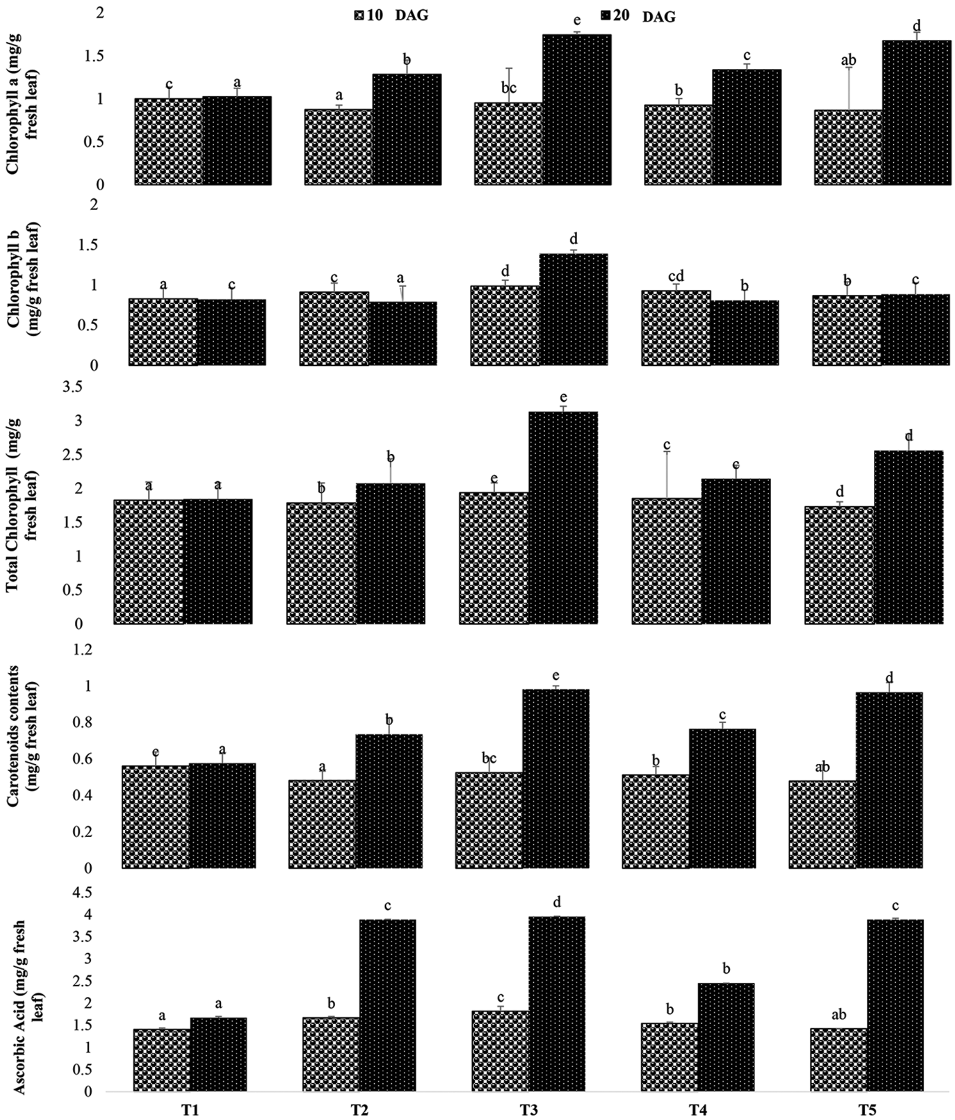

Figure 4. Effectiveness of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on chlorophyll a, b, and total chlorophyll, carotenoids, and ascorbic acid contents (mg/g fresh leaf) of *Cicer arietinum L.* cultivars. Mean± standard deviation of three replicates is shown by thin vertical bars. T1 is the control, T2 is 25 parts per million (ppm) TiO_2, T3 is 50 ppm TiO_2, T4 is 25 ppm ZnO, and T5 is 50 ppm ZnO.
Note: Values within same letter are not significantly different $p<0.05$, according to Duncan's multiple range test.
Abbreviation: DAG: Days after germination.

showed the least value of ascorbic acid content compared to the control (Figure 4). As plants matured, their physiological processes progressed, resulting in higher ascorbic acid content at 20 DAG compared to 10 DAG.

4. Conclusion

The global rising population and environmental stresses are contributing to agricultural losses and food crises. At present, environmental stresses and their mitigations are the main research objectives in the agriculture sector. Nanotechnology emerges as one of the best tools for agricultural sustainability. It improves crop protection, nutrient transport, and soil management, forming the basis of plant growth and yield production strategies. Therefore, the present study was conducted to assess the effectiveness of ZnO and TiO_2 nanoparticles on the germination and growth of chickpeas. The result of the study showed that TiO_2 is more effective in promoting seed germination than ZnO nanoparticles. Both nanoparticles showed positive effects on the growth and biomass of the plants, with 50 ppm treatments stimulating greater root elongation, shoot growth, and biomass than 25 ppm.

In conclusion, TiO_2 proved more effective than ZnO nanoparticles for seed germination, while both nanoparticles showed dose-dependent results for the growth and biomass of plants. This study aimed to evaluate the mechanisms and efficacy of nanoparticles in promoting seed germination. The findings suggest that nanotechnology could be a useful tool for sustainable agriculture, ultimately helping to reduce the global food crisis. In addition, the study concluded that field research is necessary to have a more comprehensive understanding of the impacts of nanoparticles on the growth, development, and health of agricultural plants.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Indra Jeet Chaudhary
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Investigation: All authors
Methodology: Anuradha Navnath Karale, Indra Jeet Chaudhary
Writing – original draft: All authors
Writing – review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All generated data are present within the manuscript.

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