RESEARCH PAPER

# Effect of zinc oxide nanoparticles and bioformulations on growth and biomass of hydroponically grown lettuce (Lactuca sativa L.)

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## ABSTRACT

Hydroponic cultivation offers precise nutrient management and accelerated growth cycles for leafy vegetables such as lettuce (Lactuca sativa L.). Zinc oxide nanoparticles (ZnO NPs) and microbial bioformulations have gained considerable attention for their potential to enhance crop growth. However, their combinatorial effects in hydroponic production systems remain poorly characterized. A greenhouse experiment was conducted during January-April 2026 at Jaya Farm, Bengaluru, Karnataka, under Nutrient Film Technique (NFT) hydroponics. The experiment was laid out in a Completely Randomized Design (CRD) with nine treatments and three replications. Treatments included individual and combined foliar applications of ZnO nanoparticles (50 and 100 ppm) and bioformulations. The treatments are $T_1$: Control, $T_2$: ZnO NPs at 50 ppm, $T_3$: ZnO NPs at 100 ppm, $T_4$: Azadirachtin at 1% + Bacillus siamensis, $T_5$: Azadirachtin at 1% + Beauveria bassiana, $T_6$: ZnO NPs at 50 ppm + Azadirachtin at 1% + Bacillus siamensis, $T_7$: ZnO NPs at 50 ppm + Azadirachtin at 1 % + Beauveria bassiana, $T_8$: ZnO NPs at 100 ppm + Azadirachtin at 1 % + Bacillus siamensis and $T_9$: ZnO NPs at 100 ppm + Azadirachtin at 1 % + Beauveria bassiana. Growth parameters including plant height, leaf characters, leaf area, leaf area index, fresh weight and total dry matter production were recorded at harvest. Among all treatments, $T_9$ (ZnO NPs 100 ppm + azadirachtin 1 % + Beauveria bassiana) outperformed all others, recording improvements over the control ($T_1$) of approximately 24.6 % in plant height, 119.2 % in leaf area, 116.7 % in leaf area index, 22.0 % in fresh weight, and 47.1 % in total dry matter production. The improved performance of $T_9$ may be associated with the combined applicationvof nano-zinc supplementation and microbial bioformulations. The results suggest that integration of ZnO NPs (100 ppm), azadirachtin and Beauveria bassiana enhanced lettuce growth and biomass production under NFT hydroponic cultivation. Futher validation across seasons and locations is needed before commercial recommendation.

**Keywords:** Azadirachtin; Bacillus siamensis; Beauveria bassiana; foliar application; nutrient film technique; plant growth promotion

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## INTRODUCTION

Lettuce (Lactuca sativa L.) is one of the most economically significant leafy vegetables in hydroponic production systems, valued for its high content of vitamins A, C, and K, folate, and dietary antioxidants [1]. Hydroponic systems have emerged as viable platforms for year-round, controlled-environment lettuce production, particularly under conditions of limited arable land and urbanization pressures [2]. The soilless nature of hydroponics allows precise management of nutrient concentrations, pH, and environmental conditions, resulting in accelerated growth cycles and enhanced resource-use efficiency compared to conventional agriculture [3, 4].

Micronutrients play a pivotal role in regulating plant metabolic processes, among which zinc (Zn) is essential for enzymatic reactions, protein synthesis, phytohormone regulation and chlorophyll biosynthesis [5]. Conventional zinc fertilizer often exhibits limited utilization efficiency because of poor absorption and rapid transformation into less available forms. Zinc oxide nanoparticles (ZnO NPs) offer superior bioavailability owing to their nanoscale dimensions, which facilitate leaf penetration and provide a greater reactive surface area for Zn ion release [6,7]. Previous studies have demonstrated that ZnO nanoparticles can improve photosynthetic efficiency, antioxidant defence, nutrient utilization and biomass accumulation in several crop species. However, plant responses depend on nanoparticle concentration, application method and environmental conditions and the optimum strategy for hydroponically grown leafy vegetables remains insufficiently understood.

Alongside nanotechnology, biological plant growth-promoting formulations have received increasing attention as environmentally sustainable alternatives to synthetic agrochemicals. Bioformulations derived from naturally occurring compounds and microorganisms have garnered attention for their capacity to modulate plant growth and suppress phytopathogens [8, 9]. Bacillus siamensis is a plant growth-promoting rhizobacterium (PGPR) producing indole-3-acetic acid (IAA), siderophores, and phosphate-solubilizing enzymes that enhance nutrient uptake and plant biomass [10]. Beauveria bassiana, an entomopathogenic fungus, upon endophytic colonization, promotes systemic resistance and stimulates growth via production of gibberellin-like compounds and auxins [11]. Azadirachtin, a limonoid extracted from neem (Azadirachta indica), possesses documented growth regulatory effects through modulation of phytohormone pathways.

Although ZnO nanoparticles and biological growth-promoting formulations have individually demonstrated beneficial effects on plant growth, most previous investigations have evaluated them independently, predominantly under soil-based cultivation systems. Information regarding their combined application in hydroponic production systems is scarce, particularly in leafy vegetables such as lettuce. Furthermore, the potential complementary effects of nano-enabled zinc nutrition and microbial bioformulations on lettuce growth and biomass production under NFT hydroponics have not been adequately investigated.

The present study addresses this research gap by evaluating the individual and combined foliar application of ZnO nanoparticles (50 and 100 ppm) with azadirachtin-based bioformulations containing Bacillus siamensis and Beauveria bassiana under NFT hydroponic cultivation. It was hypothesized that the integration of ZnO nanoparticles with bioformulations would enhance lettuce growth and biomass production more effectively than other treatment applied individually.

## MATERIALS AND METHODS

A greenhouse experiment was conducted during January-April 2026 at Jaya Farm, Galipooje, Doddaballapura Taluk, Bengaluru, Karnataka, under controlled environmental conditions using a Nutrient Film Technique (NFT) hydroponic system. Romaine lettuce (Lactuca sativa L. var. longifolia) seeds (NS 10412, Namdhari Seeds Pvt. Ltd.) were sown in cocopeat-filled trays and transplanted at the 3-4 leaf stage into PVC NFT channels with a spacing of 30 × 20 cm. The nutrient solution was prepared following Hoagland and Arnon [12], by mixing potassium nitrate, calcium nitrate, magnesium sulphate and monopotassium phosphate. The solution had [N: 210 ppm, P: 31 ppm, K: 235 ppm, Ca: 200 ppm, Mg: 48 ppm, Fe: 5 ppm, Zn: 0.5 ppm]. Nutrient solution from a 5000 L reservoir was continuously circulated through food-grade PVC channels using a submersible pump, maintaining pH 6.5 and EC 1.8 dS $m^{-1}$ throughout the crop growth period [16]. Each treatment plot contained two NFT rows, resulting in 18 rows per replication and a total of 54 rows in the entire experiment (Fig. 1). The experiment was laid out in a Completely Randomized Design (CRD) with 9 treatments and 3 replications. The treatments were as follows: $T_1$: Control, $T_2$: ZnO NPs at 50 ppm, $T_3$: ZnO NPs at 100 ppm, $T_4$: Azadirachtin at 1% + Bacillus siamensis, $T_5$: Azadirachtin at 1% + Beauveria bassiana, $T_6$: ZnO NPs at 50 ppm + Azadirachtin at 1% + Bacillus siamensis, $T_7$: ZnO NPs at 50 ppm + Azadirachtin at 1% + Beauveria bassiana, $T_8$: ZnO NPs at 100 ppm + Azadirachtin at 1% + Bacillus siamensis and $T_9$: ZnO NPs at 100 ppm + Azadirachtin at 1% + Beauveria bassiana. Azadirachtin, Bacillus siamensis, and Beauveria bassiana were not included as fully independent single-component treatments in this design, since azadirachtin always co-occurred with one of the two microbial agents; this constrains the extent to which component-specific or interactive contributions can be statistically isolated (see Study Limitations). Zinc oxide nanoparticles (ZnO NPs) used in the study were synthesized at the Department of Electronic Engineering, SRM Institute of Science and Technology, Kattankulathur. The chemical precipitation method was followed for synthesizing ZnO nanoparticles. The nanoparticles possessed an average particle size of <100 nm, specific surface area of 65 $m^2$ $g^{-1}$, density of 5.606 g $cm^{-3}$, and a foliar suspension viscosity of 0.8872 cP. Comprehensive physicochemical characterization was performed and is presented in Fig. 2. Dynamic light scattering (DLS) analysis yielded a Z-average hydrodynamic diameter of 82.43 nm (PDI = 0.278; intercept = 0.721; result quality: Good) (Fig. 3.), with a single dominant peak at 6.964 d.nm (100% intensity), confirming that the particle population was predominantly within the nanoscale range with acceptable dispersion characteristics. Scanning electron microscopy (SEM) revealed predominantly quasi-spherical to irregular particles occurring as clustered nanoparticle assemblies with moderate aggregation, a morphology consistent with commercial ZnO nanoparticle formulations. Transmission electron microscopy (TEM) confirmed that primary particle dimensions were below 100 nm, with clustered aggregation typical of ZnO nanoparticles (Fig. 2.). The apparent particle dimensions observed in SEM are attributable to inter-particle aggregation and agglomeration; primary nanoscale dimensions are corroborated by the DLS hydrodynamic diameter and TEM measurements. X-ray diffraction (XRD) analysis (Fig. 3.) identified major diffraction peaks at approximately 2θ = 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, and 62.8°, corresponding to the hexagonal wurtzite crystalline structure of ZnO, with no major impurity peaks detected, thereby confirming crystalline phase identity and structural purity. The bioformulations, namely Bacillus siamensis and Beauveria bassiana, were synthesized and mass multiplied at the Central Laboratory, Department of Entomology, SRM College of Agricultural Sciences. Bacillus siamensis acts as a plant growth-promoting rhizobacterium, while Beauveria bassiana exhibits endophytic colonization and growth-promoting effects. Azadirachtin with botanical pesticidal and growth regulatory properties was procured from the Farm Store, SRMCAS. Foliar application of the treatments was imposed at 10 and 25 DAT. Growth observations including plant height (cm), leaf characters (leaf length, leaf breadth, number of leaves, plant spread), leaf area, leaf area index, SPAD value, fresh weight and total dry matter production were recorded at harvest. The data were analysed statistically by Analysis of Variance (ANOVA) using R software (R version 4.3.0) and treatment means were compared using critical difference (CD) at the 5% level of probability [13]. Although the nine treatments reflect a 3 × 3 arrangement of ZnO nanoparticle level (0, 50, 100 ppm) and bioformulation package (none, azadirachtin + Bacillus siamensis, azadirachtin + Beauveria bassiana), treatments were analysed as nine discrete levels of a single factor rather than as a two-factor factorial; consequently, the formal statistical interaction between ZnO nanoparticle level and bioformulation package was not partitioned or tested in the current analysis, and comparisons among treatment combinations should be interpreted descriptively rather than as evidence of a tested interaction. Tests of ANOVA assumptions (normality of residuals and homogeneity of variance) were not explicitly reported; explicit assumption testing is recommended in future analyses.

**RESULTS**

Plant height differed significantly among the treatments at harvest stage. The highest plant height (35.27 cm) was recorded in Treatment $T_9$ (ZnO nanoparticles at 100 ppm + azadirachtin 1 % + Beauveria bassiana), whereas the lowest value (28.30 cm) was observed in $T_1$ (control) treatment (Fig. 4, Table 1).

The highest leaf length and leaf breadth (27.04 cm and 16.38 cm, respectively) were recorded in $T_9$ (ZnO nanoparticles at 100 ppm + azadirachtin 1 % + Beauveria bassiana), while the lowest values (20.42 cm and 9.91 cm) were observed in $T_1$ (control) (Fig. 4, Table 1).

Plant spread was significantly influenced by the treatments. The maximum plant spread (34.05 cm) was recorded in $T_9$ (ZnO nanoparticles at 100 ppm + azadirachtin 1 % + Beauveria bassiana), whereas the minimum (27.36 cm) was recorded in $T_1$ (Fig. 4, Table 1).

$T_9$ (ZnO nanoparticles at 100 ppm + azadirachtin 1% + Beauveria bassiana) recorded the highest leaf area (310.61 cm² plant⁻¹) and leaf area index (0.52), while the lowest values (141.73 cm² plant⁻¹ and 0.24, respectively) were recorded in $T_1$ (control) (Fig. 4, Table 2).

Fresh weight was significantly influenced by the treatments, the highest value of fresh weight (268.75 g plant⁻¹) was recorded in $T_9$ whereas the lowest (220.32 g plant⁻¹) was observed in $T_1$ (control) (Table 3, Fig. 4).

Total dry matter production differed significantly among the treatments. $T_9$ (ZnO nanoparticles at 100 ppm + azadirachtin 1 % + Beauveria bassiana) recorded the highest TDMP (18.80 g plant⁻¹), whereas the lowest value (12.78 g plant⁻¹) was recorded in $T_1$ (control) (Fig. 4, Table 3).

SPAD values, an index of relative leaf chlorophyll content, were highest in $T_9$ (ZnO nanoparticles at 100 ppm + azadirachtin 1 % + Beauveria bassiana), whereas the lowest value was observed in the T1 control treatment (Table 2 and Fig. 5.).

**DISCUSSION**

The taller plant height was observed in T9 (ZnO nanoparticles at 100 ppm + azadirachtin 1% + Beauveria bassiana) may be associated with improved zinc availability following foliar application of ZnO nanoparticles. Zinc has been reported to participate in auxin biosynthesis, including the conversion of tryptophan to indole-3-acetic acid (IAA), which is associated with cell elongation in meristematic tissue [14]. Beauveria bassiana has been reported to produce gibberellin-like metabolites during endophytic colonization, which may further contribute to the stem elongation observed [11]. However, hormonal and enzymatic activity were not directly evaluated in the present study, these explanations remain interpretive and should be confirmed through targeted biochemical or molecular analyses in future work.

The increased leaf length and leaf breadth observed in T9 may be associated with the role of zinc RNA polymerase activity and ribosomal protein synthesis, which are involved in cell division and differentiation [5]. Adequate zinc supply has also been reported to maintain membrane integrity and support leaf cell expansion. Azadirachtin has been described in earlier studies as influencing cytokinin-like signalling pathways, thereby promoting lateral cell proliferation [7]. Since these biochemical and hormonal responses were not directly measured in the present study, the proposed mechanism remains hypothetical.

The greater plant spread recorded in T9 may be associated with improved canopy development resulting from enhanced nutrient availability. Zinc has been reported to activate carbonic anhydrase, an enzyme involved in $CO_2$ hydration during photosynthesis, while Bacillus siamensis-based bioformulations have been reported to improve phosphorus and potassium uptake [8, 9].

The higher leaf area and leaf area index recorded in T9 may be attributed with the influence of ZnO nanoparticles on chloroplast development and photosynthesis-related processes reported in earlier studies [7]. A larger photosynthetically active surface may favour greater interception of solar radiation and biomass accumulation. Furthermore, endophytic colonization Beauveria bassiana has been associated with improved stomatal regulation and water-use efficiency [17], which may have supported sustained leaf expansion.

The increased fresh weight observed in T9 may be due to improved plant water relations and nutrient availability. Zinc contributes to membrane stability and aquaporin function, which may facilitate water movement within plant tissues [2, 15]. The continuous nutrient film in the NFT system ensured a continuous supply of water and nutrients, while azadirachtin has been reported to influence stomatal behaviour, which may have contributed to water retention [11].

The higher total dry matter production recorded in T9 might be due to improved zinc availability from ZnO nanoparticles and enhanced nutrient acquisition through microbial bioformulations.

Zinc has been reported to activate several metalloenzyme involved in glycolysis and the tricarboxylic acid cycle, thereby supporting biomass production [6]. Beauveria bassiana endophytic colonization has been associated with increased root exudate production and nutrient acquisition in previous reports [7, 11], which may have additionally supported dry matter accumulation. The combined application of ZnO nanoparticles and bioformulation components produced the greatest combined treatment effect on total dry matter production observed in this study; because azadirachtin, Bacillus siamensis and Beauveria bassiana were not tested as single-component treatments independent of one another, the relative contribution of each component and the presence of a true statistical interaction could not be determined.

The higher SPAD value observed in T9 attributed with the reported role of zinc in maintaining chloroplast integrity and activating photosynthetic enzymes such as Rubisco and superoxide dismutase (SOD) [5, 14]. Zinc-mediated nitrate reductase activity has also been reported to support nitrogen assimilation and chlorophyll precursor availability. Beauveria bassiana has been reported to delay chlorophyll degradation through secondary metabolite production [11]. Since these biochemical and physiological assays were outside the scope of this study, all physiological interpretations below are based on associations with previously published literature and are not direct measurements obtained in this experiment; they should be regarded as hypotheses for future targeted investigation rather than confirmed mechanisms.

**Study Limitations**

This study was conducted as a single growing season (January–April 2026), at one location (Jaya Farm, Bengaluru, Karnataka), using a single lettuce cultivar (Romaine lettuce, NS 10412) under a Nutrient Film Technique (NFT); therefore, validation across multiple seasons, locations, cultivars and hydroponic systems is required.

- Physiological interpretations regarding hormonal, enzymatic, and gene-expression mechanisms are based on prior literature and were not directly measured in this study; targeted biochemical and molecular assays are required to confirm these proposed mechanisms.
- The individual effects of azadirachtin, Bacillus siamensis and Beauveria bassiana could not be distinguished because they were not evaluated as separate treatment components.
- The experiment included three replications per treatments and future studies with larger sample sizes would improve the robustness of the findings.
- With three replications per treatment across nine treatments, the experiment had a relatively modest sample size, which may limit statistical power to detect smaller treatment differences.

**CONCLUSION**

Under the specific conditions of this single-season greenhouse trial, combined foliar application of ZnO nanoparticles at 100 ppm with azadirachtin 1% and Beauveria bassiana ($T_9$) resulted in the greatest increases in growth and biomass parameters of hydroponically grown Romaine lettuce relative to the untreated control, with improvements ranging from approximately 22 % to 119 % across the measured parameters, the largest relative gains being observed in leaf area and leaf area index. These findings indicate that combined application of nano-zinc and bioformulation-based treatments has the potential to enhance lettuce productivity under NFT hydroponic conditions. However, because azadirachtin, Bacillus siamensis, and Beauveria bassiana were not evaluated as independent single-component treatments, the relative contribution of each component, and whether their combined effects reflect additive or genuinely interactive responses, could not be determined from the present design. The physiological mechanisms proposed to explain these growth responses are based on previously published literature rather than direct biochemical measurement in this study and require confirmation through targeted assays. Given that this trial was conducted at a single location, in a single season, using a single lettuce cultivar and hydroponic configuration, the findings should be regarded as preliminary.

**REFERENCES**

1. Murray JJ, Basset G, Sandoya G. Nutritional benefits of lettuce consumed at recommended portion sizes. EDIS. 2021;2021(3). https://doi.org/10.32473/edis-hs1416-2021.

2. Rajendran S, Domalachenpa T, Arora H, Li P. Hydroponics: exploring innovative sustainable technologies and applications across crop production. Heliyon. 2024;10:e26823. https://doi.org/10.1016/j.heliyon.2024.e26823.

3. Swain A, Chatterjee S, Viswanath M, Roy A. Hydroponics in vegetable crops: a review. Pharma Innov J. 2021;10:629–634.

4. Tuxun A, Xiang Y, Shao Y, Son JE, Yamada M, Yamada S, et al. Soilless cultivation: precise nutrient provision and growth environment regulation under different substrates. Plants. 2025;14(14):2203. https://doi.org/10.3390/plants14142203.

5. Hamzah Saleem M, Usman K, Rizwan M, Al Jabri H, Alsafran M. Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. Front Plant Sci. 2022;13:1033092. https://doi.org/10.3389/fpls.2022.1033092.
6. Yadav A, Yadav K, Abd-Elsalam KA. Exploring the potential of nanofertilizers for sustainable agriculture. Plant Nano Biol. 2023;5:100044. https://doi.org/10.1016/j.nanob.2023.100044.
7. Francisco M de, Fernandes-Silva P, Durães L, Romeiro A, Álvarez-Torrellas S, Almendros P. Influence of the application of different zinc oxide nanoparticles on a lettuce crop grown in an acidic Mediterranean soil. Horticulturae. 2024;10:681. https://doi.org/10.3390/horticulturae10070681.
8. Stegelmeier AA, Rose DM, Joris BR. The use of PGPB to promote plant hydroponic growth. Plants. 2022;11:2783. https://doi.org/10.3390/plants11212783.
9. Ikiz B, Dasgan HY, Gruda NS. Utilizing the power of plant growth promoting rhizobacteria on reducing mineral fertilizer, improved yield, and nutritional quality of Batavia lettuce in a floating culture. Sci Rep. 2024;14:1616. https://doi.org/10.1038/s41598-024-51818-w.
10. Artyszak A, Gozdowski D. The effect of growth activators and plant growth-promoting rhizobacteria (PGPR) on the soil properties, root yield, and technological quality of sugar beet. Agronomy. 2020;10:1262. https://doi.org/10.3390/agronomy10091262.
11. Vogelmann ES, Júnior JB, Tiruneh GA. Bio-product as a nutritional approach for sustainable hydroponic lettuce cultivation. Discov Appl Sci. 2025;7:881.
12. Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. Circ Calif Agric Exp Stn. 1940;347:1–32.
13. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. 2nd ed. New York: John Wiley & Sons; 2010. 680 p.
14. Song U, Kim J. Zinc oxide nanoparticles: a potential micronutrient fertilizer for horticultural crops with little toxicity. Hortic Environ Biotechnol. 2020;61:625–631. https://doi.org/10.1007/s13580-020-00254-2.
15. Zhang J, Gao F, Xie J, Li J, Wang C, Zhang X, Han K. Zinc oxide nanoparticles reduce cadmium accumulation in hydroponic lettuce (Lactuca sativa L.) by increasing photosynthetic capacity and regulating phenylpropane metabolism. Ecotoxicol Environ Saf. 2024;285:117033. https://doi.org/10.1016/j.ecoenv.2024.117033.
16. Yang T, Samarakoon U, Altland J, Ling P. Influence of electrical conductivity on plant growth, nutritional quality, and phytochemical properties of kale and collard grown using hydroponics. Agronomy. 2024;14:2704. https://doi.org/10.3390/agronomy14112704.
17. Macuphe N, Oguntibeju OO, Nchu F. Evaluating the endophytic activities of Beauveria bassiana on the physiology, growth, and antioxidant activities of extracts of lettuce (Lactuca sativa L.). Plants. 2021;10(6):1178. https://doi.org/10.3390/plants10061178.

Table 1. Mean leaf length (cm), leaf breadth (cm), plant height (cm), and plant spread (cm) of hydroponically grown lettuce at harvest, as affected by ZnO nanoparticle and bioformulation treatments. Values are means of three replications. SEd = standard error of difference between means; CD (5%) = critical difference at the 5% probability level.

| Treatment | Leaf length (cm) | Leaf breadth (cm) | Plant height (cm) | Plant spread (cm) |
|---|---|---|---|---|
| $T_1$- Control | 20.42 | 9.91 | 28.30 | 27.36 |
| $T_2$- ZnO Nanoparticle at 50 ppm | 22.31 | 10.66 | 29.80 | 28.15 |
| $T_3$- ZnO Nanoparticle at 100 ppm | 22.00 | 11.39 | 30.53 | 28.94 |
| $T_4$- Azadirachtin at 1 % + *Bacillus siamensis* | 22.84 | 11.82 | 31.17 | 29.73 |
| $T_5$- Azadirachtin at 1 % + *Beauveria bassiana* | 23.01 | 12.25 | 31.92 | 30.41 |
| $T_6$- ZnO Nanoparticle at 50 ppm + Azadirachtin at 1 % + *Bacillus siamensis* | 24.17 | 13.60 | 32.87 | 31.88 |
| $T_7$- ZnO Nanoparticle at 50 ppm + Azadirachtin at 1 % + *Beauveria bassiana* | 24.86 | 14.14 | 33.34 | 32.40 |
| $T_8$- ZnO Nanoparticle at 100 ppm + Azadirachtin at 1 % + *Bacillus siamensis* | 25.93 | 15.43 | 34.60 | 33.57 |
| $T_9$- ZnO Nanoparticle | 27.04 | 16.38 | 35.27 | 34.05 |

| | | | | |
|---|---|---|---|---|
| at 100 ppm + Azadirachtin at 1 % + *Beauveria bassiana* | | | | |
| Sed | **0.83** | **0.61** | **1.56** | **1.22** |
| CD (5%) | **1.74** | **1.27** | **3.26** | **2.55** |

Table 2. Mean leaf area (cm²), leaf area index, and SPAD value of hydroponically grown lettuce at harvest, as affected by ZnO nanoparticle and bioformulation treatments. Values are means of three replications. SEd = standard error of difference between means; CD (5%) = critical difference at the 5% probability level.

| Treatment | Leaf area ($cm^2$) | Leaf area index | SPAD |
|---|---|---|---|
| $T_1$- Control | 141.73 | 0.24 | 30.92 |
| $T_2$- ZnO Nanoparticle at 50 ppm | 166.52 | 0.28 | 32.27 |
| $T_3$- ZnO Nanoparticle at 100 ppm | 176.31 | 0.29 | 32.78 |
| $T_4$- Azadirachtin at 1 % + *Bacillus siamensis* | 189.63 | 0.32 | 34.43 |
| $T_5$- Azadirachtin at 1 % + *Beauveria bassiana* | 197.35 | 0.33 | 35.26 |
| $T_6$- ZnO Nanoparticle at 50 ppm + Azadirachtin at 1 % + *Bacillus siamensis* | 230.19 | 0.38 | 38.48 |
| $T_7$- ZnO Nanoparticle at 50 ppm + Azadirachtin at 1 % + *Beauveria bassiana* | 246.14 | 0.41 | 38.59 |
| $T_8$- ZnO Nanoparticle at 100 ppm + Azadirachtin at 1 % + *Bacillus siamensis* | 280.92 | 0.47 | 42.03 |
| $T_9$- ZnO Nanoparticle at 100ppm + Azadirachtin at 1 % + *Beauveria bassiana* | 310.61 | 0.52 | 44.81 |
| SEd | **11.71** | **0.02** | **2.62** |
| CD (5%) | **24.43** | **0.05** | **5.47** |

**Table 3.** Mean fresh weight (g plant⁻¹) and total dry matter production (g plant⁻¹) of hydroponically grown lettuce at harvest, as affected by ZnO nanoparticle and bioformulation treatments. Values are means of three replications. SEd = standard error of difference between means; CD (5%) = critical difference at the 5% probability level.

| Treatment | Fresh weight (g/plant) | Total dry matter production (g/plant) |
|---|---|---|
| $T_1$- Control | 220.32 | 12.78 |
| $T_2$- ZnO Nanoparticle at 50 ppm | 229.67 | 13.34 |
| $T_3$- ZnO Nanoparticle at 100 ppm | 232.86 | 13.70 |
| $T_4$- Azadirachtin at 1 % + *Bacillus siamensis* | 239.63 | 14.37 |
| $T_5$- Azadirachtin at 1 % + *Beauveria bassiana* | 244.26 | 14.65 |
| $T_6$- ZnO Nanoparticle at 50 ppm + Azadirachtin at 1 % + *Bacillus siamensis* | 251.38 | 16.34 |
| $T_7$- ZnO Nanoparticle at 50 ppm + Azadirachtin at 1 % + *Beauveria bassiana* | 259.52 | 16.87 |
| $T_8$- ZnO Nanoparticle at 100 ppm + Azadirachtin at 1 % + *Bacillus siamensis* | 266.23 | 18.12 |
| $T_9$- ZnO Nanoparticle at 100 ppm + Azadirachtin at 1 % + *Beauveria bassiana* | 268.75 | 18.80 |
| SEd | **11.77** | **0.47** |
| CD (5%) | **24.55** | **0.98** |

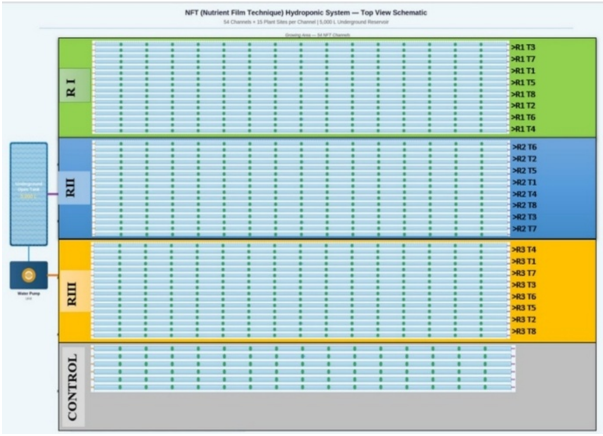
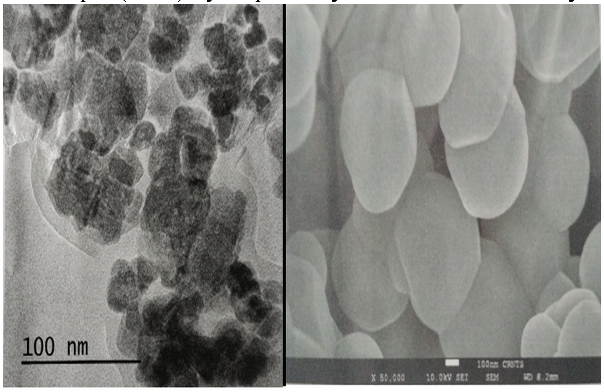



Fig. 1. Layout of the experimental Nutrient Film Technique (NFT) hydroponic system used in the study.




TEM Analysis of ZnO Nanoparticles. SEM Analysis of ZnO Nanoparticles.

Fig. 2. Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM) images of the synthesized zinc oxide nanoparticles (ZnO NPs), showing particle morphology and size.

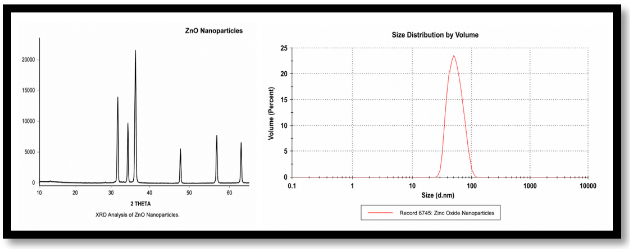



Fig. 3. Characterization of zinc oxide nanoparticles (ZnO NPs) used in the study. (A) X-ray diffraction (XRD) pattern showing characteristic diffraction peaks corresponding to the hexagonal wurtzite crystalline structure of ZnO, confirming phase purity and crystallinity. (B) Dynamic light scattering (DLS) particle size distribution indicating a mean hydrodynamic particle size of 82.43 nm with a polydispersity index (PDI) of 0.278, confirming the nanoscale nature of the particles.

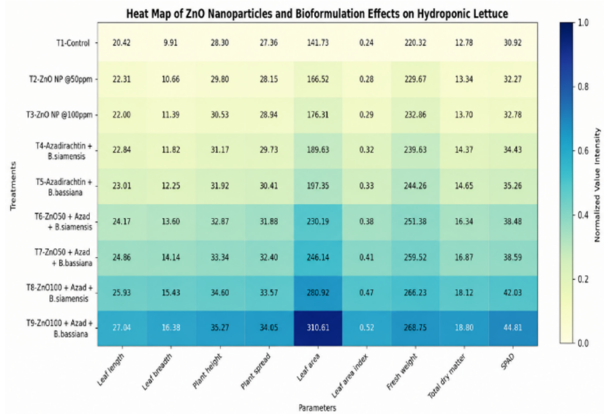



Fig. 4. Heat map illustrating the relative effect of ZnO nanoparticle and bioformulation treatments on leaf length, leaf breadth, plant height, plant spread, leaf area, leaf area index, fresh weight, total dry matter production, and SPAD value at harvest stage of hydroponically grown lettuce.

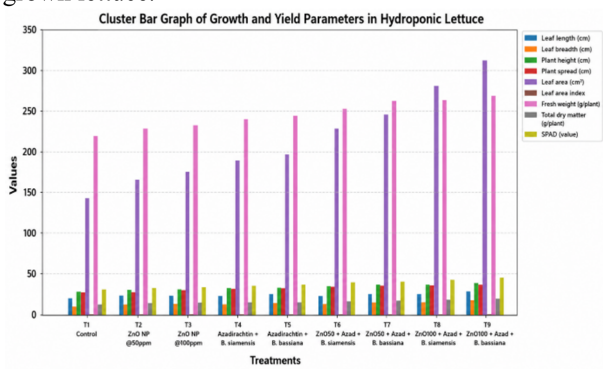



Fig. 5. Clustered bar graph comparing the effect of ZnO nanoparticle and bioformulation treatments on leaf length, leaf breadth, plant height, plant spread, leaf area, leaf area index, fresh weight, total dry matter production, and SPAD value at harvest stage of hydroponically grown lettuce. Error bars represent standard error of the mean.