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EFFECT OF NANOSTRUCTURES BIOSTIMULANTS AND BIOISOLATES ON MORPHOLOGICAL, PHYSIOLOGICAL AND ANATOMICAL CHARACTERISTICS OF BASIL (*OCIMUM* SPECIES)

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ABSTRACT
The present study evaluated the effects of nanostructured zinc-based nano-biostimulants and nano-bioisolates on the morphological, physiological, and anatomical characteristics of basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.). A factorial randomized complete block design (RCBD) was employed with two species and thirteen treatments, including control and three concentrations (25, 100, and 250 mg L^{-1}) of nano-biostimulants and nano-bioisolates, applied through soil drenching. Data were analyzed using Fisher's analysis of variance (ANOVA). Tukey's test was used to compare treatment means at a 5% probability level. The results demonstrated that all nano-enabled treatments significantly ($P \leq 0.05$) enhanced plant growth, physiological efficiency, and root anatomical development compared to the control. Among treatments, sodium nitrophenolate-based nano-biostimulants (SNP-Zn NPs) showed the highest effectiveness, resulting in maximum plant height (192.78 cm), branching (193.26), leaf area (400.58 cm^2), fresh biomass (489.83 g), and dry weight (169.64 g) in *O. basilicum*. Physiological parameters, including photosynthetic rate (24.21 µmol m^{-2} s^{-1}), stomatal conductance (400.58 mmol m^{-2} s^{-1}), transpiration rate (20.25 mmol m^{-2} s^{-1}), and chlorophyll content (49.47 µmol m^{-2}), were significantly improved under SNP-Zn NP treatments. Root anatomical traits such as epidermal thickness (4001.83 µm), aerenchyma cell area (2219.50 µm^2), metaxylem area (501.78 µm^2), and phloem area (24.21 µm^2) were also markedly enhanced, indicating improved structural and functional efficiency. Linalool-based nano-bioisolates ranked second, followed by eucalyptol-based formulations, whereas chlormequat chloride-based nano-biostimulants exhibited comparatively lower responses. Overall, *O. basilicum* showed higher absolute values, while *O. tenuiflorum* exhibited greater proportional responses. These findings highlight the potential of functionalized Zn NPs, particularly sodium nitrophenolate-based nano-biostimulants, for improving crop performance and advancing sustainable agricultural practices.

KEYWORDS: Nano-biostimulants, Nano-bioisolates, Zn NPs, Basil, *Ocimum basilicum* L., *Ocimum tenuiflorum* L., Plant physiology and Root anatomy

INTRODUCTION
Basil, a member of the *Ocimum* genus, is a treasured herb prized for its distinctive aroma and flavour (Dhama et al., 2023). Basil, a fragrant and aromatic herb, belongs to the family Lamiaceae and shares its botanical lineage with well-known plants such as mint and rosemary. This versatile herb has been cultivated by numerous cultures around the world for centuries. Across different societies, basil has found diverse applications, making it an integral component of traditional culinary and medicinal practices. It is widely used in the cosmetic industry owing to its rich content of essential oils and bioactive compounds such as linalool, eugenol, and methyl chavicol. In addition to its industrial importance, basil is recognized for its antioxidant, antimicrobial, and therapeutic properties, making it a valuable crop in both agricultural and medicinal sectors (Zlotek et al., 2021; Ahmed et al., 2022). However, the productivity and quality of basil are often influenced by environmental stresses, nutrient limitations, and inefficient management practices, which necessitate the development of innovative and sustainable approaches to enhance plant growth and performance (Arinaitwe et al., 2025; Shahrajabian et al., 2020).
The basil genus encompasses various plant species, including *Ocimum basilicum* L. (sweet basil), *Ocimum tenuiflorum* L. (holy basil), and others, each possessing distinct flavour profiles, aromas, and health benefits (Gavric et al., 2023). Holy basil is characterized by its narrow, green leaves with a slightly serrated edge. This plant may exhibit a more upright and compact growth habit compared to other basil species. Sweet basil shows a wide range of physical

differences, including variations in leaf size, shape, color, and texture. It's global popularity has led to its widespread cultivation in many countries around the world (Kumar et al., 2023; Salehi et al., 2024).

In recent years, the application of nanotechnology in agriculture has gained significant attention as a promising strategy to improve crop productivity, nutrient use efficiency, and stress tolerance. Nanoparticles (NPs) possess unique physicochemical properties such as high surface area, enhanced reactivity, and controlled release behavior, which enable efficient interaction with plant systems (Khan et al., 2023). Among various nanomaterials, zinc (Zn) based NPs have been extensively studied due to their essential role in plant metabolism. Zn is a vital micronutrient involved in enzymatic activation, chlorophyll synthesis, protein metabolism, and regulation of plant growth hormones. The application of Zn NPs has been reported to enhance plant growth, photosynthetic activity, and nutrient uptake while improving tolerance against abiotic stresses (Dimkpa et al., 2022; Gupta et al., 2024).

The integration of nanotechnology with plant growth regulators and bioactive compounds has led to the development of nanostructured biostimulants, which offer a novel approach for sustainable crop production. Synthetic biostimulants such as sodium nitrophenolate (SNP) and chlormequat chloride (CCC) have been widely used to regulate plant growth, improve physiological processes, and enhance crop productivity. SNP is known to stimulate cell division, enzyme activity, and photosynthetic efficiency, whereas CCC acts as a growth retardant by inhibiting gibberellin biosynthesis and promoting compact plant growth (Rademacher, 2021; Zulfiqar et al., 2022). When combined with NPs, these compounds exhibit enhanced efficiency due to improved delivery and bioavailability at the cellular level.

In addition to synthetic biostimulants, plant-derived bioisolates such as linalool and eucalyptol have emerged as important natural compounds with significant biological activity. Linalool, an oxygenated monoterpene commonly found in basil essential oil (EO), is known for its antimicrobial, antioxidant, and growth-promoting properties. Similarly, eucalyptol (1,8-cineole), a major component of eucalyptus EO, exhibits strong bioactive and stress-mitigating effects in plants (Nazzaro et al., 2021; Santos et al., 2023). The incorporation of these bioisolates into NPs systems enhances their stability, bioavailability, and effectiveness, thereby improving plant physiological responses and overall performance.

Recent studies have demonstrated that nano-enabled formulations can significantly influence plant morphological, physiological, and anatomical characteristics. These effects include improved plant height, biomass accumulation, leaf area, photosynthetic rate, stomatal conductance, and internal tissue development (Elbanna et al., 2024; Khaliq et al., 2023). Furthermore, NPs based treatments have been reported to modify root anatomical structures, enhancing water and nutrient transport efficiency and improving plant adaptability under varying environmental conditions. Despite these advancements, limited research has been conducted on the combined effects of nanostructured synthetic biostimulants and plant-derived bioisolates on basil species, particularly with respect to their integrated impact on growth, physiology, and anatomical traits.

Therefore, the present study was conducted to evaluate the effects of nanostructured biostimulants and bioisolates loaded with Zn NPs on the morphological, physiological, and anatomical characteristics of basil (*Ocimum* species). This research aims to provide a comprehensive understanding of nano-enabled bio-stimulant and bio-isolates applications and their potential role in enhancing plant performance and sustainable agricultural production.

MATERIALS AND METHODS

This experiment was carried out at the Gardening Wing, Lalazar Nursery, University of Agriculture, Faisalabad, Pakistan, which is located at 31° 25′ 7.374″ N and 73° 4′ 44.7924″ E. In this experiment, four different nanostructured biostimulants and bioisolates loaded with Zn NPs were applied to two basil species, sweet basil (*Ocimum basilicum* L.) and holy basil (*Ocimum tenuiflorum* L.), using the soil drenching method. Basil seeds were procured from Mohibbat Company, Iran. The synthesis and preparation of nano-structured biostimulants and bioisolates were carried out in the Nano and Biomaterials Laboratory, Department of Chemistry, University of Agriculture, Faisalabad.

The experiment was designed using a factorial randomized complete block design (RCBD) with three replications. Two factors were included: Factor A consisted of two basil species, while Factor B comprised thirteen treatments. The treatments included control and three concentrations (25, 100, and 250 mg L^{-1}) of SNP-Zn NPs, CCC-Zn NPs, Linalool-Zn NPs, and Eucalyptol-Zn NPs. Each treatment was applied to both species in all replications. A total of 78 experimental plots were established, with each experimental unit consisting of ten plants. Seeds of both basil species were sown in earthen pots containing a mixture of loamy soil, farmyard manure, and sand. Proper irrigation and nursery management practices were followed to ensure uniform germination and healthy seedling growth. Seedlings were transplanted into the field after four to five weeks under well-prepared soil conditions.

The first application of treatments was performed two days after transplanting, followed by six applications at two-week intervals. Data were recorded prior to each application. Plants were grown under open field conditions using standard agronomic and cultural practices. Plants were spaced at 30 cm × 60 cm (plant-to-plant × row-to-row). Each plot measured 1.83 m × 3.05 m. Within each replication, two parallel strips were maintained for both basil species, and corresponding treatments were arranged side by side to facilitate direct comparison of species × treatment interactions under uniform environmental conditions.

Table 1. Treatments and concentration levels used in the experiment.

. Treatments	Levels
T_0	Control
T_1	25 mg L^{-1} SNP-Zn NPs
T_2	100 mg L^{-1} SNP-Zn-NPs
T_3	250 mg L^{-1} SNP-Zn NPs
T_4	25 mg L^{-1} CCC-Zn NPs
T_5	100 mg L^{-1} CCC-Zn NPs
T_6	250 mg L^{-1} CCC-Zn NPs
T_7	25 mg L^{-1} Linalool-Zn NPs
T_8	100 mg L^{-1} Linalool-Zn NPs
T_9	250 mg L^{-1} Linalool-Zn NPs
T_{10}	25 mg L^{-1} Eucalyptol-Zn NPs
T_{11}	100 mg L^{-1} Eucalyptol-Zn NPs
T_{12}	250 mg L^{-1} Eucalyptol-Zn NPs

Zn NPs

Zn NPs were synthesized using zinc nitrate $Zn(NO_3)_2$ as a precursor under controlled laboratory conditions. The precursor salt was dissolved in distilled water and continuously stirred at 700 rpm at 35°C to ensure complete dissolution. During the reaction, Zn ions underwent hydrolysis leading to the formation of zinc hydroxide, which upon drying at 45°C for 6-8 hours was converted into ZnO NPs. The synthesized NPs were collected and stored in airtight containers to maintain their stability and prevent contamination.

SNP-Zn NPs

For the preparation of SNP-Zn NPs, SNP was dissolved in distilled water under controlled temperature and stirring conditions. $Zn(NO_3)_2$ solution was gradually added to the mixture to facilitate controlled nucleation and NPs formation. Sodium sulfate was used as a stabilizing agent, and the pH was adjusted to near neutral conditions. The reaction mixture was stirred for an extended period, followed by centrifugation and drying at 45°C for 6-8 hours to obtain stable NPs.

CCC-Zn NPs

CCC-Zn NPs were prepared by dissolving CCC in distilled water under continuous stirring at 35°C. $Zn(NO_3)_2$ solution was added gradually to form Zn organic complexes, while sodium hydroxide was used to regulate pH conditions. Sodium sulfate was added to stabilize the system, and the mixture was stirred for several hours to ensure complete NPs formation. The final product was centrifuged and dried at 45°C for 6-8 hours before storage.

Linalool-Zn NPs

Fresh basil leaves were collected for the extraction of EO through hydro-distillation under controlled temperature conditions (95-100°C). The extracted EO was subjected to fractional distillation under reduced pressure to isolate linalool. The purified linalool was then used for the synthesis of Linalool-Zn NPs. Linalool was emulsified with Tween-20 in distilled water, followed by gradual addition of $Zn(NO_3)_2$ solution under continuous stirring. Sodium sulfate was added as a stabilizing agent, and pH was adjusted to neutral using sodium hydroxide. The reaction mixture was stirred, centrifuged, and dried at 45°C to obtain uniform NPs.

Eucalyptol-Zn NPs

Similarly, fresh eucalyptus leaves were used for essential EO distillation under reduced pressure. Eucalyptol-Zn NPs were synthesized by emulsifying eucalyptol with Tween-20 in distilled water, followed by dropwise addition of $Zn(NO_3)_2$ solution under continuous stirring. Sodium sulfate was used to stabilize the system, and pH was maintained at neutral using sodium hydroxide. The reaction mixture was centrifuged and dried at 45°C to obtain stable NPs.

METHODOLOGY OF ROOT ANATOMY

For anatomical analysis, root samples were collected from each treatment and preserved in 70% alcohol. Sections were prepared using standard procedures and stained with safranin and fast green. The anatomical characteristics of basil roots were examined by the following steps i.e., preservation of materials, sectioning of samples and staining by using standard procedure (Berhin et al., 2019). Microscopic observations were made to record anatomical parameters including epidermal thickness, aerenchyma cell area, metaxylem cell area, and phloem cell area.

Data were collected on morphological, physiological, and anatomical parameters using standard procedures. Morphological attributes included plant height, number of branches, number of leaves, leaf length, leaf width, leaf area, fresh weight, and dry weight. Physiological parameters such as photosynthetic rate, transpiration rate, and stomatal conductance were measured using an IRGA system (LCi-SD), while leaf chlorophyll content was determined using a digital chlorophyll meter (CCM-200 plus). The collected data were statistically analyzed using Statistix 8.1 software. Fisher's analysis of variance (ANOVA) technique was applied, and treatment means were compared using Tukey's test at a 5% probability level (Steel et al., 1997).

RESULTS

Morphological Parameter Plant Height (cm)

The plant height of both basil species (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.) was significantly influenced ($P \leq 0.05$) by nanostructured biostimulants and bioisolates loaded with Zn NPs. Analysis of variance indicated that species, treatments, and their interaction had a highly significant effect on plant height, reflecting the differential responsiveness of both species to nano-enabled formulations. Under control conditions, sweet basil exhibited a higher plant height (60.83 cm) compared to holy basil (56.67 cm). However, the application of Zn NPs significantly enhanced plant height in both species, demonstrating the strong growth-promoting potential of nano-biostimulants.

Among all treatments, SNP-Zn NPs showed the most pronounced effect on plant height, with a clear concentration-dependent increase. In sweet basil, the maximum plant height (192.78 cm) was recorded at 250 mg L^{-1}, followed by 174.03 cm at 100 mg L^{-1} and 151.25 cm at 25 mg L^{-1}. Similarly, in holy basil, the highest plant height (162.19 cm) was observed at 250 mg L^{-1}, followed by 141.06 cm and 128.86 cm at 100 and 25 mg L^{-1}, respectively.

Linalool-Zn NPs ranked as the second most effective treatment. In sweet basil, the highest plant height (180.83 cm) was recorded at 250 mg L^{-1}, followed by 162.39 cm and 138.61 cm at 100 and 25 mg L^{-1}, respectively. In holy basil, plant height reached 121.50 cm at the highest concentration, followed by 109.89 cm and 103.31 cm at lower concentrations.

Eucalyptol-Zn NPs also significantly improved plant height, although the effect was comparatively moderate. In sweet basil, plant height ranged from 157.44 cm at 25 mg L^{-1} to 172.11 cm at 250 mg L^{-1}. Similarly, in holy basil, the highest plant height (101.00 cm) was recorded at 250 mg L^{-1}, followed by 97.56 cm and 92.14 cm at lower concentrations.

CCC-Zn NPs showed the least stimulatory effect among all treatments. In sweet basil, plant height increased to 141.67 cm at 250 mg L^{-1}, while in holy basil, the maximum height recorded was 89.92 cm. The relatively lower response under CCC treatments may be attributed to its growth-retarding nature, which limits excessive cell elongation. Overall, the treatments followed a consistent trend in enhancing plant height, with SNP-Zn NPs showing the greatest effect, followed by linalool-Zn NPs, eucalyptol-Zn NPs, and CCC-Zn NPs. In all cases, sweet basil exhibited higher plant height compared to holy basil, indicating species-specific variation in response to nano-biostimulants.

Number of branches (count)

The number of branches in both basil species was significantly affected ($P \leq 0.05$) by nanostructured biostimulants and bioisolates loaded with Zn NPs. The interaction between species and treatments was also significant, indicating differential responsiveness of both species to the applied formulations. Under control conditions, sweet basil produced a higher number of branches (60.61) compared to holy basil (55.89). However, all Zn NPs treatments significantly enhanced branching in both species.

SNP-Zn NPs showed the highest increase in branch number with a clear concentration-dependent trend. In sweet basil, the maximum number of branches (193.26) was recorded at 250 mg L^{-1}, followed by 173.68 and 151.08 at 100 and 25 mg L^{-1}, respectively. Similarly, in holy basil, the highest number of branches (163.40) was observed at 250 mg L^{-1}, followed by 141.69 and 128.95 at lower concentrations.

Linalool-Zn NPs ranked second, significantly increasing branch number in both species. In sweet basil, the highest value (181.61) was recorded at 250 mg L^{-1}, followed by 160.46 and 137.54. In holy basil, the maximum number of branches (120.83) was observed at the highest concentration, followed by 109.30 and 102.77.

Eucalyptol-Zn NPs also improved branching, although the magnitude was moderate. In sweet basil, branch number ranged from 157.15 to 172.04 across concentrations, whereas in holy basil, it ranged from 91.71 to 101.67.

CCC-Zn NPs exhibited the lowest increase in branch number. In sweet basil, the maximum value (141.56) was recorded at 250 mg L^{-1}, while in holy basil, the highest value was 89.64. Overall, treatments followed a consistent trend of effectiveness: SNP-Zn NPs > linalool-Zn NPs > eucalyptol-Zn NPs > CCC-Zn NPs. Sweet basil consistently produced a higher number of branches than holy basil across all treatments.

Number of leaves per plant (count)

The number of leaves per plant was significantly influenced ($P \leq 0.05$) by Zn NPs treatments, with a significant interaction between species and treatments. Under control conditions, sweet basil produced 205.06 leaves, while holy basil produced 199.47 leaves. The application of nanostructured biostimulants resulted in a substantial increase in leaf number in both species.

SNP-Zn NPs produced the highest number of leaves across all treatments. In sweet basil, the maximum number of leaves (860.81) was recorded at 250 mg L^{-1}, followed closely by 841.92 and 833.72 at 100 and 25 mg L^{-1}, respectively. Similarly, in holy basil, leaf number reached 848.58 at the highest concentration, followed by 831.67 and 818.92.

Linalool-Zn NPs also significantly enhanced leaf production. In sweet basil, leaf number ranged from 825.69 to 851.58 across concentrations, while in holy basil, it ranged from 787.28 to 808.11. Eucalyptol-Zn NPs resulted in moderate increases. In sweet basil, the maximum number of leaves (799.22) was recorded at 250 mg L^{-1}, whereas in holy basil, it reached 779.64.

CCC-Zn NPs showed comparatively lower and inconsistent responses. In sweet basil, leaf number increased gradually with concentration, reaching 699.86 at 250 mg L^{-1}. However, in holy basil, the response was irregular, with the highest value (497.28) observed at 250 mg L^{-1}. Overall, SNP-Zn NPs were the most effective in enhancing leaf production, followed by linalool, eucalyptol, and CCC treatments. Sweet basil consistently produced more leaves than holy basil.

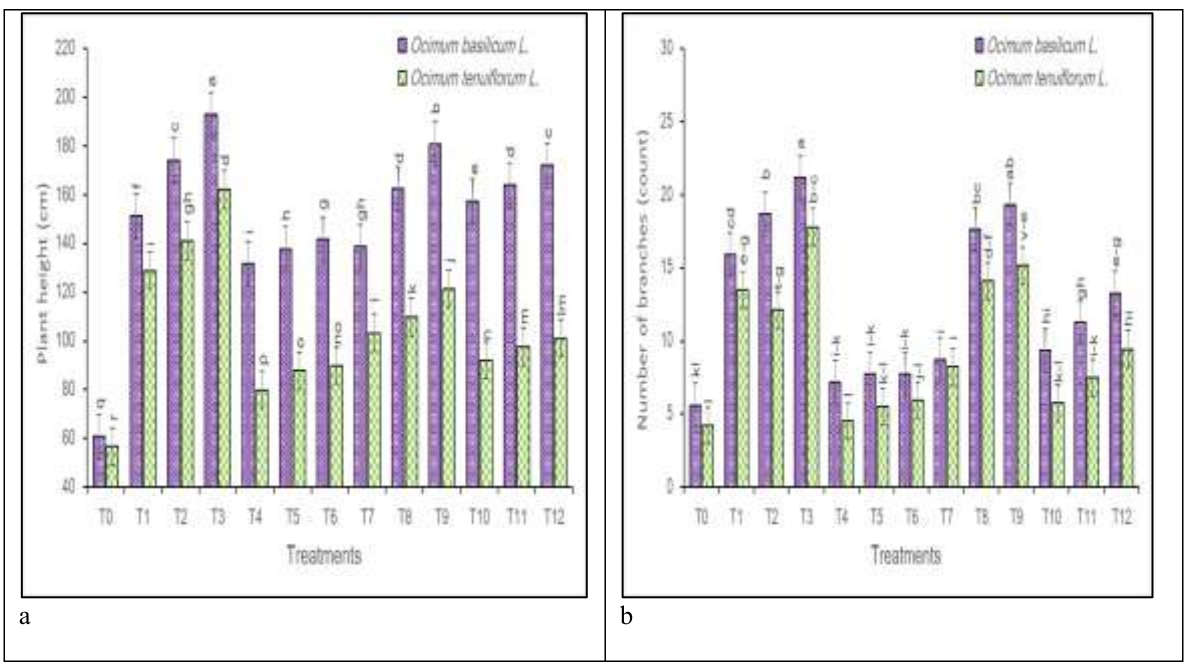
Leaf length (cm)

Leaf length was significantly affected ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, leaf length was 5.00 cm in sweet basil and 3.00 cm in holy basil. All treatments significantly increased leaf length in both species.

SNP-Zn NPs showed the highest increase. In sweet basil, maximum leaf length (10.00 cm) was recorded at 250 mg L^{-1}, followed by 9.00 and 8.00 cm. In holy basil, leaf length reached 9.00 cm at the highest concentration.

Linalool-Zn NPs also significantly improved leaf length. In sweet basil, leaf length ranged from 7.00 to 9.00 cm, while in holy basil, it ranged from 3.75 to 6.00 cm. Eucalyptol-Zn NPs produced moderate increases. In sweet basil, leaf length ranged from 5.50 to 7.67 cm, whereas in holy basil, it ranged from 4.00 to 4.33 cm.

CCC-Zn NPs showed the least improvement. In sweet basil, leaf length increased to 7.00 cm at the highest concentration, while in holy basil, responses were inconsistent. Overall, SNP-Zn NPs showed the greatest enhancement, followed by linalool, eucalyptol, and CCC treatments. Sweet basil exhibited longer leaves than holy basil across all treatments.



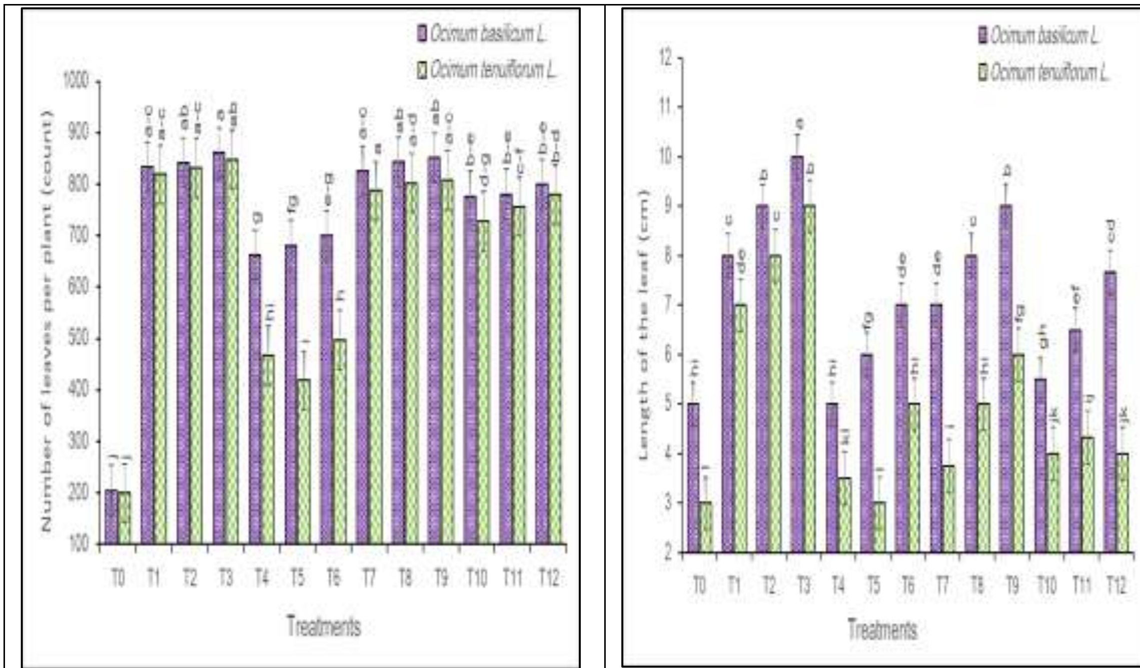


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Figure 1. Effect of different chemical treatments of two basil species (a)plant height, (b) number of branches, (c) number of leaves and (d) leaf length.

Leaf width (cm)

Leaf width was significantly influenced ($P \leq 0.05$) by nanostructured Zn NPs treatments and their interaction with species. Under control conditions, leaf width was 4.00 cm in sweet basil and 2.00 cm in holy basil. All treatments significantly increased leaf width in both species.

SNP-Zn NPs resulted in the highest increase. In sweet basil, leaf width reached 8.00 cm at 250 mg L^{-1}, while in holy basil, it reached 5.00 cm. Linalool-Zn NPs showed similar effectiveness. In sweet basil, leaf width ranged from 6.00 to 8.00 cm, whereas in holy basil, it ranged from 3.00 to 5.00 cm. Eucalyptol-Zn NPs produced moderate increases. In sweet basil, leaf width ranged from 4.50 to 6.51 cm, while in holy basil, the response was weaker, with a maximum of 3.00 cm.

CCC-Zn NPs showed the least and most variable response. In sweet basil, leaf width increased up to 7.00 cm, whereas in holy basil, responses were inconsistent across concentrations. Overall, treatments followed the order: SNP-Zn NPs > linalool-Zn NPs > eucalyptol-Zn NPs > CCC-Zn NPs. Sweet basil consistently exhibited greater leaf width than holy basil.

Total leaf area (cm²)

Total leaf area was significantly influenced ($P \leq 0.05$) by nanostructured Zn NPs treatments, with a significant interaction between species and treatments. Under control conditions, sweet basil exhibited a leaf area of 98.22 cm^2, whereas holy basil showed 80.78 cm^2. All treatments significantly enhanced leaf area in both species.

SNP-Zn NPs produced the highest leaf area. In sweet basil, the maximum value (400.58 cm^2) was recorded at 250 mg L^{-1}, followed by 366.69 and 380.03 cm^2 at 100 and 25 mg L^{-1}, respectively. Similarly, in holy basil, leaf area reached 384.64 cm^2 at the highest concentration, followed by 370.47 and 362.08 cm^2.

Linalool-Zn NPs ranked second, significantly increasing leaf area in both species. In sweet basil, leaf area ranged from 359.31 to 394.53 cm^2, while in holy basil, it ranged from 352.39 to 387.94 cm^2. Eucalyptol-Zn NPs also enhanced leaf area, although the response was moderate and non-linear. In sweet basil, the highest leaf area (386.75 cm^2) was observed at 100 mg L^{-1}, while in holy basil, the maximum (383.47 cm^2) was also recorded at the same concentration.

CCC-Zn NPs showed comparatively lower and variable responses. In sweet basil, leaf area increased gradually with concentration, reaching 300.81 cm^2 at 250 mg L^{-1}. In contrast, holy basil exhibited a decline at higher concentration, with the lowest value recorded at 250 mg L^{-1}. Overall, SNP-Zn NPs were the most effective in enhancing leaf area, followed by linalool, eucalyptol, and CCC treatments. Sweet basil consistently produced greater leaf area than holy basil.

Fresh weight (g)

Fresh biomass was significantly affected ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, sweet basil recorded 200.00 g fresh weight, while holy basil produced 59.44 g. The application of nanostructured biostimulants significantly increased fresh biomass in both species.

SNP-Zn NPs produced the highest fresh weight. In sweet basil, fresh weight increased from 450.83 g at 25 mg L^{-1} to 489.83 g at 250 mg L^{-1}. In holy basil, fresh weight reached 390.83 g at the highest concentration. Linalool-Zn NPs ranked second. In sweet basil, fresh weight ranged from 409.44 to 430.83 g, while in holy basil, it ranged from 308.31 to 330.97 g. Eucalyptol-Zn NPs showed moderate increases. In sweet basil, fresh weight ranged from 380.75 to 400.89 g, whereas in holy basil, it ranged from 270.75 to 299.36 g.

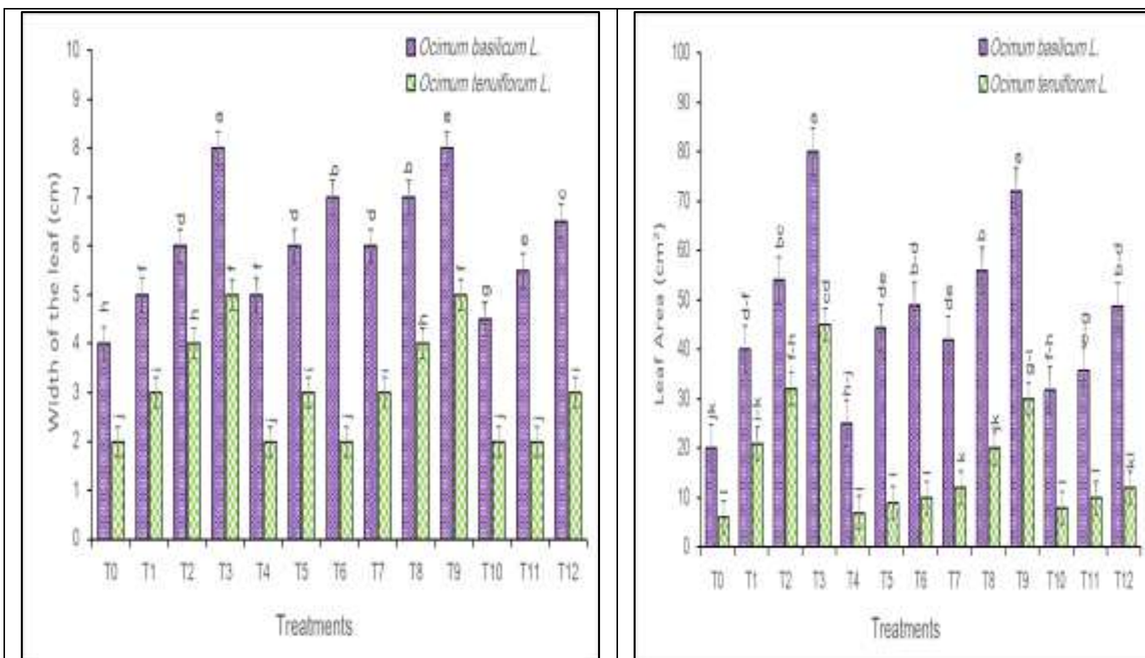
CCC-Zn NPs exhibited the lowest and most variable response. In sweet basil, fresh weight increased to 341.56 g at the highest concentration, while in holy basil, responses were inconsistent across concentrations. Overall, SNP-Zn NPs showed the greatest enhancement in fresh biomass, followed by linalool, eucalyptol, and CCC. Sweet basil consistently recorded higher fresh weight than holy basil.

Dry weight (g)

Dry weight was significantly influenced ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, dry weight was 59.11 g in sweet basil and 24.44 g in holy basil. All treatments significantly increased dry biomass in both species.

SNP-Zn NPs resulted in the highest dry weight. In sweet basil, dry weight reached 169.64 g at 250 mg L^{-1}, while in holy basil, it reached 70.00 g at the same concentration. Linalool-Zn NPs showed strong enhancement. In sweet basil, dry weight ranged from 118.89 to 139.58 g, whereas in holy basil, it ranged from 108.75 to 127.86 g. Eucalyptol-Zn NPs produced moderate increases. In sweet basil, dry weight ranged from 109.22 to 129.31 g, while in holy basil, it ranged from 47.44 to 60.72 g.

CCC-Zn NPs exhibited comparatively lower and species-dependent responses. In sweet basil, dry weight increased up to 141.67 g, whereas in holy basil, the increase was limited and less responsive to concentration changes. Overall, SNP-Zn NPs were the most effective in enhancing dry biomass, followed by linalool, eucalyptol, and CCC treatments. Sweet basil consistently accumulated higher dry weight than holy basil.



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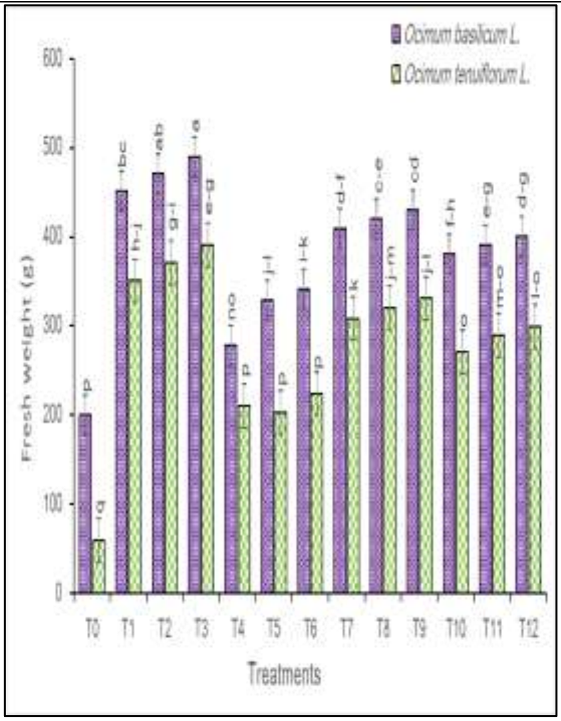

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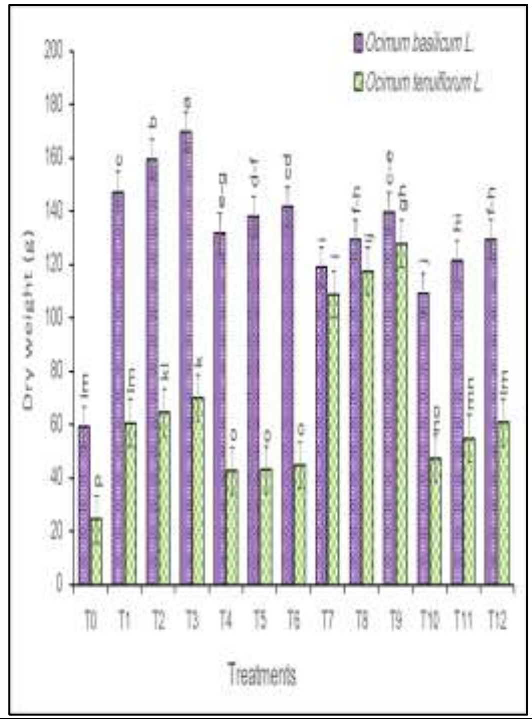

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Figure 2. Effect of different chemical treatments of two basil species, (a) width of leaf, (b) leaf area, (c) fresh weight and (d) dry weight.

Physiological Parameters

Photosynthetic rate (µmol m⁻² s⁻¹)

Photosynthetic rate was significantly influenced ($P \leq 0.05$) by nanostructured Zn NPs treatments, with a significant interaction between species and treatments. Under control conditions, sweet basil exhibited a higher photosynthetic rate (5.92 µmol m^{-2} s^{-1}) compared to holy basil (4.19 µmol m^{-2} s^{-1}). Application of Zn NPs significantly enhanced photosynthetic activity in both species. SNP-Zn NPs produced the highest photosynthetic rate. In sweet basil, the maximum value (24.21 µmol m^{-2} s^{-1}) was recorded at 250 mg L^{-1}, followed by 17.11 and 14.89 µmol m^{-2} s^{-1} at 100 and 25 mg L^{-1}, respectively. Similarly, in holy basil, the highest rate (19.17 µmol m^{-2} s^{-1}) was observed at the highest concentration, followed by 15.00 and 11.69 µmol m^{-2} s^{-1}.

Linalool-Zn NPs ranked second. In sweet basil, photosynthetic rate ranged from 14.08 to 23.67 µmol m^{-2} s^{-1}, while in holy basil, it ranged from 12.00 to 21.69 µmol m^{-2} s^{-1}. Eucalyptol-Zn NPs showed moderate improvements. In sweet basil, the maximum value (14.50 µmol m^{-2} s^{-1}) was observed at 100 mg L^{-1}, whereas in holy basil, the highest value (16.33 µmol m^{-2} s^{-1}) was recorded at 250 mg L^{-1}.

CCC-Zn NPs exhibited variable responses. In sweet basil, the highest value (22.25 µmol m^{-2} s^{-1}) was recorded at 100 mg L^{-1}, while in holy basil, the increase was comparatively low, with a maximum of 7.00 µmol m^{-2} s^{-1}. Overall, SNP-Zn NPs showed the greatest enhancement in photosynthetic rate, followed by linalool, eucalyptol, and CCC. Sweet basil consistently exhibited higher photosynthetic activity than holy basil.

Transpiration rate (mmol m⁻² s⁻¹)

Transpiration rate was significantly affected ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, transpiration rate was 3.86 mmol m^{-2} s^{-1} in sweet basil and 3.00 mmol m^{-2} s^{-1} in holy basil. SNP-Zn NPs produced the highest increase in transpiration rate. In sweet basil, the maximum value (8.42 mmol m^{-2} s^{-1}) was recorded at 250 mg L^{-1}, while in holy basil, a substantial increase was observed, reaching 20.25 mmol m^{-2} s^{-1} at the same concentration.

Linalool-Zn NPs also significantly increased transpiration. In sweet basil, transpiration rate increased up to 7.00 mmol m^{-2} s^{-1}, while in holy basil, it showed more than a twofold increase over control. Eucalyptol-Zn NPs showed moderate and concentration-dependent responses. In sweet basil, the highest values were observed at intermediate concentration, whereas in holy basil, maximum transpiration was recorded at the highest dose.

CCC-Zn NPs exhibited irregular responses. In sweet basil, an unusually high value was recorded at the lowest concentration, while other concentrations showed moderate increases. In holy basil, only slight increases were observed across treatments. Overall, SNP-Zn NPs produced the greatest increase in transpiration, followed by linalool, eucalyptol, and CCC treatments. Sweet basil showed higher absolute values, whereas holy basil showed greater proportional increases.

Stomatal conductance (mmol m^{-2} s^{-1})

Stomatal conductance was significantly influenced ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, stomatal conductance was 98.22 mmol m^{-2} s^{-1} in sweet basil and 80.78 mmol m^{-2} s^{-1} in holy basil. SNP-Zn NPs produced the highest increase in stomatal conductance. In sweet basil, the maximum value (400.58 mmol m^{-2} s^{-1}) was recorded at the highest concentration, while in holy basil, conductance values exceeded 360 mmol m^{-2} s^{-1} across treatments.

Linalool-Zn NPs showed similar effectiveness. In sweet basil, stomatal conductance reached 394.53 mmol m^{-2} s^{-1}, while in holy basil, it reached 387.94 mmol m^{-2} s^{-1}. Eucalyptol-Zn NPs resulted in moderate increases. In sweet basil, the highest values were observed at intermediate concentration, while in holy basil, increases were consistent across concentrations.

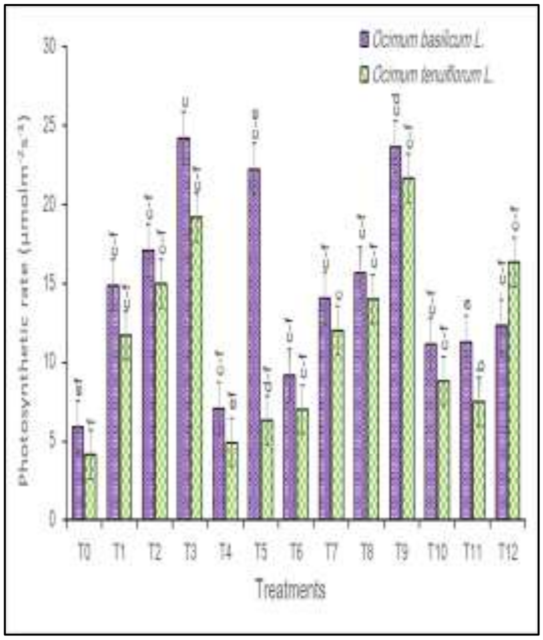
CCC-Zn NPs exhibited the lowest response. In sweet basil, conductance increased gradually, whereas in holy basil, only minor increases were observed. Overall, SNP-Zn NPs showed the greatest enhancement in stomatal conductance, followed by linalool, eucalyptol, and CCC. Sweet basil consistently exhibited higher conductance than holy basil.

Total leaf chlorophyll content (µmol m^{-2})

Total leaf chlorophyll content was significantly affected ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, chlorophyll content was 14.58 µmol m^{-2} in sweet basil and 10.86 µmol m^{-2} in holy basil. SNP-Zn NPs produced the highest chlorophyll content. In sweet basil, chlorophyll reached 49.47 µmol m^{-2} at 250 mg L^{-1}, followed by 47.50 and 45.69 µmol m^{-2} at lower concentrations. In holy basil, the maximum value (38.03 µmol m^{-2}) was recorded at the highest concentration.

Linalool-Zn NPs ranked second. In sweet basil, chlorophyll ranged from 44.11 to 47.97 µmol m^{-2}, while in holy basil, it ranged from 27.47 to 31.08 µmol m^{-2}. Eucalyptol-Zn NPs showed moderate increases. In sweet basil, chlorophyll ranged from 35.50 to 40.53 µmol m^{-2}, whereas in holy basil, it ranged from 19.08 to 22.00 µmol m^{-2}.

CCC-Zn NPs showed the lowest enhancement. In sweet basil, chlorophyll reached 36.75 µmol m^{-2}, while in holy basil, the maximum value was 17.72 µmol m^{-2}. Overall, SNP-Zn NPs were the most effective in enhancing chlorophyll content, followed by linalool, eucalyptol, and CCC treatments. Sweet basil consistently showed higher chlorophyll content than holy basil.



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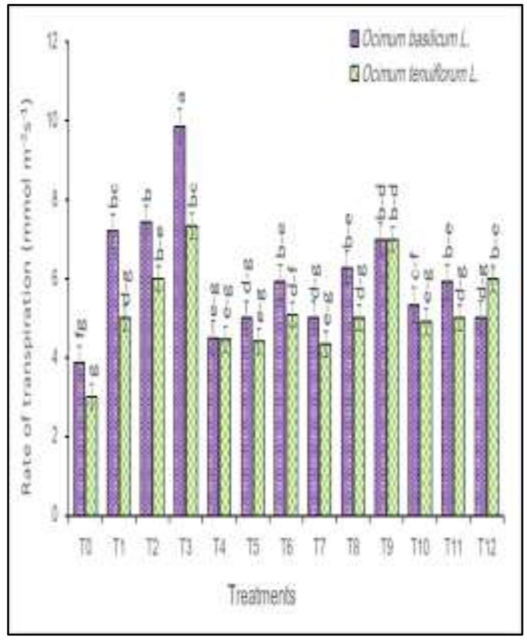

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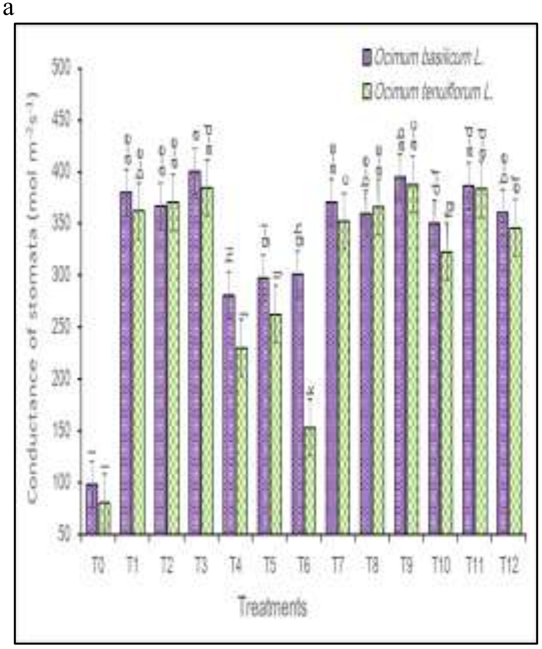

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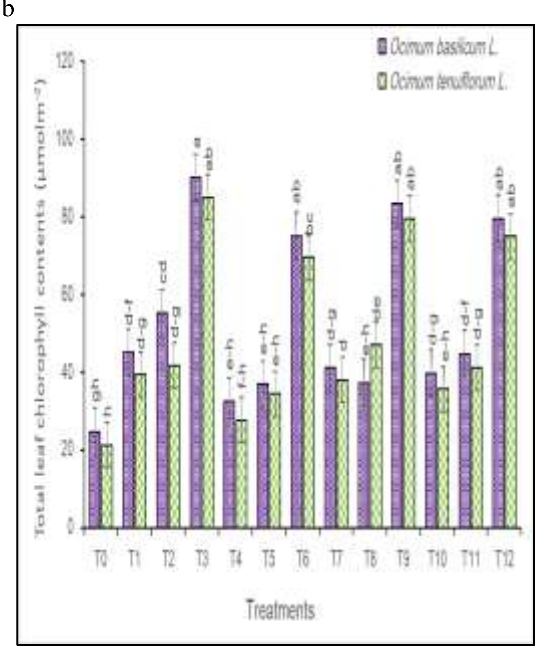

d

Figure 3. Effect of different chemical treatments of two basil species, (a) photosynthetic rate, (b)rate of transpiration, (c) stomatal conductance and (d) leaf chlorophyll contents.

Root Anatomical Parameters

Epidermal thickness (µm)

Root epidermal thickness was significantly influenced ($P \leq 0.05$) by nanostructured Zn NPs treatments, with a significant interaction between species and treatments. Under control conditions, sweet basil exhibited higher epidermal thickness (2002.44 µm) compared to holy basil (1501.78 µm). Application of Zn NPs significantly increased epidermal thickness in both species. SNP-Zn NPs produced the highest epidermal thickness. In sweet basil, thickness increased from 3401.22 to 4001.83 µm across increasing concentrations. Similarly, in holy basil, epidermal thickness reached 3002.11 µm at the highest concentration.

Linalool-Zn NPs ranked second. In sweet basil, epidermal thickness ranged from 3601.28 to 3800.81 µm, while in holy basil, it reached up to 3131.42 µm. Eucalyptol-Zn NPs showed moderate increases. In sweet basil, thickness ranged from 3201.47 to 3502.14 µm, whereas in holy basil, it ranged from 2301.86 to 2501.33 µm.

CCC-Zn NPs exhibited the lowest response. In sweet basil, thickness reached 3301.50 µm, while in holy basil, it increased up to 2351.50 µm. Overall, SNP-Zn NPs were the most effective in enhancing epidermal thickness, followed by linalool, eucalyptol, and CCC treatments. Sweet basil consistently exhibited higher epidermal thickness than holy basil.

Aerenchyma cell area (µm²)

Aerenchyma cell area was significantly affected ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, sweet basil recorded higher aerenchyma area (902.36 µm²) than holy basil (803.94 µm²). SNP-Zn NPs produced the highest increase. In sweet basil, aerenchyma area increased up to 2219.50 µm² at 250 mg L^{-1}, while in holy basil, it reached 1815.42 µm². Linalool-Zn NPs showed strong enhancement. In sweet basil, values ranged from 1751.69 to 1997.14 µm², whereas in holy basil, they reached up to 1751.81 µm². Eucalyptol-Zn NPs resulted in moderate increases. In sweet basil, values ranged from 1641.61 to 1705.56 µm², while in holy basil, they ranged from 1612.72 to 1651.47 µm².

CCC-Zn NPs exhibited comparatively lower increases. In sweet basil, aerenchyma area reached 1553.22 µm², whereas in holy basil, it reached 1503.08 µm². Overall, SNP-Zn NPs showed the greatest enhancement, followed by linalool, eucalyptol, and CCC. Sweet basil consistently exhibited larger aerenchyma area than holy basil.

Metaxylem cell area (µm²)

Metaxylem cell area was significantly influenced ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, sweet basil exhibited slightly higher metaxylem area (201.19 µm²) compared to holy basil (195.94 µm²). SNP-Zn NPs produced the highest increase. In sweet basil, metaxylem area reached 501.78 µm² at the highest concentration, while in holy basil, it reached 405.58 µm². Linalool-Zn NPs ranked second. In sweet basil, values ranged from 410.75 to 451.00 µm², while in holy basil, they reached up to 351.39 µm².

Eucalyptol-Zn NPs showed moderate increases. In sweet basil, values ranged from 385.22 to 407.44 µm², whereas in holy basil, they ranged from 290.81 to 330.58 µm². CCC-Zn NPs exhibited the lowest response. In sweet basil, metaxylem area reached 380.75 µm², while in holy basil, it reached 321.28 µm². Overall, SNP-Zn NPs showed the greatest enhancement in metaxylem area, followed by linalool, eucalyptol, and CCC treatments. Sweet basil consistently exhibited higher values than holy basil.

Phloem cell area (µm²)

Phloem cell area was significantly affected ($P \leq 0.05$) by Zn NPs treatments and their interaction with species. Under control conditions, sweet basil recorded slightly higher phloem area (3.86 µm²) than holy basil (3.00 µm²). SNP-Zn NPs produced the highest increase. In sweet basil, phloem area reached 24.21 µm² at the highest concentration, while in holy basil, it reached 19.17 µm². Linalool-Zn NPs also showed strong enhancement. In sweet basil, values reached 23.67 µm², whereas in holy basil, they reached 21.69 µm². Eucalyptol-Zn NPs resulted in moderate increases. In sweet basil, values reached 12.31 µm², while in holy basil, they increased up to 16.33 µm² at the highest concentration.

CCC-Zn NPs exhibited variable but lower responses. In sweet basil, the maximum value was recorded at intermediate concentration, while in holy basil, gradual increases were observed across treatments. Overall, SNP-Zn NPs showed the greatest enhancement in phloem area, followed by linalool, eucalyptol, and CCC treatments. Sweet basil exhibited higher absolute values, whereas holy basil showed greater proportional increases.

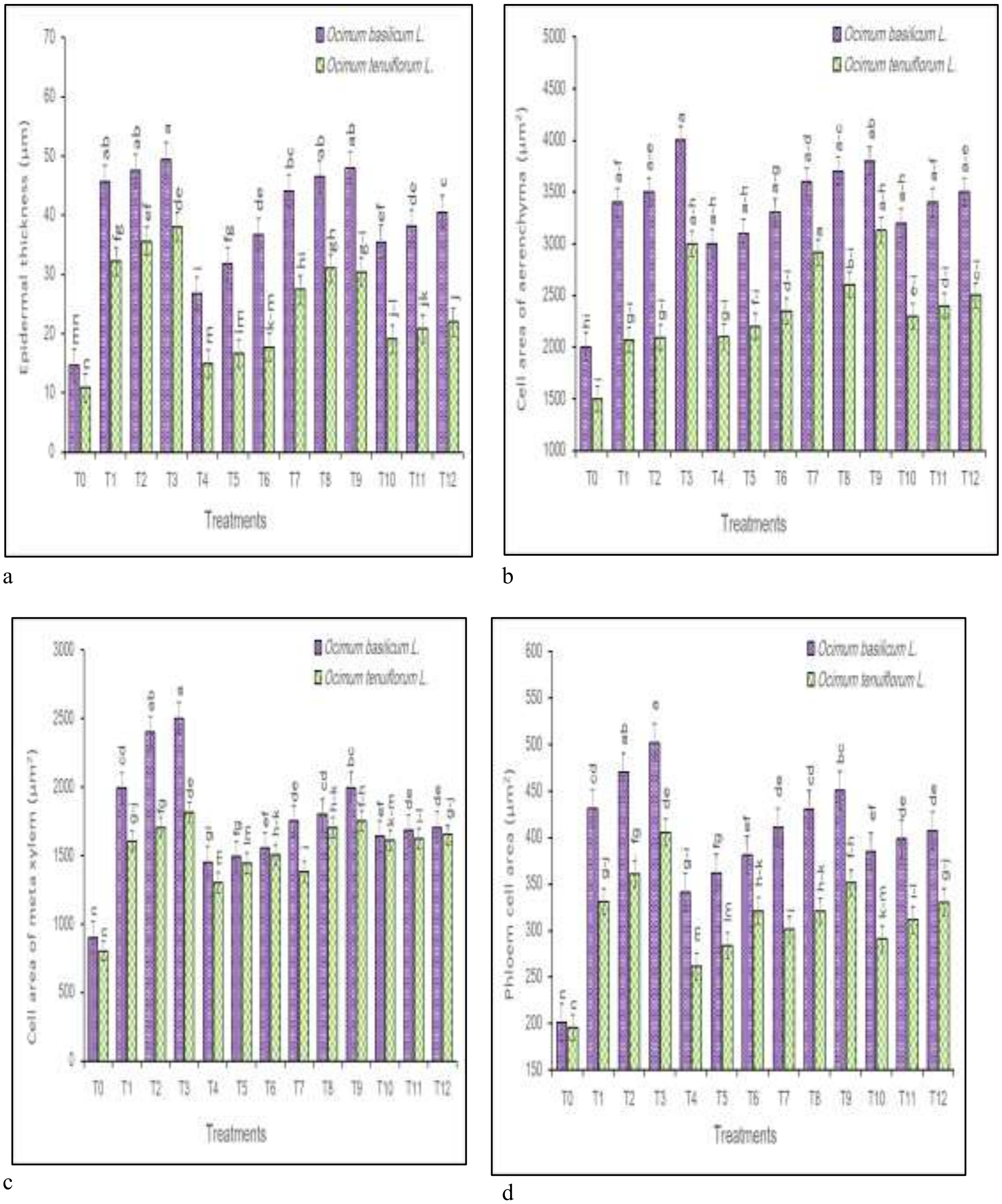

Figure 4. Effect of different chemical treatments of two basil species, (a) epidermal thickness, (b) cell area of aerenchyma, (c) cell area of meta xylem and (d) phloem cell area.

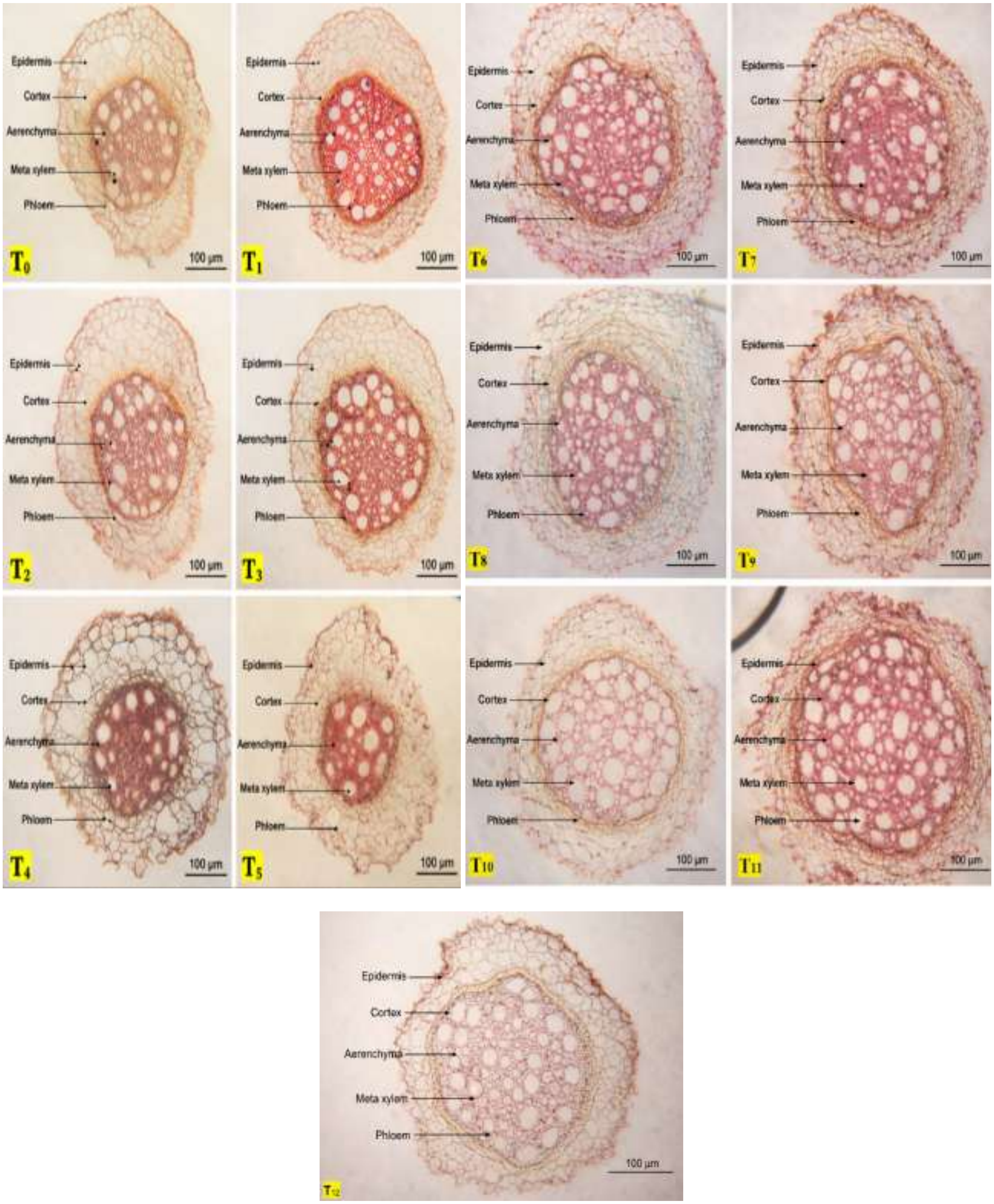

Figure 5. Root anatomical cross sections of Sweet basil under different treatments (T_0- T_{12}).

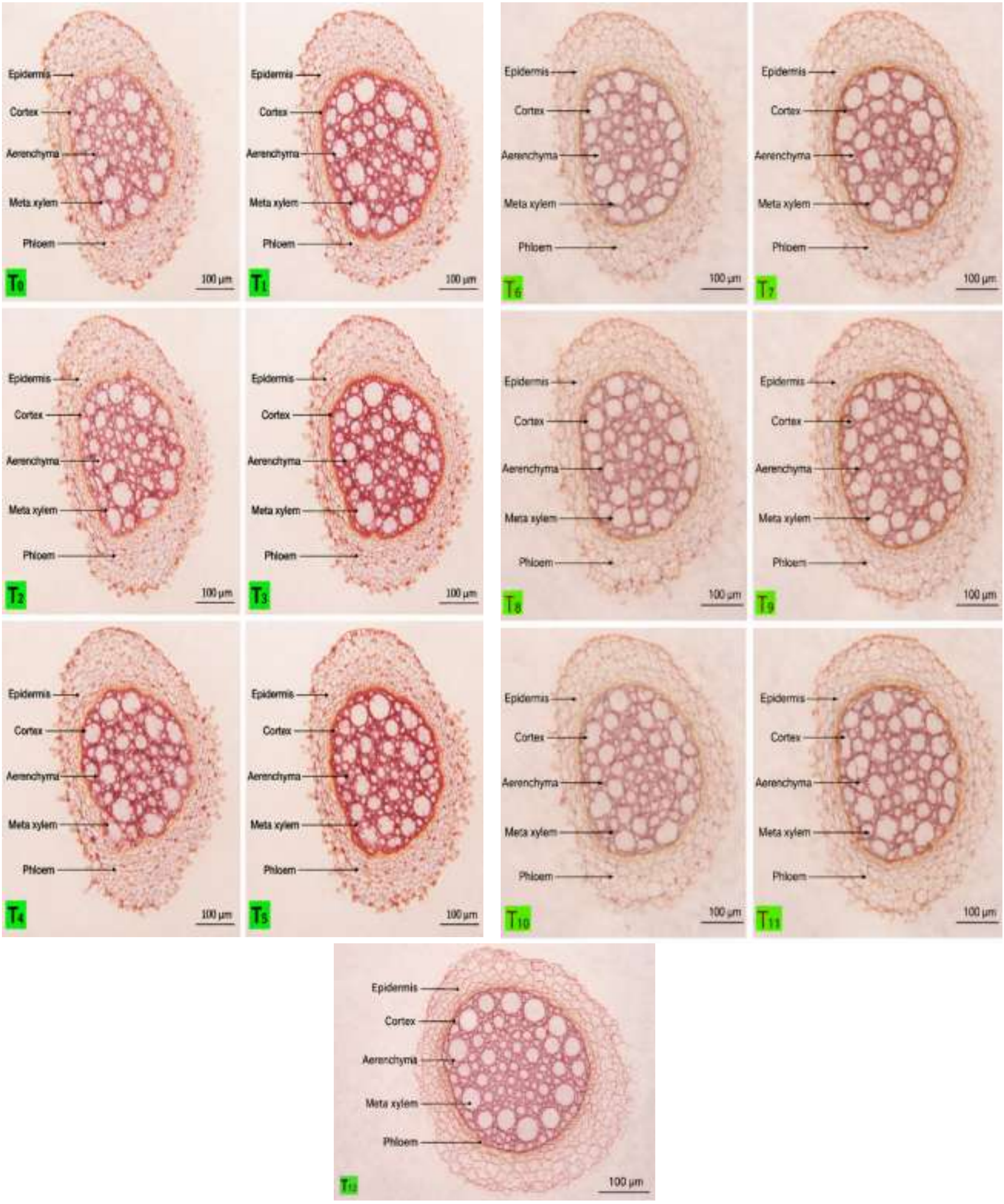

Figure 6. Root anatomical cross sections of holy basil under different treatments (T_0- T_{12}).

DISCUSSION

Morphological Parameters

Morphological development was strongly improved across all Zn NP treatments, though the extent varied with the functionalizing ligand. The most substantial gains were observed under SNP-functionalized Zn NPs, which is consistent with recent findings showing that nitrophenolate-based biostimulants enhance plant growth by stimulating auxin and gibberellin-mediated pathways and improving carbon assimilation (Liu et al., 2025; Rouphael and Colla, 2020). These enhanced hormonal and metabolic activities explain the exceptional vegetative growth observed in sweet basil under SNP treatments. Similar improvements in plant height, branching, and biomass accumulation have been widely reported in crops treated with Zn-based nanomaterials, where Zn acts as a cofactor for enzymatic activity and promotes cell division and elongation (Ciriello et al., 2022; Gupta et al., 2024).

Linalool-functionalized Zn NPs also promoted remarkable vegetative expansion. The strong performance of linalool may be attributed to its amphiphilic nature, which enhances NPs stability and facilitates penetration through plant cuticles. Plant derived bioisolates such as linalool have been shown to improve membrane permeability, nutrient uptake, and metabolic activity, thereby supporting enhanced growth and biomass production (Elbanna et al., 2024; Rouphael and Colla, 2020). The significant increases in plant height, branching, and leaf production observed in this study indicate that linalool acted not only as a stabilizing ligand but also as a bioactive compound enhancing Zn delivery and utilization.

Eucalyptol-functionalized Zn NPs produced moderate but consistent improvements in morphological traits. This comparatively lower response suggests that eucalyptol provides a controlled stimulatory effect, likely by improving membrane dynamics and facilitating gradual nutrient transport without strongly activating hormonal pathways. Similar moderate responses under nano-enabled treatments have been reported, where intermediate concentrations often produce optimal growth due to balanced nutrient availability and reduced physiological stress (Gupta et al., 2024). The non-linear responses observed in some traits further support the idea of concentration-dependent NPs efficiency.

CCC-functionalized Zn NPs exhibited comparatively weaker effects on morphological traits, particularly in holy basil. This response is expected because chlormequat chloride is a well-known growth regulator that inhibits gibberellin biosynthesis and reduces excessive shoot elongation. Previous studies have confirmed that CCC promotes compact growth and regulates plant architecture rather than enhancing vegetative expansion (Qin et al., 2024; Zhang et al., 2024). The differential response between sweet basil and holy basil suggests species-specific sensitivity to hormonal regulation and nano-delivered growth modulators.

Leaf development responded strongly to Zn NP treatments, with significant increases in leaf number and size across all treatments. Zn plays a critical role in maintaining meristematic activity, enzyme function, and protein synthesis, all of which are essential for leaf initiation and expansion (Ciriello et al., 2022). The rapid increase in leaf production under SNP and linalool treatments indicates enhanced metabolic activity and improved nutrient availability. Similarly, leaf area expansion observed in this study is consistent with previous reports showing that Zn NPs improve cell enlargement, chlorophyll synthesis, and vascular transport, leading to enhanced leaf development (Gupta et al., 2024; Elbanna et al., 2024).

Overall, the morphological responses observed in this study highlight the synergistic interaction between Zn NPs and functionalizing ligands. SNP-Zn NPs emerged as the most effective treatment, followed by linalool, eucalyptol, and CCC. These findings emphasize that the effectiveness of nano-biostimulants depends not only on the nutrient element but also on the chemical nature of the ligand, which governs nutrient delivery, metabolic activation, and hormonal regulation in plants.

Physiological Parameters

The significant enhancement in photosynthetic capacity observed across all functionalized Zn NP treatments indicates improved physiological efficiency in both *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. Zn is directly involved in carbonic anhydrase activity, chlorophyll maintenance, and the functional stability of the photosynthetic apparatus, and Zn bio fortification in basil has already been shown to improve gas-exchange traits and pigment-related responses (Ciriello et al., 2022; Gupta et al., 2024). The marked increase in photosynthetic rate in the present study therefore suggests that nano-enabled Zn delivery improved both carbon assimilation and overall metabolic activity in basil leaves.

The strong rise in stomatal conductance further confirms that Zn NP treatments enhanced gas-exchange regulation. ZnO NPs have been reported to improve stomatal attributes, photosynthetic induction, and transpiration behavior under stress conditions, indicating that nano-supplied Zn can strengthen guard cell function and stomatal responsiveness (Ahmed et al., 2023; Shabbir et al., 2023). This agrees with the present findings, where improved stomatal conductance was closely associated with greater photosynthetic activity in treated plants.

The chlorophyll gains recorded in this study also support the interpretation that functionalized Zn NPs improved pigment biosynthesis and chloroplast performance. Recent work in common bean showed that ZnO NPs improve plant growth together with biochemical traits and nutrient homeostasis, while broader analyses of nanomaterials in plants indicate that NPs can enhance chlorophyll accumulation, photosystem efficiency, and antioxidant balance when applied within an effective dose range (Li et al., 2024; Nongbet et al., 2022). These mechanisms are consistent with the substantial increase in chlorophyll content observed in both basil species.

The superior response under SNP-functionalized Zn NPs suggests that the ligand chemistry strongly influenced nutrient efficiency and downstream physiology. Nitrophenolate-based stimulation likely increased metabolic activity beyond the effect of Zn alone, promoting stronger photosynthetic performance, pigment accumulation, and assimilate production. A similar principle has been emphasized in recent nano-stimulant literature, where surface chemistry determines NPs reactivity, transport, and physiological effectiveness in crop plants (Bekah et al., 2025; Al-Mamun et al., 2021).

Linalool-functionalized Zn NPs also produced pronounced physiological enhancement, especially in photosynthetic rate and stomatal conductance. Although direct reports on linalool-Zn NPs in basil are limited, recent basil work with NPs based stimulation and broader bio-stimulant literature both support the view that bioactive natural compounds can improve membrane functionality, redox homeostasis, and nutrient use efficiency, thereby sustaining high photosynthetic performance (Elbanna et al., 2024; Zulfiqar et al., 2023). This likely explains why linalool ranked immediately after SNP in the present study.

Eucalyptol-functionalized Zn NPs induced moderate but consistent physiological gains, suggesting a more controlled stimulatory effect. This pattern is in line with studies showing that NPs mediated delivery can improve physiological performance without necessarily maximizing all parameters at the highest concentration. In coriander, nano Zn application enhanced photosynthetic rate, chlorophyll content, water-use traits, and productivity under drought, indicating that the physiological advantage of Zn-based nanomaterials often depends on balanced dosage and regulated uptake (Mahmoud et al., 2023; Alenezi et al., 2022).

The positive shifts in physiology were translated directly into greater biomass accumulation, confirming that improved gas exchange and chlorophyll content resulted in stronger assimilate production and carbon allocation. Recent studies in tea and olive have shown that ZnO NPs can enhance photosynthetic traits, growth performance, antioxidant status, and biomass formation, which closely matches the higher fresh and dry weights recorded in the present experiment (Chen et al., 2024; Regni et al., 2022).

The substantial proportional gains shown by holy basil indicate that this species retained considerable physiological plasticity, even though its absolute values were generally lower than those of sweet basil. Similar responses have been documented in NPs based studies, where ZnO NPs improved physiological and growth traits most strongly in plants with lower baseline performance, suggesting that nano-delivered micronutrients can partially overcome inherent physiological limitations (Nongbet et al., 2022; Gupta et al., 2024).

Overall, the physiological responses observed in this study demonstrate that functionalized Zn NPs significantly enhanced gas exchange, chlorophyll biosynthesis, and biomass production in basil. Among the treatments, SNP-Zn NPs showed the strongest effect, followed by linalool, eucalyptol, and CCC, confirming that ligand chemistry governs the magnitude of physiological stimulation and the efficiency with which nano-delivered Zn is translated into plant performance.

Root Anatomical Parameters

The present findings clearly demonstrate that nanostructured Zn-based formulations significantly improved root anatomical characteristics of *Ocimum basilicum L.* and *Ocimum tenuiflorum L.*, indicating enhanced structural development and functional efficiency of the root system. The marked increase in epidermal thickness under Zn NP treatments reflects improved protective capacity and absorptive potential, which can be attributed to the role of Zn in regulating enzymatic activity, membrane stability, and cell division processes (Imtiaz et al., 2021; Kumar et al., 2023). The superior performance of SNP-Zn NPs suggests that SNP enhances hormonal signaling pathways, particularly auxin- and gibberellin-mediated cell expansion, thereby promoting epidermal proliferation, as also supported by previous reports on NPs induced anatomical improvements (Faizan et al., 2021; Ameen et al., 2022). Linalool-Zn NPs also significantly enhanced epidermal thickness, likely due to terpene-mediated modulation of membrane permeability and cellular signaling, whereas CCC-Zn NPs showed comparatively lower responses, consistent with its inhibitory effect on gibberellin biosynthesis and cell elongation (Iqbal et al., 2022; Zafar et al., 2023).

Similarly, the substantial increase in aerenchyma cell area indicates improved root aeration and adaptive plasticity, which facilitates oxygen diffusion and enhances metabolic efficiency under varying environmental conditions (Yadav et al., 2022). The pronounced effect of SNP-Zn NPs on aerenchyma development may be associated with enhanced reactive oxygen species signaling and programmed cell differentiation pathways, which are known to regulate intercellular space formation (Rizwan et al., 2021). Linalool-based treatments also exhibited strong responses,

supporting the role of bioactive terpenes in cell wall remodeling and tissue differentiation (Ali et al., 2023), while CCC-Zn NPs exhibited relatively limited enhancement due to their growth regulating nature (Sharma et al., 2022).
Furthermore, the significant increase in metaxylem cell area under Zn NP treatments highlights improved vascular differentiation and water transport capacity, as Zn plays a crucial role in lignin biosynthesis and xylem development (Hussain et al., 2021; Patel et al., 2024). The enhanced metaxylem development observed with SNP-Zn NPs indicates improved vascular tissue formation through coordinated hormonal and enzymatic regulation, which has been widely reported in NPs based studies (Nair et al., 2022). Likewise, phloem cell area was significantly enhanced, suggesting improved assimilate transport and carbohydrate distribution, as Zn is directly involved in phloem loading and metabolic processes (Kaur et al., 2021).

SNP-Zn NPs exhibited the highest improvement, followed by linalool and eucalyptol treatments, indicating their effectiveness in enhancing transport tissues, while CCC-Zn NPs showed variable responses due to their regulatory role in plant growth. Overall, the consistent superiority of SNP-Zn NPs across all anatomical parameters confirms their strong potential as nano-biostimulants for improving root structure and function, thereby enhancing nutrient uptake, water transport, and stress tolerance. Additionally, the observed species-specific responses, where *O. basilicum* exhibited higher absolute anatomical values and *O. tenuiflorum* showed greater proportional increases, highlight inherent genetic variability in response to nanomaterials, which should be considered in future precision agriculture strategies (Khan et al., 2024; Raliya et al., 2021).

CONCLUSION

The present study demonstrated that nanostructured Zn based nano-biostimulants and nano-bioisolates significantly enhanced the morphological, physiological, and root anatomical characteristics of basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.), highlighting their potential in sustainable crop production. The application of functionalized Zn NPs through soil drenching markedly improved plant growth traits, including plant height, branching, leaf development, and biomass accumulation, along with physiological attributes such as photosynthetic rate, stomatal conductance, transpiration, and chlorophyll content. Furthermore, substantial improvements in root anatomical features, including epidermal thickness, aerenchyma formation, metaxylem development, and phloem area, indicated enhanced structural organization and functional efficiency of the root system.
Among the tested formulations, SNP-Zn NPs consistently exhibited the highest effectiveness across all parameters, suggesting their strong role in stimulating hormonal activity, nutrient utilization, and metabolic processes. Linalool-based nano-bioisolates ranked second, reflecting the importance of plant-derived bioactive compounds in improving plant performance through enhanced membrane functionality and physiological regulation. Eucalyptol-based treatments showed moderate yet consistent improvements, whereas CCC based nano-biostimulants exhibited comparatively lower responses due to their growth-regulating nature.
Species-specific responses were also evident, with *O. basilicum* showing higher absolute values across most traits, while *O. tenuiflorum* exhibited greater proportional responsiveness, indicating inherent genetic variability in response to nano-enabled treatments. Overall, the findings confirm that the effectiveness of nano-biostimulants depends not only on the nutrient element but also on the nature of the functionalizing ligand. The integration of Zn NPs with synthetic biostimulants and plant-derived bioisolates represents a promising strategy for enhancing crop productivity, improving physiological efficiency, and strengthening root architecture under sustainable agricultural systems.

Consent for publication/Ethical standards

All authors have reviewed and approved the final version of the manuscript for submission and publication. The work presented in this study complies with ethical standards, and all authors have provided their unanimous consent for publication.

Authors' contribution

This study constitutes a part of the Ph.D. research of the first author, Ali Ahmad. The experimental design, data analysis, organization, and interpretation were carried out by Ali Ahmad in collaboration with Ahsan Akram. The manuscript was primarily written by Ali Ahmad, while Adnan Younis and Muhammad Asif Hanif contributed to critical revision and proofreading of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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