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Effect of nitrogen nano-fertilizer concentrations on growth and some agro-physiological performance of *Withania coagulans*, *Echinacea purpurea*, and *Valeriana officinalis* in an aeroponic system

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Abstract

Objective: Nitrogen is a critical macronutrient for plant growth and development, and the application of nitrogen in nanoscale form has emerged as a promising strategy to enhance nutrient use efficiency and crop productivity. This study investigated the effects of different concentrations of nitrogen nano-fertilizer on some traits of three medicinal plants, *Withania coagulans*, *Echinacea purpurea*, and *Valeriana officinalis*, cultivated in an aeroponic system.
Methods: The trials were performed in a completely randomized design using three replications. The foliar application of nitrogen nano-fertilizer (0, 1000, and 2000 mg/L) was performed at 20, 40, and 60 days after transferring to the aeroponic system. Morphological traits, including fresh and dry weights of shoots and roots, as well as the height of shoots and roots, and the number of leaves, were measured. Additionally, photosynthetic pigments consisting of chlorophylls (a and b) and carotenoids were recorded.
Results: The results demonstrated a dose-dependent effect of nitrogen nano-fertilizer on all measured traits across the three species. In *Valeriana officinalis*, the highest concentration significantly increased root length, leaf number, shoot dry weight, plant height, and root dry weight, compared to the control. Similarly, *Echinacea purpurea* and *Withania coagulans* showed significant improvements in vegetative growth and biomass accumulation, although the magnitude of response varied among species. Photosynthetic pigments were also enhanced under nano-fertilizer treatment, with chlorophyll a, chlorophyll b, and carotenoids reaching their highest values at 2000 mg/L in all species, suggesting improved photosynthetic capacity and photoprotection. Comparison of these species revealed that *Valeriana officinalis* had the highest growth improvement, while *Echinacea purpurea* showed more root development and pigment accumulation.
Conclusion: Nitrogen nano-fertilizer proved to be an effective tool for promoting vegetative growth, biomass accumulation, and photosynthetic efficiency in the medicinal plants grown under controlled aeroponic conditions. This study provided insights for the sustainable cultivation of high-value medicinal crops and supports the use of nanoscale nutrients to improve plant productivity.

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Introduction

Medicinal plants are important resources for ornamental, pharmaceutical, nutritional, and economic use due to their bioactive compounds and therapeutic properties. Among these is purple coneflower (*Echinacea purpurea* L.) from the Asteraceae and native to North America (Gupta *et al*. 2023). It is now cultivated in many temperate regions worldwide, including Europe, Canada, China, and parts of Asia. Purple coneflower prefers well-drained soils, with sandy or loamy soils as ideal types. It is relatively drought-tolerant once established. Various parts of this plant, including roots and aerial biomass, contain valuable compounds such as polyphenols, particularly caffeic acid derivatives, and flavonoids, including quercetin and kaempferol (Senica *et al.* 2019; Petrova *et al.* 2023). The initial use of this herbal medicine is its immune-boosting properties, which are commonly used to prevent or reduce the duration of cold, flu, and other infections. Purple coneflower is widely used for treating urinary infections, wound healing, and immune system enhancement, and it also exhibits antioxidant and anti-inflammatory activity (Hudson 2012; Geszke-Moritz *et al.* 2023).

Valeriana officinalis L. from the Valerianaceae, is a native species of European and Asian regions, and grows well in temperate and humid regions (Penzkofer and Heuberger 2020). Valerian prefers a well-drained fertile soil and a sunny to partially shaded location. Moist soil is ideal, but it does not tolerate waterlogging. It is usually propagated by seeds or root division, but roots are the main harvested part, typically in the second or third year of growth when they are richest in active compounds. Valerian roots contain essential oils of considerable pharmaceutical importance, including valerenal, α-pinene, β-pinene, and isovaleric acid (Raj *et al.* 2023). Roots and rhizomes of valerian possess sedative, anxiolytic, antidepressant, nervine-strengthening, and anti-insomnia properties, and pharmaceutical formulations such as anti-migraine drops, valerian syrup, and capsules exploit these effects (Akram *et al.* 2019; Muhetaer *et al.* 2025). Valerenic acids, valepotriates, and various sesquiterpenes are the main bioactive constituents responsible for sedative and anxiolytic effects.

Another economically and pharmaceutically significant species is *Withania coagulans* Dunal (Stocks), commonly known as paneer-bad, belonging to the Solanaceae family and native to the

Middle East and Indian subcontinent (Yasmin *et al.* 2025). It is also cultivated in arid and semi-arid regions with suitable conditions. This species tolerates poor soils and prefers well-drained sandy or loamy soils. *W. coagulans* is tolerant to heat and drought, making it suitable for stabilizing shifting sands (Iqbal *et al.* 2023). It is propagated by seeds or cuttings, and the plant is hardy and does not require intensive care. Harvesting is usually done from mature plants, especially for fruits and roots. Its main uses include antidiabetic effects to aid in decreasing blood glucose levels and enhancing insulin effectiveness (Sampathkumar *et al.* 2019). Also, it is used for hepatoprotective effects for supporting liver health, and has some anti-inflammatory and antioxidant capabilities, which aid in decreasing the harmful effects of oxidative stress. Its various parts, like fruits, roots, and leaves, show anti-stress, antitumor, and antimicrobial capabilities, which are also traditionally used to coagulate milk in cheese-making (Nazish *et al.* 2022).

Among the various factors that strongly impact the quantity and quality of medicinal plants is the appropriate application of nutrients throughout their growth and development. Nitrogen is a key element in this regard, as it contributes to the formation of amino acids, proteins, and enzymes, and plays a critical role in plant productivity in both natural and agricultural ecosystems (Zayed *et al.* 2023; Daryabari *et al.* 2025). Nitrogen is also the main constituent of chlorophyll, and its deficiency reduces fresh weight and essential oil content, whereas optimal application enhances biomass and bioactive compound production (Valipour *et al.* 2021; Ghasembaghlou *et al.* 2022). Previous studies have demonstrated that nitrogen fertilization improves yield, essential oil content, and physiological traits in various medicinal plants (Alhasan *et al.* 2021; Hao *et al.* 2024; Rostaei *et al.* 2024).

In recent years, advanced technologies have been increasingly applied to improve the quality of agricultural products. Among these, nanotechnology has been found to be a promising strategy to enhance both the quantity and quality of crops. One of its key applications in agriculture is the use of nano-fertilizers, in which the particle size of nutrients is less than 100 nm (Avila-Quezada *et al.* 2022). Such fertilizers allow for gradual nutrient release, reduce leaching, and enhance nutrient uptake efficiency. Foliar application of nano-fertilizers provides rapid nutrient availability, bypassing soil-related limitations, while nanoscale properties improve solubility, membrane penetration, and catalytic activity (Gholinezhad *et al.* 2024; Shah *et al.* 2025). Determining the optimal nitrogen rate is crucial to maximize yield while avoiding environmental damage, as excessive application can increase costs and lead to groundwater contamination.

Production of medicinal plants under controlled environments, such as aeroponic systems, offers opportunities to improve quality, purity, adaptability, and biomass production on a commercial scale, particularly for species with valuable roots (López-Valdez *et al.* 2022; Dsouza *et al.* 2025). Based on these considerations, the current research aimed to study the effects of nitrogen nano-fertilizer on selected growth traits of *Echinacea purpurea*, *Withania coagulans*, and *Valeriana officinalis* under aeroponic cultivation.

Materials and Methods

Trials

Seed samples of *Valeriana officinalis* and *Echinacea purpurea* were bought from Pakanbazr Co., Iran and the seed sample of *Withania coagulans* was collected from its natural habitat in Sistan and Baluchistan Province, Iran. These three seed samples were washed with distilled water, treated with sulfuric acid, and rinsed with distilled water for three times, for use in three distinct trials. The disinfected seeds were placed in Petri dishes in a germinator for germination, and obtained seedlings were transferred to small pots containing peat moss. At the four-leaf stage, the plants were transferred to an aeroponic system in a greenhouse at Malayer (34°18′N; 48°49′E), Iran, maintained under 25/20 °C day and night temperature, with a relative humidity of 60%. The aeroponic system comprised a seed chamber, nutrient solution tank, spray nozzles, pumps for nutrient solution spraying, and a control system according to Movahedi and Moieni (2024). The composition of the applied nutrient solution is provided in Table 1. Three weeks after plant establishment in the aeroponic system, nitrogen nano-fertilizer treatments were applied at three concentrations 0, 10, and 20 mg/L. This nano-fertilizer contained 20% nitrogen with particle sizes of 23-80 nm and was obtained from Sepehr Parmis Company, Iran. Foliar applications of the nano-fertilizer were performed three times, at 20, 40, and 60 days after transferring to the aeroponic system. All of the above-mentioned tasks were performed for three species, *Valeriana officinalis*, *Echinacea purpurea*, and *Withania coagulans*, in three separate trials.

Table 1. Concentration of the solution used in the aeroponic system.

Element	Concentration (mg/L)	Element	Concentration (mg/L)
K	200	Fe	1.00
N	190	Mn	0.50
Ca	150	B	0.50
S	70	Zn	0.15
Mg	45	Cu	0.10
P	35	Mo	0.05

Morphological traits

Morphological traits were measured four months after planting. Fresh and dry weights of shoots and roots were measured via a digital balance with 0.01 g precision. Dry weight was measured after placing samples in an oven at 70 °C for 48 hours. The height of shoots and roots was determined in cm via a stainless steel ruler. The number of leaves per plant was counted individually.

Photosynthetic pigments

For pigment analysis, 0.5 g of tissue samples was ground in a mortar using liquid nitrogen. Twenty milliliters of 80% acetone were added, and were centrifuged at 6000 rpm for 10 min. An aliquot of the supernatant was placed in a spectrophotometer cuvette (Analytical Jena Specord 205, Jena, Germany), and absorbance was recorded at 663, 645, and 470 nm for chlorophylls (a and b), and carotenoids, respectively. Pigment concentrations were calculated using the suggested methods of Arnon (1967).

Statistical analysis

The completely randomized design was used in all three trials with three replications. The Shapiro-Wilk test confirmed the normality of experimental errors for all measured traits. After analysis of variance (ANOVA), comparison of means was performed via the multiple range test of Duncan. Data were analyzed using SPSS 21.0 software.

Results and Discussion

Withania coagulans

According to ANOVA, the effects of treatments were highly significant on all studied traits (data are not shown). Application of nitrogen nano-fertilizer significantly increased the leaf number in *W. coagulans* (Table 2), and the highest concentration (20 mg/L) produced the greatest number of leaves (23.2), which was significantly higher than the 10 mg/L treatment (18.2) and the control (17.1). Thus, nitrogen nano-fertilizer enhanced the vegetative growth of *W. coagulans* in a dose-dependent manner. Our findings align with the report of Namdeo *et al.* (2023), who found that nano-form of nitrogen enhances the early vegetative growth of *W. coagulans*, whereas foliar application of conventional nitrogen had lower effects on growth and yield traits, indicating superior canopy development with nano-nitrogen delivery.

Both dry and fresh weights of shoots were influenced by the nitrogen nano-fertilizer (Table 2), and the highest dosage of nitrogen nano-fertilizer (20 mg/L) recorded the highest shoot dry weight

(18.1 g) and fresh weight (47.67 g), compared to 10 mg/L (15.2 and 35.95 g dry and fresh weight of shoots, respectively), and the control (12.7 and 30.91 g dry and fresh weight of shoots, respectively). This suggests that nitrogen nano-fertilizer promotes shoot growth and overall biomass accumulation. According to Rathnayaka *et al.* (2018), full replacement with nano-nitrogen led to higher shoot dry weight and yield relative to the standard urea treatments in rice.

Root growth of *W. coagulans* responded positively to nitrogen nano-fertilizer, and 20 mg/L treatment had the highest dry (6.9 g) and fresh root weight (21.1 g), whereas the control had the lowest values (4.32 and 15.8 g dry and fresh weight of roots, respectively) (Table 2). These results demonstrate enhanced root development, which could improve nutrient and water uptake efficiency. Moderate or high levels of nitrogen in cotton improved root length, highlighting the role of nitrogen in enhancing root morphological development (Chen *et al.* 2020). However, nano-fertilizers are known to promote nutrient uptake and utilization due to their small particle size, enabling rapid penetration, controlled release, and efficient translocation, reinforcing the mechanisms underlying our observed root growth response.

Root length showed an increase with nitrogen nano-fertilizer application (Table 3), measuring 36.3 cm at 10 mg/L, 41.6 cm at 20 mg/L, and 32.67 cm in the control. Similar to root biomass trend, root elongation increased at higher nitrogen levels, possibly due to simultaneous thickening and elongation regarding overall growth. Improved root length, root density, and yield of most crops were found when nano-nitrogen was applied as compared to controls or reduced conventional nitrogen rates (Upadhyay *et al.* 2023). Also, nitrogen fertilization show higher root length density and root surface/volume with adequate nitrogen supports the biological plausibility that supplying nitrogen stimulates root development (Luo *et al.* 2024). Thus, our findings indicated that increased root biomass under nano-nitrogen usage increases water and nutrient absorption capacity, contributing to plant vigor and resilience.

Plant height was also affected by nitrogen nano-fertilizer, with the control showing the shortest plants (62.45 cm), while 10 and 20 mg/L treatments measured 66.17 cm and 85.3 cm, respectively (Table 3). This pattern suggests that under higher nitrogen conditions, plants may allocate resources preferentially to elongation. Similar reports of seedling elongation have been reported in *W. coagulans*, where foliar nano-urea improved height and yield traits relative to conventional fertilization (Namdeo *et al.* 2023). Nano-fertilizers promote resource allocation toward elongation under higher nitrogen availability, likely through enhanced nitrogen use efficiency and modulation of growth regulators such as auxins and cytokinins.

Photosynthesis pigments (chlorophylls a and b, and carotenoids) of *W. coagulans* increased with nitrogen nano-fertilizer application. Chlorophyll a increased from 1.057 mg/g (at the control) to 1.45 mg/g (at 20 mg/L), chlorophyll b from 0.71 mg/g to 0.92 mg/g, and carotenoids from 0.97 mg/g to 1.18 mg/g (Table 3). These increases indicate enhanced photosynthetic capacity and photoprotection with higher nitrogen availability. Application of nano-fertilizers significantly affected the levels of total chlorophyll, chlorophylls a and b, and carotenoids in lettuce leaves (Zyadeh *et al.* 2025).

In total, nitrogen nano-fertilizer positively influenced morpho-physiological traits of *W. coagulans* in the aeroponic system, and the 20 mg/L treatment consistently caused the highest leaf number, shoot and root biomass, and photosynthetic pigment content, demonstrating a dose-dependent improvement in growth and physiological performance of this species.

Table 2. Mean comparison of nitrogen nano-fertilizer concentrations for leaf number, and dry and fresh weights of shoots and roots of *Withania coagulans* in the aeroponic system.

Nano-nitrogen treatments	Leaf number	Dry weight of shoots (g)	Fresh weight of shoots (g)	Dry weight of roots (g)	Fresh weight of roots (g)
Control	17.1 c	12.7 c	30.91c	4.32 c	15.8 c
10 mg/L	18.2 b	15.2 b	35.95 b	5.6 b	18.47 b
20 mg/L	23.2 a	18.1 a	47.67 a	6.9 a	21.1 a

Means followed by different letter(s) are significantly different at the 0.05 level of probability, using Duncan's multiple range test.

Table 3. Mean comparison of nitrogen nano-fertilizer concentrations for root length, plant height, and photosynthetic pigments of *Withania coagulans*, in the aeroponic system.

Nano-nitrogen treatments	Root length (cm)	Plant height (cm)	Carotenoids (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
Control	32.67 c	62.45 c	0.97 c	1.057 c	0.71 b
10 mg/L	36.3 b	66.17 b	1.16 b	1.15 b	0.75 b
20 mg/L	41.6 a	85.3 a	1.18 a	1.45 a	0.92 a

Means followed by different letter(s) are significantly different at the 0.05 level of probability, using Duncan's multiple range test.

Echinacea purpurea

ANOVA indicated highly significant treatment effects on all traits studied (data not shown). Nitrogen nano-fertilizer significantly increased the number of leaves in *E. purpurea*, and the highest concentration (20 mg/L) produced the greatest leaf number (18.5), which was significantly higher than both the 10 mg/L treatment (15.72) and the control (12.75), indicating a clear dose-dependent enhancement of vegetative growth (Table 4). Similarly, Ahmadi *et al.* (2020) found that using nitrogen slow-release fertilizers at higher dosages (150 mM) significantly increased plant growth characteristics, including leaf number, in *E. purpurea*.

Dry and fresh weights of shoots were positively affected by increasing nano-fertilizer concentration, and 20 mg/L treatment showed the highest dry weight (25.1 g) and fresh weight (80.87 g), compared to 10 mg/L (21.92 and 71.02 g dry and fresh weight of shoots, respectively) and the control (18.34 and 51.97 g dry and fresh weight of shoots, respectively) (Table 4). Bonomelli (2005) also reported that nitrogen fertilization improved biomass accumulation and nutrient uptake in *E. purpurea*, indicating a positive relationship between nitrogen levels and vegetative growth. These findings suggest that nitrogen application, particularly at optimal concentrations, can enhance vegetative growth in *E. purpurea*, supporting our observations.

Plant height was significantly enhanced with increasing nano-fertilizer concentrations (Table 5), and the tallest plants were observed at 20 mg/L (68.45 cm), compared to 10 mg/L (53.97 cm) and the control (46.1 cm), indicating improved vegetative growth under higher nitrogen availability. Similarly, research on cucumber demonstrated a 31% increase in plant height with 6 ml/L liquid nano-nitrogen, potassium, and phosphorus application (Goyal *et al.* 2025). This study supports again the idea that nitrogen nano-fertilizer promotes vegetative growth in *E. purpurea*, leading to increased plant height. In a three-year field experiment, the effects of various nutrient applications, including nitrogen levels, on the growth characteristics and essential oil properties of *E. purpurea* were evaluated, and it was found that nitrogen fertilization, particularly at 150 kg N/ ha, positively affected plant height, yield, and essential oil production (Soltanbeigi and Maral 2022).

Root growth reacted positively to nitrogen nano-fertilizer, and 20 mg/L treatment recorded the highest dry (20.1 g) and fresh root weight (57.2 g), while the control had the lowest values (15.68 and 41.3 g dry and fresh root weight, respectively) (Table 4). This demonstrates that nitrogen nano-fertilizer enhances root development, potentially improving water and nutrient uptake. These results align with findings from other studies demonstrating that increasing nitrogen availability enhances root biomass in *E. purpurea*. For example, despite differing fertilization methods, nitrogen treatments such as ammonium nitrate elevated both fresh and dry root weights (Kizil and Toncer 2013). Thus, observed increases in the root fresh and dry weight under nano-fertilizer application reflect a consistent, dose-dependent promotion of root growth.

Root length increased significantly with nano-fertilizer application, measuring 25.52 cm in the control, 35.5 cm at 10 mg/L, and 39.3 cm at 20 mg/L nano-fertilizer (Table 5). This indicates that higher nitrogen levels favor root elongation, supporting greater anchorage and nutrient absorption capacity. Luo *et al.* (2024) observed that applying nitrogen fertilizer at a rate of 225 kg ha^{-1}

significantly increased the length, surface, and volume of roots, compared to the treatment without nitrogen fertilization. This study supports the idea that nitrogen nano-fertilizer promotes root elongation in *E. purpurea*, enhancing its anchorage and nutrient uptake capabilities.

Chlorophyll a, chlorophyll b, and carotenoids were enhanced with increasing nitrogen nano-fertilizer. Chlorophyll a increased from 1.15 mg/g (control) to 1.45 mg/g (20 mg/L), chlorophyll b from 0.55 mg/g to 0.66 mg/g, and carotenoids from 1.27 mg/g to 1.61 mg/g when 20 mg/L nitrogen nano-fertilizer was applied (Table 5). These results suggest enhanced photosynthetic capacity and photoprotection in response to higher nitrogen availability.

In general, nitrogen nano-fertilizer positively affected the morpho-physiological traits of *E. purpurea* in the aeroponic system, and 20 mg/L nitrogen nano-fertilizer treatment consistently produced the highest leaf number, biomass, root length, plant height, and pigment content, demonstrating a dose-dependent improvement in growth and physiological performance.

Table 4. Mean comparison of nitrogen nano-fertilizer concentrations for leaf number, and dry and fresh weights of shoots and roots of *Echinacea purpurea*, in the aeroponic system.

Nano-nitrogen treatments	Leaf number	Dry weight of shoot (g)	Fresh weight of shoot (g)	Dry weight of root (g)	Fresh weight of root (g)
Control	12.75 c	18.34 c	51.97 c	15.68 c	41.3 c
10 mg/L	15.72 b	21.92 b	71.02 b	17.82 b	44.87 b
20 mg/L	18.5 a	25.1 a	80.87 a	20.1 a	57.2 a

Means followed by different letter(s) are significantly different at the 0.05 level of probability, using Duncan’s multiple range test.

Table 5. Mean comparison of nitrogen nano-fertilizer concentrations for root length, plant height, and photosynthetic pigments of *Echinacea purpurea*, in the aeroponic system.

Nano-nitrogen treatments	Root length (cm)	Plant height (cm)	Carotenoids (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
Control	25.52 c	46.1 c	1.27 b	1.15 c	0.55 c
10 mg/L	35.5 b	53.97 b	1.33 b	1.32 b	0.62 ab
20 mg/L	39.3 a	68.45 a	1.61 a	1.45 a	0.66 a

Means followed by different letter(s) are significantly different at the 0.05 level of probability, using Duncan’s multiple range test.

Valeriana officinalis

ANOVA revealed significant differences among treatments for all investigated traits (data not shown). Nitrogen nano-fertilizer significantly increased the number of leaves in *V. officinalis*. The highest concentration (20 mg/L) produced the highest leaf number (34.2), followed by 10 mg/L (27.5) and the control (23.75) (Table 6). This demonstrates a dose-dependent enhancement of vegetative growth with nano-fertilizer application. In some field trials, foliar application of nano-nitrogen improved leaf number and yield in lettuce (Sharaf-Eldin *et al.* 2022).

Dry and fresh weight of shoots were positively affected by nitrogen nano-fertilizer, so the 20 mg/L treatment showed the highest shoot dry weight (25.59 g) and fresh weight (92.17 g), compared to 10 mg/L concentration (21.75 g dry weight and 73.67 g fresh weight) and the control (13.73 g dry weight and 57.87 g fresh weight) (Table 6). Thus, we observed improved biomass accumulation and shoot vigor under higher nitrogen availability. In a study, foliar nano-N application in potato improved shoot growth and fresh biomass compared to the traditional urea fertilization and suggested that nano-N promotes more efficient nitrogen uptake and assimilation, leading to higher photosynthetic activity and greater shoot vigor (Al-Juthery and Al-Maamouri 2020).

Plant height was significantly enhanced by nitrogen nano-fertilizer, increasing from 43.22 cm in the control to 60.9 cm at 20 mg/L (Table 7), with an intermediate height at 10 mg/L (52.02 cm). This reflects improved vegetative growth and overall plant development under higher nitrogen availability. In wheat, nano-nitrogen contributed to taller plants even at reduced fertilizer doses, highlighting improved nitrogen use efficiency (Kumar *et al.* 2024b). The increase in height observed in V. *officinalis* suggests that nano-nitrogen provides more efficient nitrogen assimilation, stimulating hormonal and metabolic processes that support vertical growth and overall plant development.

The 20 mg/L treatment showed the highest dry (21.04 g) and fresh root weight (80.8 g), while the control had the lowest values (15.43 g dry weight and 59.67 g fresh weight, respectively). Such enhanced root biomass suggested improved nutrient and water uptake capacity (Table 6). Similar observation has been reported in rice, where nano-nitrogen application improved root biomass and nutrient uptake efficiency compared with the conventional fertilization under salt stress (Kumar *et al.* 2024a). The reduced nitrogen rates combined with nano-nitrogen produced greater root biomass in soybean and maize demonstrating that nano-formulations enhance nutrient use efficiency and root development (Ghobashi and Ismail 2022). The improved root biomass observed in *V. officinalis* therefore suggests enhanced nutrient and water acquisition capacity, which in turn supports greater shoot vigor and overall plant performance.

Root length increased significantly with higher nitrogen nano-fertilizer concentration, from 25.07 cm in the control to 35.97 cm at 20 mg/L (Table 7). This indicates that nitrogen enrichment promotes root elongation, supporting better anchorage and absorption efficiency. Comparable enhancements have been reported in fodder oats, where nano-nitrogen improved root elongation compared with the conventional urea fertilization (Chethan Babu *et al*. 2025). In mustard, nano-nitrogen spray stimulated root growth by enhancing nitrogen assimilation and auxin activity (Al-Asally and Al-Hijemy 2023).

The elongation of roots observed in *V. officinalis* indicates improved anchorage, nutrient and water uptake, and suggests that nano-nitrogen fertilization supports a more efficient and resilient root system, which in turn contributes to overall plant vigor.

Photosynthetic pigments, including carotenoids, chlorophyll a, and chlorophyll b, increased by nano-fertilizer application. Chlorophyll a increased from 1.17 mg/g (control) to 1.38 mg/g (20 mg/L), chlorophyll b from 0.39 mg/g to 0.54 mg/g, and carotenoids from 1.08 mg/g to 1.28 mg/g (Table 7). These enhancements suggest improved photosynthetic capacity and photoprotection under elevated nitrogen levels. Nitrogen plays a central role in chlorophyll biosynthesis, and its efficient delivery through nanoparticles ensures pigment accumulation (Mony *et al.* 2022). Elevated carotenoids further suggest enhanced antioxidant protection, reducing photodamage so the observed pigment increase is likely underpinning the higher biomass accumulation and overall plant vigor associated with nano-nitrogen application.

In general, nitrogen nano-fertilizer significantly improved the morpho-physiological traits of *V. officinalis* in an aeroponic system. The 20 mg/L treatment consistently produced the highest values for leaf number, shoot and root biomass, plant height, root length, and photosynthetic pigments, demonstrating the improvement in growth and physiological performance of this species.

Table 6. Mean comparison of nitrogen nano-fertilizer concentrations on leaf number, and dry and fresh weights of Shoots and roots of *Valeriana officinalis* in the aeroponic system.

Nano-nitrogen treatments	Leaf number	Dry weight of shoot (g)	Fresh weight of shoot (g)	Dry weight of root (g)	Fresh weight of root (g)
Control	23.75 c	13.73 c	57.87 c	15.43 c	59.67 c
10 mg/L	27.5 b	21.75 b	73.67 b	17.53 b	71.7 b
20 mg/L	34.2 a	25.59 a	92.17 a	21.04 a	80.8 a

Means followed by different letter(s) are significantly different at the 0.05 level of probability, using Duncan’s multiple range test.

Table 7. Mean comparison of nitrogen nano-fertilizer concentrations for root length, plant height, and photosynthetic pigments of *Valeriana officinalis*, in the aeroponic system.

Nano-nitrogen treatments	Root length (cm)	Plant height (cm)	Carotenoids (mg/g)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
Control	25.07 c	43.22 c	1.08 c	1.17 c	0.39 c
10 mg/L	28.6 b	52.02 b	1.12 b	1.24 b	0.42 b
20 mg/L	35.97 a	60.9 a	1.28 a	1.38 a	0.54 a

Means followed by different letter(s) are significantly different at the 0.05 level of probability, using Duncan’s multiple range test.

Comparison of species

Across all three species mentioned, nitrogen nano-fertilizer consistently enhanced vegetative growth, root development, plant height, and photosynthetic pigment content, demonstrating a dose-dependent response, with the highest concentration (20 mg/L) producing the most pronounced improvements. Leaf number and shoot biomass increased in response to higher nitrogen nano-fertilizer concentrations in all three species. These findings align with previous studies reporting that nano-nitrogen improves vegetative growth by enhancing nitrogen use efficiency and photosynthetic activity (Bhardwaj *et al.* 2025; Chauhan *et al.* 2025). The dose-dependent increases in shoot biomass suggest that higher nitrogen availability promotes protein synthesis, enzymatic activity, and vegetative vigor. Root biomass and length were also positively affected by nitrogen nano-fertilizer. While *V. officinalis* and *E. purpurea* showed clear increases in root biomass, *W. coagulans* exhibited a moderate increase in root biomass. This may indicate a species-specific allocation strategy. Enhanced root systems in all species likely improved nutrient and water uptake, contributing to overall plant vigor, consistent with previous observations in cereals (Maqbool *et al.* 2022), and other medicinal plants (Xue *et al.* 2018; Miransari *et al.* 2022).

All three species responded with increased plant height under higher nitrogen nano-fertilizer concentrations, whereas *W. coagulans* showed the largest relative increase (from 62.45 to 85.3 cm), suggesting somewhat stronger elongation response than *E. purpurea* and *V. officinalis*. These differences may reflect species-specific growth habits and sensitivity to nitrogen availability, and are in agreement with studies demonstrating that nano-fertilizers enhance shoot elongation by improving nitrogen assimilation and modulating growth regulators such as auxin and cytokinin (Dey and Sadhukhan 2024; Ali *et al.* 2025).

Chlorophyll a and b, and carotenoids increased across all three species, with the highest values observed under 20 mg/L nano-fertilizer. *V. officinalis* and *E. purpurea* exhibited higher absolute carotenoids and chlorophyll a content than *W. coagulans*, consistent with their more vigorous vegetative growth. Enhanced pigment levels indicate improved photosynthetic capacity, photoprotection, and antioxidant potential, which likely contributed to the observed increases in biomass (Simkin *et al.* 2022; Songserm *et al.* 2024). While all three species benefited from nitrogen nano-fertilizer, *V. officinalis* displayed the strongest growth response, indicated by higher leaf number, shoot biomass, and root development. *E. purpurea* showed intermediate responses, and *W. coagulans* exhibited relatively higher shoot elongation and moderate root elongation. These differences may be attributed to inherent species traits, growth strategies, and nutrient allocation patterns. The differential response also highlights the importance of optimizing nitrogen doses based on species-specific physiology to maximize growth and metabolite accumulation.

Conclusion

This study demonstrated that foliar application of nitrogen nano-fertilizers significantly enhances morpho-physiological traits of *W. coagulans*, *E. purpurea*, and *V. officinalis* cultivated in the aeroponic system. Across all species, the highest concentration of nitrogen nano-fertilizer (20 mg/L) produced the most pronounced improvements in leaf number, shoot and root biomass, plant height, root length, and photosynthetic pigment content, indicating a dose-dependent response. These findings highlight the potential of nitrogen nano-fertilizers as an effective tool for improving the growth, productivity, and physiological efficiency of medicinal plants in controlled cultivation systems. Optimizing nano-fertilizer concentrations according to species-specific responses can maximize benefits, supporting sustainable and high-yield production of valuable medicinal crops.

Conflict of Interest

The authors declare that they have no conflict of interest.

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