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Mitigating drought stress in *Catharanthus roseus* along with nano-iron enriched algal fertilizer application: a sustainable approach for enhanced growth and secondary metabolite production

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Abstract

Algal biomass enriched with iron nanoparticles, termed as Nano-Phyco Fertilizer (NPF) offers a novel and sustainable strategy to improve plant growth and secondary metabolite production in *Catharanthus roseus*, particularly under drought conditions. This study evaluated the effects of NPF and NPF combined with drought stress (NPF+DS) on plant morphology, growth parameters, and the biosynthesis of key alkaloids including vinblastine, vincristine, catharanthine, and vindoline. While NPF alone significantly enhanced growth traits such as shoot and root length, leaf number, and biomass accumulation, it did not markedly increase alkaloid content. In contrast, the NPF+DS treatment not only improved growth performance relative to drought-stressed controls but also significantly elevated secondary metabolite levels. Total alkaloid content increased by 3.5-fold, with vinblastine, vincristine, catharanthine, and vindoline levels rising by 4.16, 3.3, 3.2, and 2.1 fold respectively, compared to the control. These findings suggest that NPF application under drought stress can mitigate the adverse effects of water scarcity while simultaneously promoting the accumulation of valuable therapeutic compounds. This dual effect makes NPF a promising, eco-friendly tool for enhancing both biomass and phytochemical yield in medicinal plant cultivation under challenging environmental conditions.

Keywords: Algae, Catharanthus roseus, drought stress, nano-phyco-fertilizer, plant growth

Introduction

Catharanthus roseus (L.) G. Don, commonly known as Madagascar periwinkle, is a well-established medicinal plant valued for its production of pharmaceutically important alkaloids such as vincristine, vinblastine, catharanthine, and vindoline (Arulvendhan et al., 2024; Tikhomiroff, & Jolicoeur, 2002). These indole alkaloids possess potent anti-cancer properties and are widely used in chemotherapy (Goswami et al., 2024; Islam et al., 2022). However, the yield of these secondary metabolites from natural sources remains low, creating a need for innovative strategies to enhance their in planta accumulation (Gholizadeh et al., 2023; Yahyazadeh et al., 2021). Recent advances in plant nanobiotechnology and stress physiology suggest that external interventions such as nano-fertilizer application and abiotic stress imposition can modulate secondary metabolism (Hassan et al., 2021; Fouad et al., 2021). Among these, iron nanoparticles (INPs) have gained attention due to their ability to enhance plant metabolic pathways, especially those involving iron-dependent enzymes (Mondal et al., 2023). Meanwhile, algal biomass, rich in phytohormones, micronutrients, and bioactive compounds, has been successfully used in organic farming systems (Hashemi et al., 2025; Sales et al., 2024). Combining both components i.e Nano-Phyco-Fertilizer (NPF), iron nanoparticle-loaded algal biomass presents a promising, sustainable biotechnological tool to enhance plant growth and metabolite production (Mondal et al., 2024a, 2024b).

In our previous work, the efficacy of NPF (from *Ulva intestinalis*) was established in rice cultivation, demonstrating significant improvements in plant growth, nutrient content, and yield (**Figure 1**A) (Mondal et al., 2024). Building upon this foundation, the present study investigates the application of this standardized NPF on *C. roseus* for the first time. Initially, four treatment sets

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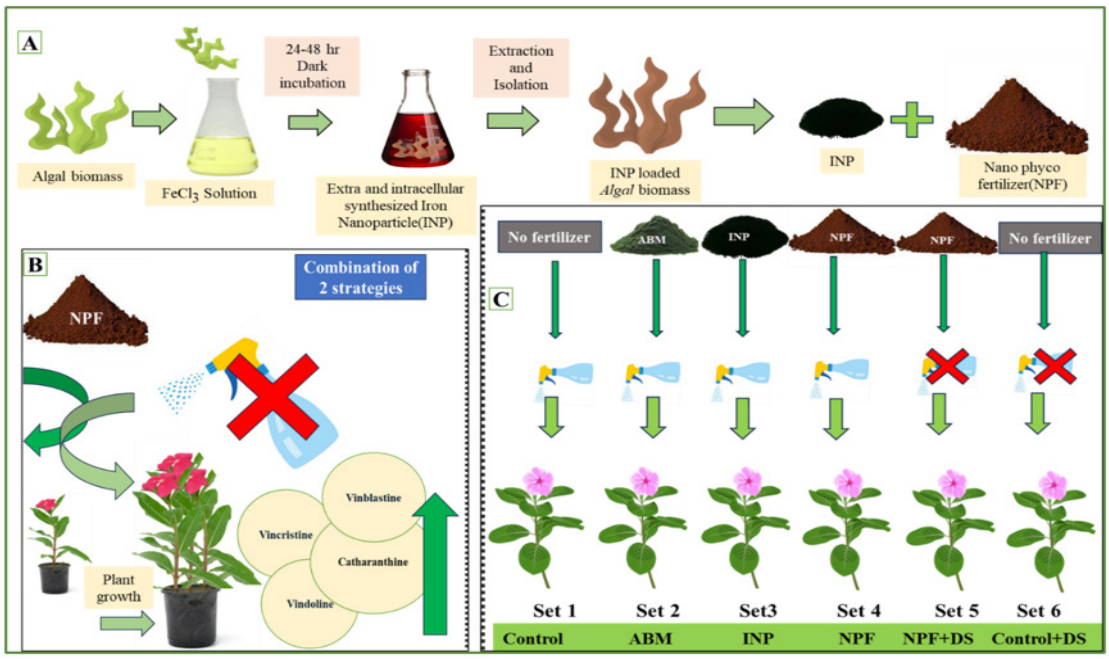

Figure 1 - Work plan of the experiment. A-Production of Nano-Phyco-Fertilizer (NPF) from *Ulva intestinalis*, B-Combining effect of NPF and Drought stress to the *Catharanthus roseus*, C- Combination of NPF and Drought stress application on different sets

were established: Control, algal biomass (ABM), iron nanoparticles (INP), and NPF. The results indicated that NPF treatment led to enhanced growth and a notable increase in total alkaloid content per plant, including vincristine, vinblastine, catharanthine, and vindoline. This increase was attributed to both improved biomass and a potential stimulation of biosynthetic pathways. To further refine this approach and increase secondary metabolite concentration per unit biomass, drought stress was introduced based on literature evidence suggesting that water limitation can upregulate plant secondary metabolism (Shil & Dewanjee 2023; Ababaf et al., 2021; Alhaithloul et al., 2019) (Figure 1B). Two additional treatment groups were designed- NPF under drought stress and drought stress alone (without any amendment), both receiving reduced irrigation (100 ml per 5 kg soil). Remarkably, the combination of NPF and drought stress led to the highest accumulation of alkaloids per gram of dry weight, suggesting a synergistic effect between nutrient enhancement and abiotic stress-induced metabolic shifts.

So, this study reports, for the first time, the combined application of Nano-Phyco-Fertilizer and drougth stresse as a novel strategy to enhance therapeutc alkalcaloid production in Catharanthus roseus. The fingings hold potential for sustainable, high-value phytopharmaceutical production using eco-friendly and cost-effective methods.

Materials and Methods

NPF preparation, Plant Material and Soil Preparation

NPF was synthesized using intact biomass of *Ulva intestinalis*. Fresh algal biomass was thoroughly washed, chopped, and incubated directly in an aqueous solution (0.01M) of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) under controlled conditions. The biosynthesis of iron nanoparticles was indicated by a visible color change of the solution. After sufficient incubation, the biomass now enriched with iron nanoparticles, was collected, air-dried, and finely powdered to obtain the final Nano-Phyco Fertilizer (NPF). This dry formulation was stored in airtight containers and used for treatment applications.

Catharanthus roseus seeds were obtained from ICAR-CIFRI, Vadodara, Gujarat, India. After

Table 1 - Detailed component of different sets)

Set Name	Description	Amount of Fertilizer	Amount of water ml/kg/day
Control	Soil without fertillizer	-	500
ABM	Dried Algal biomass	29	500
INP	Iron nanoparticles	2	500
NPF	Nano phyco fertilizer	29	500
NPF +DS	Nano-Phyco-Fertilizer with drought stress	29	100
Control + DS	Soil without fertilizer with drought stress	-	100

germination, seedlings were transplanted into 10 L water-leach-proof plastic pots filled with 5 kg of soil collected from conventional paddy fields of Tamluk, Purba Medinipur, West Bengal (22.2858° N, 87.9189° E). The soil was autoclaved, air-dried, and sieved through a 3 mm mesh before use. Its physico-chemical properties were recorded as follows: inorganic nitrogen 92 mg/kg, phosphorus 7.4 mg/kg, potassium 85 mg/kg, available organic carbon 9.8 g/kg, iron 7.2 mg/kg, and pH 5.3. The pots were initially irrigated with 1 L of water to ensure proper soil settlement. Six treatment sets were designed to evaluate the role of nano-phycofertilizer (NPF) under both normal and drought conditions. The first four sets included: Control (no amendment), ABM (algal biomass at 29 g/kg soil), INP (iron nanoparticles at 2 g/kg soil), and NPF (iron nanoparticle-loaded algal biomass at 29 g/kg soil), with each receiving 500 ml water per day per 5 kg of soil. These application doses were standardized based on *Mondal et al., 2024*. To assess drought stress effects, two additional sets were established: a drought control (no amendment) and a drought + NPF treatment, both receiving only 100 ml water per day per 5 kg of soil (Table 1) (Figure 1C).

Assessment of Growth Parameters

Growth-related parameters were recorded to evaluate the effect of different treatments on *Catharanthus roseus*. Measurements were taken at 60 days after treatment (i.e., 10 weeks after transplantation). Plant height (shoot length), root length and Main stem diameter were measured using a measuring scale. The number of leaves per plant and leaf area were manually counted. Fresh weights of shoots and roots were recorded immediately after harvesting. Dry weights were obtained by oven-drying the samples at 50 ± 2 °C until a constant weight was achieved.

Biochemical Compounds Estimation and Stress-Related Enzyme Assays

Leaf samples were collected from each treatment group at 60 days after treatment for biochemical analyses. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were measured spectrophotometrically following Arnon (1949) and Lichtenthaler and Wellburn (1983). Iron content in the leaf tissues was estimated using atomic absorption spectroscopy (ICP-OES) after acid digestion. Glutathione content was quantified according to the method of Griffith (1980). Proline accumulation, as an indicator of osmotic stress, was estimated following Bates et al. (1973). Total alkaloid content was determined using a standard acid–base extraction method and expressed as mg/g dry weight (Gupta et al., 2021).

In addition to biochemical parameters, stress-related enzyme activities were assessed. Catalase (CAT) activity was measured following Aebi (1984) by monitoring the decomposition of hydrogen peroxide at 240 nm. Lipid peroxidation was evaluated by estimating malondialdehyde (MDA) content through the thiobarbituric acid reactive substances (TBARS) method (Heath and Packer, 1968). Ascorbate peroxidase (APX) activity was measured following Nakano and Asada (1981) by monitoring the decrease in absorbance at 290 nm due to ascorbate oxidation. Superoxide dismutase (SOD) activity was determined according to Beauchamp and Fridovich (1971), based on the inhibition of nitroblue tetrazolium (NBT) reduction. All enzyme extractions were carried out in ice-cold phosphate buffer (50 mM, pH 7.0), and spectrophotometric readings were taken using a UV–Vis spectrophotometer [Hitachi U-2900 (Tokyo, Japan)].

Secondary Metabolite Determination

The major secondary metabolites, namely vinblastine, vincristine, catharanthine, and vindoline, were quantified using High-Performance Liquid Chromatography (HPLC) analysis. At the end of the treatment period, leaf samples were harvested, washed thoroughly with distilled water, and shade-dried. The dried samples were finely powdered and subjected to methanolic extraction. The extracts were filtered through a 0.22 µm syringe filter before HPLC injection. Quantitative analysis was performed using a reverse-phase C18 column, with a mobile phase consisting of acetonitrile and water (containing 0.1% formic acid) in a gradient mode. The flow rate was maintained at 1.0 mL/min, and detection was carried out at a wavelength of 254 nm. Standard compounds of vinblastine, vincristine, catharanthine, and vindoline were used for calibration, and the quantification of metabolites in the samples was based on the corresponding standard curves.

Statistical analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software (version 8.4.3, GraphPad Software, San Diego, California, USA). One-way analysis of variance (ANOVA) was used to assess the significance of differences among the treatment groups, followed by Tukey's multiple comparison test. Differences were considered statistically

significant at $p \leq 0.05$, and significance levels were represented as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

Result and Discussion

Plant Growth Parameters

The shoot length of *Catharanthus roseus* was significantly affected by the different treatments (**Figure 2**). The highest shoot length was observed in the NPF group (108 cm), followed by NPF+DS (98 cm), showing a growth increase of 31 cm and 21 cm, respectively, which corresponds to a 40.26% and 27.27% increase compared to the Control group. The ABM and INP treatments also showed enhanced shoot lengths of 82 cm and 79 cm, with growth increases of 5 cm (6.49%) and 2 cm (2.60%), respectively. In contrast, the Control+DS treatment resulted in a decrease in shoot length, with a reduction of 6 cm (–7.79%), highlighting the negative impact of drought stress when applied alone (**Figure 3**-A).

The root length in the various treatments showed significant variation, with the NPF group exhibiting the highest root length (7.6 cm), followed by NPF+DS (6.9 cm), reflecting growth increases of 1.7 cm (22.88%) and 1 cm (13.56%), respectively, compared to the Control (5.9 cm). The ABM and INP treatments also resulted in improved root lengths of 6.6 cm and 5.2 cm, showing increases of 0.7 cm (11.86%) and 0.4 cm (6.78%), respectively. In contrast, the Control+DS treatment led to a reduction in root length (5.0 cm), with a decrease of 0.9 cm (–15.25%) compared to the Control. Notably, when comparing NPF+DS with Control+DS, the NPF+DS treatment exhibited significantly better root growth under drought stress conditions, with an increase of 1.9 cm (38%) in root length (Figure 3B).

The leaf number across various treatments exhibited considerable variation. In the NPF treatment, the leaf number ranged from 81 to 87, with the highest values observed. The NPF+DS treatment showed a range of 60-68 leaves as well. The ABM and INP treatments showed leaf numbers ranging from 63 to 69 and 43 to 51, respectively. The Control group exhibited a leaf number range of 38 to 43, while the Control+DS treatment showed the lowest range, from 26 to 31 leaves. When comparing NPF+DS to Control+DS, the NPF+DS treatment consistently demonstrated a higher leaf number, indicating that the application of NPF helped mitigate the negative effects of drought stress on leaf development (Figure 3C).

The main stem diameter across various treatments showed notable variation, with the NPF treatment exhibiting the largest stem diameter (7.2 mm), followed by the INP treatment (6.7 mm). The ABM treatment also showed an increased diameter of 6.6 mm, while the Control group had a smaller diameter of 3.8 mm. The NPF+DS treatment demonstrated a slightly smaller diameter (6.7 mm), while the Control+DS treatment exhibited the smallest stem diameter of 2.9 mm (Figure 3F).

The NPF treatment resulted in the highest fresh weight at 79 g, showing an impressive 118.33% increase

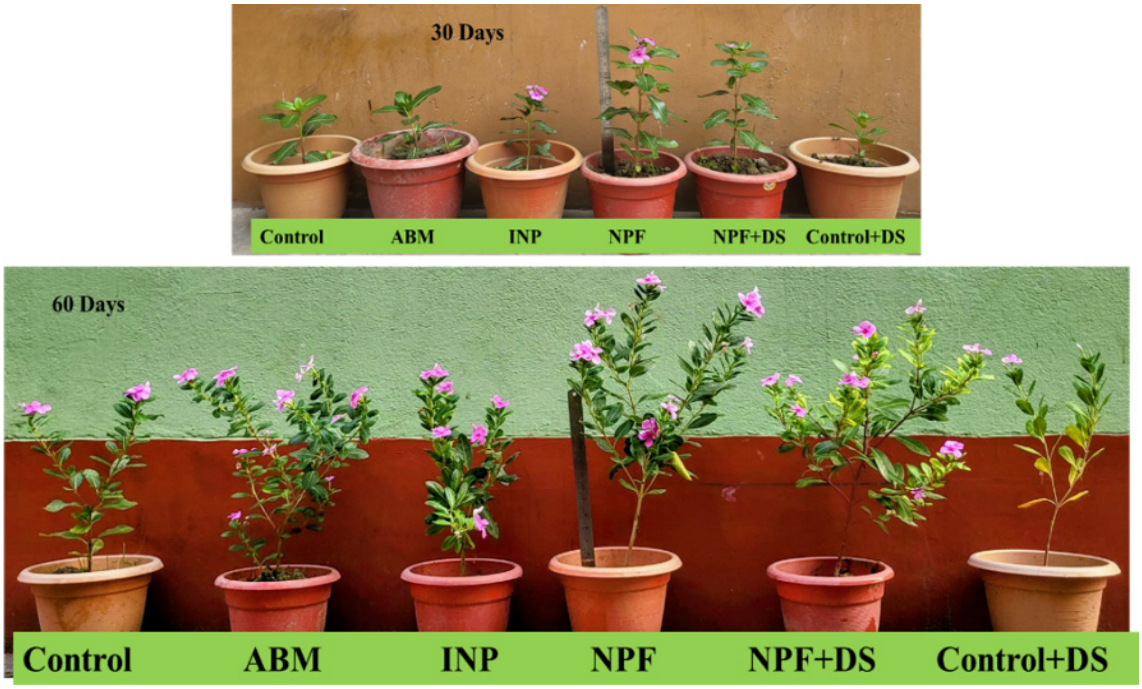

Figure 2 - Different experimental sets of *C. roseus* after 15 days and 30 days.

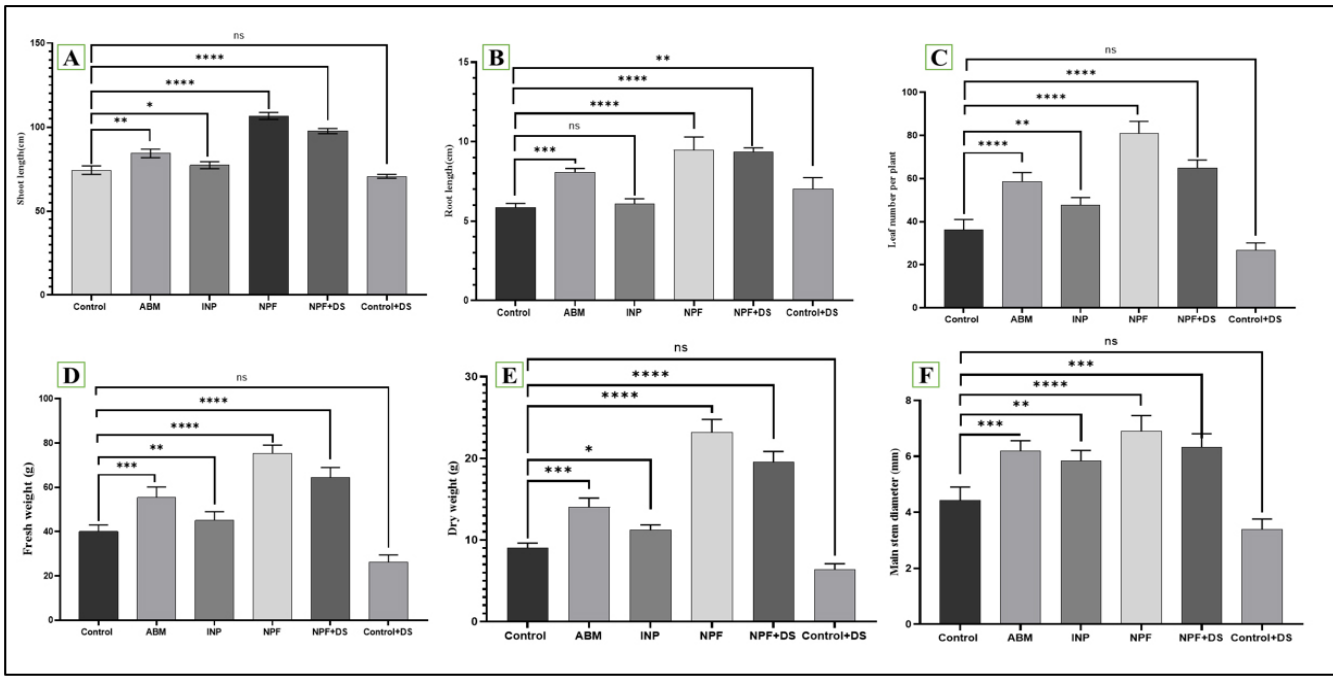

Figure 3 - Morphological parameters of *C. roseus* after 60 days. A-Shoot length, B- Root length. C- Leaf number per plant, D- Fresh weight, E- Dry weight, F- Fresh weight.

over the Control group, which weighed 36 g. The NPF+DS treatment also demonstrated a considerable increase with a fresh weight of 69 g, reflecting a 91.67% rise compared to the Control. Plants treated with ABM and INP had fresh weights of 59 g and 49 g, respectively, which corresponded to increases of 63.89% and 36.11%. On the other hand, the Control+DS group exhibited the lowest fresh weight at 28 g, marking a decrease of 22.22% relative to the Control (Figure 3D). In terms of dry weight, the NPF group again showed the highest value at 26.5 g, representing a remarkable 187.50% increase over the Control's 9.2 g. The NPF+DS treatment also yielded a substantial dry weight of 20.6 g, showing a 124.46% increase compared to the Control. The ABM and INP treatments resulted in dry weights of 15.4 g and 11.1 g, reflecting increases of 67.39% and 20.65%, respectively. Conversely, the Control+DS treatment had the lowest dry weight at 7.1 g, a reduction of 22.83% when compared to the Control (Figure 3E).

Biochemical Analysis

The biochemical composition of *Catharanthus roseus* under different treatments showed significant variations in various parameters. The chlorophyll content ranged from 2.41 mg/g in Control+DS to 4.66 mg/g in the NPF treatment, highlighting an increase in chlorophyll levels in plants treated with algal-based treatments, particularly NPF, which showed a 29.44% higher value compared to the Control (3.6 mg/g). In contrast, Control+DS demonstrated a notable decline in chlorophyll content by 33.61% (**Figure 4A**). This suggests that the stress imposed by drought (Control+DS) may have hindered chlorophyll production, while treatments like NPF helped sustain chlorophyll synthesis under normal conditions and stress.

Similarly, carotenoid content, which ranged from 0.19 mg/g in Control+DS to 0.38 mg/g in NPF, showed a marked increase (57.89%) in the NPF group compared to the Control (0.25 mg/g). This suggests that NPF may play a crucial role in improving plant antioxidant potential, contributing to better adaptation to stress conditions. On the other hand, the Control+DS group had a significant reduction in carotenoid content by 24%, possibly due to stress-induced degradation of these vital compounds (Figure 4B).

The iron content also demonstrated an interesting pattern. The highest value was recorded in the NPF treatment at 216 mg/kg, showing a dramatic increase of more than two fold compared to the Control (28 mg/kg) (Figure 4D). This indicates the effective uptake and accumulation of iron in plants treated with NPF, likely facilitated by the bioavailability of nanoparticles. Both NPF+DS (201 mg/kg) and INP (106 mg/kg) treatments also showed considerable improvements in iron content compared to Control (28 mg/kg), suggesting a positive effect of iron-based treatments on nutrient accumulation, even under drought stress conditions.

Alkaloid content, a key secondary metabolite in *Catharanthus roseus*, showed varying results. The highest value was observed in Control+DS (3.56%), while

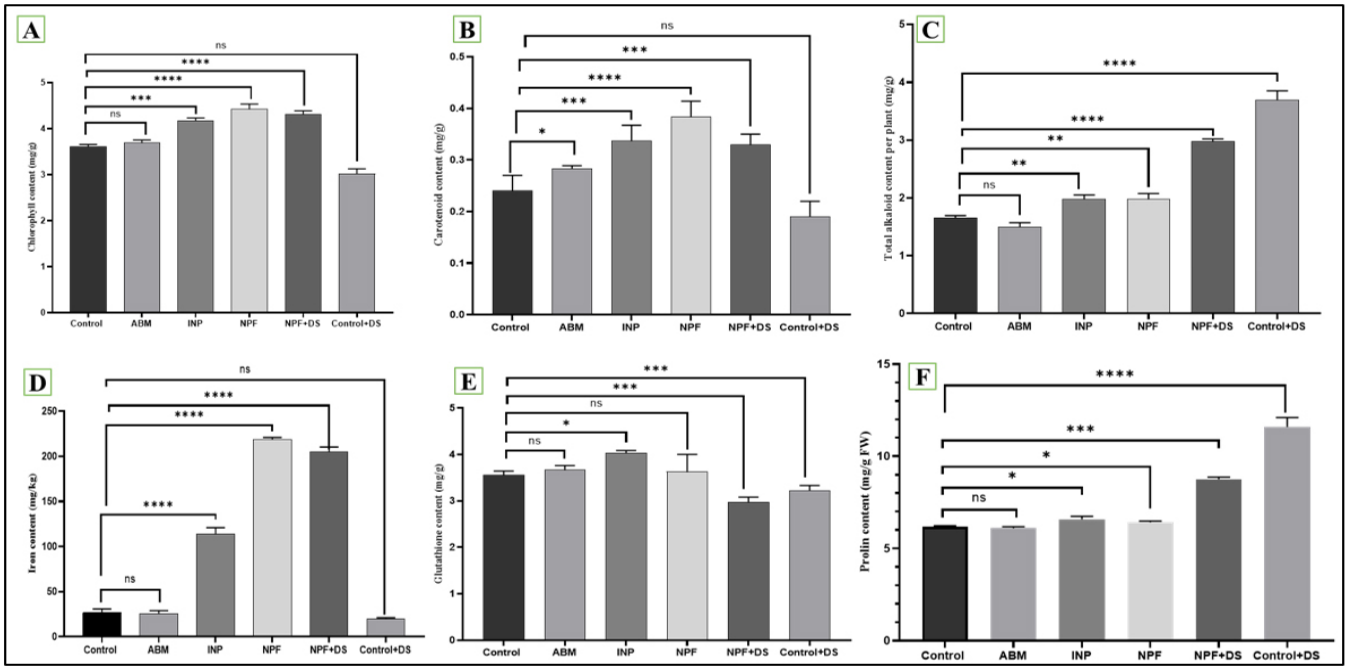

Figure 4 - Biochemical parameters of *C. roseus* after 60 days. A-Chlorophyll content, B – Carotenoid content, C- Total alkaloid content, D- Iron content, E- Glutathione content, F- Proline content.

the NPF treatment (1.88%) exhibited a decrease of 11.76% compared to the Control (1.69%). This suggests that drought stress could stimulate alkaloid production, possibly as a defensive mechanism. Interestingly, the NPF+DS treatment had a higher alkaloid content (3.02%) than NPF alone, pointing to an interaction between the priming effect of NPF and drought stress that may enhance alkaloid synthesis (Figure 4C).

Glutathione content, an important antioxidant, showed the highest value in NPF+DS (4.33 µg/g), with a significant increase of 18.26% compared to the Control (3.66 µg/g). This suggests that NPF can enhance the plant's ability to combat oxidative stress, especially under drought conditions. However, the Control+DS treatment had the lowest glutathione content (2.01 µg/g), indicating a depletion of antioxidants under drought stress (Figure 4E).

Proline, a key osmolyte involved in stress tolerance, showed the highest value in the Control+DS (11.95 mg/g FW), a 90.67% increase compared to the Control (6.24 mg/g FW). This is expected, as proline is known to accumulate in plants under osmotic stress, contributing to cellular protection. The NPF+DS treatment also exhibited a notable increase (8.65 mg/g FW), further emphasizing the role of NPF in enhancing drought tolerance (Figure 4F).

Stress-Related Enzyme Assays

The stress-related enzyme activities reflected the overall impact of the treatments on oxidative stress management. Catalase (CAT), which plays a key role in detoxifying hydrogen peroxide, showed the highest activity in Control+ DS (0.288 mmol/mg protein), marking a significant increase of 84.62% compared to the Control (0.156 mmol/mg protein). This increase suggests that drought stress triggered a more robust antioxidant defense response in the plants. However, the NPF+DS treatment (0.201 mmol/mg protein) showed an increase of 28.85% over the Control (0.156 mmol/mg protein), indicating that NPF may enhance catalase activity, albeit at a lower level than in drought-stressed plants (**Figure 5**A).

Lipid peroxidase (LPX), an enzyme associated with membrane damage due to oxidative stress, also showed a clear increase under stress conditions. The highest LPX activity was observed in Control+DS (0.228 mmol/mg protein), which was a significant 58.33% higher than the Control (0.144 mmol/mg protein). The NPF+DS treatment (0.198 mmol/mg protein) also showed a marked increase of 37.5% compared to the Control, suggesting that NPF could help mitigate membrane damage under drought stress (Figure 5B).

Superoxide dismutase (SOD), which is involved in scavenging reactive oxygen species (ROS), showed the highest activity in Control+DS (4.49 µmol/mg FW), an increase of 26.21% compared to the Control (3.56 µmol/mg FW). This increase in SOD activity reflects the plants' enhanced ability to combat oxidative stress under drought conditions. The NPF+DS treatment (4.02 µmol/mg FW) exhibited a 12.92% increase in SOD activity compared to the Control, further supporting the notion that NPF can aid in stress tolerance (Figure 5C).

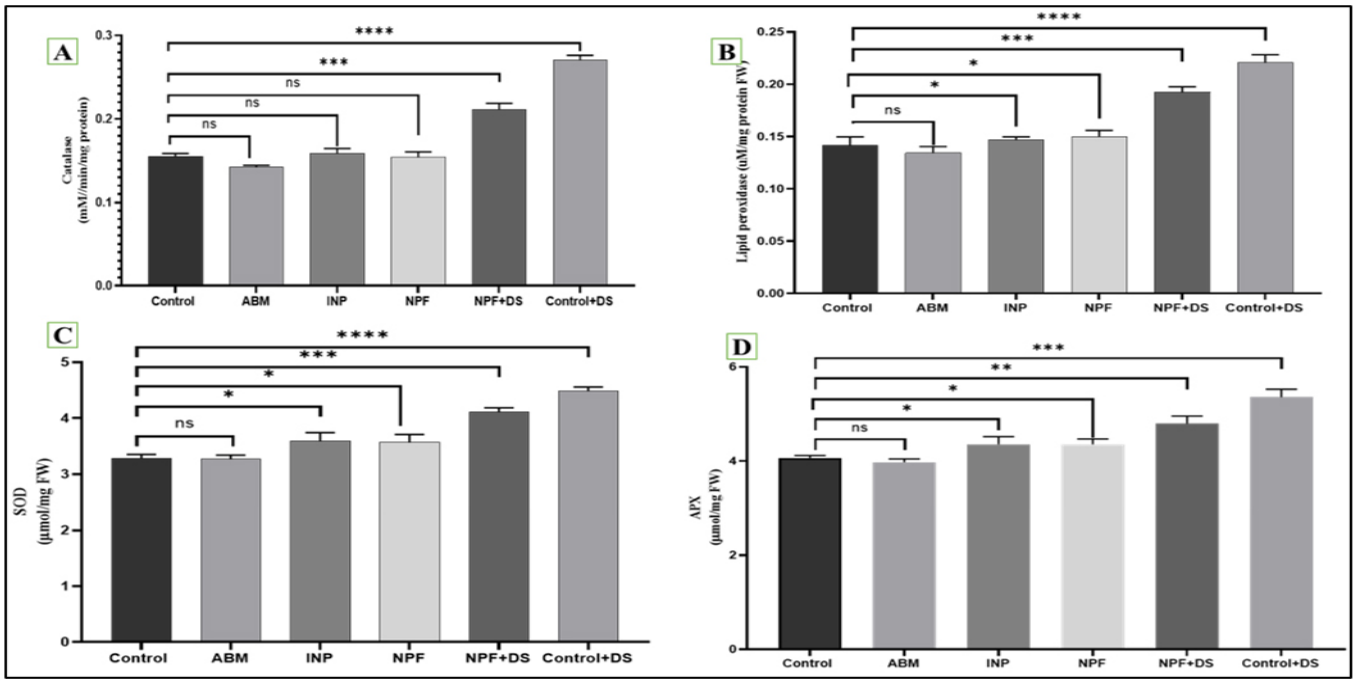

Figure 5 - Stress-related enzymatic assays in *C. roseus* after 60 days. A- Catalase activity, B- Lipid peroxide activity, C- Superoxide dismutase activity, D- Ascorbate peroxidase activity.

Ascorbate peroxidase (APX), another important antioxidant enzyme, showed an increase in activity under all treatments, with the highest value recorded in Control + DS (5.21 µmol/mg FW), reflecting a 30.43% increase compared to the Control (4.01 µmol/mg FW). The NPF+DS treatment (4.65 µmol/mg FW) exhibited an 11.16% increase in APX activity, indicating that NPF can enhance the antioxidant defense, particularly in plants under stress (Figure 5D).

Secondary Metabolite Analysis

The secondary metabolites in *Catharanthus roseus*, particularly the alkaloids vinblastine, vincristine, catharanthine, and vindoline, were significantly affected by the different treatments. These compounds are crucial for the medicinal properties of the plant, especially in the context of anticancer activity.

Vinblastine content ranged from 3.6 µg/g in the Control group to 8.8 µg/g in the control+DS treatment, exhibiting an increase of 144.44%. This suggests a substantial enhancement in vinblastine production under drought stress, which may be a stress-induced defence mechanism. However, the NPF and NPF+DS treatments showed the most remarkable increases. The NPF+DS treatment (7.4 µg/g) showed a 105.56% increase compared to the Control (3.6 µg/g), while NPF alone (5.3 µg/g) exhibited a 47.22% increase (**Figure 6**A).

Vincristine, another important alkaloid, showed a steady increase across all treatments. The Control+DS group exhibited the highest content of vincristine at 25.16 µg/g, reflecting a substantial 52.91% increase compared to the Control (16.48 µg/g). However, the NPF+DS treatment, with 23.98 µg/g, demonstrated a similar significant increase of 50.63%. Both these treatments outperformed other treatments like NPF (19.55 µg/g) and ABM (19.28 µg/g), indicating that the combined NPF and DS treatment had a particularly strong effect on vincristine biosynthesis (Figure 6B).

Catharanthine content, which plays an essential role in vincristine synthesis, was highest in the NPF+DS treatment at 2.14 µg/g, reflecting a 29.09% increase compared to the Control (1.66 µg/g). This suggests that NPF treatment, especially under drought stress, could enhance the production of catharanthine, potentially leading to increased vincristine production. While the NPF treatment also showed an increase in catharanthine (1.69 µg/g), it was the combined effect of NPF and drought stress that provided the most substantial improvement in alkaloid synthesis (Figure 6C).

Vindoline, a precursor for vinblastine and vincristine, demonstrated a progressive increase across all treatments, with the highest content recorded in the Control+DS treatment at 1.78 µg/g, a 45.90% increase compared to the Control (1.22 µg/g). The NPF+DS treatment (1.69 µg/g) also showed a 38.52% increase, while the NPF group exhibited a more moderate 17.21% increase, reaching 1.44 µg/g (Figure 6D).

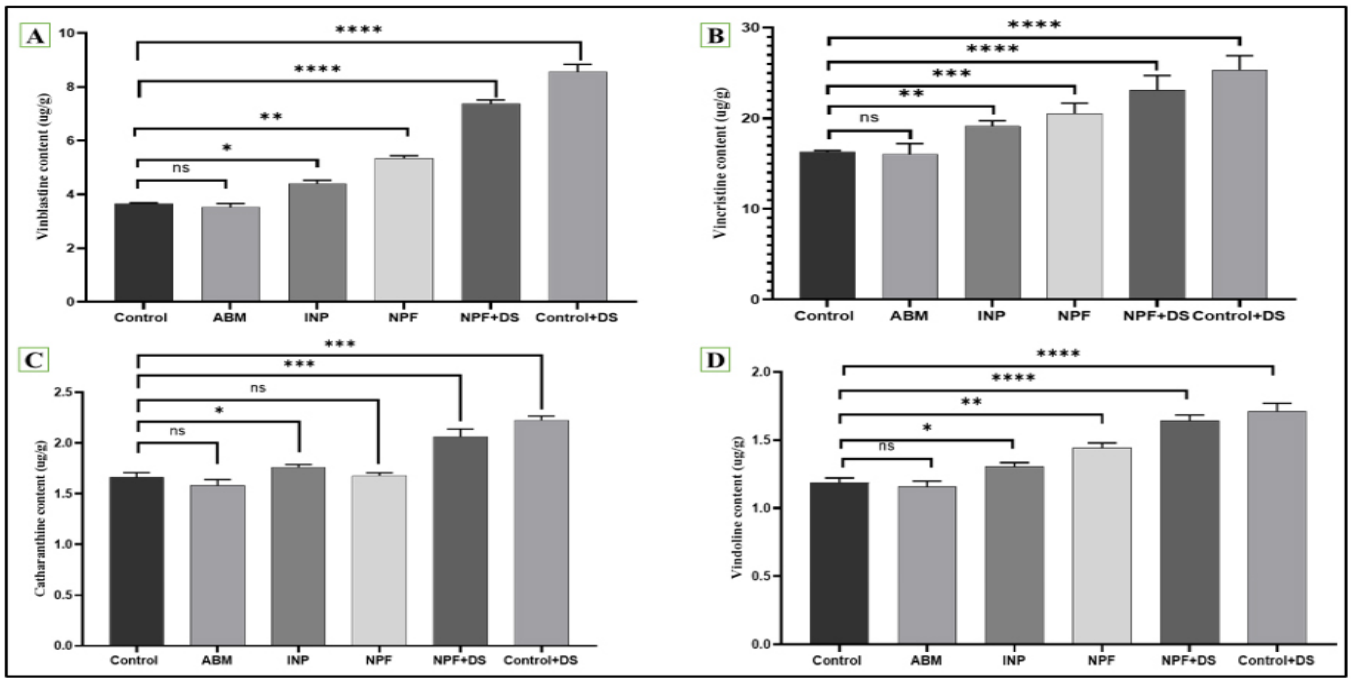

Figure. 6 - Secondary metabolites production in *C. roseus* after 60 days. A- Vinblastine content, B-Vincristine content, C- Catharanthine content, D- Vindoline content.

Enhanced Alkaloid and Secondary Metabolite Production Per Plant

The combined application of NPF and drought stress (NPF+DS) significantly enhanced the production of secondary metabolites in *Catharanthus roseus*. The total alkaloid content reached its peak in the NPF+DS treatment at 0.622 g per plant, marking a remarkable increase over NPF alone (0.498 g) and far exceeding the Control (0.156 g). Vinblastine production achieved its highest value in the NPF+DS group at 159.3 µg per plant, representing a 4.81-fold increase compared to the Control (33.12 µg) (**Figure 7A**). Similarly, vincristine production in NPF+DS plants reached 499.5 µg per plant, corresponding to a 3.29-fold increase over the Control (151.6 µg) (Figure 7B). For catharanthine, the NPF+DS treatment recorded 49.34 µg per plant, reflecting a 3.23-fold rise compared to the Control (15.27 µg) (Figure 7C). Vindoline production also showed a substantial enhancement under NPF+DS conditions, with 41.26 µg per plant, a 2.15-fold increase over the Control (19.22 µg) (Figure 7D). Compared to NPF alone, vinblastine and vindoline contents further improved by 15.76% and 11.49%, respectively, under NPF+DS, while vincristine exhibited a minimal decline (1.34%) yet remained significantly higher than all other treatments.

Several studies have investigated the potential of various nanoparticles to enhance both vegetative growth and secondary metabolite accumulation in *Catharanthus roseus*. Among them, silver nanoparticles have demonstrated a remarkable ability to stimulate the biosynthesis of key alkaloids such as vincristine and vinblastine, primarily through the modulation of oxidative stress pathways and the activation of secondary metabolic routes (Fouad et al., 2021). Zinc oxide nanoparticles and magnesium nanoparticles have also been reported to improve biomass and alkaloid content by promoting photosynthetic activity and nutrient assimilation (Sepehri et al 2025a). Similarly, titanium dioxide nanoparticles enhanced chlorophyll content, antioxidant activity, and vindoline accumulation in treated plants (Abed et al, 2023; Abyari et al., 2023).

Only a limited number of studies have explored the application of iron nanoparticles (INPs) in *Catharanthus roseus*, despite the growing interest in nanotechnology-based interventions in medicinal plants. Available reports suggest that INPs can positively influence plant growth by enhancing chlorophyll synthesis, nutrient uptake, and antioxidant activity (Askary et al., 2017). However, comprehensive studies on the mechanistic roles of INPs in modulating secondary metabolite biosynthesis pathways in *Catharanthus* remain scarce. This highlights the novelty and importance of the present investigation in filling this critical research gap.

On the other hand, although limited, emerging research has highlighted the potential of algal-based biofertilizers in improving the growth and metabolic activity of *Catharanthus roseus*. Seaweed extracts, particularly from *Sargassum*, *Gracilaria*, and *Ulva*

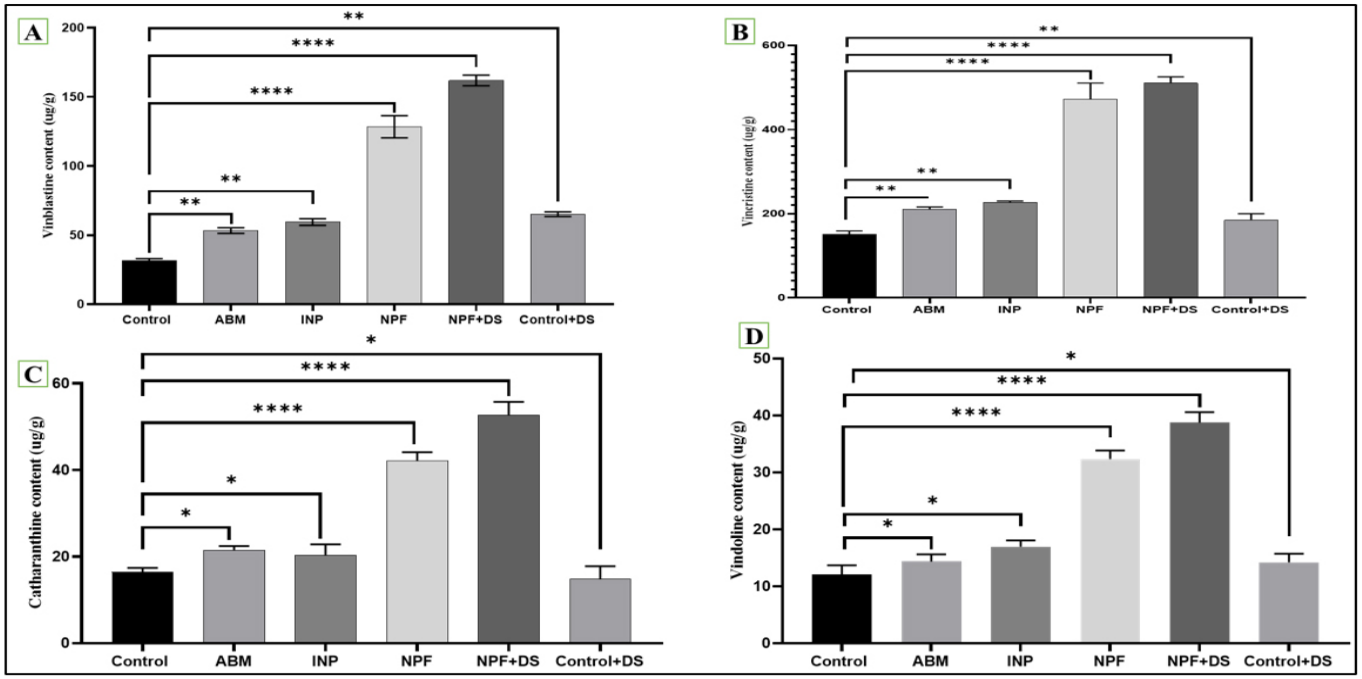

Figure 7 - Important secondary metabolites from one Catharanthus plant. A-Vinblastine content, B-Vincristine content, C-Catharanthine content, D- Vindoline content

species, have been reported to stimulate vegetative growth and enhance alkaloid production in *C. roseus* by supplying essential micronutrients, phytohormones, and elicitors (Hashemi et al., 2025). These algal formulations improve root architecture, chlorophyll content, and antioxidant enzyme activity, thereby promoting both primary and secondary metabolism. Additionally the algal extract application significantly increased vindoline and catharanthine accumulation, possibly due to the synergistic action of algal-derived cytokinins and mineral content. Such studies support the notion that algal fertilizers can serve as sustainable biostimulants for medicinal plants like *Catharanthus*, with potential to replace or supplement synthetic inputs (Kularathne et al.,2021; El-Beltagi et al., 2023).

The concept of Nano Phyco-Fertilizer (NPF), which combines the advantages of algal bioactive compounds with nanoparticle delivery systems, has gained momentum in sustainable agriculture and medicinal plant research. Algae are naturally rich in micronutrients, amino acids, vitamins, and plant growth-promoting substances like auxins, cytokinins, and gibberellins, which can enhance plant development and secondary metabolism. When fortified with nanoparticles—such as iron, zinc, or copper—these algal matrices can serve as efficient carriers, facilitating slow and targeted nutrient release while minimizing environmental losses. Recent studies have demonstrated that NPFs can improve nutrient use efficiency, photosynthetic activity, antioxidant response, and metabolite biosynthesis in crops like rice, tomato, and maize (Mondal et al., 2024a; 2024b). In the context of *Catharanthus roseus*, the present study provides pioneering evidence that iron-based NPF not only sustains plant growth under drought stress but also promotes the biosynthesis of key alkaloids, highlighting its potential as a dual-action biostimulant and elicitor. This innovative approach aligns with the global push for eco-friendly fertilizers that support high-value medicinal plant cultivation under resource-limited conditions.

The significant improvement in plant growth parameters observed under NPF and NPF+DS treatments highlights the potential of iron nanoparticle-loaded bioformulations to enhance plant vigor, even under stress conditions. The marked increase in shoot length, root length, leaf number, fresh and dry weight under these treatments compared to the control suggests that NPF application promotes better nutrient uptake and physiological efficiency. Interestingly, the NPF+DS treatment also maintained superior growth compared to drought-stressed controls, indicating that the fortified nanoparticles may impart a protective advantage against abiotic stress. It is well known that nanoparticles can modulate antioxidant defense systems, reduce oxidative damage, and maintain cellular integrity during stress, aligning well with the current findings (Sepehri et al., 2025; Hassan et al., 2021). The slight decline in some growth parameters under drought stress alone (Control+DS) further strengthens the view that NPF supplementation can act as a buffer against environmental stresses. c Thus, the integration of nanoparticle-based bioformulation

with drought stress management appears promising for maintaining growth performance under challenging conditions.

The application of nanoparticle-fortified bioformulations (NPF) demonstrated a significant positive impact on the overall plant growth and vigor. In all morphological parameters like shoot and root length, leaf number, fresh and dry biomass, plants treated with NPF alone showed substantial improvements compared to the control. This clearly suggests that the integration of iron nanoparticles into the plant system facilitated better nutrient acquisition, enhanced photosynthetic efficiency, and improved physiological functioning, ultimately resulting in healthier, more robust plants. Previous studies also highlight similar trends, where nano-iron applications promoted vegetative growth by stimulating chlorophyll biosynthesis and efficient resource allocation (Mondal et al., 2024a, 2024b; Feng et al., 2022;El-Desouky et al., 2021).

On the other hand, drought stress (DS) alone, although effective in enhancing the biosynthesis of secondary metabolites such as vinblastine, vincristine, catharanthine, and vindoline, led to a noticeable compromise in plant growth (Mall et al., 2022). This is a well-documented phenomenon, where drought-induced oxidative stress triggers the activation of secondary metabolism as a survival mechanism (Shil & Dewanjee, 2022; Aguirre-Becerra et al., 2021). However, the trade-off between enhanced metabolite production and reduced biomass often limits the commercial feasibility of using drought as an elicitor.

What makes the present study truly novel is the strategic combination of NPF and drought stress. When NPF was applied alongside drought conditions (NPF+DS), plants not only maintained their growth performance but also exhibited a remarkable surge in secondary metabolite content. Unlike the drought-stressed controls, where plant vitality visibly declined, the NPF+DS plants sustained active growth, indicating that nanoparticle supplementation provided the necessary physiological support to withstand water scarcity. The enhanced antioxidative enzyme activities under NPF+DS further underscore the role of nanoparticles in mitigating stress-induced damage, preserving membrane stability, and sustaining metabolic processes even under adverse conditions.

Moreover, literature suggests that application of iron nanoparticles (INP) and nanoparticle-fortified bioformulations can stimulate the activities of enzymes that require iron as a cofactor (Rezayian et al., 2023; Sutariya et al., 2021). The increase in available iron likely boosts the functionality of these iron-dependent enzymes, which in turn may accelerate secondary metabolite biosynthesis pathways, contributing further to the elevated production of therapeutic compounds observed in the present study.

This dual advantage- simultaneous promotion of biomass and valuable secondary metabolites, offers a promising and commercially applicable approach for medicinal plant cultivation. By integrating nanoparticle bioformulations into drought management strategies, it becomes possible to maximize both plant productivity and phytochemical yield without compromising either. Such a technique holds potential not only for *Catharanthus roseus* but also for a wide range of high-value medicinal plants where secondary metabolite accumulation is crucial for therapeutic and industrial use.

Conclusion

The integration of iron nanoparticle-loaded bioformulations (NPF) with drought stress management has proven to be a highly effective and innovative strategy for boosting both plant growth and secondary metabolite production in *Catharanthus roseus*. The combined application of NPF and drought stress resulted in remarkable improvements across both morphological and biochemical parameters, demonstrating a synergistic effect on plant vigor and secondary metabolite accumulation. The combined NPF and drought stress treatment significantly boosted secondary metabolite accumulation in *Catharanthus roseus*, with total alkaloids increasing 3.5-fold, vinblastine 4.16-fold, vincristine 3.3-fold, catharanthine 3.2-fold, and vindoline 2.1-fold compared to the control, despite a slight reduction in vincristine compared to NPF alone. These results collectively highlight the strong potential of the combined NPF and drought stress treatment to significantly boost secondary metabolite accumulation in *Catharanthus roseus*.

The findings from this study clearly demonstrate that NPF not only supports plant growth by enhancing nutrient uptake and physiological efficiency, but it also significantly boosts the biosynthesis of important secondary metabolites under drought stress. The combination of NPF and drought stress resulted in a balanced outcome, with enhanced plant growth and increased production of valuable alkaloids, surpassing the effects of NPF or drought stress alone. This approach could revolutionize agricultural practices, especially in regions facing water scarcity, and contribute to the development of more resilient, high-yielding medicinal plants.

Future research should aim to further explore

the long-term effects of this combined treatment on soil health, plant-microbe interactions, and other medicinal species to broaden the applicability of this technique. With continued investigation, the use of nanoiron-based algal fertilizer combined with drought management may become an essential part of sustainable agricultural practices in the cultivation of high-value medicinal crop.

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Authors contribution

Anwesha Mondal: Writing original draft, data acquisition, preparation of Figureures, analysis. Santanu Paul: Supervision, writing, editing, reviewing.

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All data generated or analysed during this study are included in this published article

Conflict of interest

There is no conflict of interest.

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