



Green-synthesized Fe₂O₃ and Ca(OH)₂ nanoparticles, Alongside Rosemary extract, Enhance growth, Antioxidant defense, and Secondary Metabolite Accumulation in *Lupinus Termis* (Forssk.)

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Abstract

Lupinus termis (Forssk.) is a valuable leguminous plant, prized for its medicinal and nutritional properties. This study aimed to investigate the biochemical and physiological responses of lupine to elicitation using biosynthesized nanoparticles (NPs) and a plant-derived extract. Iron oxide (Fe₂O₃) and calcium hydroxide (Ca(OH)₂) NPs were synthesized via rosemary (*Salvia rosmarinus* Spenn.) leaf polyphenols. The efficacy of green synthesized NPs was compared to rosemary extract as separate elicitor treatments in a greenhouse experiment to increase plant growth and metabolism. Both the NPs and rosemary extract significantly enhanced growth, biomass and photosynthetic attributes, whereas the NPs induced greater growth activity. All treatments reduced malondialdehyde levels while increasing hydrogen peroxide, ascorbic acid, total antioxidant activity, and the activities of polyphenol oxidase and peroxidase. Additionally, Ca(OH)₂ NPs increased the levels of key polyphenols constituents (ellagic acid, syringenic acid and kaempferol); whereas Fe₂O₃ NPs increased quercetin and myricetin accumulation. Rosemary treatment induced the highest levels of chlorogenic acid and naringin. Both NPs and rosemary extract increased key alkaloids (lupinine and angustifoline) to remarkable levels, which correlated with secondary metabolism and PAL activation. Furthermore, NPs boosted endogenous auxins (IAA) and gibberellins (GA) via modulating abscisic acid (ABA) and salicylic acid (SA) levels, thereby improving hormonal homeostasis and growth. Because of its bioactive phenolic derivatives, rosemary markedly increased SA levels while lowering GA and ABA, which strengthened plant defense. These findings demonstrate that green-synthesized Fe₂O₃ and Ca(OH)₂ NPs, as well as rosemary extract, improved *L. termis* growth, stress tolerance, and nutraceutical compounds.

Keywords Alkaloids · Antioxidants · Nanoparticles · Phenolics · Photosynthesis · Phytohormones

1 Introduction

White lupine (*Lupinus termis* Forssk.) is a significant pulse crop cultivated in diverse environments. Its seeds are an excellent nutritional source, providing 13% oil and 33–40% crude protein (Sadak and Ramadan 2021). This nutritional profile renders white lupine a valuable addition to diets, offering several significant health benefits, including promoting cell renewal, regulating blood glucose levels, lowering uric acid and cholesterol levels, and enhancing intestinal

transit owing to its high fiber and trace element contents (Jimenez-Lopez 2020; Prusinski 2017). In addition, the phytochemicals found in *L. termis* play crucial roles in preventing chronic inflammation-associated diseases, such as diabetes and hypertension, because of their antioxidant, anti-hyperlipidemic, and anti-inflammatory properties (Andor et al. 2016). Therefore, the exploration of physiological and biochemical responses of lupine to various elicitation treatments is a major field, with the goal of increasing the levels of its valuable secondary metabolites.

In agriculture, nanotechnology is emerging as a sustainable approach to reduce chemical inputs and address challenges such as nutrient delivery and disease management, contributing to improved plant health and productivity (Singh et al. 2021). Nanoparticles (NPs) are used in molecular pharming, both in vivo and in vitro, to stimulate

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secondary metabolite production from plants (Lala 2021). NPs can mediate the overproduction of reactive oxygen species (ROS), which act as effective elicitors of the innate immune response in plants (Chandra et al. 2015). Elicitor-driven stress acclimation can also intersect chaperone networks, providing a mechanistic bridge between mild elicitation (e.g., NP-triggered ROS waves) and durable stress resilience (Wang et al. 2023b). Converging with nanoparticle-based strategies, beneficial plant-derived extracts and microbial inoculants can optimize plant performance. For example, *Bacillus subtilis* improved water-use efficiency, nutrient acquisition, and yield via rhizosphere microbiome optimization in cotton (Ren et al. 2025). These biostimulants are complementary, low-input levers that can be integrated with green-synthesized nano-elicitors.

A wide range of inorganic metals and metallic oxide NPs, such as zinc, iron, sulfur, calcium, copper, etc., can be applied for seed priming or foliar applications to promote healthy growth of plants (Kasim et al. 2017; Saad-Allah and Ragab 2020). When applied as a sustainable supply of Fe, magnetic iron nanoparticles can elicit antioxidant defenses and the synthesis of key flavonoids in *Dracocephalum kotschy* hairy root cultures (Nourozi et al. 2019). Mohsen et al. (2016) reported that chemically synthesized Ca NPs enhanced the antioxidant capacity, early growth and phenolic content of lupine. Similarly, calcium nanoparticles are vital for maintaining cell wall integrity and enhancing crop yield and quality more effectively than conventional calcium sources are (Davarpanah et al. 2018). In this context, Ca^{2+} modulates the transcription of target genes by modifying the phosphorylation state of certain transcription factors, thereby eliciting secondary metabolism (Lala 2021). Further exploration of innovative sustainable approaches is needed to ensure their superior benefits and safe application with minimal adverse impacts.

To overcome the risks of chemical methods, eco-friendly green synthesis is favored for nanoparticle production (Ragab and Saad-Allah 2020). Rosemary (*Salvia rosmarinus* L. Spenn.) has been exploited in green synthesis methods, leveraging the rich secondary metabolite content. It is a woody herb native to the Mediterranean region, from the Lamiaceae family, with fragrant and needle-like leaves (Bakirel et al. 2008). Its leaf extract is rich in polyphenolic compounds, which are responsible for more than 90% of the extract's antioxidant activity, serving as reducing and capping agents for NPs synthesis (Moroda et al. 2025). In addition to phenolic-rich extracts such as *S. rosmarinus*, other plant biopolymers can also function as potent bio-elicitors and immunomodulators. For example, a polysaccharide from *Alocasia cucullata* was recently purified and structurally

characterized, demonstrating robust immune-stimulatory activity (Gong et al. 2024). These bioactive compounds can prime defense signaling, complementing polyphenol-mediated redox buffering and suggesting broader design rules for phytochemical-assisted nanoparticle synthesis and plant biostimulation. Thus, the green fabricated NPs derived from rosemary extract might conserve beneficial characteristics and positively influence plant physiology in agro-systems.

Although previous work by Mohsen et al. (2016) explored the effects of chemical calcium hydroxide NPs on lupine metabolic activities, their green-synthesized forms compared to rosemary extract have not been studied as eliciting agents in this species. The present study targeted simple and cost-effective synthesis of iron oxide (Fe_2O_3) and calcium hydroxide ($\text{Ca}(\text{OH})_2$) NPs as micro and macronutrients using rosemary leaf extract. The potential benefits of sustainable rosemary-derived NPs and their application are hypothesized to boost biomass, bioactive constituents, antioxidant defense, and hormonal modulation in white lupine. Therefore, transferring rosemary's antioxidant properties could impart nano-fertilizing properties to improve lupine productivity and reduce the risk of chemical inputs in agro-systems.

2 Materials and Methods

2.1 Preparation and Characterization of Rosemary Leaf Extracts

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (USA) and Elnasr company (Cairo, Egypt) unless otherwise specified. Fresh rosemary leaves were obtained from the local market, washed with distilled water, and then 10 g of the leaves were ground in 100 ml of distilled water (10% w/v). The mixture was incubated at 60 °C for 10 min and allowed to cool. After cooling, the mixture was centrifuged (Centurion Scientific CR2000) at 4000 rpm for 20 min and filtered through Whatman No. 1 filter paper. The resulting rosemary extract was freshly used as a bio-reducing agent for nanoparticle preparation or diluted to 2.5% (v/v; 2.5 ml of extract per 100 ml of water) for the main pot experiment. A preliminary experiment was conducted prior to the main trial to select doses that enhanced seedling vigor without causing phytotoxicity (Table S1), which is consistent with the ranges used in comparable studies on legumes and medicinal plants (Elsherif et al. 2025; Kasim et al. 2017; Mohsen et al. 2016). The tested concentration ranges were 50–200 mg L^{-1} for Fe_2O_3 ; 50–200 mg L^{-1} for $\text{Ca}(\text{OH})_2$; and 2.5–10% (v/v) for rosemary extract).

For the identification of bioactive components in rosemary leaf extract, gas chromatography-mass spectrometry (GC-MS) analysis was performed. The leaf extract was evaporated in the vacuum chamber of a rotary evaporator (taistic SHZ-D(III) water aspirator). The leaf extract residues were extracted with 1,4-dioxane. The analysis was conducted via an Agilent HP-6890 gas chromatograph coupled with an Agilent HP-5973 mass selective detector, equipped with a 30 m $\times$ 0.25 mm HP-5 MS fused silica column with a 0.25 μ m film thickness from 40 to 440 m z^{-1} (Asadollahi-Baboli and Aghakhani 2014). For elemental analysis, 20 ml aliquot of rosemary leaf extract was subjected to acid digestion via a $HNO_3:H_2O_2$ mixture (5:3, v/v) to estimate mineral contents using inductively coupled plasma optical emission spectroscopy (ICP-OES; Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA).

2.2 Green Synthesis of Nanoparticles

The green synthesis of both iron and calcium nanoparticles was carried out using rosemary extract due to the method described by (Kasim et al. 2017). The solutions of both ferric chloride dehydrate (0.2 M) and calcium chloride dehydrate (0.2 M) were prepared separately in 500 ml of distilled water. To each of these solutions, 400 ml of rosemary extract was added under continuous stirring on a magnetic stirrer for 2 h thereafter, the pH was adjusted to 10, where either brown or pale white precipitates were formed. Following NPs formation, the precipitated pellets were thoroughly washed three times with distilled water and ethanol before drying and purified, eliminating free extract and unreacted. The samples were then centrifuged and oven-dried to ensure the complete removal of residual aqueous components. The removal of residual phytochemical groups was demonstrated by further characterization methods, indicating successful purification.

2.3 Characterization of the Nanoparticles

The biosynthesis of Fe_2O_3 and $Ca(OH)_2$ NPs was confirmed by light absorption using a UV–visible spectrophotometer (Shimadzu 240 UV-Visible spectrophotometer). The X-ray diffraction patterns of the dried Fe_2O_3 and $Ca(OH)_2$ NPs were determined by an X-ray diffractometer (GNR-APD 2000 pro, Central Lab at Tanta University). The size and morphology of green synthesized Fe_2O_3 and $Ca(OH)_2$ NPs were determined using a transmission electron microscopy (TEM) (HR-TEM, JEM-2100, Jeol, Japan, Agricultural Research Center). The chemical composition of the synthesized nano-powders was studied using FT-IR spectrophotometer (Bruker Tenor 27, Central Lab, Tanta University). For the main pot experiment, Fe_2O_3 NPs were resuspended

to a final concentration of 100 mg L^{-1} , whereas $Ca(OH)_2$ NPs were re-suspended to 50 mg L^{-1} . Both suspensions exhibited stability for a minimum of 72 h without observable aggregation, hence enabling uniform administration during seed priming and foliar spraying. The physico-chemical characteristics of green-synthesized Fe_2O_3 and $Ca(OH)_2$ NPs are summarized in Table 3.

2.4 Pot Experiment

2.4.1 Plant Material and Treatments

In the present study, *Lupinus termis* Forssk (cv. Giza 1) was utilized as an experimental plant. The certified seeds were supplied by the Agricultural Research Center, Giza, Egypt, and selected for apparent morphological uniformity. Before the main pot trial, surface sterilization of the seeds was applied using 5% sodium hypochlorite for 5 min, followed by 5 times thoroughly rinsing with distilled water. During the growing season (October 2023), the seeds were allocated into four treatment groups. Both seed priming and foliar spraying were applied to all groups to ensure consistency of elicitor exposure.

For priming, each group was soaked for 6 h under continuous shaking to prevent aggregation in either one of the following solutions: (1) distilled water (control), (2) 100 mg L^{-1} Fe_2O_3 NPs, (3) 50 mg L^{-1} $Ca(OH)_2$ NPs, (4) 2.5% (v/v) rosemary extract. The concentrations of nanoparticles and plant extract were chosen based on preliminary dose-response experimental trials. Following priming, the soaked seeds were air-dried and then planted in plastic pots (30 cm diameter $\times$ 35 cm depth) filled with 10 kg of soil (air-dried, sieved through a 2-mm mesh). The soil had a clay-sandy texture (2:1 w/w), a pH of 8, an EC of 0.47 dS m^{-1} , and 0.9% organic matter. Elemental analysis (per kg soil) showed 2.6 g of N, 13 mg P, 17 mg of K, 14 mg of Mg, 64 mg of Ca, 4 mg of Fe, 0.8 mg of Zn, 0.7 mg of Mn and 0.18 mg of Cu.

Ten seeds were planted per pot to ensure adequate germination. Once the seedlings reached the first true leaf stage, they were thinned to six uniform seedlings per pot to standardize spacing and biomass competition. The plants were grown under a 10-h photoperiod, 28/16°C day/night temperature, and 65% relative humidity. Throughout the experiment, each pot received approximately 560 ml of water once per week which was equivalent to 70% of the soil's field capacity. The pots were watered uniformly to avoid moisture gradients. Each treatment consisted of three biological replicates, with each replicate represented by one pot containing six uniformly grown seedlings.

On day 15 after sowing, all treatment groups received an additional foliar dose of their corresponding priming

solutions. Foliar treatments were applied using a hand sprayer at 200 ml per pot, once daily for three consecutive days. The application was performed uniformly to ensure complete coverage. Seedlings were harvested at 21 days of growth. The freshly harvested seedlings were separated into roots and shoots, washed thoroughly with tap water several times followed by deionized water, and stored at -80°C . For dry matter analysis, the plant samples were oven-dried at 60°C until constant weight was reached.

2.4.2 Quantification of Growth Criteria and Photosynthesis Parameters

The collected 21-days old seedlings were collected to measure growth criteria (lengths, fresh and dry weights for both roots and shoots, and leaf area). All growth attributes were recorded from the six seedlings per pot, and their mean value was considered one biological replicate, resulting in 3 for each parameter. The technical replicates for the biochemical assays were conducted in triplicate for each biological replicate.

To estimate the photosynthetic pigments, the second expanded leaf tissue sample (0.1 g) was extracted with acetone (85%) according to Arnon (1949) method for chlorophylls and Horvath et al. (1972) method for carotenoids. The absorbance was measured using a UV/visible spectrophotometer and expressed as mg g^{-1} dry weight (d.wt). Furthermore, the photosynthetic activity (Fv/Fm) of dark-adapted leaves was measured using an OS-30p chlorophyll fluorometer (Hudson, NH03051, USA) to evaluate photosystem II (PSII) performance.

2.4.3 Evaluation of Oxidative Stress Parameters

The levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were recorded as stress biomarkers in fresh leaf tissues according to (Velikova et al. 2000) and (Heath and Packer 1968), respectively. The content of H_2O_2 was measured in 0.1% trichloroacetic acid (TCA) plant extract. The TCA extract was mixed with 1 M potassium iodide and phosphate buffer (pH 7.0). To record H_2O_2 in $\mu\text{mol g}^{-1}$ fresh weight (f.wt), the absorbance was read at 390 nm. The content of MDA, as a product of lipid peroxidation, was assessed in fresh leaves by extraction in a 5% (w/v) TCA solution. To this extract, 0.67% (w/v) thiobarbituric acid (TBA) was added. The mixture was then boiled for 20 min and immediately cooled. The samples absorbance was measured at 532 and 600 nm. To calculate the MDA content ($\mu\text{mol g f.wt}^{-1}$), the extinction coefficient ($155\text{ mM}^{-1}\text{ cm}^{-1}$) was utilized.

2.4.4 Determination of Ascorbic Acid and Antioxidant Activity

The non-enzymatic antioxidant ascorbic acid (ASA) was estimated due to the method of Oser (1979). Leaf tissues (0.1 g) were homogenized in 5% (w/v) sulfosalicylic acid and centrifuged at 10,000 rpm for 15 min. The tissue extract was incubated with 2 ml of 2% Na-molybdate, 2 ml of 0.15 N H_2SO_4 , and 1 ml of 1.5 mM Na_2HPO_4 at 60°C for 40 min. The absorbance was recorded at 660 nm to estimate ASA as mg g d.wt^{-1} via a calibration curve prepared with standard ascorbic acid.

The total antioxidant activity (TAA) was determined in the shoot tissues via phosphomolybdate assay (Prieto et al. 1999). The ethanolic extract was mixed with the reagent consisted of 28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid. The mixture was incubated at 95°C for 90 min then cooled. The absorbance was measured at 695 nm to express TAA as ascorbic acid equivalent (mg g d.wt^{-1}).

To evaluate the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity, a procedure modified from Brand-Williams et al. (1995) was used. To 0.1 ml of shoot ethanolic extract, 3.9 ml of a DPPH solution (30 mg l^{-1} in methanol) was added. The mixture was incubated in the dark for 1 h at room temperature. The decrease in absorbance was measured at 517 nm against a blank sample. The percentage of scavenged DPPH $^{\bullet}$ was calculated using the following equation $\text{DPPH scavenging \%} = \frac{A_o - A_s}{A_o} \times 100$ where A_o is the blank absorbance and A_s is the sample absorbance.

2.4.5 Estimation of peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity

Fresh lupine leaves were homogenized in cold K-phosphate buffer (pH 7) to extract enzymes, followed by centrifugation at 10,000 rpm for 20 min. Peroxidase activity (POX, EC 1.11.1.7) was assayed following the method of Kato and Shimizu (1987). The reaction mixture comprised the enzyme extract, 0.1 M sodium phosphate buffer (pH 5.8), 7.2 mM guaiacol, and 11.8 mM H_2O_2 . The absorbance was detected by tracking the oxidation of guaiacol by H_2O_2 at 470 nm with the aid of an extinction coefficient of $26.6\text{ mM}^{-1}\text{ cm}^{-1}$. The activity was expressed as $\mu\text{M g f.wt}^{-1}\text{ min}^{-1}$.

Polyphenol oxidase (PPO, EC 1.10.3.1) activity was assayed using the method described by Hassan et al. (2017). The assay depends on detecting purpurogallin formation to express the activity as $\mu\text{M g f.wt}^{-1}\text{ min}^{-1}$. The reaction mixture, including the enzyme extract, 0.1 M phosphate buffer (pH 6.0), and 0.02 M pyrogallol, was incubated for 5 min at 25°C . The reaction was stopped by adding 2.5 N H_2SO_4 .

To estimate PPO activity, the absorbance was measured at 420 nm, and the extinction coefficient was $26.40 \text{ mM}^{-1} \text{ cm}^{-1}$.

Phenylalanine ammonia lyase (PAL, EC 4.3.1.24) activity was measured using a modified method from El-Shora (2002). The reaction mixture consisted of 100 mM Tris-HCl buffer (pH 8.5) with 1 mM 2-mercaptoethanol, 50 mM L-phenylalanine, and enzyme extract. This mixture was incubated at 30 °C for 15 min. The reaction was then terminated by the addition of 6 M HCl. The absorbance was measured at 290 nm and PAL specific activity was calculated as $\mu\text{M g f.wt}^{-1} \text{ min}^{-1}$.

2.4.6 Detection of Secondary Metabolites

The total phenolic content was quantified using the method described by Jindal and Singh (1975). Folin-Ciocalteu reagent was mixed with the shoot ethanolic extract and 20% Na_2CO_3 . After 35 min, the absorbance was measured at 650 nm. A standard curve was prepared using different concentrations of gallic acid to determine the total phenolic content, expressed in mg g d.wt^{-1} .

The total flavonoid content in the dry shoot samples was detected via colorimetric aluminum chloride following the method of Chang et al. (2002). The ethanolic extract was mixed with, 10% aluminum chloride, 1 M potassium acetate, and 95% ethanol. Before recording the absorbance at 415 nm, the mixture was incubated at room temperature for 30 min. For the blank, 10% aluminum chloride was replaced with distilled water. A calibration curve was prepared using quercetin as a standard flavonoid to determine the flavonoid content, expressed in mg g d.wt^{-1} .

The total alkaloid content was quantitatively measured using the method described by Fazel et al. (2010). Briefly, 10^{-4} M bromocresol green (BG) solution was prepared by heating 69.8 mg of BG in 2 N sodium hydroxide until complete dissolution, then diluted to 1000 ml. A mixture of plant ethanolic extract, BG, and phosphate buffer (pH 4.7) was exposed to continuous shaking for 5 min and left to stand for 10 min. The absorbance of the resulting complex was then measured at 470 nm. To estimate total alkaloid content (mg g d.wt^{-1}), a calibration curve was prepared using atropine as a standard.

2.4.7 Quantitative Analyses of phenolics, Flavonoids and Alkaloids

The dry shoot samples were extracted using absolute ethanol and subjected to high-performance liquid chromatography (HPLC) analysis using an Agilent 1260 series (Oliva et al. 2015). Eight phenolic compounds (chlorogenic acid, ellagic acid, pyrogallol, syringic acid, ferulic acid, cinnamic

acid, benzoic acid, and salicylic acid) and nine flavonoids were detected by HPLC. The standard compound mixtures were employed for quantification of phenolic and flavonoid compounds as shown in HPLC chromatogram (Fig. S1). Three major alkaloids (lupinine, sparteine, and angustifoline) were estimated via HPLC and their standard curve is shown Fig S2. The sample extract (5 μl) was injected in an Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm) and the column temperature was kept at 40 °C. The separation was performed using a mobile phase consisting of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml min^{-1} . The mobile phase was programmed sequentially in a gradient: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A); 15–16 min (82% A); and 16–20 (82%A). The multi-wavelength detector was adjusted to 280 nm.

2.4.8 Quantification of Endogenous Phytohormones

Fresh lupine shoots were extracted with 80% methanol for 24 h at 4 °C, then filtered following the protocol of Nessem et al. (2023) to estimate the endogenous phytohormones, including indole-3-acetic acid (IAA), gibberellic acid 3 (GA), and abscisic acid (ABA). The extract was evaporated to an aqueous phase, adjusted to pH 8.6, and partitioned with ethyl acetate to separate acidic phytohormones. Further acidification to pH 2.8 allowed additional partitioning with ethyl acetate, and the resulting organic phase was used for hormone quantification. High-performance liquid chromatography (HPLC, Waters 1525 Binary HPLC Pump) was employed to determine the concentrations of IAA, GA, and ABA. Retention times were confirmed against authentic standards (IAA, GA, and ABA) and quantified from standard curves (Fig S3).

2.4.9 Statistical Analysis

The data were statistically analyzed using one-way ANOVA with CoStat software (CoHort, v.6.311) and are presented as means $\pm$ the standard deviation (SD). The statistical analysis was conducted under the standard assumptions of normality, homogeneity of variances, and independence of observations. The least Significant Difference (LSD) test at $p < 0.05$ was applied as the post-hoc comparison to indicate the statistical significance among treatment means. Furthermore, Pearson's correlation analysis was performed using GraphPad Prism version 10 to evaluate relationships among phytochemical constituents and growth parameters. Principal component analysis (PCA) was performed using XLSTAT software to visualize treatment differentiation and variation in polyphenol and alkaloid profiles.

3 Results

3.1 Characterization of Rosemary Extract

In Table 1, nitrogen, potassium, and calcium dominated the mineral ion composition of rosemary extract, revealing robust nutritional profile of 19.97, 8.80, and 10 mg g⁻¹, respectively. The presence of P and Mg was represented by moderate levels. Micronutrients such as Na, Fe, Zn, Mn, and Cu were found at relatively low but consistent amounts.

A GC-MS chromatogram was used to detect rosemary extract phytochemicals (Fig. 1). Many phytochemicals, including phenolics, terpenes, organic acids, fatty acids, and nitrogen-containing compounds, are listed in Table 2. The major detected compounds were rosmanol, carnosol, ursolic acid, camphor, limonene, 1,8-cineole, borneol, and octadecanoic acid. Also, a quinone derivative 2,5-cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethylethyl) and

Table 1 The elemental analysis of Rosemary (*Salvia Rosmarinus* Spenn.) extract

Mineral ion	Content (mg g d.wt ⁻¹)
N	19.96±0.32
P	2.30±0.07
K	8.80±0.30
Ca	10.0±0.20
Mg	2.09±0.19
Na	0.20±0.04
Fe	0.10±0.003
Zn	0.020±0.0006
Mn	0.050±0.002
Cu	0.005±0.001

glycine, N-[4-[trimethylsilyl]oxy] benzoyl]-, methyl ester, were minorly represented.

3.2 Characterization of the Green Synthesized Nanoparticles

UV-visible absorption spectra of green synthesized Fe₂O₃ and Ca(OH)₂ NPs are shown in Fig. 2. A distinctive absorption peak at 318 nm is characteristic of Fe₂O₃ NPs (Fig. 2a), and a strong peak at 316 nm is characteristic of Ca(OH)₂ NPs (Fig. 2b). TEM photographs showed rod-shaped Fe₂O₃ NPs with an average size of 23.83 nm (Fig. 2c) and spherical Ca(OH)₂ NPs with an average size of 30.26 nm (Fig. 2d).

XRD pattern of Fe₂O₃ NPs (Fig. 3a) reveals peak positions with 2θ values of 31.55°, 34.18°, 35.39°, 45.3°, and 53.83°, corresponding to hexagonal Fe₂O₃ NPs (021), (101), (022), (004), and (132), respectively. Further, Ca(OH)₂ NPs (Fig. 3b) exhibit peak positions with 2θ values of 18.01°, 34.05°, 36.5°, 45.49°, 47.05°, 50.76°, 54.30°, and 64.13°, corresponding to (001), (106), (011), (012), (110), (111), (200), and (013), respectively. The calculated crystallite size of Fe₂O₃ is 29.87 nm, whereas that of Ca(OH)₂ NPs is 28.53 nm.

FT-IR analysis of Fe₂O₃ NPs showed absorption peaks at 3449, 1636, 1466, 1357, 613 and 397 cm⁻¹ (Fig. 3c). Further, 3642, 3426.26, 2922.48, 2854.40, 1444.54, 548.79, 390.91 and 313.98 cm⁻¹ were distinctly associated with Ca(OH)₂ NPs (Fig. 3d). Table 3 summarizes the key physicochemical properties of the resulting green-synthesized Fe₂O₃ and Ca(OH)₂ NPs.

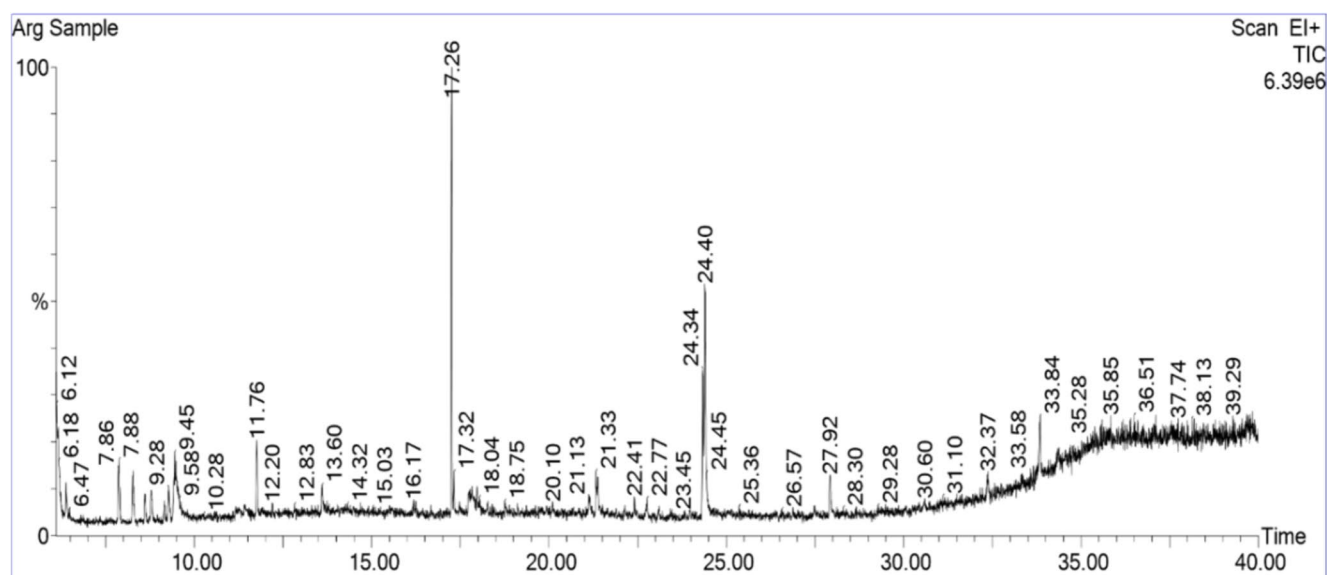


Fig. 1 GC-MS chromatogram of rosemary (*Salvia rosmarinus* Spenn) extract

Table 2 Phytochemical components of Rosemary extract by GC-MS

No.	Compound name	Molecular Formula	Retention time (RT)	Area%
1	2-Cyclopenten-1-one, 3-methyl-	C ₆ H ₈ O	6.384	0.639
2	Limonene	C ₁₀ H ₁₆	7.879	1.288
3	1,8-Cineole	C ₁₀ H ₁₈ O	8.274	1.146
4	Benzaldehyde, 4-methyl-	CH ₃ C ₆ H ₄ CHO	8.604	0.543
5	2-Butoxyethyl acetate	C ₈ H ₁₆ O ₃	8.789	0.683
6	2-Ethyl-3-methoxy-2-cyclopentenone	C ₈ H ₁₂ O ₂	9.155	0.296
7	Ethylene glycol diallyl ether	C ₈ H ₁₄ O ₂	9.280	0.707
8	Camphor	C ₁₀ H ₁₆ O	9.450	1.066
9	1,8-Cineole	C ₁₀ H ₁₈ O	9.480	1.610
10	1,2-Dimethoxy-ethene	C ₄ H ₁₀ O ₂	11.296	0.271
11	Octanoic Acid	C ₈ H ₁₆ O ₂	11.441	0.443
12	1,2-Propanediamine, N,N'-dimethyl-, (S)-	C ₁₆ H ₂₆ N ₇ O ₆ P	11.481	0.241
13	Borneol	C ₁₀ H ₁₈ O	11.761	1.064
14	1-Methylcyclopropanemethanol	C ₅ H ₁₀ O	12.826	0.252
15	Nonanoic acid	C ₉ H ₁₈ O ₂	13.601	0.474
16	Carnosol	C ₂₀ H ₂₆ O ₄	17.258	4.284
17	2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1- dimethylethyl)	C ₁₄ H ₂₀ O ₂	17.323	0.376
18	1-Propanol, 2,2-dimethyl-	C ₅ H ₁₂ O	17.743	0.423
19	Octanoic acid, 7-oxo-	C ₈ H ₁₄ O ₃	17.828	0.516
20	Benzoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	C ₁₇ H ₂₆ O ₃	18.038	0.233
21	Benzoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydrox	C ₁₅ H ₂₂ O ₃	18.038	0.233
22	5-Methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	C ₁₆ H ₁₂ O ₃	18.754	0.285
23	4(3 H)-Quinazolinone, 2-amino-3-(2-pyridinylmethyl)-	C ₁₅ H ₁₄ N ₄ O ₁	20.104	0.303
24	Furan, 2-(2-furanylmethyl)-5-methyl	C ₁₀ H ₁₀ O ₂	21.140	0.565
25	n-Decanoic acid	C ₁₀ H ₂₀ O ₂	21.330	0.553
26	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	C ₁₅ H ₂₂ O ₂	21.380	0.527
27	6-Hepten-1-ol, 3-methyl	C ₈ H ₁₆ O	22.410	0.299
28	Ursolic Acid	C ₃₀ H ₄₈ O ₃	22.770	0.456
29	Octadecane, 1-(ethenyloxy)-	C ₂₀ H ₄₀ O	23.105	0.315
30	Ursolic Acid	C ₃₀ H ₄₈ O ₃	24.341	2.083
31	Rosmanol	C ₂₀ H ₂₆ O ₅	24.396	4.471
32	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	27.922	0.872
33	2,5-Dihydroxyacetophenone, bis(trimethylsilyl) ether	C ₁₄ H ₂₄ O ₃ Si ₂	34.765	0.327
34	Sinapic acid	C ₁₁ H ₁₂ O ₅	34.800	0.235
1. 35	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl	C ₁₃ H ₂₂ OSi ₂	37.551	0.290
2. 36	Trimethyl[4-(1,1,3,3,-tetramethylbutyl) phenoxy] silane	C ₁₇ H ₃₀ OSi	37.851	0.246
3. 37	Benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	C ₁₄ H ₂₄ O ₃ Si ₂	38.417	0.271
38	Glycine, N-[4-[(trimethylsilyl)oxy] benzoyl]-, methyl ester	C ₁₃ H ₁₉ NO ₄ Si	39.682	0.257
39	Sinapic acid	C ₁₁ H ₁₂ O ₅	39.827	0.266

3.3 Pot Experiment

3.3.1 Growth Parameters

Table 4 shows that Fe₂O₃ NPs, Ca(OH)₂ NPs, and rosemary extract improved lupine seedling growth. Compared to the control, treatment with Ca(OH)₂ NPs resulted in greater increases in root and shoot lengths by 107% and 27%, respectively. In terms of biomass accumulation, Ca(OH)₂ NPs showed the highest increase in root fresh weight (87%). As for Fe₂O₃ NPs, it caused the greatest shoot biomass, causing fresh and dry weights to increase by 49.7% and

71%, respectively. All treatments increased leaf area, with Fe₂O₃ NPs showed the greatest increase (113%), followed by Ca(OH)₂ NPs (81%).

3.3.2 Photosynthetic Parameters

Table 5 shows that Fe₂O₃ NPs led to the greatest chl a, chl b, carotenoids, and total pigments compared to the control by 58%, 113%, 74%, and 93%, respectively. Applying Ca(OH)₂ NPs and rosemary extract significantly enhanced photosynthetic pigments to considerable levels comparable to the control but less than Fe₂O₃ NPs. Further, all treatments

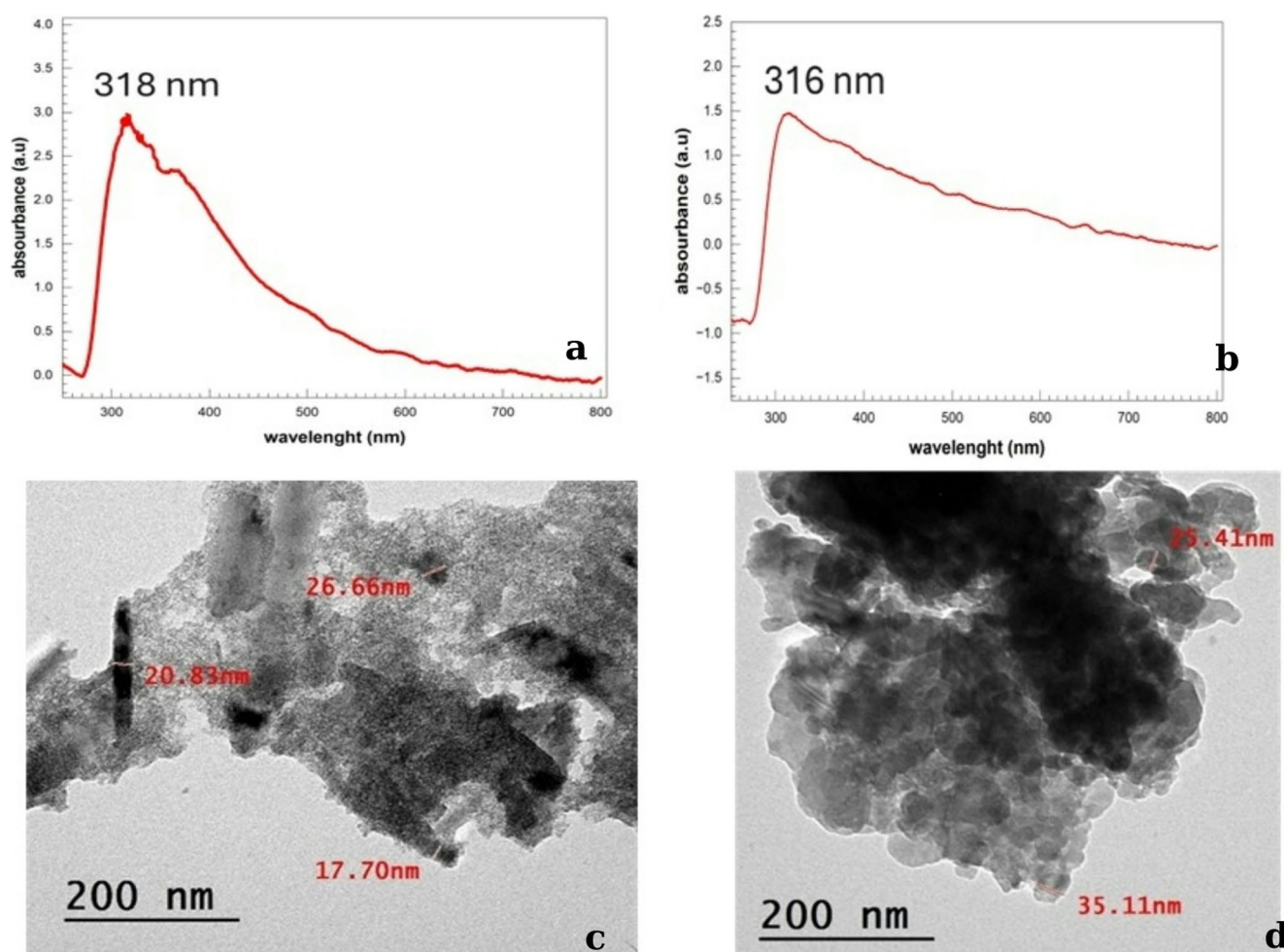


Fig. 2 UV–VIS absorption spectrum of green-synthesized iron oxide nanoparticles (Fe₂O₃) (a) and calcium hydroxide nanoparticles (Ca(OH)₂) (using rosemary extract) (b); and TEM photographs showed a distinct peak of Fe₂O₃ NPs (c) and Ca(OH)₂ NPs (d)

considerably increased photosynthetic activity without significant differences between treatments.

3.3.3 Oxidative Stress Parameters

Figure 4 shows the effects of Fe₂O₃ NPs, Ca(OH)₂ NPs, or rosemary extract on H₂O₂ and MDA in 21-days old *L. termis* seedlings. Compared with the control, NPs and rosemary extract increased H₂O₂ and reduced MDA. The greatest H₂O₂ level was 35% with Ca(OH)₂ NPs. The substantial MDA reduction compared to the control was detected with rosemary extract.

3.3.4 Ascorbic Acid and Antioxidant Activity

The treatments of Fe₂O₃ and Ca(OH)₂ NPs significantly elevated ASA levels to the highest levels by 10% and 12% above the control (Fig. 5). Additionally, Ca(OH)₂ NPs

showed the greatest improvements in antioxidant activity (63%). Regarding DPPH scavenging activity, all eliciting treatments showed enhanced levels over the control but without significant differences between treatments.

3.3.5 Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase Activity

Figure 6 shows how Fe₂O₃ NPs, Ca(OH)₂ NPs, and rosemary extract differently modulated enzyme activity. Compared with the control, all treatments increased PPO activity, with Fe₂O₃ and Ca(OH)₂ NPs showing the largest increases. Rosemary treatment increased POX activity by 89.7% more than the control with no significant variations in POX activity by NPs. Furthermore, all treatments significantly increased PAL activity, whereas Fe₂O₃ and Ca(OH)₂ NPs had the greatest increases (72% and 80%, respectively).

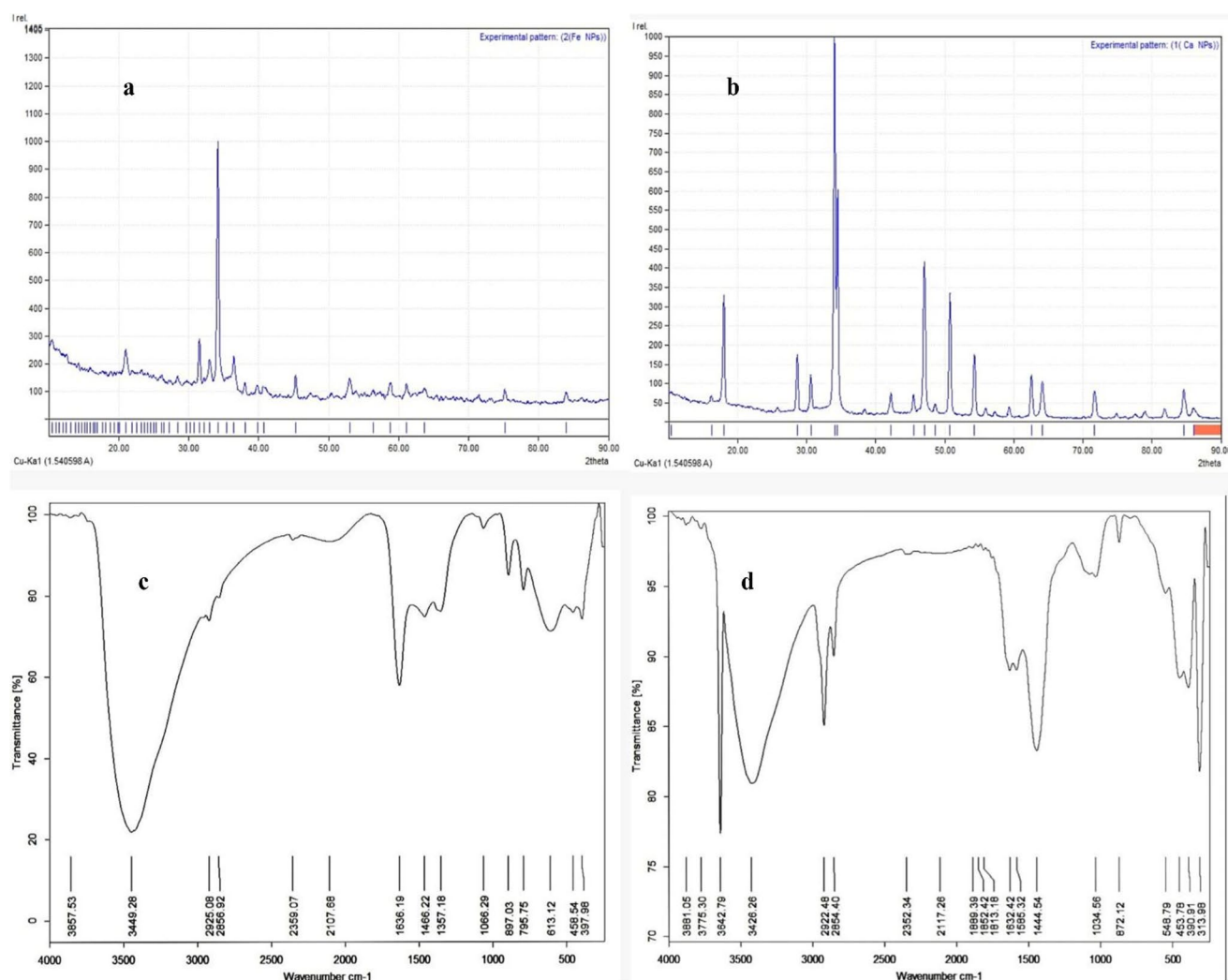


Fig. 3 X-ray diffraction pattern of green-synthesized Fe_2O_3 NPs (a) and $\text{Ca}(\text{OH})_2$ NPs (b); and FT-IR spectra of green-synthesized Fe_2O_3 NPs (c) and $\text{Ca}(\text{OH})_2$ NPs (d)

Table 3 Physicochemical characteristics of green-synthesized Fe_2O_3 and $\text{Ca}(\text{OH})_2$ NPs using Rosemary extract

Parameter	Fe_2O_3 Nanoparticles	$\text{Ca}(\text{OH})_2$ Nanoparticles
Color	brown	pale white
UV-VIS absorption peak	318 nm	316 nm
Morphology (TEM)	rod-shaped	spherical
Average particle size (TEM)	23.83 nm	30.26 nm
Crystallite size (XRD)	29.87 nm	28.53 nm
Capping phytochemicals (FTIR)	phenolics, flavonoids, diterpenes	phenolics, terpenoids, organic acids
Final applied concentration	100 mg L^{-1}	50 mg L^{-1}
Suspension stability (72 h)	stable	stable

3.3.6 Secondary Metabolites

Figure 7 shows that all exogenous treatments increased bioactive compounds. Nano fertilizing treatments had the highest levels of secondary metabolites and $\text{Ca}(\text{OH})_2$ NPs showed the highest increases of total phenolics (164%), flavonoids (22.8%), and alkaloids (189%). Rosemary extract increased their contents, although not as much as NPs.

3.3.7 Quantitative Determination of Phenolic and Flavonoid Compounds

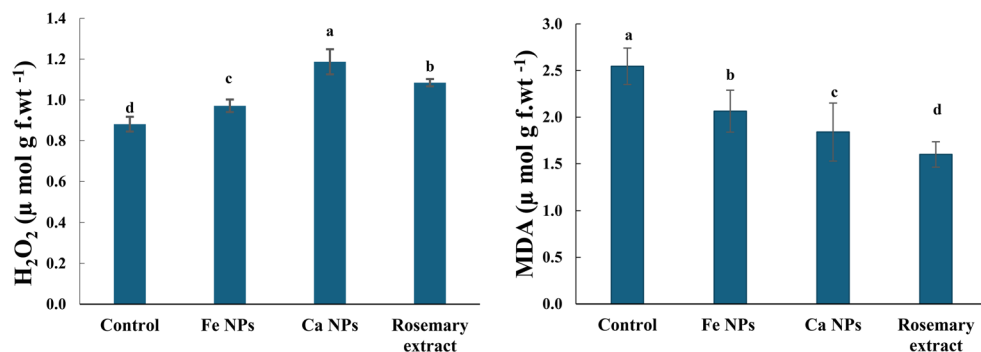
Table 6 and Fig S4 show that Fe_2O_3 NPs, $\text{Ca}(\text{OH})_2$ NPs, and rosemary extract substantially modulated the levels of phenolic and flavonoid constituents compared to the control. Interestingly, $\text{Ca}(\text{OH})_2$ NPs increased syringenic acid level by 150%. The largest increase in total polyphenol

Table 4 Effect of green-synthesized Fe NPs (100 Mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 Mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and Rosemary extract (2.5% v/v) on growth parameters of 21-day old *L. termis* seedlings. Data represent mean±SD (n=3). Different letters indicate significant differences (p<0.05) among treatments according to LSD test

Treatments	Length (cm organ ⁻¹)		Fresh weight (g organ ⁻¹)		dry weight (g organ ⁻¹)		Leaf Area (cm ² leaf ⁻¹)
	Root	Shoot	Root	Shoot	Root	Shoot	
Control	2.54±0.42 ^b	22.6±0.80 ^c	0.110±0.01 ^d	2.812±0.23 ^d	0.024±0.004 ^c	0.221±0.010 ^c	4.02±0.37 ^d
Fe NPs	4.86±0.57 ^a	27.2±1.3 ^a	0.207±0.02 ^b	4.195±0.23 ^a	0.049±0.004 ^a	0.389±0.029 ^a	8.58±1.2 ^a
Ca NPs	5.26±0.56 ^a	28.6±1.2 ^a	0.327±0.03 ^a	3.855±0.48 ^b	0.041±0.004 ^b	0.306±0.021 ^b	7.27±0.73 ^b
Rosemary extract	4.72±0.65 ^a	25.4±1.4 ^b	0.172±0.02 ^c	3.062±0.13 ^c	0.027±0.006 ^c	0.228±0.011 ^c	6.44±0.17 ^c

Table 5 Effect of green-synthesized Fe NPs (100 Mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 Mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and Rosemary extract (2.5% v/v) on photosynthetic parameters of 21-days old *L. termis* seedlings. Data represent mean±SD (n=3). Different letters indicate significant differences (p<0.05) among treatments according to LSD test

Treatments	Chl a (mg g d.wt ⁻¹)	Chl b (mg g d.wt ⁻¹)	Carotenoids (mg g d.wt ⁻¹)	Total Pigments (mg g d.wt ⁻¹)	Photosynthetic activity
Control	5.39±0.34 ^c	2.44±0.20 ^d	1.77±0.076 ^b	9.63±0.335 ^c	0.777±0.001 ^b
Fe NPs	8.53±0.17 ^a	5.20±0.28 ^a	3.08±0.18 ^a	16.60±0.061 ^a	0.831±0.006 ^a
Ca NPs	7.97±0.15 ^b	3.80±0.17 ^b	2.84±0.025 ^a	14.61±0.078 ^b	0.830±0.005 ^a
Rosemary extract	8.43±0.13 ^a	3.28±0.07 ^c	2.912±0.15 ^a	14.71±0.088 ^b	0.826±0.002 ^a

Fig. 4 Effect of green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v) on H₂O₂ and MDA levels of 21-day old *L. termis* seedlings. Data represent mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) among treatments according to LSD test

contents (phenolics and flavonoids) was seen with Ca(OH)₂ NPs (52%), followed by Fe₂O₃ NPs (27%), and rosemary extract (25%). Notably, rosemary extract markedly increased chlorogenic acid (107%), salicylic acid (267%), whereas Ca(OH)₂ NPs increased ellagic acid (122%), and kaempferol (150%). Pyrogallol and catechin, missing in the control, were found following treatments, particularly with Ca(OH)₂-NPs. Compared to the control, Fe₂O₃ NPs boosted quercetin and myricetin accumulation by 246% and 192%, respectively, whereas rosemary extract produced the greatest naringin levels (294%).

3.3.8 Quantitative Determination of Alkaloid Compounds

Figure 8 and Fig. S5 show the levels of key alkaloids (lupinine, sparteine, and angustifoline). Relative to the control, Ca(OH)₂ NPs increased lupinine content by 104%, followed

by rosemary extract (78%) and Fe₂O₃ NPs (16.5%). However, sparteine was present at the highest level with Fe₂O₃ NPs (15.57 μg g d.wt⁻¹) and other treatments decreased its content. Treatments with Ca(OH)₂ and Fe₂O₃ NPs resulted in the greatest angustifoline content (14.18 and 13.53 μg g d.wt⁻¹, respectively).

3.3.9 Quantitative Determination of Phytohormones

The effects of Fe₂O₃, Ca(OH)₂ NPs, or rosemary extract treatments on growth hormones are shown in Fig. 9 and Fig. S6. The greatest increase of GA level (22% above control) was detected with Fe₂O₃ and Ca(OH)₂ NPs. Compared to the control, rosemary extract decreased GA content by 2.2%. All treatments significantly increased IAA content but decreased ABA content. Compared with the control, Ca(OH)₂ NPs increased IAA levels by 122%.

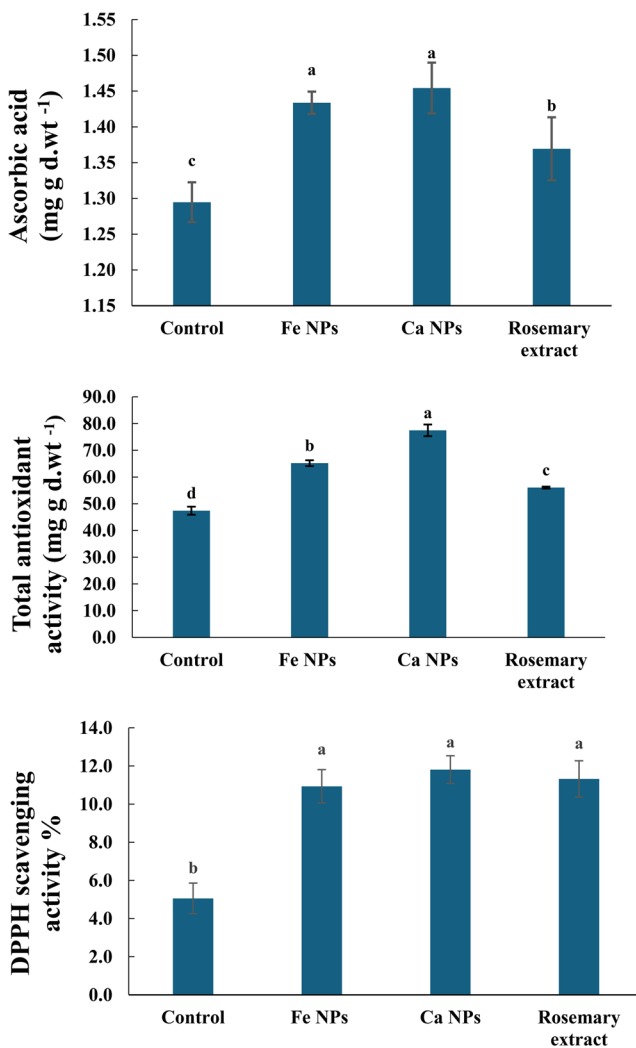


Fig. 5 Effect of green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v) on ascorbic acid and antioxidant activity of 21-day old *L. termis* seedlings. Data represent mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) among treatments according to LSD test

3.3.10 Pearson Correlation Analysis

Figure 10 shows considerable synergy between morphological, physiological, and biochemical parameters in *L. termis* seedlings treated with Fe₂O₃ NPs, Ca(OH)₂ NPs, and rosemary extract. Significantly, shoot and root biomass parameters were correlated positively with photosynthetic pigments like chl a, chl b, and carotenoids. Secondary metabolites such as phenolics, flavonoids, and alkaloids were correlated strongly ($r > 0.85$) with TAA, DPPH scavenging activity, and PAL activity. However, MDA was negatively correlated with chlorophyll, biomass characteristics, phenolics, flavonoids, and IAA. Also, IAA and GA were associated positively with growth indices, photosynthetic

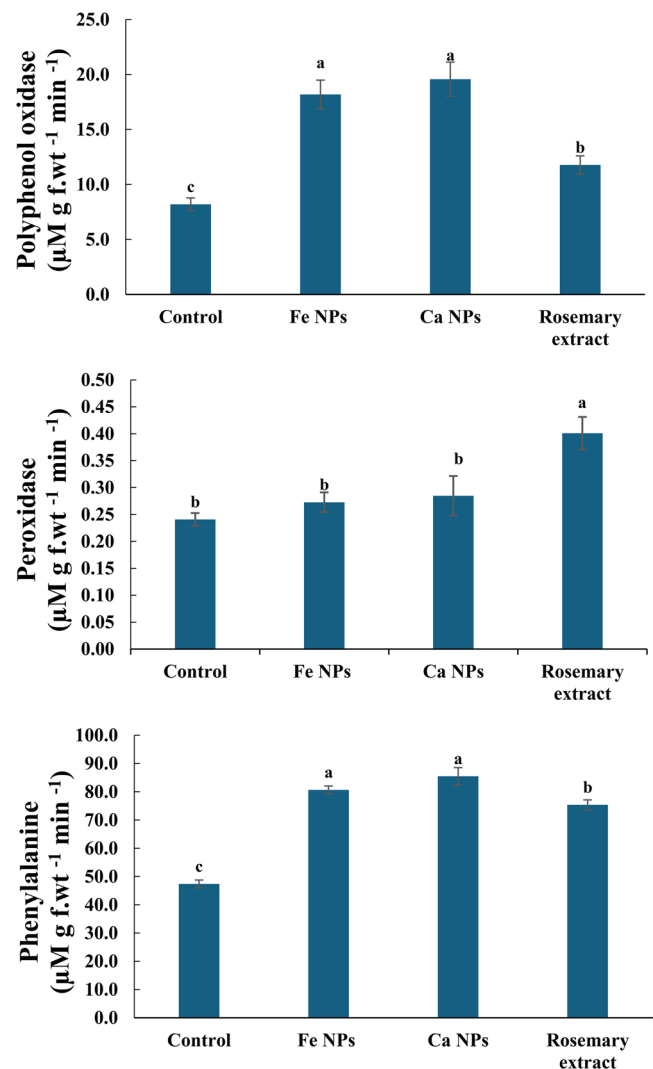


Fig. 6 Effect of green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v) on PPO, POX, and PAL activity of 21-day old *L. termis* seedlings. Data represent mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) among treatments according to LSD test

pigments, antioxidant capacity, and negatively associated with MDA and ABA.

3.3.11 Principal Component Analysis (PCA)

Figure 11 (a) shows phenolic and flavonoids PCA following the treatments. First three principal components (PC1, PC2, and PC3) explained 98.92% of variation. PC1 and PC2 supplied 38.69% and 34.27% of variance, whereas PC3 contributed 25.96%. PCA biplot of PC1 and PC2 (72.96% variance) clustered treatments by metabolite profile. Fe₂O₃ NPs were connected to PC2 and metabolites (ferulic acid, rutin, and naringin). Ca(OH)₂ NPs strongly correlated with PC1 and substances (kaempferol, ellagic acid, and

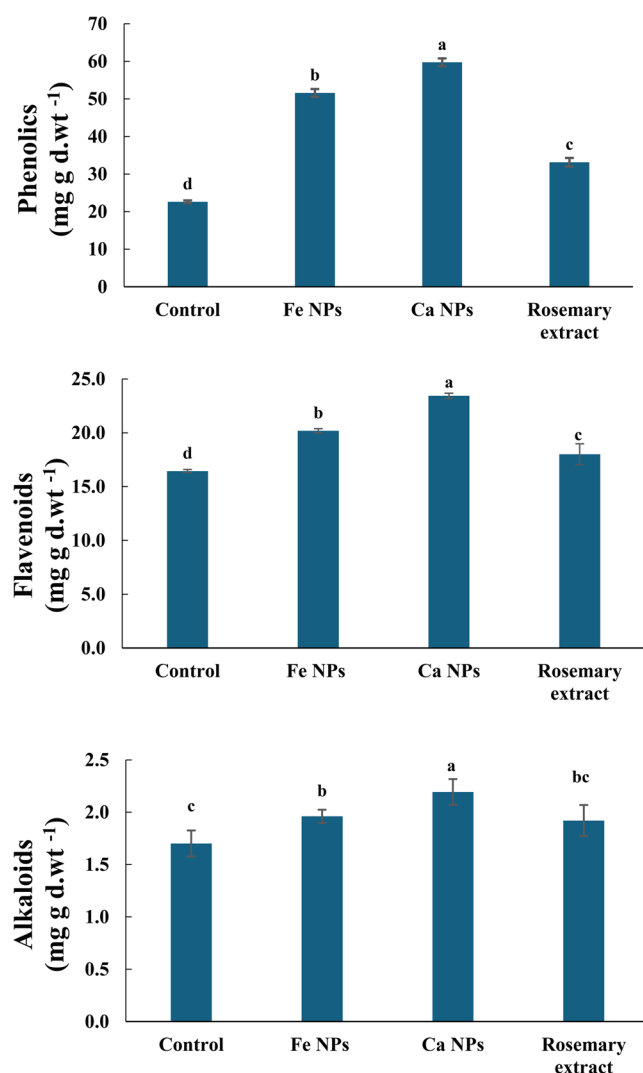


Fig. 7 Effect of green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v) on secondary metabolites of 21-day old *L. termis* seedlings. Data represent mean $\pm$ SD (n = 3). Different letters indicate significant differences (p < 0.05) among treatments according to LSD test

chlorogenic acid). Rosemary, coupled with chlorogenic acid, quercetin, and salicylic acid, moderately affected PC1 and PC2. Syringenic acid, luteolin, and catechin-related control are grouped along PC3.

PCA of lupinine, angustifoline, and sparteine under NPs and rosemary extract compared to the control is shown in Fig. 11 (b). Both PC1 and PC2 explained 98.6% of variation (67.86% and 30.74%). Fe₂O₃ NPs strongly associated with PC2, showing elevated angustifoline and sparteine. Ca(OH)₂ NPs aligned with PC1, reflecting higher lupinine. Rosemary had high PC1 but negative PC2 values, suggesting lower angustifoline. Control samples clustered negatively on both axes, indicating reduced metabolites.

4 Discussion

4.1 The Nutritional Value of the Rosemary Extract

The overproduction of secondary metabolites and antioxidants is one of several defensive strategies that plant crops have evolved to employ in their defense against environmental stress. Chemical, biological, or nano elicitors can induce the production of these metabolites, which enhance plant defensive systems such as plant-based extracts (Elsherif et al. 2025). The mineral composition of rosemary extract revealed major representation of macro- (N, K, Ca, P and Mg) and micro-elements (Na, Fe, Zn, Mn and Cu), suggesting its beneficial effects as a biostimulant. GC-MS analysis of rosemary extract revealed predominant phenolic compounds, terpenes, organic acids, fatty acids, and nitrogen-containing compounds. Phenolic diterpenes or triterpenes (carnosol, rosmanol, and ursolic acid), which have potent antioxidant and anti-inflammatory activities, can mitigate oxidative stress (Klancnik et al. 2009). Other bioactive monoterpenes, including limonene, 1,8-cineole, camphor, and borneol could enrich the pharmacologic potential of the extract, contributing to its therapeutic properties (Soliman et al. 2024). Additionally, medium-chain fatty acids and fatty acid esters such as octadecanoic acid esters octanoic, nonanoic, and decanoic acids can serve as potent antioxidants to combat oxidative damage (Qadir et al. 2020; Ragab et al. 2025a). Thus, plant-based natural extracts, including rosemary, which are rich in bioactive constituents, can serve as effective biostimulants and contribute to NPs production. As a result, their incorporation into farming systems presents a promising avenue for improving crop yield and quality.

4.2 Characterization of Green Synthesized Nanoparticles Using Rosemary Extract

The diverse bioactive constituents of rosemary extract present it as potent reducing and capping agent for NPs synthesis. Green synthesis via rosemary extract produced rod-shaped Fe₂O₃ NPs and spherical shaped Ca(OH)₂ NPs. In the UV–VIS spectrum, the characteristic absorption peaks of Fe₂O₃ NPs and Ca(OH)₂ NPs appeared at 318 and 316 nm, in agreement with previous findings (Lakshminarayanan et al. 2021) for Fe₂O₃ NPs and (Darroudi et al. 2011; El-Gogary 2020) for Ca(OH)₂ NPs. In contrast to their bulk counterparts, bio-engineered Fe₂O₃ and Ca(OH)₂ NPs are suggested to have an eliciting potential.

The XRD pattern of Fe₂O₃ NPs revealed the most intense peak appeared at 34.18°, which align with the previous studies used plant extracts for the green synthesis of α -Fe₂O₃ such as *Plectranthus* (Ramesh et al. 2019). Further, XRD pattern of Ca(OH)₂ NPs displayed sharp diffraction peaks,

Table 6 Quantitative determination of phenolics and flavonoids of 21-day old *L. termis* seedlings treated with green-synthesized Fe NPs (100 Mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 Mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and Rosemary extract (2.5% v/v). Data represent mean ± SD (*n* = 3). Different letters indicate significant differences (*p* < 0.05) among treatments according to LSD test

No	Category	Compounds	Control ($\mu\text{g g d.wt}^{-1}$)	Fe NPs	Ca NPs	Rosemary extract	RT min
1	Phenolics	Chlorogenic acid	8.1 $\pm$ 0.36 ^b	6.2 $\pm$ 0.2 ^c	4.3 $\pm$ 0.3 ^d	12.5 $\pm$ 0.3 ^a	3.0
2		Ellagic acid	20.5 $\pm$ 0.5 ^b	18.7 $\pm$ 0.6 ^c	25 $\pm$ 0.41 ^a	13.8 $\pm$ 0.4 ^d	5.0
3		Pyrogallol	ND	15.2 $\pm$ 0.2 ^b	23.3 $\pm$ 0.3 ^a	12.4 $\pm$ 0.3 ^c	6.0
4		Syringenic acid	6.2 $\pm$ 0.25 ^c	14 $\pm$ 0.34 ^b	15.5 $\pm$ 0.3 ^a	14 $\pm$ 0.4 ^b	8.0
5		Ferulic acid	5.8 $\pm$ 0.7 ^b	7.85 $\pm$ 0.8 ^a	5.0 $\pm$ 0.53 ^c	4.0 $\pm$ 0.10 ^d	13.0
6		Cinnamic acid	5.6 $\pm$ 0.6 ^b	2.1 $\pm$ 0.3 ^c	6.7 $\pm$ 0.2 ^a	2.0 $\pm$ 0.05 ^c	2.04
7		Benzoic acid	15.7 $\pm$ 0.7 ^a	6.7 $\pm$ 1.1 ^c	14.3 $\pm$ 0.3 ^b	15.8 $\pm$ 0.4 ^a	15.77
8	Flavonoids	Salicylic acid	3.54 $\pm$ 0.5 ^b	3.3 $\pm$ 0.5 ^b	2.09 $\pm$ 0.1 ^c	13 $\pm$ 0.3 ^a	12.95
9		7-OH Flavone	5.0 $\pm$ 0.19 ^b	4.67 $\pm$ 0.6 ^c	10.0 $\pm$ 0.3 ^a	5.22 $\pm$ 0.1 ^b	2.0
10		Apegenin	ND	4.67 $\pm$ 0.7 ^a	ND	3.74 $\pm$ 0.1 ^b	4.0
11		Querecetin	5.97 $\pm$ 0.3 ^b	14.7 $\pm$ 0.3 ^a	ND	14.8 $\pm$ 0.4 ^a	14.77
12		Rutin	ND	4.1 $\pm$ 0.38 ^a	ND	ND	10.00
13		Myricetin	7.14 $\pm$ 0.3 ^c	13.7 $\pm$ 0.6 ^a	8.2 $\pm$ 0.2 ^b	8.55 $\pm$ 0.2 ^b	11.0
14		Naringin	5.06 $\pm$ 0.7 ^c	ND	12.1 $\pm$ 0.5 ^b	14.9 $\pm$ 0.4 ^a	14.86
15		Kaempferol	10.0 $\pm$ 0.3 ^c	15.7 $\pm$ 0.7 ^b	25 $\pm$ 0.6 ^a	4.88 $\pm$ 0.1 ^d	17.5
16		Catechin	ND	1.9 $\pm$ 0.38 ^b	11.9 $\pm$ 0.2 ^a	ND	16.2
17		Luteolin	12.6 $\pm$ 0.8 ^a	ND	5.9 $\pm$ 0.15 ^b	ND	20.8
Total content			111.4 $\pm$ 1.1 ^d	141.7 $\pm$ 5.2 ^b	169 $\pm$ 1.4 ^a	139.5 $\pm$ 3.4 ^c	

ND non detected

revealing their crystallographic planes. The crystallite sizes were estimated using the Debye–Scherrer equation: $D = K\lambda / (\beta \cos \theta)$; where D is the average crystallite size, K is the shape factor (0.9), λ is the X-ray wavelength (0.15406 nm), β is the full width at half maximum in radians, and θ is the Bragg angle. The calculated average crystallite sizes were 29.87 nm for Fe₂O₃ nanoparticles and 28.53 nm for Ca(OH)₂ NPs.

The FTIR spectra of both Fe₂O₃ NPs and Ca(OH)₂ NPs revealed distinct absorption bands indicative of key functional groups, indicating the presence of both organic and inorganic functional groups. The peaks of 3642 and 3426 cm⁻¹ appeared in Ca(OH)₂ NPs spectrum, and the broad peak at 3449 cm⁻¹ for Fe₂O₃ NPs were attributed to the O–H stretching vibrations which confirmed the presence of phenolic groups (Ragab and Saad-Allah 2020; Tanpure et al. 2022). An absorption peak at 1636 cm⁻¹, for Fe₂O₃ NPs, was assigned to C=C stretching, while peaks within the 1000–1066 cm⁻¹ range corresponded to C–O–C and O–H groups (Farshchi et al. 2018). Notably, bands at 424 and 625 cm⁻¹ were attributed to Fe–O stretching vibrations, suggesting the formation of iron oxide nanoparticles (Iram et al. 2010; Xu et al. 2013). For Ca(OH)₂ NPs, the peaks at 1444 and 872 cm⁻¹ were associated with C–O bonds, indicating the probable carbonation of Ca(OH)₂ (Liu et al. 2010). Additionally, a peak at 2854 cm⁻¹ indicated the presence of -CH₂- groups due to C–H stretching vibrations, whereas medium-intensity bands at 548 and 400 cm⁻¹ may correspond to Ca-based hydroxides (Molla et al. 2022; Tanpure

et al. 2022). In this context, phenolic compounds, the major constituents of rosemary extract, are known to play a role in the reduction and stabilization of metal ions.

4.3 Plant Growth Interactions

The results of pot experiment revealed significant increases in growth, biomass and photosynthetic parameters (chl *a*, chl *b*, carotenoids, total pigments and photosynthetic activity) due to NPs and rosemary extract applications. Notably, Fe₂O₃ NPs exhibited the highest stimulatory effect on plant biomass and photosynthetic pigment levels. The significant role of Fe₂O₃ NPs is attributed to the role of iron as a structural and catalytic cofactor in proteins and enzymes involved in pigment synthesis and electron transport (Eid et al. 2021; Mohammadi et al. 2018). Similarly, Ca(OH)₂ NPs significantly improved growth parameters, reflecting calcium's signaling functions and structural role in cell walls and membranes, thus enhancing cell division and cell enlargement (El-Shenody et al. 2023). Compared to their bulk, the enhanced performance of NPs is largely due to their nano-size, surface area, and improved penetration, leading to more efficient nutrient transport (Wang et al. 2023a). Similarly, metal-based NPs are known to enhance energy conversion efficiency in photosynthetic systems (Govorov and Carmeli 2007; Kasim et al. 2017), resulting in increased photosynthetic rates and biomass accumulation. Because root system architecture governs seedling establishment and nutrient foraging, elicitor impacts should be interpreted

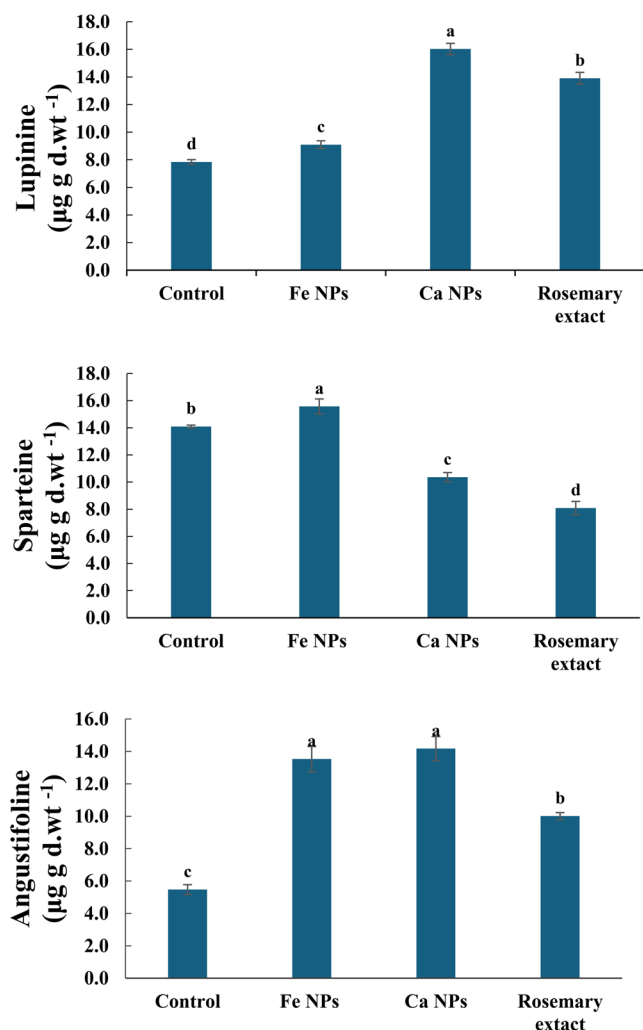


Fig. 8 Effect of green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v) on quantitative determination of alkaloids compounds of 21-day old *L. termis* seedlings. Data represent mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) among treatments according to LSD test

considering developmental regulators. For example, transcription factors such as WOX family have been implicated in modulating adventitious root development in fiber crops (Abubakar et al. 2023). Although not assayed here, such axes provide a developmental context for interpreting root elongation responses to Fe- and Ca-based nano-elicitors in lupine.

Plant-derived biostimulants can improve photosynthesis, nutrient availability, and water use efficiency, boosting plant growth and quality. Rosemary extract improved plant growth and photosynthetic performance, likely due to its phenolic and bioactive constituents such as carnosol, rosmarinic acid, α -pinene, camphor, and eucalyptol, which are known for their antioxidant, antimicrobial, and growth-promoting properties (Arranz et al. 2015; Habtemariam

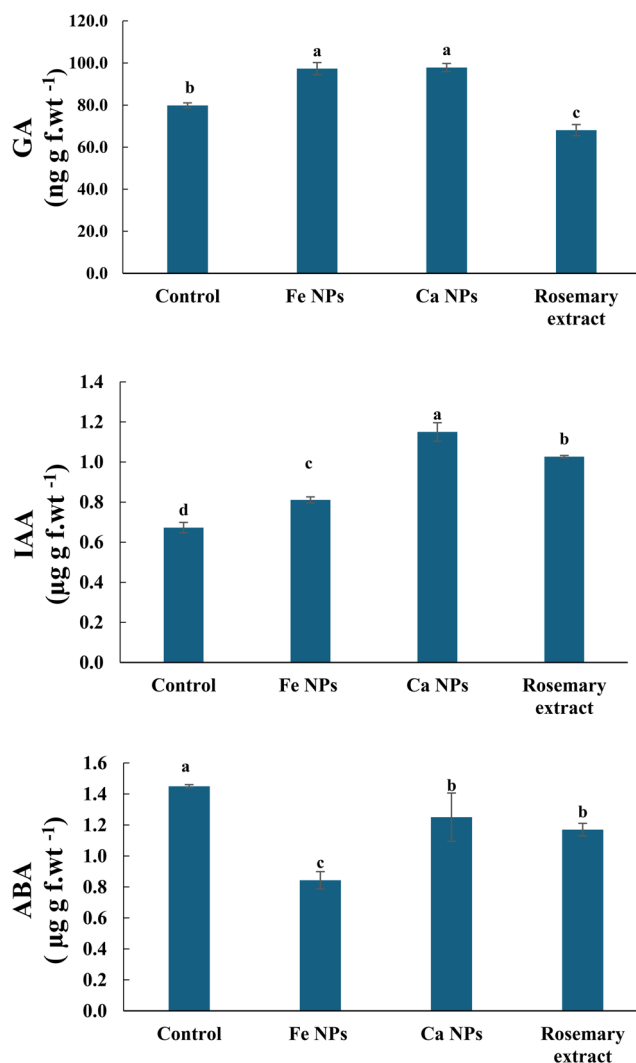
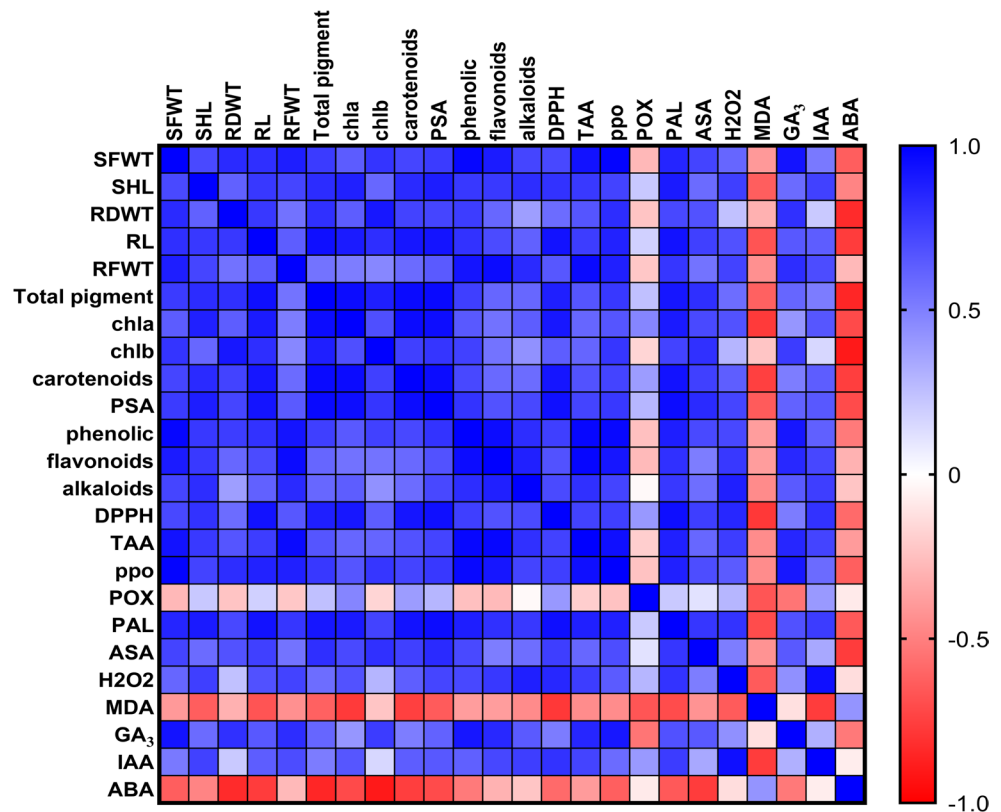


Fig. 9 Effect of green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v) on phytohormones of 21-day old *L. termis* seedlings. Data represent mean ± SD (n = 3). Different letters indicate significant differences (p < 0.05) among treatments according to LSD test

2016). Rosemary oil improved root development, and nutrient uptake, reducing the fertilizer demand of tomato (Souri and Bakhtiarizade 2019). These findings align with previous studies that reported an increase in photosynthetic pigments following the use of eucalyptus leaf extract (Abd-Elkader et al. 2022). These findings support the use of green-synthesized NPs and biostimulants for sustained plant growth. The improved growth can be attributed to the synergistic effect of bioactive substances, activation of physiological processes, and enhanced nutrient delivery. However, rosemary extract contributed positively to plant development, the application of NPs exhibited the highest recorded growth attributes owing to their physicochemical properties and efficient uptake compared to crude extracts.

Fig. 10 Pearson correlation heat map of morphological, physiological, and biochemical parameters of *L. termis* seedlings treated with green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v). SFWT: shoot fresh weight; SHL: shoot length; RDWT: root dry weight; RFWT: root fresh weight; RL: root length; PSA: total antioxidant activity via phosphomolybdate assay



4.4 ROS Signaling and the Antioxidant Response

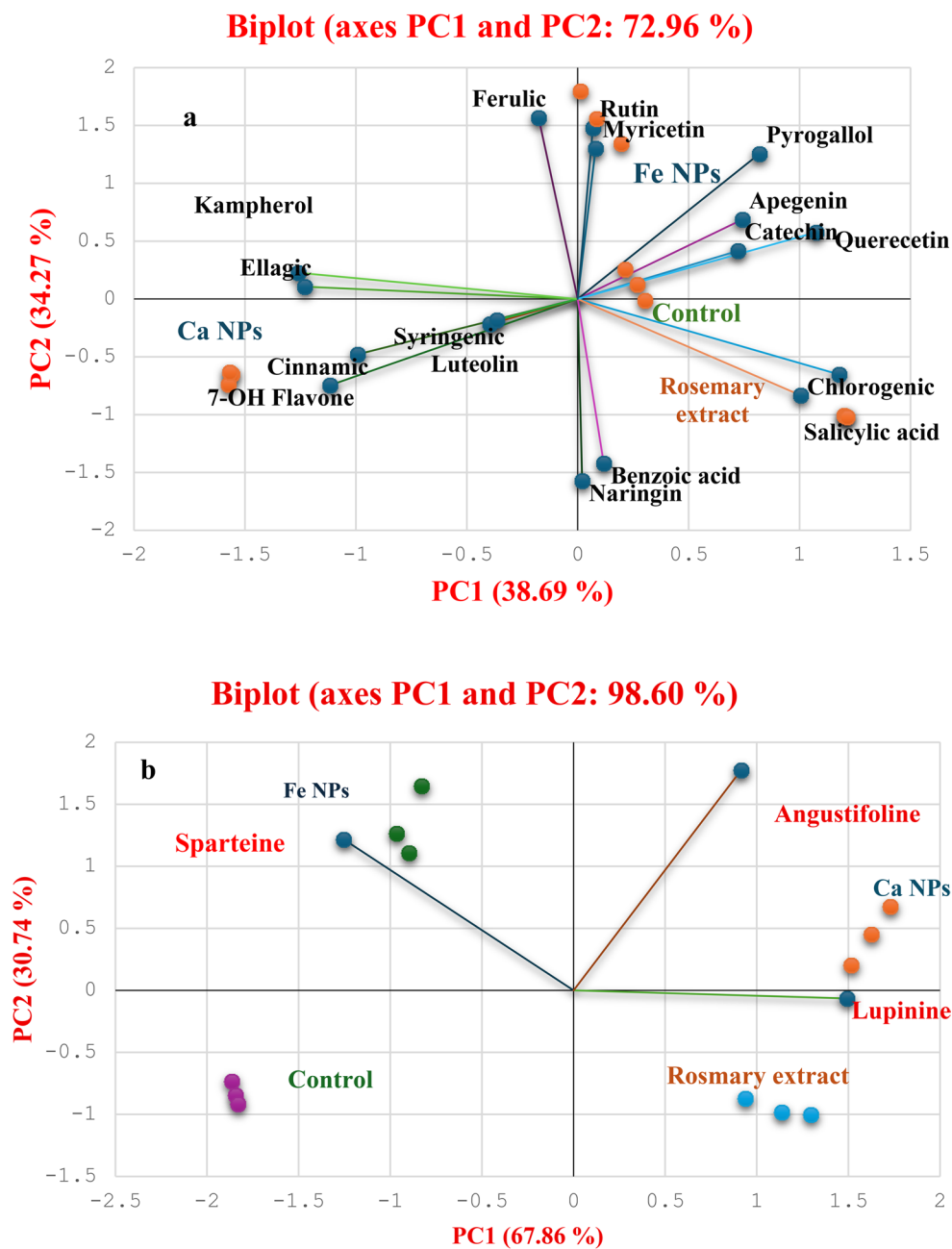
Stress markers (MDA and H₂O₂) were recorded to assess the oxidative status following NPs and biostimulant application. All eliciting treatments increased H₂O₂ content, but decreased MDA levels as compared to the control. In this regard, Fe₂O₃ and Ca(OH)₂ NPs might activate a controlled release of ROS, particularly H₂O₂, which functions as a secondary messenger in plant signaling pathways. Adequate hydrogen peroxide levels can modulate plant responses to environmental and internal stimuli, augmenting seed germination, photosynthesis, and growth (Nazir et al. 2020). Rosemary-induced H₂O₂ can be attributed to its phenolics, flavonoids, and terpenoids, acting as mild elicitors of oxidative signaling to enhance stress tolerance (Apel and Hirt 2004; Ragab et al. 2025b). The concurrent reduction in MDA indicated that elicitor-induced H₂O₂ were effectively detoxified, preventing membrane damage and maintaining cellular integrity via enhanced antioxidant systems. Particularly, Ca contributes to membrane stabilization and ion balance, modifying leakage and oxidative damage. However, Ca in nanoscale enhances calcium bioavailability and uptake, further supporting these events (Ayyaz et al. 2024). Due to high content of polyphenols (Table 2), rosemary extract exhibited a notable scavenging potential to induce a primed antioxidant state in plant cells, thereby reducing lipid peroxidation (Bozin et al. 2007). Thus, the moderate

increase of H₂O₂ enables plants to better cope with potential stress without leading to oxidative damage.

A significant increase in ASA content was recorded in response to Fe₂O₃ NPs, Ca(OH)₂ NPs and rosemary extract treatments which correlated with increased total antioxidant activity and DPPH scavenging activity. Exposure to Ca(OH)₂ NPs induced comparatively greater increases in antioxidant activity because of increased production of not only ASA but also phenolic and flavonoid compounds. Likewise, similar enhancements in antioxidant capacity were reported with Ca-based NPs (Badihi et al. 2021; Mohsen et al. 2016) and with Fe₃O₄ NPs (Rastegaran et al. 2022). Rosemary extract improved the antioxidant activity due to its rich phytochemical profile including phenolic diterpenes and triterpenoids as confirmed by GC-MS analysis, thereby enhancing plant growth and defense responses.

The induced antioxidant status in lupine seedlings caused by the application of NPs and biostimulants can be linked to the activation of PPO and effective relieve of lipid peroxidation and MDA. As a secondary messenger, Ca²⁺ can facilitate calcium-dependent signaling pathways and regulate antioxidant enzymes (Ayyaz et al. 2024). Increased availability of nanoscale Fe increased H₂O₂ production and activated catalase and ascorbate peroxidase in *Dracocephalum kotschy* (Khanizadeh et al. 2024). Among the treatments, rosemary extract exhibited the highest POX activity. This response is attributed to its rich phytochemical composition, which

Fig. 11 PCA of the interrelationships among phenolic and flavonoid compounds (a) and alkaloids constituents (b) in *L. termis* seedlings treated with green-synthesized Fe NPs (100 mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and rosemary extract (2.5% v/v)



includes phenolics and terpenoids, stimulating antioxidant activities. These results align with those of Aziz et al. (2022) who reported that rosemary extracts significantly enhance enzymatic antioxidant responses. The present results demonstrated that nano and bio-eliciting can improve cell redox machinery, provoking defense responses.

4.5 Secondary Metabolism

PAL is a key enzyme in the shikimate pathway, that initiates the synthesis of phenolic compounds and related precursors. A significant increase in PAL activity was recorded across all

treatments, with Ca(OH)₂ and Fe₂O₃ NPs showing the highest increase among treatments. Nano-eliciting may enhance antioxidant capacity and processes linked to secondary metabolite generation via controlled ROS burst (Khanizadeh et al. 2024). The high reactivity of Fe₂O₃ or Ca(OH)₂ NPs allow them to act as catalysts, modulating intracellular biochemical pathways such as phenylpropanoid biosynthesis and enhancing antioxidant activity (Asadi-Kavan et al. 2020). Calcium acts as a secondary messenger, triggering signal transduction pathways that lead to increased downstream events and phenolics accumulation. Iron functions as a co-factor in enzymatic redox reactions and contributes

to the formation of both primary and secondary metabolites. The application of rosemary extract activated PAL, which potentially correlated to the promotion of secondary metabolism. Similarly, priming with natural extracts such as date palm seeds extract was reported to elevate PAL activity through the overexpression of *PAL* gene, thereby enhancing phenolics and flavonoids levels (Elsherif et al. 2025). Not only NPs but also rosemary extract initiated a regulated ROS signaling cascade, which can stimulate downstream metabolic pathways, including PAL upregulation and augmented phenylpropanoid and shikimate pathways (Fig. 12).

In our study, both NPs and rosemary extract increased the production of secondary metabolites including total phenolics, flavonoids, and alkaloids. These findings suggest that NPs treatments are more effective at stimulating secondary metabolism than rosemary extract are. Increasing the availability of Fe can promote source-sink carbohydrate balance which is critical for secondary metabolites production, via stimulating photosynthesis and metabolite translocation (Khanizadeh et al. 2024). The enhanced production of phenolics, flavonoids, and alkaloids is associated with their increased biosynthetic routes and directing carbon flow from primary to secondary metabolism. A positive correlation was recorded between phenolics accumulation and upregulated transcript levels of key enzymes involved in secondary metabolism (El-Naggar and Read

2010). Both Fe_2O_3 and $\text{Ca}(\text{OH})_2$ NPs exerted their effects via nutrient-mediated metabolic activation, while rosemary extract increased antioxidant activity through its rich phytochemicals and its dual roles as a natural antioxidant and bio-stimulant. Overall, these results suggest that activation of phenolics biosynthesis contributed to the increased antioxidant capacity of *L. termis* plants elicited by NPs or biostimulant.

The quantitative analysis using HPLC revealed significant modulation in secondary metabolites due to Fe_2O_3 NPs, $\text{Ca}(\text{OH})_2$ NPs and rosemary extract in *L. termis* seedlings. Notably, Fe_2O_3 NPs significantly elevated quercetin, myricetin, and syringic acid contents, highlighting its main effect on flavonoid biosynthesis. Iron activates key enzymes in flavonoid pathways, including flavone synthase I, flavonol synthase, and anthocyanidin synthase, which require Fe^{2+} , 2-oxoglutarate, and ascorbate for catalytic activity (Busche et al. 2021). The observed increases in ferulic acid further support the idea that Fe_2O_3 NPs promote the accumulation of phenolic compounds, intensifying metabolic activity along these routes. Interestingly, reductions in cinnamic and benzoic acids suggest the selective stimulation of specific branches within phenylpropanoid pathway, potentially accompanied by the suppression of other routes.

Among all treatments, $\text{Ca}(\text{OH})_2$ NPs resulted in the most extensive enhancement in, syringenic acid, ellagic acid,

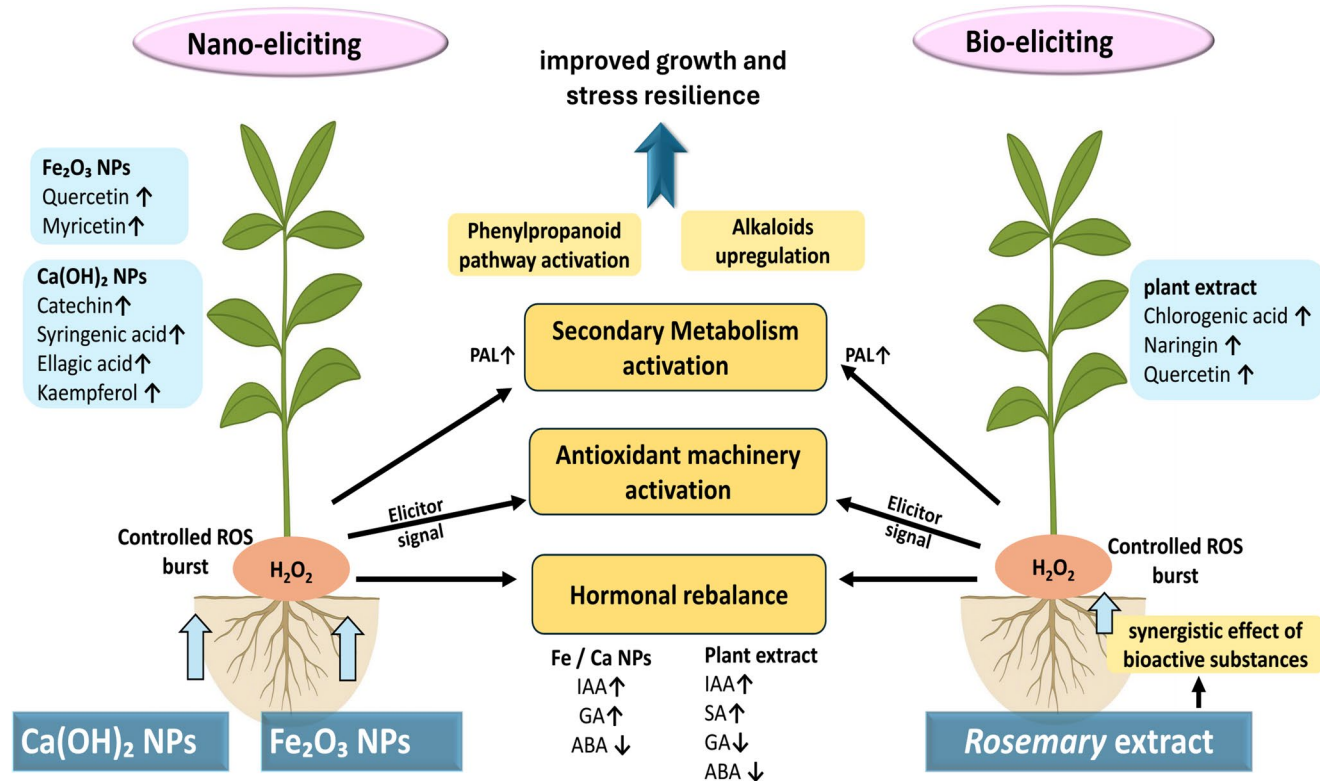


Fig. 12 Mechanistic model of *L. termis* response to green synthesized 100 mg L⁻¹ Fe_2O_3 NPs, 50 mg L⁻¹ $\text{Ca}(\text{OH})_2$ NPs, and 2.5% (v/v) rosemary extract

syringic acid, and catechin levels, along with flavonoids such as kaempferol and 7-hydroxyflavone. $\text{Ca}(\text{OH})_2$ NPs role is ascribed for enhanced ion balance, thereby activating phenylpropanoid pathway, which plays a pivotal role in the biosynthesis of phenolics and flavonoids. These results are consistent with previous studies indicating that Ca NPs enhanced secondary metabolism and upregulated antioxidant enzymes (Nazir et al. 2022). The bioactive constituents of rosemary may act as elicitors or signaling agents that upregulate genes associated with secondary metabolism. The application of rosemary extract resulted in elevated levels of chlorogenic acid, salicylic acid, quercetin, and naringin which are known for their antioxidant and antimicrobial effects. The marked increase in naringin suggests specific induction of flavanone biosynthetic pathway. These observations are in line with previous studies demonstrating the biostimulant properties of rosemary-derived polyphenols (Moghaddam and Mehdizadeh 2017).

Nano and bio-eliciting led to an increase in the key alkaloids lupanine and angustifoline in *L. termis* plants. In early plant development, alkaloid biosynthesis increases markedly during seed germination, suggesting a regulatory role. In Fabaceae species, quinolizidine alkaloids (Qas) also serve as crucial nitrogen storage compounds, especially in legumes that depend on atmospheric N fixation (Cely-Veloza et al. 2023). $\text{Ca}(\text{OH})_2$ NPs caused the most substantial accumulation of lupanine and angustifoline. Beyond their physiological roles in plants, many Qas have demonstrated pharmacological potential in humans. For instance, lupanine has been reported to stimulate insulin secretion and modulate gene expression in a glucose-dependent manner in diabetic rats (Wiedemann et al. 2015). Unlike other treatments, Fe_2O_3 NPs potentially favored sparteine synthesis. Fe_2O_3 NPs can elicit cell membrane receptors, thus initiating signaling events, including rapid ion flux, activation of transcription factors, and ROS burst (Ghorbanpour and Askary 2024). Further, the quantitative enhancement of phenolics, flavonoids, and alkaloids in lupine is consistent with their scavenging roles and stress signaling. These bioactive secondary metabolites are critically involved in plant defense mechanisms and stress adaptation.

4.6 Growth-promoting Phytohormones

Treatments with Fe_2O_3 and $\text{Ca}(\text{OH})_2$ NPs significantly enhanced the production of growth-promoting phytohormones including GA and IAA compared to the control, but decreased ABA levels. These findings suggest that NPs can act as hormonal elicitors, promoting plant growth and development through nutrient-mediated activation. Plant hormones respond differently to nanoparticle concentrations (Vankova et al. 2017). Adequate levels of NPs may

cause mild stress stimuli that activate adaptive physiological responses by increasing auxins and gibberellins (Yakhin et al. 2017; Iavicoli et al. 2018). In this context, induced GA and IAA levels contribute to ROS generation, functioning as secondary messengers within hormone-dependent signaling cascades. For example, gibberellin- and auxin-induced H_2O_2 production can regulate plant growth and stomatal opening responses (Ishibashi et al. 2012). In contrast, ABA is typically associated with growth inhibition, senescence, and stress responses as observed with a negative correlation with most physiological traits. A significant reduction in ABA was recorded after the treatment with NPs and rosemary extract, which could contribute to the improved growth and delayed senescence. A similar result was recorded in peanut plants treated with Fe_2O_3 NPs, which presented a significant reduction in ABA (Rui et al. 2016). Similarly, Mustafa et al. (2021) reported that application of TiO_2 NPs increased IAA and GA levels and decreased ABA levels.

Notably, unlike the NPs treatments, the rosemary extract produced a marginal decrease in GA levels (−2.2%), while simultaneously increasing IAA accumulation. Its bioactive components could be regulating agent for defense signaling pathways and plant growth (Li et al. 2022). Therefore, rosemary-derived elicitors may stimulate auxin-associated growth without increasing GA-dependent pathways by modulating hormones biosynthesis or turnover. On the other hand, the rosemary extract caused a remarkable accumulation of endogenous salicylic acid (Table 6). Salicylic acid (SA) is synthesized primarily in the shikimate pathway via phenylalanine deamination by PAL to yield trans-cinnamic acid then benzoic acid through several enzymatic reactions. Finally, benzoic acid is hydroxylated to form SA (Arif et al. 2020). The rosemary extract has rich contents of polyphenols and benzoic acid derivatives (Table 1) which can act as signaling molecules or precursors to SA biosynthesis. PAL activation also contributes to SA biosynthesis via the phenylalanine SA biosynthesis route. The increased SA may cause a reduction in GA levels, as SA has been shown to antagonize GA signaling, partly through the stabilization of DELLA proteins (the negative regulators of GA signaling). This antagonism could shift the plant's metabolic state from growth promotion to defense activation due to the interaction of the SA receptor NPR1 with the GA receptor GID1 (Yu et al. 2022). Therefore, the rosemary extract can modulate plant hormone balance to promote defense activation and growth regulation through complex hormonal crosstalk and antioxidant support (Fig. 2). The integrated comparison of physiological, biochemical, and hormonal responses across treatments is compiled to relate the key outcomes and eliciting potential of Fe_2O_3 NPs, $\text{Ca}(\text{OH})_2$ NPs, and rosemary extract (Table 7).

Table 7 Major physiological and biochemical responses of 21-day old *L. termis* seedlings elicited by green-synthesized Fe NPs (100 Mg L⁻¹ iron oxide nanoparticles, Fe₂O₃), Ca NPs (50 Mg L⁻¹ calcium hydroxide nanoparticles, Ca(OH)₂), and Rosemary extract (2.5% v/v)

Category	Fe NPs	Ca NPs	Rosemary extract	Interpretation
Growth performance	Highest shoot biomass, leaf area	Highest length, root biomass	Moderate increase of all growth parameters	Enhanced early vegetative growth and biomass
Photosynthetic attributes	Highest Chl a, Chl b, and carotenoids	Moderate increase of Chl a, Chl b, and carotenoids	Highest Chl a Moderate increase of Chl b, and carotenoids	Improved light-harvesting efficiency and photoprotection
Oxidative stress	Moderate increase of H ₂ O ₂ Moderate decrease of MDA	Highest H ₂ O ₂ Moderate decrease of MDA	Moderate increase of H ₂ O ₂ Lowest MDA	Controlled ROS signaling Enhanced redox homeostasis
Antioxidant systems	Highest PPO Moderate increase of ASA, and total antioxidant activity	Highest PPO, ASA, and total antioxidant activity	Highest POX Moderate increase of PPO, ASA, and total antioxidant activity	Strengthened detoxification and antioxidant defense
Secondary metabolism	Highest PAL Moderate increase of total phenolics, flavonoids, and alkaloids	Highest PAL Highest total phenolics, flavonoids, and alkaloids	Moderate increase of PAL, total phenolics, flavonoids, and alkaloids	Activation of phenylpropanoid pathway
Phytochemicals	Highest sparteine and angustifoline	Highest lupinine, and angustifoline	Highest chlorogenic acid, naringin, and quercetin	Enhanced nutraceutical value
Hormonal regulation	Highest GA Moderate increase of IAA Lowest ABA	Highest GA, and IAA Moderate decrease of ABA	Highest SA Moderate increase of IAA Moderate decrease of ABA, and GA	Growth-promoting hormonal shift

The current study was performed in controlled greenhouse pots to ensure cause-effect interpretation and decrease confounding variability. Field conditions with various abiotic variables, soil organic matter, and rhizosphere microbial interactions may alter green elicitor responses differently. Thus, future long-term studies with wider concentration ranges are needed to confirm the agricultural application of these elicitors. Also, a full phenolic characterization of rosemary extract should be performed in future studies.

5 Conclusion

The present work indicates that green-synthesized iron oxide and calcium hydroxide nanoparticles, as well as rosemary plant extract, can serve as effective bio-elicitors that improve growth and metabolism of lupine (Fig. 2). The elicitors enhanced plant performance by stimulating antioxidant defenses, improving both enzymatic and non-enzymatic protection, and activating secondary metabolic pathways. All treatments increased hydrogen peroxide, which acted as a controlled signaling molecule rather than a damaging oxidant, thereby reducing lipid peroxidation and promoting redox stability. The nanoparticles and rosemary extract enhanced the accumulation of valuable phenolic compounds, flavonoids, and quinolizidine alkaloids, indicating the activation of the phenylpropanoid and alkaloid biosynthetic pathways. Although both elicitors were effective, the nanoparticles tended to induce greater increases in several physiological and metabolic traits than did rosemary extract. A favorable hormonal balance was recorded as

marked by elevated levels of auxin and gibberellin, alongside reduced abscisic acid, which contributes to improved growth and stress resilience. In contrast, rosemary extract facilitated the accumulation of salicylic acid, highlighting its polyphenolic composition and its impact on defense-related signaling. Taken together, these findings indicate that green-synthesized nanoparticles and rosemary extract represent promising, eco-friendly tools for enhancing early growth, metabolic activity, and stress modulation in lupine, but further field validation is needed.

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Author Contributions GR involved in conceptualization, methodology, software, data curation, validation and reviewing. AE involved in conceptualization, visualization, investigation validation, software, original draft preparation, and editing. MO involved in methodology, data curation, conceptualization, visualization, supervision validation and reviewing. SD involved in conceptualization, visualization and investigation.

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Data Availability The authors declare that the data presented in this manuscript is their own work. All the data collected in this experiment were given in the manuscript and supplementary data.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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References

- Abd-Elkader DY, Mohamed AA, Feleafel MN, Al-Huqail AA, Salem MZM, Ali HM, Hassan HS (2022) Photosynthetic pigments and biochemical response of zucchini (*Cucurbita Pepo* L.) to Plant-Derived Extracts, Microbial, and potassium silicate as biostimulants under greenhouse conditions. *Front Plant Sci* 13:879545. <https://doi.org/10.3389/fpls.2022.879545>
- Abubakar AS, Wu Y, Chen F, Zhu A, Chen P, Chen K, Qiu X, Huang X, Zhao H, Chen J, Gao G (2023) Comprehensive analysis of WUSCEL-Related homeobox gene family in Ramie (*Boehmeria nivea*) indicates its potential role in adventitious root development. *Biology (Basel)* 12:1475. <https://doi.org/10.3390/biology12121475>
- Andor B, Danciu C, Alexa E, Zupko I, Hogeia E, Cioca A, Corico-vac D, Pinzaru I, Pătrașcu JM, Mioc M, Cristina RT, Soica C, Dehelean C (2016) Germinated and ungerminated seeds extract from two *Lupinus* species: biological compounds characterization and in vitro and in vivo evaluations. *Evid-Based Compl Alt* 2016:7638542. <https://doi.org/10.1155/2016/7638542>
- Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55:373–399. <https://doi.org/10.1146/annurev.arplant.55.031903.141701>
- Arif Y, Sami F, Siddiqui H, Bajguz A, Hayat S (2020) Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. *Environ Exp Bot* 175:104040. <https://doi.org/10.1016/j.envexpbot.2020.104040>
- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol* 24:1–15. <https://doi.org/10.1104/pp.24.1.1>
- Arranz E, Mes J, Wichers HJ, Jaime L, Mendiola JA, Reglero G, Santoyo S (2015) Anti-inflammatory activity of the basolateral fraction of Caco-2 cells exposed to a Rosemary supercritical extract. *J Funct Foods* 13:384–390. <https://doi.org/10.1016/j.jff.2015.01.015>
- Asadi-Kavan Z, Khavari-Nejad RA, Iranbakhsh A, Najafi F (2020) Cooperative effects of iron oxide nanoparticle ($\alpha\text{-Fe}_2\text{O}_3$) and citrate on germination and oxidative system of evening primrose (*Oenothera biennis* L.). *J Plant Interact* 15:166–179. <https://doi.org/10.1080/17429145.2020.1774671>
- Asadollahi-Baboli M, Aghakhani A (2014) Rapid analysis of *Origanum Majorana* L. fragrance using a nanofiber sheet, gas chromatography with mass spectrometry, and chemometrics. *J Sep Sci* 37:990–996. <https://doi.org/10.1002/jssc.201301355>
- Ayyaz A, Zhou Y, Batool I, Hannan F, Huang Q, Zhang K, Shahzad K, Sun Y, Farooq MA, Zhou W (2024) Calcium nanoparticles and abscisic acid improve drought Tolerance, mineral nutrients uptake and Inhibitor-Mediated photosystem II performance in *Brassica Napus*. *J Plant Growth Regul* 43:516–537. <https://doi.org/10.1007/s00344-023-11108-7>
- Aziz E, Batool R, Akhtar W, Shahzad T, Malik A, Shah MA, Iqbal S, Rauf A, Zengin G, Bouyahya A et al (2022) Rosemary species: a review of phytochemicals, bioactivities and industrial applications. *S Afr J Bot* 151:3–18. <https://doi.org/10.1016/j.sajb.2021.09.026>
- Badihi L, Gerami M, Akbarinoddeh D, Shokrzadeh M, Ramezani M (2021) Physio-chemical responses of exogenous calcium nanoparticle and Putrescine polyamine in saffron (*Crocus sativus* L.). *Physiol Mol Biol Plants* 27:119–133. <https://doi.org/10.1007/s12298-020-00923-x>
- Bakirel T, Bakirel U, Keleş OÜ, Ülgen SG, Yardibi H (2008) In vivo assessment of antidiabetic and antioxidant activities of Rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. *J Ethnopharmacol* 116:64–73. <https://doi.org/10.1016/j.jep.2007.10.039>
- Bozin B, Mimica-Dukic N, Samojlik I, Jovin E (2007) Antimicrobial and antioxidant properties of Rosemary and Sage (*Rosmarinus officinalis* L. and *salvia officinalis* L., Lamiaceae) essential oils. *J Agric Food Chem* 55:7879–7885. <https://doi.org/10.1021/jf0715323>
- Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol* 28:25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Busche M, Acatay C, Martens S, Weisshaar B, Stracke R (2021) Functional characterisation of banana (*Musa* spp.) 2-Oxoglutarate-Dependent dioxygenases involved in flavonoid biosynthesis. *Front Plant Sci* 12:701780. <https://doi.org/10.3389/fpls.2021.701780>
- Cely-Veloza W, Kato MJ, Coy-Barrera E (2023) Quinolizidine-Type alkaloids: Chemodiversity, Occurrence, and bioactivity. *ACS Omega* 8:27862–27893. <https://doi.org/10.1021/acsomega.3c02179>
- Chandra S, Chakraborty N, Dasgupta A, Sarkar J, Panda K, Acharya K (2015) Chitosan nanoparticles: A positive modulator of innate immune responses in plants. *Sci Rep* 5:15195. <https://doi.org/10.1038/srep15195>
- Chang C-C, Yang M-H, Wen H-M, Chern J-C (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 10:178–182. <https://doi.org/10.38212/2224-6614.2748>
- Darroudi M, Ahmad MB, Zak AK, Zamiri R, Hakimi M (2011) Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-Light. *Int J Mol Sci* 12:6346–6356. <https://doi.org/10.3390/ijms12096346>
- Davarpanah S, Tehranifar A, Abadía J, Val J, Davarynejad G, Aran M, Khorassani R (2018) Foliar calcium fertilization reduces fruit cracking in pomegranate (*Punica granatum* cv. Ardestani). *Sci Hortic* 230:86–91. <https://doi.org/10.1016/j.scienta.2017.11.023>
- Eid EM, Shaltout KH, Alamri SAM, Alrumman SA, Hussain AA, Sewelam N, Ragab GA (2021) Sewage sludge enhances tomato growth and improves fruit-yield quality by restoring soil fertility. *Plant Soil Environ* 67:514–523. <https://doi.org/10.17221/205/2021-PSE>
- El-Gogary M (2020) Ecofriendly synthesis of calcium nanoparticles with biocompatible *Rosmarinus officinalis* extract on physiological and immunological effects in broiler chickens. *Egypt Poult Sci J* 40:81–102. <https://doi.org/10.21608/epsj.2020.78748>
- El-Naggar HM, Read PE (2010) PAL gene activity and Rosmarinic acid production in Rosemary genotypes. *J Herbs Spices Med Plants* 16:83–89. <https://doi.org/10.1080/10496475.2010.481907>
- El-Shenody RA, Elshobary ME, Ragab GA, Huo S, Essa D (2023) Towards biorefinery: exploring the potential of seaweed-derived biodiesel and its residual biomass in improving the traits of *Eruca vesicaria* (L.) Cav. *S Afr J Bot* 155:361–371. <https://doi.org/10.1016/j.sajb.2023.02.029>
- El-Shora HM (2002) Properties of phenylalanine ammonia-lyase from marrow cotyledons. *Plant Sci* 162:1–7. [https://doi.org/10.1016/S0168-9452\(01\)00471-X](https://doi.org/10.1016/S0168-9452(01)00471-X)

- Elsherif DE, Ragab GA, Sobhy SE, Hafez EE, Khalifa AM (2025) Enhanced biochemical and morphological parameters in *Eruca vesicaria* by applications of date-palm seed extract. *J Soil Sci Plant Nutr* 25:666–679. <https://doi.org/10.1007/s42729-024-02159-0>
- Farshchi HK, Azizi M, Jaafari MR, Nemati SH, Fotovat A (2018) Green synthesis of iron nanoparticles by Rosemary extract and cytotoxicity effect evaluation on cancer cell lines. *Biocatal Agric Biotechnol* 16:54–62. <https://doi.org/10.1016/j.bcab.2018.07.017>
- Fazel S, Hamidreza M, Rouhollah G, Verdian-Rizi M (2010) Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *J Appl Hortic* 12:69–70. <https://doi.org/10.56808/3027-7922.2196>
- Ghorbanpour M, Askary F (2024) Comparative study on the morphophysiological responses and alkaloids content in *Eschscholzia californica* cham to foliar applied α -Fe₂O₃ NPs and ferric ions. *Ind Crops Prod* 214:118484. <https://doi.org/10.1016/j.indcro.2024.118484>
- Gong H, Tan X, Hou J, Gong Z, Qin X, Nie J, Zhu H, Zhong S (2024) Separation, purification, structure characterization, and immune activity of a polysaccharide from *Alocasia cucullata* obtained by freeze-thaw treatment. *Int J Biol Macromol* 282:137232. <https://doi.org/10.1016/j.ijbiomac.2024.137232>
- Govorov AO, Carmeli I (2007) Hybrid structures composed of photo-synthetic system and metal nanoparticles: plasmon enhancement effect. *Nano Lett* 7:620–625. <https://doi.org/10.1021/nl062528t>
- Habtemariam S (2016) The therapeutic potential of Rosemary (*Rosmarinus officinalis*) diterpenes for alzheimer's disease. *Evid-Based Complement Altern Med* 2016(2680409). <https://doi.org/10.1155/2016/2680409>
- Hassan A, Saber N, Abdulaziz H (2017) Changes of enzymatic antioxidants and minerals content of broad bean (*Vicia Faba* L.) plants in response to chilling stress and reaclimation. *Egypt J Bot* 57:539–553. <https://doi.org/10.21608/ejbo.2017.489.1018>
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125:189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Horvath G, Kissimon J, Faludi-Dániel Á (1972) Effect of light intensity on the formation of carotenoids in normal and mutant maize leaves. *Phytochemistry* 11:183–187. [https://doi.org/10.1016/S0031-9422\(00\)89987-2](https://doi.org/10.1016/S0031-9422(00)89987-2)
- Iavicoli I, Leso V, Fontana L, Calabrese EJ (2018) Nanoparticle exposure and hormetic dose-responses: an update. *Int J Mol Sci* 19:805. <https://doi.org/10.3390/ijms19030805>
- Iram M, Guo C, Guan Y, Ishfaq A, Liu H (2010) Adsorption and magnetic removal of neutral red dye from aqueous solution using Fe₃O₄ Hollow nanospheres. *J Hazard Mater* 181:1039–1050. <https://doi.org/10.1016/j.jhazmat.2010.05.119>
- Ishibashi Y, Tawaratsumida T, Kondo K, Kasa S, Sakamoto M, Aoki N, Zheng SH, Yuasa T, Iwaya-Inoue M (2012) Reactive oxygen species are involved in gibberellin/ abscisic acid signaling in barley aleurone cells. *Plant Physiol* 158:1705–1714. <https://doi.org/10.1104/pp.111.192740>
- Jimenez-Lopez JC (2020) Narrow-leafed lupin (*Lupinus angustifolius* L.) β -conglutin: A multifunctional family of proteins with roles in plant defence, human health benefits, and potential uses as functional food. *Legum Sci* 2:e33. <https://doi.org/10.1002/leg.333>
- Jindal KK, Singh RN (1975) Phenolic content in male and female carica papaya: a possible physiological marker for sex identification of vegetative seedlings. *Physiol Plant* 33:104–107. <https://doi.org/10.1111/j.1399-3054.1975.tb03774.x>
- Kasim Y, AboKassem E, Ragab G (2017) Ameliorative effect of yeast Extract, IAA and Green-synthesized nano zinc oxide on the growth of Cu-stressed *Vicia Faba* seedlings. *Egypt J Bot* 57:1–16. <https://doi.org/10.21608/ejbo.2017.885.1065>
- Kato M, Shimizu S (1987) Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves: phenolic-dependent peroxidative degradation. *Canad J Bot* 65:729–735. <https://doi.org/10.1139/b87-097>
- Khanizadeh P, Mumivand H, Morshedloo MR, Maggi F (2024) Application of Fe₂O₃ nanoparticles improves the growth, antioxidant power, flavonoid content, and essential oil yield and composition of *Dracocephalum kotschy* Boiss. *Front Plant Sci* 15:1475284. <https://doi.org/10.3389/fpls.2024.1475284>
- Klancnik A, Guzej B, Kolar MH, Abramovič H, Možina SS (2009) In vitro antimicrobial and antioxidant activity of commercial Rosemary extract formulations. *J Food Prot* 72:1744–1752. <https://doi.org/10.4315/0362-028X-72.8.1744>
- Lakshmnarayanan S, Shereen MF, Niraimathi KL, Brindha P, Arumugam A (2021) One-pot green synthesis of iron oxide nanoparticles from *Bauhinia tomentosa*: characterization and application towards synthesis of 1, 3 diolein. *Sci Rep* 11:8643. <https://doi.org/10.1038/s41598-021-87960-y>
- Lala S (2021) Nanoparticles as elicitors and harvesters of economically important secondary metabolites in higher plants: A review. *IET Nanobiotechnol* 15:28–57. <https://doi.org/10.1049/nbt.12005>
- Li A, Sun X, Liu L (2022) Action of Salicylic acid on plant growth. *Front Plant Sci* 13:878076. <https://doi.org/10.3389/fpls.2022.878076>
- Liu T, Zhu Y, Zhang X, Zhang T, Zhang T, Li X (2010) Synthesis and characterization of calcium hydroxide nanoparticles by hydrogen plasma-metal reaction method. *Mater Lett* 64:2575–2577. <https://doi.org/10.1016/j.matlet.2010.08.050>
- Moghaddam M, Mehdizadeh L (2017) Chemistry of essential oils and factors influencing their constituents. *Soft chemistry and food fermentation*. Elsevier, pp 379–419
- Mohammadi M, Majnoun Hoseini N, Chaichi MR, Alipour H, Dashtaki M, Safikhani S (2018) Influence of nano-iron oxide and zinc sulfate on physiological characteristics of peppermint. *Commun Soil Sci Plant Anal* 49:2315–2326. <https://doi.org/10.1080/0103624.2018.1499766>
- Mohsen AA, El-Kemary M, Dowidar SMA, Abo-Hamad SA, Elhaak MA, Khalaf BMM (2016) Impact of nano calcium hydroxide and calcium oxide on some metabolic activities and phenolic compounds of *Lupinus termis* seedlings. *Delta J Sci* 37:215–227. <https://doi.org/10.21608/djs.2016.139956>
- Molla MR, Begum MHA, Farhad SFU, Asadur Rahman ASM, Tanvir NI, Bashar MS, Bhuiyan RH, Alam MS, Hossain MS, Rahman MT (2022) Facile extraction and characterization of calcium hydroxide from paper mill waste sludge of Bangladesh. *R Soc Open Sci* 9:220681. <https://doi.org/10.1098/rsos.220681>
- Moroda MD, Leta Deressa T, Tiwikrama AH, Chala TF (2025) Green synthesis of copper oxide nanoparticles using *Rosmarinus officinalis* leaf extract and evaluation of its antimicrobial activity. *Next Mater* 7:100337. <https://doi.org/10.1016/j.nxmte.2024.100337>
- Mustafa H, Ilyas N, Akhtar N, Raja NI, Zainab T, Shah T, Ahmad A, Ahmad P (2021) Biosynthesis and characterization of titanium dioxide nanoparticles and its effects along with calcium phosphate on physicochemical attributes of wheat under drought stress. *Ecotoxicol Environ Saf* 223:112519. <https://doi.org/10.1016/j.ecoenv.2021.112519>
- Nazir F, Fariduddin Q, Khan TA (2020) Hydrogen peroxide as a signaling molecule in plants and its crosstalk with other plant growth regulators under heavy metal stress. *Chemosphere* 252:126486. <https://doi.org/10.1016/j.chemosphere.2020.126486>
- Nazir MM, Noman M, Ahmed T, Ali S, Ulhassan Z, Zeng F, Zhang G (2022) Exogenous calcium oxide nanoparticles alleviate cadmium toxicity by reducing Cd uptake and enhancing antioxidative capacity in barley seedlings. *J Hazard Mater* 438:129498. <https://doi.org/10.1016/j.jhazmat.2022.129498>

- Nessem AA, Mohsen AA, Atlam A, Ragab GA (2023) Effect of priming with natural plant extract on the Growth, hormonal Status, and yield value of *Triticum aestivum* (L.) grown under lead and nickel stress. Egypt J Bot 63:159–174. <https://doi.org/10.21608/ejbo.2022.129151.1943>
- Nourozi E, Hosseini B, Maleki R, Mandoulakani BA (2019) Iron oxide nanoparticles: a novel elicitor to enhance anticancer flavonoid production and gene expression in *Dracocephalum kotschy* hairy-root cultures. J Sci Food Agric 99:6418–6430. <https://doi.org/10.1002/jsfa.9921>
- Oliva M, Ovadia R, Perl A, Bar E, Lewinsohn E, Galili G, Oren-Shamir M (2015) Enhanced formation of aromatic amino acids increases fragrance without affecting flower longevity or pigmentation in *Petunia × hybrida*. Plant Biotechnol J 13:125–136. <https://doi.org/10.1111/pbi.12253>
- Oser BLL (1979) Hawk's physiological chemistry, 14th edn. McGraw-Hills, New York
- Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269:337–341. <https://doi.org/10.1006/abio.1999.4019>
- Prusinski J (2017) White lupin (*Lupinus albus* L.) - Nutritional and health values in human nutrition - A review. Czech J Food Sci 35:95–105. <https://doi.org/10.17221/114/2016-CJFS>
- Qadir A, Aqil M, Ali A, Ahmad FJ, Ahmad S, Arif M, Khan N (2020) GC-MS analysis of the methanolic extracts of *Smilax China* and *Salix Alba* and their antioxidant activity. Turk J Chem 44:352–363. <https://doi.org/10.3906/KIM-1907-5>
- Ragab GA, Saad-Allah KM (2020) Green synthesis of sulfur nanoparticles using *Ocimum Basilicum* leaves and its prospective effect on manganese-stressed *Helianthus annuus* (L.) seedlings. Ecotoxicol Environ Saf 191:110242. <https://doi.org/10.1016/j.ecoen.2020.110242>
- Ragab GA, Nessem AA, Elshobary ME, Henjes J, Razzaky EO (2025a) Unraveling the physiological and ultrastructural responses of wheat to combat Cobalt stress and the protective role of *Jania Rubens* related to antioxidant defense and cellular integrity. Front Plant Sci 16:1621482. <https://doi.org/10.3389/fpls.2025.1621482>
- Ragab GA, Saad-Allah KM, Nessem AA (2025b) Mitigating arsenate-Induced phytotoxicity in *Fenugreek* seedlings using Garlic extract: insights into Photosynthesis, arsenate Uptake, antioxidative machinery and ultrastructure. J Soil Sci Plant Nut 25:4091–4111. <https://doi.org/10.1007/s42729-025-02386-z>
- Ramesh R, Yamini V, Rajkumar D, John Sundaram S, Lakshmi D, Khan FLA (2019) Biogenic synthesis of a-Fe₂O₃ nanoparticles using *Plectranthus amboinicus* leaf extract. In: Materials Today: Proceedings. Elsevier Ltd, pp 453–458
- Rastegaran Z, Hassanpour H, Ziyadi H (2022) Synthesized Fe₃O₄ nanoparticles induce antioxidant activity and total phenolic and flavonoid contents in *Matricaria Chamomilla* seedlings. Iran J Plant Physiol 12:4003–4011. <https://doi.org/10.22034/ijpp.2021.1919085.1281>
- Ren P, Zhou B, Bi Y, Chen X, Yao S, Yang X (2025) *Bacillus subtilis* can promote cotton phenotype, yield, nutrient uptake and water use efficiency under drought stress by optimizing rhizosphere microbial community in arid area. Ind Crops Prod 227:120784. <https://doi.org/10.1016/j.indcrop.2025.120784>
- Rui M, Ma C, Hao Y, Guo J, Rui Y, Tang X, Zhao Q, Fan X, Zhang Z, Hou T, Zhu S (2016) Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). Front Plant Sci 7:815. <https://doi.org/10.3389/fpls.2016.00815>
- Saad-Allah KM, Ragab GA (2020) Sulfur nanoparticles mediated improvement of salt tolerance in wheat relates to decreasing oxidative stress and regulating metabolic activity. Physiol Mol Biol Plants 26:2209–2223. <https://doi.org/10.1007/s12298-020-0089-8>
- Sadak MS, Ramadan AAEM (2021) Impact of melatonin and Tryptophan on water stress tolerance in white lupine (*Lupinus termis* L.). Physiol Mol Biol Plants 27:469–481. <https://doi.org/10.1007/s12298-021-00958-8>
- Singh RP, Handa R, Manchanda G (2021) Nanoparticles in sustainable agriculture: an emerging opportunity. J Control Release 329:1234–1248. <https://doi.org/10.1016/j.jconrel.2020.10.051>
- Soliman MM, Elsaba YM, Soliman MSA, Ahmed EZ (2024) Composition and antimicrobial activity of *Rosmarinus officinalis* L. and *Artemisia monosperma* L. leaf essential oils and methanolic extracts from plants grown in normal and saline habitats in Egypt. Sci Rep 14:7342. <https://doi.org/10.1038/s41598-024-57301-w>
- Souri MK, Bakhtiarzade M (2019) Biostimulation effects of Rosemary essential oil on growth and nutrient uptake of tomato seedlings. Sci Hortic 243:472–476. <https://doi.org/10.1016/j.scienta.2018.08.056>
- Tanpure S, Ghanwat V, Shinde B, Tanpure K, Lawande S (2022) The eggshell waste transformed green and efficient synthesis of K-Ca(OH)₂ catalyst for room temperature synthesis of Chalcones. Polycycl Aromat Compd 42:1322–1340. <https://doi.org/10.1080/10406638.2020.1776740>
- Vankova R, Landa P, Podlipna R, Dobrev PI, Prerostova S, Langhansova L, Gaudinova A, Motkova K, Knirsch V, Vanek T (2017) ZnO nanoparticle effects on hormonal pools in *Arabidopsis Thaliana*. Sci Total Environ 593:535–542. <https://doi.org/10.1016/j.scitotenv.2017.03.160>
- Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151:59–66. [https://doi.org/10.1016/S0168-9452\(99\)00197-1](https://doi.org/10.1016/S0168-9452(99)00197-1)
- Wang D, He H, Wei C (2023a) Cellular and potential molecular mechanisms underlying transovarial transmission of the obligate symbiont *sulcia* in cicadas. Environ Microbiol 25:836–852. <https://doi.org/10.1111/1462-2920.16310>
- Wang YX, Yu TF, Wang CX, Wei JT, Zhang SX, Liu YW, Chen J, Zhou Y, Bin, Chen M, Ma YZ, Lan JH, Zheng JC, Li F, Xu ZS (2023b) Heat shock protein TaHSP17.4, a TaHOP interactor in wheat, improves plant stress tolerance. Int J Biol Macromol 246:125694. <https://doi.org/10.1016/j.ijbiomac.2023.125694>
- Wiedemann M, Gurrola-Díaz CM, Vargas-Guerrero B, Wink M, García-López PM, Düfer M (2015) Lupanine improves glucose homeostasis by influencing KATP channels and insulin gene expression. Molecules 20:19085–19100. <https://doi.org/10.3390/molecules201019085>
- Xu P, Zeng G, Huang D, Hu S, Feng C, Lai C, Zhao M, Huang C, Li N, Wei Z, Xie G (2013) Synthesis of iron oxide nanoparticles and their application in *Phanerochaete Chrysosporium* immobilization for Pb(II) removal. Colloids Surf Physicochem Eng Asp 419:147–155. <https://doi.org/10.1016/j.colsurfa.2012.10.061>
- Yakhin OI, Lubyantov AA, Yakhin IA, Brown PH (2017) Biostimulants in plant science: A global perspective. Front Plant Sci 7(2049). <https://doi.org/10.3389/fpls.2016.02049>
- Yu X, Cui X, Wu C, Shi S, Yan S (2022) Salicylic acid inhibits Gibberellin signaling through receptor interactions. Mol Plant 15:1759–1771. <https://doi.org/10.1016/j.molp.2022.10.001>