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Functional nanoparticles enhance wheat resilience to nickel toxicity through morpho-physiological, antioxidant, and agronomic modulation

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Introduction: Nickel (Ni) contamination poses a serious threat to crop productivity by inducing oxidative stress and disrupting physiological and metabolic processes. This study explores green nanotechnology as a sustainable strategy for mitigating heavy metal stress to support climate-resilient agriculture (SDG 13).

Methods: We evaluated the efficacy of *Withania coagulans*-mediated, green-synthesized silver (Ag), copper oxide (CuO), and CuO-Ag bimetallic nanoparticles (NPs) in alleviating Ni-induced stress in two wheat (*Triticum aestivum* L.) cultivars: SKD-1 and Borlaug-16. NPs were applied at 250 ppm via seed priming and foliar spraying.

Results: NPs treatments significantly improved photosynthetic performance; total chlorophyll reached 36.9 µg/g FW in SKD-1 and 21.1 µg/g FW in Borlaug-16. Antioxidant defense was reinforced, with superoxide dismutase activities up to 37 U/g FW and catalase activities up to 0.8 U/g FW, while malondialdehyde (MDA) content reduced to 1.3 µmol/g FW. Ni translocation was restricted via root sequestration, limiting grain accumulation to 0.2 mg/kg. Agronomic performance improved, with spike lengths of 12.4 cm, biological yields of 2.4 g/plant, and grain yields of 1.0 g/plant. Furthermore, phytochemical enrichment was observed, with total phenolics at 51.4 mg GAE/g DW and total flavonoids at 448.8 mg QE/g DW.

Discussion: CuO-Ag bimetallic NPs were the most effective in enhancing wheat resilience by limiting Ni translocation and sustaining yield. Future research should prioritize optimizing delivery strategies, elucidating molecular mechanisms of NP-mediated gene expression, and evaluating the scalability of these materials in diverse field-scale environments.

KEYWORDS

agronomic performance, antioxidant, climate resilience, nanotechnology, nickel toxicity, SDGs, sustainable agriculture, wheat

1 Introduction

Heavy metal contamination is a chronic and growing challenge for sustainable agriculture due to the non-biodegradable nature of metal pollutants and their potential for long-term accumulation in the terrestrial environment (Rehman et al., 2025; Yadav et al., 2025). Anthropogenic activities such as industrial effluent discharge, urban expansion, mining activities, and excessive application of agrochemicals, are contributing significantly to the sources of influx of toxic metals into the arable soil, thereby precipitating a decline in the soil's health, interfering with microbial life, and reducing the crop yield (Mubeen et al., 2024; Saeed et al., 2025; Yasir et al., 2025). Among these contaminants, nickel (Ni) is especially pervasive in origin from industrial processes, mining operations, and overuse of phosphate fertilizers and pesticides. Elevated Ni levels are readily taken up by plants, in which they interfere with vital physiological functions, nutrient uptake, and growth and biomass formation. The long-term accumulation of Ni in the tissues of edible plants is a significant human health concern, as chronic exposure induces neurotoxicity and reproductive disorders and increases the risk of morbidity from oxidative stress-induced cellular damage (Kumar et al., 2025b).

Within wheat, (*Triticum aestivum* L.) one of the most widely cultivated cereal plants in the world, it has been documented that Ni toxicity may compromise photosynthetic efficiency, alter the integrity of the cell membrane, and cause excessive generation of reactive oxygen species (ROS). ROS accumulation subsequently leads to damage of cell macromolecules and inhibits metabolism pathways which are key component for growth and development (Khan et al., 2020; Saleh, 2024; Anas et al., 2026). Wheat plants are able to counteract oxidative stress naturally via antioxidant defense mechanisms that include a series of enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POD), as well as non-enzymatic antioxidants that include phenolics, flavonoids and ascorbic acid (Rao et al., 2025). However, the severity of Ni stress often outpaces these innate defenses reaching a point of irreversible cell damage resulting in huge yield losses. This is a critical issue in that calls are made for innovative and sustainable mitigation

strategies that can combine plant resilience while also preserving environmental integrity (Zhang et al., 2024).

Nanotechnology has become a potentially great approach for overcoming heavy metal stress in agriculture, as it holds novel solutions for improving crop performance under unfavorable conditions (Bibi et al., 2024; Ansari et al., 2026; Khan et al., 2026). Engineered nanoparticles (NPs) of sizes below 100 nanometer have unique physicochemical properties (comprising high surface to volume ratios, high reactivity, and targeted interaction with biological systems) which make them efficient modulators of stress responses in plants (Hassan et al., 2018; Kumari et al., 2024; Abbasi et al., 2025; Qadri et al., 2026). Recent investigations have shown that metallic NPs as copper oxide (CuO) and silver (Ag) can enhance plant growth under stress by augmenting the activities of antioxidant enzymes, stabilizing cellular membranes, and enhancing the uptake of nutrients, which will subsequently result in improving the physiological status and yield components (Kumar et al., 2025a). For instance, green-synthesized CuO NPs were significantly demonstrated to improve the unfavorable effects of cadmium stress induced in wheat through the enhancement of antioxidant defenses and reduction of oxidative damage to plant tissues. Likewise, nano-fertilizers such as ZnO NPs have been reported to increase chlorophyll content, enzymatic antioxidant activity and biomass production in wheat exposed to abiotic stressors (Ochoa-Chaparro et al., 2026). Bimetallic nanoparticles have shown significant promise in various environmental applications, particularly in water pollution control. Recent studies highlight their superior performance over monometallic particles due to synergistic effects between the two metals, which enhance catalytic and removal abilities for contaminants such as heavy metals, organic compounds, and dyes. Bimetallic nanoparticles, including those used in water treatment (Fu et al., 2015; Khan et al., 2025a, b, c), exhibit higher efficiency in removing contaminants due to their unique structural properties, such as core-shell configurations and alloy compositions (Loza et al., 2020; Khan et al., 2025d). Furthermore, bimetallic metal-organic frameworks (BMOFs) have emerged as highly effective adsorbents, with exceptional adsorption capabilities that allow them to tackle complex environmental challenges (Kayani and Mohammed, 2025). These enhanced properties make bimetallic nanoparticles more efficient and versatile in heavy metal remediation and other environmental applications compared to their monometallic counterparts.

Bimetallic nanoparticles such as Ag-Cu composites, are a recent innovation over their monometallic counterparts because of synergistic effects resulting from the co-occurrence of two metals promoting increased redox activity, stability and ROS-scavenging efficiency (Ragavendran et al., 2024). These dual metal systems have shown better performance in terms of stress mitigation by improving catalytic property and bioavailability at the same time while better counteracting the oxidative stress caused by heavy metals (Hu et al., 2024). The green synthesis of such nanoparticles using plant extracts or bioactive phytochemicals is particularly attractive because it avoids the use of toxic chemical reagents and reduces environmental impact, while providing capping or stabilizing compounds with inherent biological activities that can

Abbreviations: BY, Biological Yield; GY, Grain Yield; T/P, Tillers per Plant; Sp/P, Spikelets per Plant; TKW, Thousand-kernal Weight; NPs, Nanoparticles; Ni, Nickel; FLA, Flag Leaf Area; HMs, Heavy Metals; H₂O₂, Hydrogen Peroxide; OH, Hydroxyl Radical; DTT, Days to Tillering; Chl_Til, Chlorophyll at Tillering Stage; DTB, Days to Booting; Chl_Boot, Chlorophyll at Booting Stage; DTH, Days to Heading; Chl_Head, Chlorophyll at Heading Stage; DTA, Days to Anthesis; Chl_Anth, Chlorophyll at Anthesis Stage; FLA_Head, Flag Leaf Area at Heading; FLA_Anth, Flag Leaf Area at Anthesis; ROS, Reactive Oxygen Species; SSI, Stress Susceptibility Index; TOL, Tolerance index; MP, Mean Productivity Index; GMP, Geometric Mean Productivity; STI, Stress Tolerance Index; YI, Yield Index; SOD, Superoxide Dismutase; POD, Peroxidase; CAT, Catalase; AsA, Ascorbic Acid; MDA, Malondialdehyde; O₂⁻, Superoxide Radical; TG, Total Sugar; TAC, Total Antioxidant Capacity; TF, Translocation Factor; YSI, Yield Stability Index; HM, and Harmonic Mean; TTC, Total Tannin Content; TanC, Total Anthocyanin Content; TPC, Total Phenolic Content; TFC, Total Flavonoid Content; FRAP, Ferric Reducing Antioxidant Power; ABTS^{•+}, 2,2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid); DPPH, 2,2-Diphenyl-1-Picrylhydrazyl.

complement the biocompatibility and functional effectiveness of the nanoparticles (Shahzadi et al., 2025).

Plant mediated synthesis of nanoparticles utilizes phytochemicals such as flavonoids, alkaloids and phenolic compounds as the reducing and stabilizing agents making eco-friendly nanoparticles with desirable particle size and surface characteristics for agricultural applications (Edo et al., 2025; Ullah et al., 2025). While the mitigation of heavy metals using monometallic nanoparticles is documented, there is a critical knowledge gap regarding the synergistic potential of bimetallic systems, specifically against Ni toxicity, which is often overshadowed by studies on Lead or Cadmium (El-Mogy et al., 2025; Naz et al., 2025). Furthermore, the role of specific medicinal plant extracts, such as *Withania coagulans*, as functional “capping” agents remains under-explored in comparative agronomic trials. The aim of this study is to investigate the effectiveness of pre-synthesized green Ag, CuO, and novel CuO-Ag bimetallic nanoparticles derived from *W. coagulans* leaf extracts in mitigating Ni stress in wheat. Unlike previous reports, this research provides a comparative analysis to determine if the bimetallic configuration offers superior antioxidant modulation and reduced metal translocation compared to its monometallic counterparts. Specifically, the study evaluates the impact of these nanoparticles on growth, antioxidant defense mechanisms, and biochemical responses through seed priming and foliar application. By addressing the specific physiological disruptions caused by Ni, this research contributes to sustainable agronomic strategies (Ahmad et al., 2025; Gorge and Abood, 2025; Qadri et al., 2026), offering eco-friendly solutions to improve crop resilience and ensure food safety by significantly reducing grain Ni accumulation.

2 Materials and methods

2.1 Experimental framework and plant material

The experiment was conducted at the Quaid-i-Azam University, Islamabad, Pakistan, in collaboration with the University of South Africa. Environmental conditions such as light intensity, temperature, and humidity were controlled to provide optimal wheat development and simulate nickel (Ni) stress. Two cultivars of wheat (*Triticum aestivum* L.), including SKD-1 (V1) and Borlaug-16 (V2), were used based on previous documents on their heavy metal tolerance and differential resilience to Ni exposure. Borlaug-16 has been claimed to show reduced Ni uptake, reduced cell injury, and increased antioxidant capacity compared with SKD-1 as showed by growth parameters, anatomical modifications, oxidative stress and enzymatic antioxidant activities during Ni treatment (Anas et al., 2023). Nickel stress was induced by the insertion of NiCl_2 into the soil matrix in a concentration of 100 mg Ni kg^{-1} soil which reflects the levels of contamination occurring within industrialized areas (Afzal et al., 2014).

Green synthesis of nanoparticles was achieved using the aqueous extract of leaves from *Withania coagulans*. Fresh leaves were collected in Village Sawans Mianwali, District Mianwali, Punjab, Pakistan, in June 2022 (32°43'44" N, 71°37'59" E; elevation 285 meters) with the explicit permission of the owner of

the land. Taxonomic identification was done by Prof. Dr. Zabta Khan Shinwari and a voucher specimen (No. 133570) was deposited in the Herbarium of Pakistan (ISL). Leaves were rinsed and shade dried and milled into a fine powder. The extract was prepared by dispersing 10 g of the powdered leaves, in 100 mL distilled water and stirring for 24 h at room temperature, filtering on Whatman No. 1 paper and storing the filtrate at 4 °C until further use (Hassan et al., 2024; Sani et al., 2024). Silver (Ag), copper oxide (CuO), and CuO-Ag bimetallic nanoparticles were produced by mixing the plant extract with metal precursors under controlled heating. Nanoparticle formation was verified by UV-Vis spectroscopy, showing surface plasmon resonance (SPR) at 425 nm for Ag-NPs, 555 nm for CuO-NPs, and 525 nm for CuO-Ag bimetallic NPs. X-ray diffraction (XRD) confirmed the crystalline nature with average crystallite sizes of 21.42 nm, 21.40 nm, and 26.29 nm, respectively. Scanning electron microscopy (SEM) images revealed spherical morphologies for Ag-NPs, flake-like structures for CuO-NPs, and a composite of cubic and spherical morphologies for the bimetallic particles. Crucially, these nanoparticles were extensively pre-characterized in our previous studies, where Fourier Transform Infrared (FTIR) spectroscopy confirmed successful phytochemical capping by bioactive metabolites (such as withanolides and phenolics) from *W. coagulans*. Additionally, Dynamic Light Scattering (DLS), Zeta potential, and stability analyses were performed to verify their size distribution and long-term stability in suspension (Khan et al., 2025b, e). These standardized nanoparticles, originally developed for biomedical applications, were subsequently utilized at a concentration of 250 ppm for the current agronomic trials. Seed priming was performed by soaking the wheat seeds in nanoparticle suspensions prior to sowing and hence increasing the tolerance to Ni stress by tolerance at the germination stage. Foliar applications were provided for vegetative and reproductive stage to enhance the uptake of nanoparticles and to reduce the stress (Das et al., 2023). Treatments were arranged as shown in Table 1.

The experimental design was a completely randomized design (CRD) using factorial combinations of the cultivar and the treatment with each treatment repeated three times. Data were

TABLE 1 Summary of experimental treatments and application methods of green-synthesized nanoparticles (Ag, CuO, and CuO-Ag) used for mitigating nickel toxicity in wheat cultivars.

Treatment code	Treatment description
C	Control (no Ni stress, no nanoparticles)
T1	Nickel stress alone (100 mg Ni kg^{-1} soil)
T2	<i>Withania coagulans</i> extract (250 ppm)
T3	<i>W. coagulans</i> extract (250 ppm) + Ni stress (100 mg kg^{-1})
T4	Ag nanoparticles (Ag-NPs, 250 ppm)
T5	Copper oxide nanoparticles (CuO-NPs, 250 ppm)
T6	CuO-Ag bimetallic nanoparticles (250 ppm)
T7	Ag-NPs (250 ppm) + Ni stress
T8	CuO-NPs (250 ppm) + Ni stress
T9	CuO-Ag NPs (250 ppm) + Ni stress

analyzed using two-point Analysis of Variance (ANOVA) in XLStat using mean separation which was carried out by the method of Least Significant Difference (LSD) at $p < 0.05$. Other statistical analyses and multiple-comparison tests were performed using GraphPad Prism. This methodology provided the opportunity to comprehensively evaluate the potential of green-synthesized nanoparticles to improve the antioxidant systems, ameliorate Ni-induced stress, and growth and agronomic performance in wheat.

2.2 Soil analysis methodology

Soil samples were air-dried and sieved through a 2 mm mesh size to standardize particle size distribution. Soil pH was determined in a 1:2.5 soil: water suspension using a calibrated pH meter, and electrical conductivity (EC) was measured in the same suspension to assess salinity. Nitrate was measured by a colorimetric method, while phosphate was measured by the Olsen method and spectrophotometric detection (Olsen and Sommers, 1982). Organic carbon content was determined using the Walkley - Black method, total organic carbon (TOC) and organic matter (OM) were determined accordingly (Pal, 2019; McClelland et al., 2021). These analyses provided a comprehensive chemical profile of the soil which was necessary to assess the baseline characteristics of the soil under a stress condition with nickel (Ni). Each parameter was assessed in triplicate to ensure precise analytical results, and the data were expressed as mean $\pm$ standard error.

2.3 Growth and agronomic analysis

Wheat growth and agronomic traits were observed in successive stages of phenology including sowing, germination, tillering, booting, heading and anthesis. Temporal metric parameters, days to germination (DTG), days to tillering (DTT), days to booting (DTB), days to heading (DTH) and days to anthesis (DTA) were systematically recorded. Chlorophyll concentrations were measured at each stage of development using a SPAD-502 chlorophyll meter (Minolta, Japan), a portable device, used for the non-destructive estimation of foliar concentration of chlorophyll. Physiological parameters, i.e. fresh weight, leaf area and leaf dimension parameters were assessed at the heading and anthesis junctures to assess the adaptive response to stress. Agronomic characteristics were measured in terms of plant height, spike length, seeds per spike, biological yield, grain yield and thousand kernel weight (TKW) in order to assess the yield performance under nickel (Ni) stress (Pask, 2012).

2.4 Analysis of Ni uptake, accumulation and translocation to upper parts

The concentrations of nickel were measured on soil (HM_Soil) as well as root (HM_Root), leaf (HM_Leaf) or grain (HM_Grain) using the technology of atomic absorption spectrophotometry on a Shimadzu AA-7000 (Japan). To elucidate Ni mobility inside the plant, translocation factors (TF) were calculated between the root/grain/leaf (TF_Root/Leaf and TF_Leaf/Grain). Bioconcentration factors (BCF) and bioaccumulation factors (BAF), were used to assess the Ni uptake efficiency and the internal accumulation (Robinson et al., 2003; Hayat et al., 2024). The translocation

factors (TF), bioconcentration factor (BCF), and bioaccumulation factor (BAF) were calculated according to Equations 1–4, respectively.

$$TF_Root/Leaf = Ni_{leaf}/Ni_{root} \quad (1)$$

$$TF_Leaf/Grain = Ni_{grain}/Ni_{leaf} \quad (2)$$

$$BCF_Soil/Root = Ni_{root}/Ni_{soil} \quad (3)$$

$$BAF_Soil/Leaf = Ni_{leaf}/Ni_{soil} \quad (4)$$

2.5 Phytochemical content analysis of leaves

The phytochemical composition was assessed by the extraction and evaluation of total phenolic content (TPC), total flavonoid contents (TFC), total anthocyanin contents (TAnC), total tannins content (TTC) and ascorbic acid (AsA). TPC was measured using the Folin-Ciocalteu colorimetric method using 0.5 mL of extract which was combined with Folin-Ciocalteu reagent and sodium carbonate solution and was incubated; absorbance was recorded at 760 nm and results were calculated into the equivalent of milligrams of gallic acid per gram dry weight (mg GAE/g DW) using gallic acid calibration curve (Rahman et al., 2017; Hassan and Sani, 2025). TFC was assessed using the aluminum chloride colorimetric assay. Briefly, leaf extracts were reacted with a solution of aluminum chloride ($AlCl_3$), incubated at room temperature, and the absorbance was determined at 510 nm. The values were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW), following the standardized protocol of Zhishen et al. (1999). TAnC was determined by the pH differential method using extracts diluted independently in buffers at pH 1.0 and pH 4.5 and the absorbance and recorded at 520 nm and 700 nm. Data were presented as cyanidin-3-glucoside equivalents per 100 g FW (Vega Arroyo et al., 2017). TTC was determined according to the protocol of Sun et al. (1998), that is, mixing the extracts with a vanilin- H_2SO_4 reagent, incubating and measuring absorbance at 500 nm as catechin, with the standard catechin serving as standard, and expressed in milligrams of catechin equivalent per gram dry weight (mg Cateching/g DW). Ascorbic acid content was analyzed by 2,4 -dinitrophenylhydrazine (DNPH) method, extracts were reacted with DNPH reagent and absorbance was measured at 520 nm, the content was determined by the standard curve of L- ascorbic acid (Desai and Desai, 2019).

2.6 Antioxidant and oxidative stress analysis of leaves

2.6.1 Total antioxidant capacity and activity

The antioxidant potential of the leaf extracts was investigated by several *in vitro* assays. Total antioxidant capacity (TAC) was measured with the phosphomolybdenum method in which the samples were mixed with phosphomolybdenum reagent and heated to ensure the reduction of Mo (VI) to Mo (V) and

consequently form a green complex of phosphate and Mo (V). Absorbance was measured at 630 nm, and ascorbic acid was used as the calibration standard (Rahman et al., 2017). Ferric reducing antioxidant power (FRAP) was measured by mixing the extract with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%), and then incubating with trichloroacetic acid (10%) and FeCl₃ (0.1%), and their absorbance was measured at 700 against ascorbic acid (Gohari et al., 2011). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was measured by adding DPPH working solution to different concentrations of the sample and recording the absorbance at a wavelength of 517 nm after incubation in the dark (Sharifi-Rad et al., 2020). Radical scavenging activity (%) was determined by the following formula:

$$\text{RSA (\%)} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] \times 100$$

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), also known as ABTS^{•+} assay, consisted of the production of ABTS^{•+} radical cations and their reaction with the sample; the resultant decline in absorbance was measured at 734 nm, with the results being expressed in terms of percent scavenging activity compared to that of butylated hydroxyanisole (BHA) standard (Ko et al., 2020).

2.6.2 Enzymatic antioxidant

Antioxidant activities were measured spectrophotometrically using enzymatic antioxidants to investigate the biochemical response of wheat towards nickel-induced stress. The activity of superoxide dismutase (SOD; EC 1.15.1.1) as an indicator of superoxide radical scavenging activity was measured at 560 nm and expressed in units per gram fresh weight (U g⁻¹ F.W.). Peroxidase (POD; EC 1.11.1.7) activity was measured at 420 nm with H₂O₂ as the electron donor and guaiacol as the substrate. Catalase (CAT; EC 1.11.1.6), which is responsible for the detoxification of hydrogen peroxide, was assayed at 240 nm (Alici and Arabaci, 2016). These enzymatic tests provided a detailed profile of the plant's potential to overcome oxidative stress associated with its toxicity to nickel.

2.6.3 Oxidative stress indicators

Oxidative stress markers were measured to quantify the extent of damage caused by nickel-induced toxicity. Concentration of hydrogen peroxide (H₂O₂) was determined by the reaction of extracts from the plant with potassium iodide in an acidic medium and absorbance at 390 nm was measured (Velikova et al., 2000). The formation of malondialdehyde (MDA) as an index of lipid peroxide formation was measured with thiobarbituric acid (TBA), and specific absorbance was measured at 532 nm while non-specific absorbance was measured at 600 nm with a result expressed in micro moles of malondialdehyde per gram fresh weight (μmol/g FW) (Ozden et al., 2009). These parameters in total accentuated the severity of oxidative damage and the integrity of lipid membranes under the nickel stress.

2.7 Grain nutrition and biochemical analysis

2.7.1 Phytochemical and nutritional content

The biochemical characteristics of the grain were analyzed, including total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC). TPC was measured using the Folin-Ciocalteu assay at 760 nm, while TFC was determined by using the aluminum chloride method at 510 nm (Rahman et al., 2017). TTC was determined by the vanillin-HCl method (Sun et al., 1998). Carbohydrate composition was questioned by the quantification of non-reducing sugars (NRS), reducing sugars (RS), total sugars (TS) and starch. RS were measured in the neutral fraction as described by Bell (1955); NRS were hydrolyzed with 6M formic acid under reflux for 2hrs to liberate monosaccharides for quantification. TS were determined spectrophotometrically against standard calibration curves (Hayat et al., 2024). The difference between TS and RS was used to determine NRS. Protein content was analyzed using the Kjeldahl methodology in which the Nitrogen content was used as an estimate of protein concentration (Jung et al., 2003).

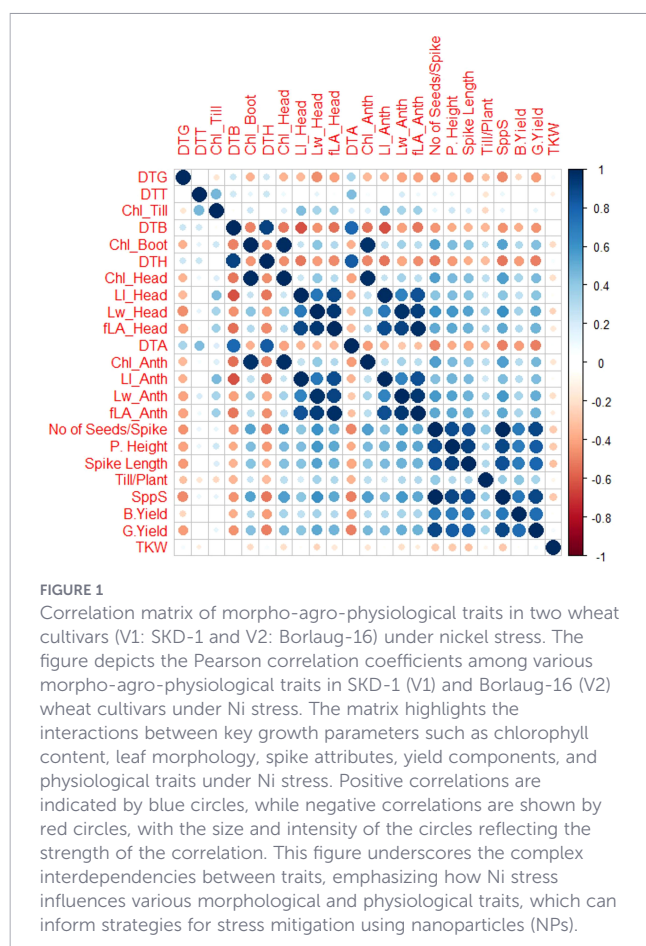
2.8 Statistical analysis

Data were analyzed using a two-way factorial Analysis of Variance (ANOVA) in RStudio (version 4.3.3) to assess the main effects of cultivars, nanoparticle treatments, and their interaction (Cultivar × Treatment). Prior to analysis, normality and homogeneity of variance were verified using Shapiro-Wilk and Levene's tests. Mean separation was performed using Fisher's Least Significant Difference (LSD) test at $p < 0.05$. All data visualizations, including boxplots and comparative graphs, were generated using Origin 2024, Minitab, and GraphPad Prism 10, ensuring a rigorous and consistent statistical approach.

3 Results

3.1 Growth stages, photosynthetic efficiency, and leaf morphometric parameters under nickel stress

The analysis showed a strong influence of the applied treatments on wheat growth stages, chlorophyll content, and leaf morphometric traits under nickel stress. The correlation matrix of morpho-agro- physiological characters for two wheat cultivars, viz. V1=SKD-1 and V2= Borlaug-16 under Ni stress is shown in Figure 1. For the SKD-1 cultivar, the T5 treatment greatly improved growth rate, such that days to booting (DTB = 59), heading (DTH = 71.3), and anthesis (DTA = 77) were significantly shorter than the Ni-stressed control (T1). Leaf morphometrics were significantly improved under T5 and there was a significantly higher maximum leaf length (LL_Head = 15.2 cm) and width (Lw_Head = 1 cm) at heading and the maximum flag leaf area at anthesis (fLA_Anth = 14.6 cm²). Treatments T6 and T5 always improved



the chlorophyll content at all the developmental stages T9 showed the highest improvements in flag leaf characteristics showing its promise in reducing the Ni-induced toxicity. In Borlaug-16, the T2 treatment had a positive effect on early-stage growth in the form of reduction of days of germination (DTG = 7.7), and significant increase of chlorophyll content at tillering (Chl_Till=42.7) and heading (Chl_Head=36). Under stress conditions, T8 gave rise to the significantly enhanced leaf morphometric parameters, such as the leaf width (Lw_Anth = 0.9 cm), flag leaf area (fLA_Anth = 7.2 cm²), and T7 had the highest level of chlorophyll content at tillering (Chl_Till = 42.5). Across both cultivars T5 and T6 had consistent performance which underlines their efficacy to improve physiological and morphological characteristics under Ni stress compared to control and untreated stress conditions.

3.1.1 Agronomic performance and stress mitigation in wheat

Application of nanoparticle treatments significantly modulated the agronomic performance of both the SKD-1 and Borlaug-16 cultivars under stress. In SKD-1, it was found that T6 treatment performed best in terms of the number of seeds per spike (23.3), plant height (24.5 cm) and spike length (12.6 cm) and biological yield (3.7 g). Grain yield was also increased (0.9 g) in addition to consistent improvements in tillers per plant (1.3) and spikelets per spike (26). T8 treatment showed further increases in plant height

(25 cm and higher) and biological yield (1.9 g/plant), indicating stress mitigation. On the contrary, T1 resulted in a marked decrease in all measured parameters, including seeds per spike (4) and spike length (5.1 cm), indicating the harmful effects of Ni toxicity. For Borlaug-16, T6 treatment also showed the superior agronomic performance as it led to the highest biological yield (2.4g) and grain yield (0.9g) of 25.5 cm height and 12.4 cm spike length with 24.7 spikelets per spike. T5 also had a positive effect on spikelets per spike (20) and plant height (22.5 cm). The Ni - stressed control (T1) resulted in heavy reduction with the of number of seed per spike reduced to 4.7 and the biological yield reduced to 0.2 g. Collectively, these results show the effectiveness of T6 and T5 treatments in mitigating the Ni-induced stress conditions where T6 was found to be the most effective treatment intervention for the two wheat cultivars.

3.2 Stress indices, phenotypic data, and productivity analysis/

Stress indices and phenotypic characteristics showed high variation among treatments for both the SKD-1 and Borlaug-16 cultivars under nickel [Ni] stress as shown in [Supplementary Table 1](#). Within the SKD-1 genotype, treatment T8 showed the best performance with an increase in grain mean productivity (GMP) of 131.3%, stress tolerance (STI) by 32%, and mean productivity index (MPI) by 135% over the control (Ni - stressed) (T1), therefore, showing the best mitigation of stress effects. This treatment also had the maximum values for yield-related phenomena, namely spike length (13.5 cm), plant height (25.1 cm), seeds per spike (24.3) and biological yield (3.8 g), as shown in [Figure 1](#). Treatments T7 and T5 showed significant performance improvements, with STI increasing by 13.6% and 25%, respectively, compared to T1, accompanied by matching increases in phenotypic characteristics. On the other hand, T1 showed the lowest performance with MPI and GMP decreasing by 60 and 72.7% against the non-stressed control (C), highlighting high susceptibility to stress. Treatments T2 (*W. coagulans* extract) and T6 (CuO-Ag bimetallic nanoparticles) resulted in moderate mitigation of stress: T2 led to an increase of 28.9% in GMP and T6 showed an improvement of 25.0% in STI compared to the control T1, together with the increase of spike length (10.20 cm) and plant height (23.8 cm). In Borlaug-16 genotype, T8 again proved to be performing the highest treatment with GMP (83.3%), STI (70.8%) and YSI (186.7%) compared to T1 with MPI increasing by 32.0%. Phenotypic characters were also enhanced to the same extent such as spike length (12.4 cm), plant height (25.5 cm) and seeds per spike (24.7). Treatments T4 (Ag-NPs) and T6 (CuO-Ag NPs) also showed interesting results; T4 showed the highest STI (29.7%) and an MPI of 27.3% compared to T1. Moderate improvements in T2 and T3 (*W. coagulans* extract and *W. coagulans* + Ni), with GMP value of 55.6% and 41.7% relative to T1, indicated their potential in the relief of Ni stress. In opposite, T1 showed a significant decrease in the productivity and tolerance indices where the GMP percentage was decreased by 41.7% and YSI decreased by 50% compared to correlation analysis ([Figure 1](#)) emphasize the relationships among chlorophyll content, leaf

morphometric and yield components parameters under Ni stress and in this context T8 is highlighted as the most effective treatment in terms of improving stress tolerance and phenotypic performance in both cultivars.

3.3 Nickel uptake, translocation, and accumulation analysis

Ni uptake and translocation were significantly modulated by the application of nanoparticles and soil amendments in both wheat cultivars (Figures 2a, b). In the SKD-1 (V1) genotype, nanoparticle treatments effectively restricted Ni accumulation in the aerial parts compared to the Ni-only stress treatment (T1). Specifically, treatments T2, T4, T5, and T6 did not induce significant increases in grain Ni, maintaining concentrations as low as 0.2 mg/kg. While treatments T3, T7, and T9 showed higher Ni retention in the roots (increasing Ni_Root by 196.4% in T9), this

served as a sequestration mechanism that limited the further translocation of toxic Ni to the grains. In the Borlaug-16 (V2) genotype, a similar trend was observed. Nanoparticle applications facilitated the stabilization of Ni in the root zone. Although T9 showed a 20.6% increase in root Ni, the overall translocation factor (TF) to the grain remained significantly lower than the control stress group. Analysis of bioconcentration factors (BCF) and bioaccumulation factors (BAF) confirmed that treatments T2, T4, and T6 were the most effective at minimizing Ni mobility. These results demonstrate that green-synthesized nanoparticles enhance wheat resilience by sequestering Nickel in the roots and soil, thereby preventing its accumulation in the grain and ensuring food safety.

3.4 Phytochemical analysis of leaves

3.4.1 TPC, TFC, TAnC, TTC and AsA

The phytochemical profiles included total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAnC), total tannin content (TTC) and ascorbic acid (AsA) were significantly different among experimental treatments applied to wheat cultivars SKD-1 and Borlaug-16 (Figure 3). In the SKD-1 cultivar, TPC and TFC showed high levels of both treatment-dependent variation. Treatment T5 showed the highest TPC (51.4 mg GAE/g DW) and the highest TFC (416. mg QE/g DW) indicating an up regulation of the production of phenolic and flavonoid biosynthetic pathways under this condition. In addition, treatments T6 and T8 also produced high levels of TPC (43.8 and 40.6 mg GAE/g DW, respectively) and TFC (448.8 and 348.7 mg QE/g DW, respectively). Contrary, treatment T1 was found to have the lowest concentrations of TPC (14 mg GAE/g DW) and TFC (219.5 mg QE/g DW) suggesting the suppressive effect of stress caused by Ni on the accumulation of secondary metabolites. For Borlaug - 16 cultivar, the TPC and TFC values were highest (48.8 and 390.3 mg GAE/g DW and 390.3 and 410.8 mg QE/g DW, respectively) in treatment T5, followed by T6, which revealed 42 and 412.8 mg GAE/g DW and 390.3 and 410.8 mg QE/g DW, respectively. The lowest TPC (15.3 mg GAE/g DW) and TFC (221.8 mg QE/g DW) values were also seen in conditions of T1 values, which again showed the detrimental effect of Ni stress on phytochemical biosynthesis.

TAnC in wheat leaves was significantly influenced by the various treatments, showing a wide range of accumulation across both varieties. In SKD-1 (V1), the control group exhibited a baseline TAnC of 1.1 mg cyanidian-3-glucoside/100g FW, which decreased to 0.5 under nickel stress (T1). The highest TAnC for this variety was recorded under T2 (4.2 mg cyanidian-3-glucoside/100g FW) and T5 (3.0 mg cyanidian-3-glucoside/100g FW), suggesting that these specific amendments significantly boost anthocyanin biosynthesis as a protective response. In Borlaug-16 (V2), the control plants showed a mean TAnC of 1.5 mg cyanidian-3-glucoside/100g FW, which also suffered a sharp decline under stress in T1 (0.5 mg cyanidian-3-glucoside/100g FW). Similar to V1, the maximum accumulation was observed in the T2 (3.1 mg cyanidian-3-glucoside/100g FW) and T6 (2.9 mg cyanidian-3-

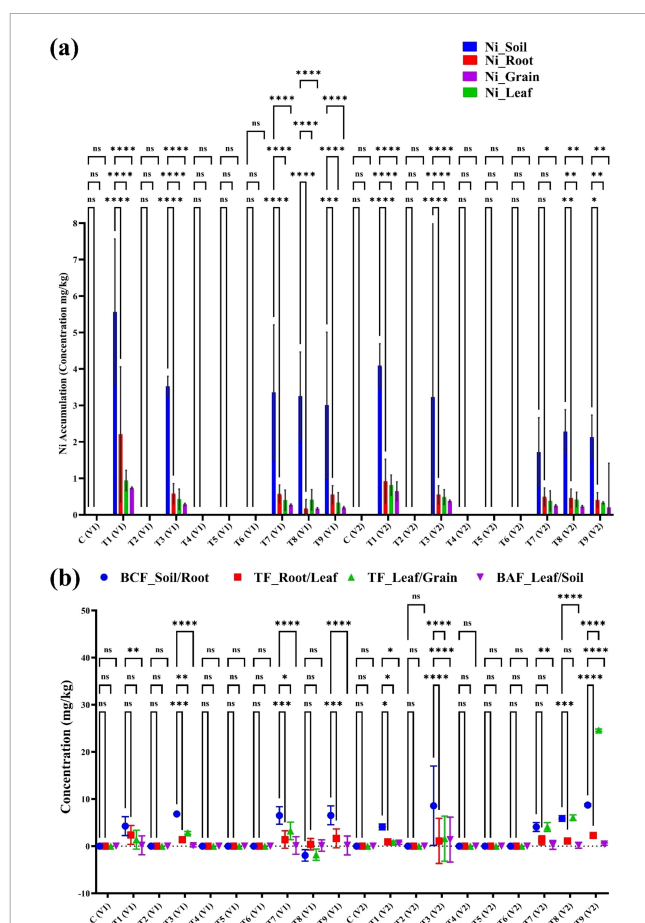


FIGURE 2
Nickel Accumulation and Translocation in SKD-1 (V1) and Borlaug-16 Cultivars (V2). **(a)** Ni accumulation (mg/kg) in soil, root, leaf, and grain tissues for both cultivars across treatments. Data were analyzed using a two-way factorial ANOVA, with statistical significance for the interaction (Cultivar × Treatment) and main effects indicated by asterisks: ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. **(b)** Translocation factors (BCF_Soil/Root, TF_Root/Leaf, TF_Leaf/Grain, and BAF_Leaf/Soil) for both cultivars, showing significant differences in Ni uptake and translocation between treatments.

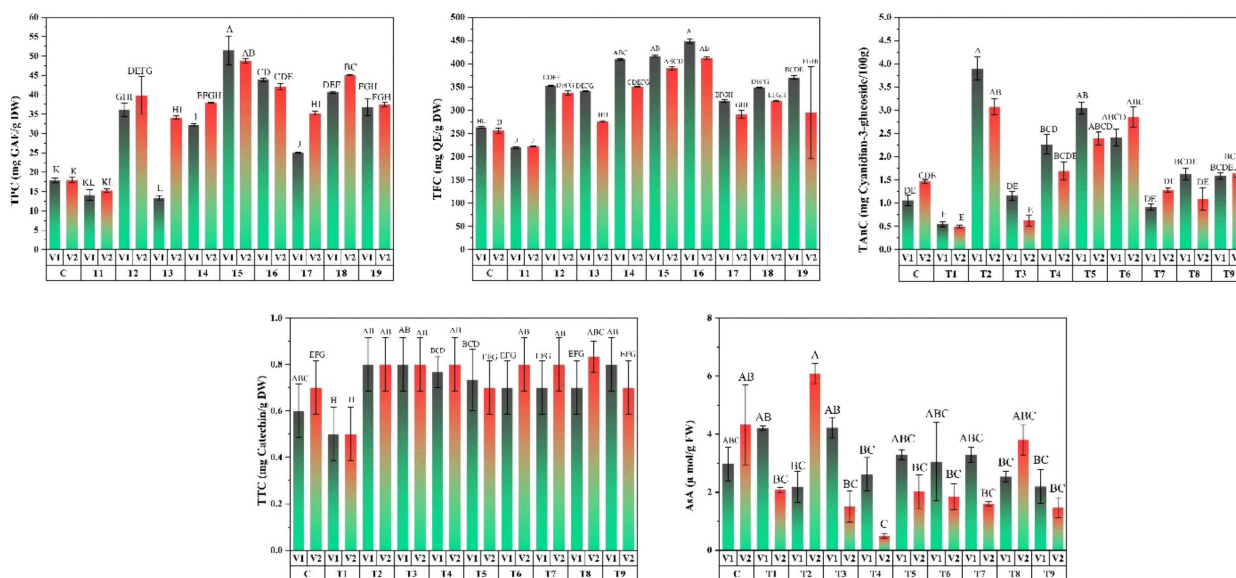


FIGURE 3

Phytochemical contents of leaves across different treatments in two wheat cultivars (V1: SKD-1, black circles; and V2: Borlaug-16, pink squares) under nickel stress. Bars represent mean $\pm$ SE (n=3). Data were analyzed using a two-way factorial ANOVA to assess the effects of cultivar (V), treatment (T), and their interaction (V $\times$ T). Different letters above the bars indicate statistically significant differences between treatments within each cultivar as determined by Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

glucoside/100g FW) treatments. Interestingly, treatment T3 resulted in the lowest accumulation for V2 (0.6 mg cyanidin-3-glucoside/100g FW), indicating a variety-specific response to that treatment. Overall, the results demonstrate that T2 and T6 treatments are the most effective at enhancing anthocyanin levels, with values maintained within a biologically plausible range of 0.5 to 4.2 mg cyanidin-3-glucoside/100g FW.

TTC revealed significant variations across different treatments in both wheat varieties. In SKD-1 (V1), the control group maintained a baseline TTC of 0.6 mg catechin/g DW. The application of nickel stress (T1) slightly reduced these levels to 0.5 mg catechin/g DW. However, the integration of nanoparticles and water-retaining agents showed a restorative effect; specifically, treatments T2, T3, T4, and T9 all recorded the highest accumulation in this variety, reaching a mean of 0.8 mg catechin/g DW. In Borlaug-16 (V2), the accumulation of tannins was slightly more pronounced. While the control and T1 groups averaged 0.7 mg catechin/g DW and 0.5 mg catechin/g DW respectively, the highest TTC was recorded under T3 and T6 treatments, both reaching 0.8 mg catechin/g DW. Treatment T8 showed an inconsistent response with a mean of 0.6 mg catechin/g DW. Overall, these results indicate that bimetallic nanoparticles and specific soil amendments effectively modulate the secondary metabolite profile of wheat under stress, maintaining tannin levels within a physiologically sound range of 0.4 to 0.8 mg catechin/g DW. Ascorbic acid content was at the highest when T2 was applied (6 μ mol/g FW) followed by T8 with 3.80 μ mol/g FW. The lowest value of TAnC and TTC was recorded under T1, indicating suppression of the processes of metabolism caused by stress; on the other hand, T4 showed the lowest result of AsA content (0.50 μ mol/g FW).

3.5 Antioxidant potential of leaves

3.5.1 TAC, FRAP, ABTS, and DPPH

The results of the antioxidant capacity, as assessed by total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), ABTS and DPPH, showed a significant variation in both wheat cultivars, SKD-1 and Borlaug-16 depending on the treatment (Figure 4). In cultivar SKD-1, the highest antioxidant potential was under T5 treatment that led to maximum TAC (786.94 mg AAE/g DW), FRAP (58 μ mol Fe^{2+} /g DW), and DPPH scavenging inhibition (52%), indicating a strong improvement under this treatment of redox homeostasis. In parallel, treatment T8 showed antioxidant responses to have increased, as TAC (720.3 mg AAE/g DW), FRAP (56.7 μ mol Fe^{2+} /g DW), and ABTS activity (35.8%) emphasizing its effectiveness in the attenuation of Ni-induced oxidative stress. On the contrary, treatment T1 was always observed to have the lowest values of the antioxidant parameters, with TAC (647.1 mg AAE/g), FRAP (2.4 μ mol Fe^{2+} /g DW), ABTS (9.7%), and DPPH (4.1%) which can be regarded as evidence of the negative effects of nickel stress on the antioxidant defense mechanism.

In cultivar Borlaug-16, the pattern of the antioxidant profile was similar and different from the profile found in SKD-1. Treatment T6 had the highest TAC (826.3 mg AAE/g DW), FRAP (314 μ mol Fe^{2+} /g DW), indicating a better ferric reduced capacity and T5 also played an important role in this capacity with the TAC (738.80 mg AAE/g DW), FRAP (304 μ mol Fe^{2+} /g DW). DPPH radical scavenging activity was maximum under treatment T2 (43.4%) and also treatment T8 (40.8%), and ABTS activity was maximum under treatment T9 (32.1%), thus the different treatments have

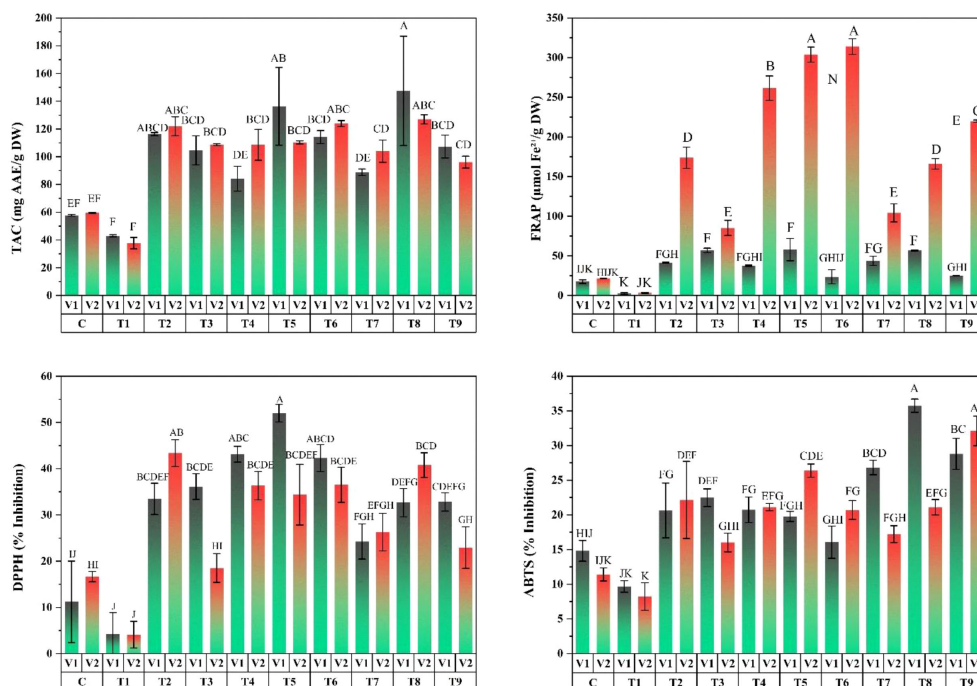


FIGURE 4

Antioxidant potential of leaf measured across different treatments in two wheat cultivars (V1: SKD-1 and V2: Borlaug-16) under nickel stress. Bars represent mean $\pm$ SE (n=3). Data were analyzed using a two-way factorial ANOVA to assess the effects of cultivar (V), treatment (T), and their interaction (V $\times$ T). Different letters above the bars indicate statistically significant differences between treatments within each cultivar as determined by Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

shown differential activation of antioxidant pathways. In agreement with what was observed in SKD-1, the antioxidant activity of treatment T1 was found to be the worst with TAC (621.2 mg AAE/g DW), FRAP (3.3 μ mol Fe²⁺/g DW), and ABTS (8.2%) and DPPH (4%) and thus indicates the severe oxidative load from nickel stress in the absence of mitigation by nanoparticles.

3.6 Enzymatic antioxidant activity of leaves

The results in Figure 5 indicated that the activities of the key enzymatic antioxidant systems such as peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) varied significantly between treatments in both the wheat cultivars, SKD-1 and Borlaug-16, indicating the existence of treatment-specific modulation of the enzymatic antioxidant defense system under nickel stress. In SKD-1 POD activity was maximum at T8 (10.5), followed by a close second at T9 (10.3) suggesting an increased ability to detoxify hydrogen peroxide under these treatments. Comparatively low POD activity was recorded in T6 (5.3) with basal enzyme activity being charged for the control treatment (3.2). In Borlaug-16 maximum POD activity was recorded in T6 (11.8) and T5 (11.3) while T9 had the lowest POD (5.3) indicating variety dependent differences in enzymatic responsiveness.

SOD activity showed strong variation on treatments in cultivars. In SKD-1, the maximum value for the peptone and the highest value obtained for the SOD was obtained with T2 (37), indicating a good response against superoxide generation under this chemical treatment, although the values of T3 (18.7) and T8 (16.1) against the superoxide anion are also high. Contrarily the lowest SOD activity

these SKD-1 was T6 (0.9) implies that there is limited activation of this enzyme under that treatment. In Borlaug-16, the maximum and minimum values SOD activity were recorded in treated 10 and 7 days old plants T9 (24.4) and T8 (24.4) and T6 (5.4) recorded lowest activity, which was found to be constant in every cultivar.

CAT activity additionally showed treatment and cultivar specific variation. In SKD-1, measurements of the highest CAT activity were found on T7 (0.8), and followed by T8 (0.6), T6 having the lowest amount of activity (0.1). In Borlaug-16, CAT activity was highest in T3 position (olive - 0.77), and there was a significant enhancement of T5 (0.8) and T7 (0.6) and lowest CAT activity was showed in T9 (0.08). Collectively, these results highlight notable and differential enzymatic responses between treatments, with specific nanoparticle-based treatments and even more so T2, T6 and T8, producing positive effects in the modulation of antioxidative defense mechanism for both wheat cultivars under conditions of stress generated by the presence of nickel.

3.7 Oxidative stress indicators (MDA and H₂O₂)

The lowest malondialdehyde (MDA) levels, indicating reduced lipid peroxidation, were observed in T8 for SKD-1 (4.5 μ mol/gFW) and Borlaug-16 (7.1 μ mol/g FW) as shown in Figure 6. Hydrogen peroxide (H₂O₂) levels were also reduced in T6 for SKD-1 (29.3 μ mol/g FW) and Borlaug-16 (29.2 μ mol/g FW). Conversely, T1 showed the highest oxidative stress markers, with elevated MDA and H₂O₂ levels across both cultivars.

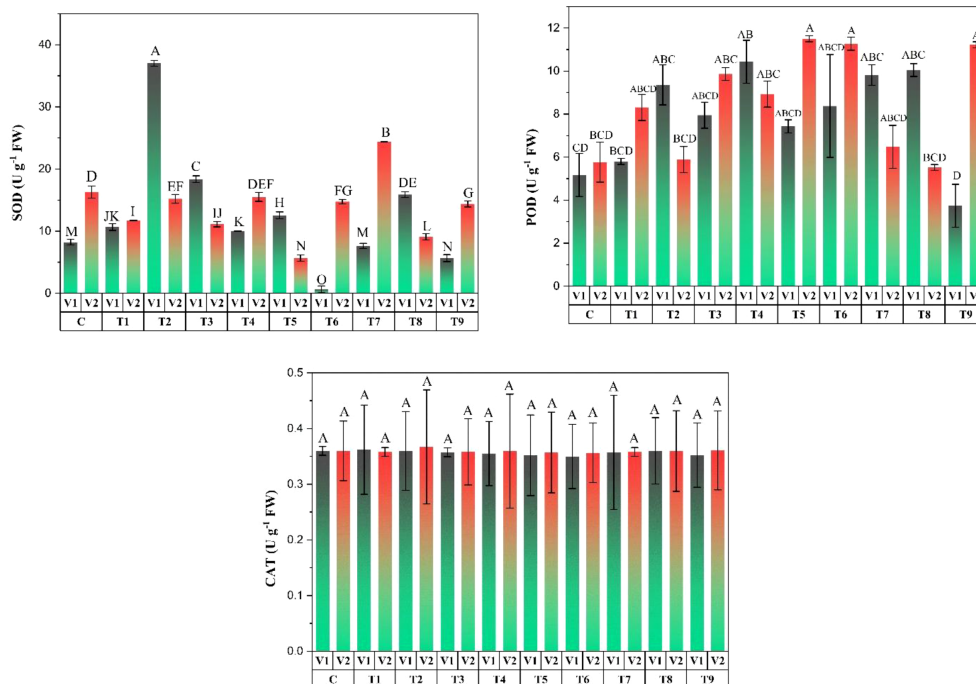


FIGURE 5

Enzymatic antioxidant activities across two wheat cultivars (V1: SKD-1 and V2: Borlaug-16) under nickel stress. Parameters include Superoxide Dismutase (SOD, U/g FW), Peroxidase (POD, U/g FW), and Catalase (CAT, U/g FW). Bars represent mean $\pm$ SE (n=3). Data were analyzed using a two-way factorial ANOVA to assess the effects of cultivar (V), treatment (T), and their interaction (V $\times$ T). Different letters above the bars indicate statistically significant differences between treatments within each cultivar as determined by Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

3.8 Photosynthetic pigments (Carotenoids, Chl a, Chl b, and total chlorophyll)

In SKD-1, T9 significantly improved carotenoid content (203.8 μ g/g FW), chlorophyll a (Chl a: 25 μ g/g FW), chlorophyll b (Chl b: 11.9 μ g/g FW), and total chlorophyll (36.9 μ g/g FW), enhanced photosynthetic efficiency as shown in Figure 7. T8 also showed strong improvements in these parameters. T2 recorded the lowest pigment levels, highlighting the detrimental effects of stress without nanoparticle mitigation. For Borlaug-16, T5 and T8 significantly enhanced carotenoid (125.5 and 91.8 μ g/g FW) and chlorophyll

contents (Total Chl: 21.07 and 20.4 μ g/g FW, respectively) as shown in Figure 7.8. T6 showed reduced pigment accumulation, with the lowest carotenoid (47.9 μ g/g FW) and total chlorophyll (10.6 μ g/g FW) levels.

3.9 Correlation analysis of phytochemical, antioxidant, and physiological parameters

Supplementary Figure 1 shows correlation analysis of phytochemical, antioxidant, and physiological parameters. Pearson correlation analysis of relationships between phytochemical,

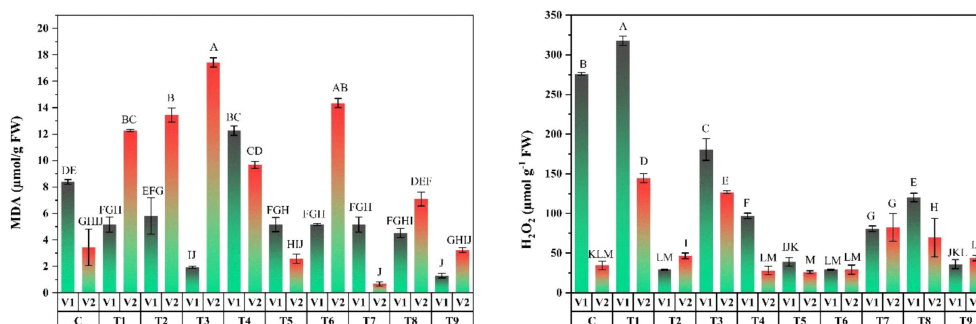


FIGURE 6

Oxidative stress markers across two wheat cultivars (V1: SKD-1 and V2: Borlaug-16) under nickel stress. Markers include Malondialdehyde (MDA, μ mol/g FW) and Hydrogen Peroxide (H₂O₂, μ mol/g FW). Bars represent mean $\pm$ SE (n=3). Statistical analysis was conducted using a two-way factorial ANOVA, specifically evaluating the effects of cultivar (V), treatment (T), and the cultivar $\times$ treatment interaction (V $\times$ T). Different letters above the bars indicate statistically significant differences between treatments within each cultivar, as determined by Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

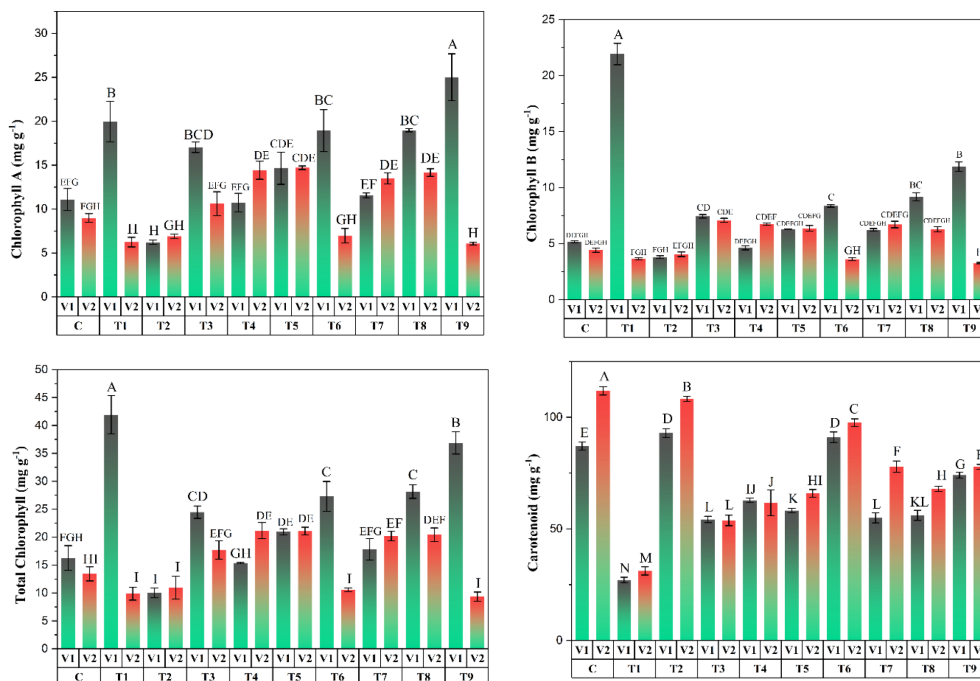


FIGURE 7

Photosynthetic pigments across two wheat cultivars (V1: SKD-1 and V2: Borlaug-16) under nickel stress. Pigments include Chlorophyll a (mg/g FW), Chlorophyll b (mg/g FW), Total Chlorophyll (mg/g FW), and Carotenoids (mg/g FW). Bars represent mean $\pm$ SE (n=3). Data were evaluated using a two-way factorial ANOVA to determine the influence of cultivar (V), treatment (T), and the interaction (V $\times$ T). Different letters above the bars indicate statistically significant differences between treatments within each cultivar according to Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

antioxidant, and physiological parameters, including TPC, TFC, TANc, TTC, TAC, FRAP, DPPH, ABTS, AsA, SOD, POD, CAT, MDA, H₂O₂, carotenoids, and chlorophyll components (Chl a, Chl b, Total C) was shown in [Supplementary Table 2](#). Strong positive correlations were observed between TPC, TFC, TANc, TTC, TAC, FRAP, DPPH, and ABTS. Chlorophyll components (Chl a, Chl b, and Total C) showed significant positive correlations with carotenoids and moderate negative correlations with MDA and H₂O₂. Antioxidant enzymes (SOD, POD, CAT) exhibited moderate correlations with AsA and negative correlations with oxidative stress markers (MDA, H₂O₂).

3.10 Harvested seeds nutritional profiling

The heatmap [Supplementary Figure 2](#) and [Supplementary Table 3](#) depict the nutritional composition of harvested seeds, showcasing the allocation of essential biochemical components. These include reducing sugars (RS), starch, non-reducing sugars (NRS), total sugars (TS), total phenolic content (TPC), proteins, total flavonoid content (TFC), and total tannin content (TTC).

3.10.1 Phytochemical contents analysis

For SKD-1, T6 recorded the highest total phenolic content (TPC: 37.1 mg GAE/g DW), total flavonoid content (TFC: 373.3 mg QE/g DW), and total tannin content (TTC: 506 mg catechin/g DW), reflecting its superior impact on phytochemical synthesis under Ni stress. Similarly, T5 showed strong results for TPC (25.3 mg GAE/g DW) and TFC (323.9 mg QE/g DW). In contrast, T1

exhibited the lowest phytochemical content, with TPC (13.2 mg GAE/g DW), TFC (163.1 mg QE/g DW), and TTC (398.5 mg catechin/g). For Borlaug-16, T6 achieved the highest TPC (27.5 mg GAE/g DW), TFC (310.8 mg QE/g DW), and TTC (485.1 mg catechin/g DW). T5 also showed elevated levels for these parameters. The lowest values were recorded in T1, with TPC (16.1 mg GAE/g DW), TFC (170.2 mg QE/g DW), and TTC (409.7 mg catechin/g DW).

3.10.2 Carbohydrates content (NRS, RS, and TS)

In SKD-1, T2 showed the highest non-reducing sugars (NRS: 3.48%) and total sugars (TS: 3.72%), while T6 exhibited balanced NRS (3.3%) and TS (3.6%). T1 had the lowest values across all carbohydrate parameters, including NRS (1.9%) and TS (2.08%). In Borlaug-16, T6 resulted in the highest TS (4.4%) and NRS (3.5%), indicating enhanced carbohydrate accumulation. T1 demonstrated the lowest RS (0.7%) and TS (3.2%), reflecting the negative impact of stress.

3.10.3 Starch and protein contents

For SKD-1, T6 recorded the highest starch content (2.8%) and protein content (16.8%), followed closely by T5 with starch (2.2%) and protein (16.6%). T1 showed the lowest starch (1.9%) and protein levels (11.5%). In Borlaug-16, T6 again excelled, achieving the highest starch (4.3%) and protein content (19.3%). T5 and T9 also showed strong results. T1 displayed reduced starch (3.5%) and protein (16.6%) levels due to stress.

3.11 Principal component analysis

A principal component analysis (PCA) biplot explained 72.5% of the total variability presented in the dataset with the first principal component (PC1) that explained 58.2% and the second principal component (PC2) which explained 14.3% of total variance (Supplementary Figure 3). PC 1 was positively associated with important productivity-related and physiological parameters, such as chlorophyll levels at all growth stages (Chl till, Chl boot, Chl head, and Chl anth) plant height, spike length and seed number per spike, which makes this PC broadly influence the agronomic traits. On the other hand, developmental timing parameters (days to germination, DTG, tillering, DTT, booting, DTB, heading, DTH and anthesis, DTA) showed negative relationships with PC 1, indicating that delayed phenological development was negatively related to productivity under stress of nickel.

The separation between the two wheat cultivars was evident in the score plot along the principal components. SKD-1 was more like characteristics of early developmental progression and stress tolerance, which is typical adaptive response to stress by Ni. In contrast Borlaug - 16 placed towards constants related to increased photosynthetic efficiency and yield formation including plant height and spike length indicating its superior agronomic performance under the applied treatments. The clear separations of cultivars in the PCA space support their differential physiological and agronomic performance, which is reflected in the greater stress mitigation ability of SKD-1 and the dominance of Borlaug-16 in the presence of stress imposed by nickel in traits associated with yield.

4 Discussion

This study reveals the potential of Ag-NPs, CuO-NPs, and CuO-Ag bimetallic NPs in overcoming nickel-induced stress in wheat cultivars both SKD-1 and Borlaug-16 by the coordinated enhancement of growth, photosynthetic efficiency, agronomical performance, and stress resistance. Growth dynamics and photosynthetic traits are important indicators of the adaptive capacity of plants to abiotic stress. The observed decreases in days to booting, heading and anthesis reveal improvements of metabolic activity and a better adaptation to stresses while improvements in morphometric traits of the leaves (e.g., length, width and area of flag leaves) have a direct impact on the photosynthesis surface area and water use efficiency under nickel stress. The high improvement in growth and leaf morphology after nanoparticle treatment in both cultivars indicates their high potential for reducing Ni toxicity. These findings are consistent with previous reports that show that nanoparticles improve photosynthetic pigments and growth characteristics in the presence of heavy metal stress and are therefore effective stress-alleviating agent (Adejumo et al., 2023; Lopes et al., 2023). Such improvements are probably attributed to the ability of CuO-NPs and CuO-Ag bimetallic NPs to enhance antioxidant enzyme activity, inhibit oxidative damage, and maintain chlorophyll integrity and thus play a complementary role in promoting leaf development and plant growth.

Agronomic traits like plant height, spike length and number of seeds per spike are good indicators of the yield potential. The significant improvement of these traits under T6 and T5 treatments suggests the efficiency of CuO-Ag bimetallic NPs and CuO-NPs in increasing the wheat productivity under Ni stress. These reported observations are consistent with previous studies involving the *in-vitro* synthesis of green nanoparticles and their use to improve nutrient uptake capacity, activation of antioxidant activity, and counter heavy metal stress and hence improved agronomic performance with reduced metal uptake levels in the grains (Alhaithloul et al., 2023). Similar trends have been reported in the literature by Alghanem et al. (2022) who demonstrated the effects of nanoparticles on enhancing the germination, seedling vigor, respiration, flowering, and photosynthesis processes and the effective regulation of the responses of heavy metal stresses. The yield improvements in current research can be explained by NPs-mediated correction of nickel-induced physiological imbalances, better assimilation of nutrients and elevated stress tolerance. On the other hand, the significant reduction in the agronomic traits under T1 in the presence of inhibitory effects of Ni toxicity on growth and yield formation. These results are in agreement with Anas et al. (2024) who reported that MnO₂NPs improved wheat agronomical and phenotypical traits as a result of limitations of heavy metals uptake, optimization of ions distribution and improving stress resilience under cadmium stress.

Stress-tolerance indices are another way to measure the productivity response under stress conditions, e.g. GMP, STI, MPI, and YSI constitute integrative measures of productivity. The high values of these indices recorded under T8 and T6 for both cultivars emphasize the high efficacy of both cultivars in reducing Ni induced stress. These results are supported by previous studies which showed that copper-based nanoparticles improve water and nutrient use efficiency, thus improving plant growth and productivity under heavy metal stress (Feigl, 2023). Furthermore, Cu-based nanoparticles have been known to lessen heavy metal accumulation in roots, shoots and the grain as well as protect the cereal crops against metal toxicity (Wang et al., 2019; Fu et al., 2022). The better performance obtained with these treatments is probably related to the capacity of stabilizing the cell metabolism, to strengthening the antioxidative defense and to preserving the photosynthetic efficiency. On the contrary, the invariably lower stress indices under T1 provide evidence of disruptive effects of Ni stress on cellular homeostasis, growth, and yield.

Ni uptake, translocation and accumulation patterns also provide further insight into stress mitigations induced by NP treatments. Elevated BCF in roots usually characterize limited metal mobility and TF describe the efficacy of metal translocations to aerial tissues. In SKD-1, T1 showed high root accumulation of Ni with limited translocation to leaves and grains which emphasized harsh effects of unmitigated stress of Ni. In contrast, other treatments (T8 and T9) led to a significant decrease of root Ni content and promoted controlled translocation from root to leaves, indicating successful detoxification strategies. These results are in line with reports that show nanoparticles decrease heavy metals bioavailability by chelation, immobilization and intracellular compartmentalization (Ahmad et al., 2024). Similar

mechanism has been observed for CuO-NPs which gets absorbed by roots and affects the metal distribution within plants (Shukla et al., 2016). In Borlaug-16, the T9 had better mitigation capacity with high BCF_Soil/Root and TF_Leaf/Grain values, indicating an efficiency in root sequestration of Ni with little translocation to grain. T8 also resulted in effective little grain Ni accumulation. Comparable results have been achieved in case of Ag-Cu bimetallic NPs, which increase nutrient transport, manage ion homeostasis, and minimize metal toxicity by increasing antioxidant defense (Al-Habeeb and Al-Bishri, 2024). Collectively, these results suggested that CuO-Ag bimetallic NPs and CuO NPs are effective in controlling the uptake of Ni and its distribution internally and minimizing toxicity and maintaining physiological function.

The improvement of antioxidant and phytochemical properties confirms the role of nanoparticles against nickel stress. Total TPC and TFC are novel indicators for secondary metabolite mediated antioxidative defense. The high value of TPC and TFC under T5 and T6 of both cultivars suggest high stimulation of biosynthesis of phenolics and flavonoids in accordance with the previous results in heavy metals stress (Abdel-Wahab et al., 2019). Similar improvements have been reported after foliar application of silver and copper nanoparticles produced by green syntheses, which has improved the antioxidant capacity and quality of the yield in *Brassica napus* L (Rameen et al., 2024). The latter improvements are probably caused by NP induced modulation of oxidative stress, enhanced enzyme activity, and an increased micronutrient availability. In contrast, the lower level of TPC and TFC under T1 reflect the inhibitory effect of nitric oxide caused by oxidative damage by Ni.

Photosynthetic pigments and antioxidant activities of enzymes are another way to verify the efficacy of the treatment with NPs. Treatments like T8 and T9 significantly increased chlorophyll, carotenoids, and enzymatic antioxidants in both cultivars that would indicate increased photosynthetic efficiency and an increased tolerance to oxidative stress. These results are consistent with those obtained in other studies that have shown that nanoparticles can increase the activities of the antioxidant enzymes and can reduce ROS-caused cell damage (Sachdev and Ahmad, 2021). Increased activities of SOD and POD under these treatments are in agreement with the finding by (Huang et al., 2022), while decreased levels of MDA and H₂O₂ indicate minimized lipid peroxidation and accumulation of ROS. The good performance of T5 and T6 can be explained by the role of these in the stabilization of chlorophyll structure, improvement of antioxidant enzymes synthesis and the redox homeostasis. Similar responses were obtained by Bibi et al. (2025) who demonstrated that green synthesized NiO-NPs enhanced the growth and the photosynthetic efficiency of wheat by enhancing antioxidative defense.

Seed biochemical analysis shows further nutritional benefits of nanoparticle treatments under the effect of nickel stress. Significant increase in TPC, TFC and TTC under T6 and T5 suggest for increased accumulation of secondary metabolites that are essential for antioxidant protection and grain quality. These results are in

line with previous research proving that nanoparticles boost secondary metabolism by reducing oxidative stress and enhancing metabolic activity (Mahmoud et al., 2021; Alhaithloul et al., 2023). On the other hand, the decrease in the phytochemicals under T1 reflects disruption of the metabolism of the plant by Ni.

Carbohydrate and starch content as well as the protein content further confirms the stress-mitigating nature of nanoparticles. T6 was able to constantly increase the non-reducing sugars, total sugars, starch and protein content, which is a demonstration of a better carbon and nitrogen metabolism as well as better nutritional quality. Similar results have been found under heavy metals stress conditions, where nanoparticles enhanced metabolic efficiency and the nutritional assimilation of nutrients (Noman et al., 2020; Hosny et al., 2022). The effectiveness of T6 and T5 is probably attributed to their capacity to stabilize metabolic enzymes, increase nutrient uptake and decrease oxidative damage. In contrast, decreased biochemical reserves during T1 indicate impaired metabolism during Ni toxicity. These results are similar to those from Lahuta et al. (2023), and Iqbal et al. (2024) who identified significant modulation of soluble carbohydrate profiles in wheat by biosynthesized AgNPs and improved carbohydrate metabolism, protein synthesis and mineral regulation by biosynthesized AgNPs in abiotic stressed wheat. Collectively, these results were conclusive on the potential of nanoparticle-based strategies to improve wheat productivity, nutritional quality and stress tolerance under condition of Ni stress.

Regarding environmental sustainability and the SDGs, the application of biogenic nanoparticles via seed priming and foliar sprays, rather than bulk soil amendment, minimizes the risk of Ag/Cu accumulation and soil persistence. The phytochemical capping derived from *W. coagulans* enhances the biocompatibility of these particles, potentially mitigating adverse effects on the soil microbiome compared to chemically synthesized counterparts. Recent innovations in green nanotechnology, such as MgO nanoparticles from *Solanum elaeagnifolium* for pest control and clove oil nanoemulsions for pathogen management, underscore the move toward safer, low-toxicity agricultural inputs (Abbas, 2024; Gorge and Abood, 2025). Similar to how copper oxide nanoparticles from *Melia azedarach* successfully combat cadmium stress in wheat (Naz et al., 2025), our bimetallic system provides a sophisticated defense against Ni toxicity (El-Mogy et al., 2025). By reducing grain Ni accumulation to safe levels (0.20 mg/kg), this approach effectively addresses food chain implications and aligns with SDG 12 (Responsible Consumption and Production), offering a sustainable alternative to conventional metal remediation strategies (Nisar et al., 2024; Ahmad et al., 2025; Anas et al., 2026).

5 Conclusion

This study highlights the efficacy of green-synthesized CuO and CuO-Ag bimetallic nanoparticles, mediated by *W. coagulans*, in mitigating Ni toxicity in wheat cultivars SKD-1 and Borlaug-16.

Nanoparticle application significantly improved plant growth and reduced Ni translocation, effectively lowering grain Ni accumulation to safe levels (0.20 mg/kg). These benefits are linked to enhanced antioxidant defense mechanisms and a higher accumulation of bioactive secondary metabolites, which maintain cellular homeostasis. Furthermore, the treatments improved the nutritional profile of harvested grains, supporting food security. The superior performance of the bimetallic system underscores the potential of synergistic nanomaterials in regulating metal homeostasis and strengthening plant resilience. By utilizing low-concentration priming and foliar strategies, this green-nanotechnology approach minimizes soil metallic loading, aligning with SDG 12 (Responsible Consumption and Production) and SDG 15 (Life on Land). While this study establishes a robust physiological and biochemical “proof of concept,” future research will prioritize molecular-level investigations, including the expression analysis of metal transporter gene families (such as IRT1, NRAMP, and HMA) and high-resolution imaging to track nanoparticle uptake and localization. Validating these nanoparticle-based approaches under field conditions, alongside their long-term impacts on the soil microbiome, will further support their adoption in sustainable, climate-resilient agronomic practices.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding authors.

Author contributions

AK: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft. MA: Methodology, Software, Visualization, Writing – review & editing. DH: Data curation, Formal analysis, Funding acquisition, Validation, Writing – review & editing. TQ: Data curation, Formal analysis, Software, Writing – review & editing. RK: Data curation, Formal analysis, Resources, Writing – review & editing. BA: Formal analysis, Resources, Writing – review & editing. AS: Data curation, Formal analysis, Resources, Validation, Writing – review & editing. MM: Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2026.1798195/full#supplementary-material>

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