

RESEARCH

Open Access



Green-synthesized cobalt oxide nanoparticles for seed nanopriming enhance chickpea growth and suppress fusarium wilt

Sadaf Anwaar^{1*}, Ayesha Arif¹, Muneeba Naseem¹, Aimen Rafaqat¹, Sheza Farooq¹, Tauseef Anwar^{2*}, Huma Qureshi³, Hossam S. El-Beltagi^{4*}, Ibtisam M. Alsudays⁵, Khalid H. Alamer⁶, Shavkat Durxadjayev⁷, Nazih Y. Rebouh⁸, Árpád Székely^{9*}, Rasha M. Alzayed¹⁰, Sondas A. Alhajouj¹⁰ and Meaad F. Alaida¹⁰

Abstract

Background Chickpea (*Cicer arietinum* L.) productivity is severely constrained by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris*, and eco-friendly seed treatments are needed. Cobalt oxide nanoparticles (CoO-NPs) were green-synthesized using *Ficus carica* leaf extract and characterized by UV–Vis, FTIR, TEM, EDS, DLS, zeta potential, XRD, and XPS analyses. Seeds were nanoprimed for 24 h with CoO-NP suspensions (0.2, 0.5, 0.7, 1.0 mg/L) or a distilled water control, grown for 25 days, and challenged with *F. oxysporum*. In vitro antifungal activity was assessed by disc diffusion. Data (n = 3 biological replicates) were analyzed by one-way ANOVA with Tukey's HSD ($p < 0.05$).

Results UV–Vis peaks at 199, 216, and 229 nm and FTIR Co–O bands at 500–700 cm^{-1} confirmed CoO-NP formation. TEM, EDS, DLS, zeta potential, XRD, and XPS further confirmed nanoscale morphology, elemental composition, colloidal behaviour, crystallinity, and surface chemical states of the synthesized CoO-NPs. Compared with the control, nanopriming at 0.5 mg/L increased shoot length and root length, while 0.7 mg/L produced the highest growth response. Disease severity decreased from 63% in infected control to 32.5% at 0.5 mg/L and 27.5% at 0.7 mg/L. Disc diffusion showed the largest inhibition zones at 0.7 mg/L (18.5 ± 0.5 mm) and 0.5 mg/L (17.0 ± 0.5 mm).

Conclusions Fig-mediated CoO-NP seed priming at 0.5–0.7 mg/L simultaneously enhanced chickpea growth and suppressed Fusarium wilt under controlled conditions. The absence of a bulk cobalt control remains an important limitation; therefore, future studies should include equivalent ionic/bulk cobalt treatments to distinguish nano-specific effects from cobalt ion effects. Field validation, dose–response refinement, nodulation assessment, and mechanistic assays, including ROS, antioxidant enzymes, and defense genes, are recommended. Biosafety work should assess nanoparticle persistence and effects on beneficial rhizosphere microbes over multiple seasons.

*Correspondence:

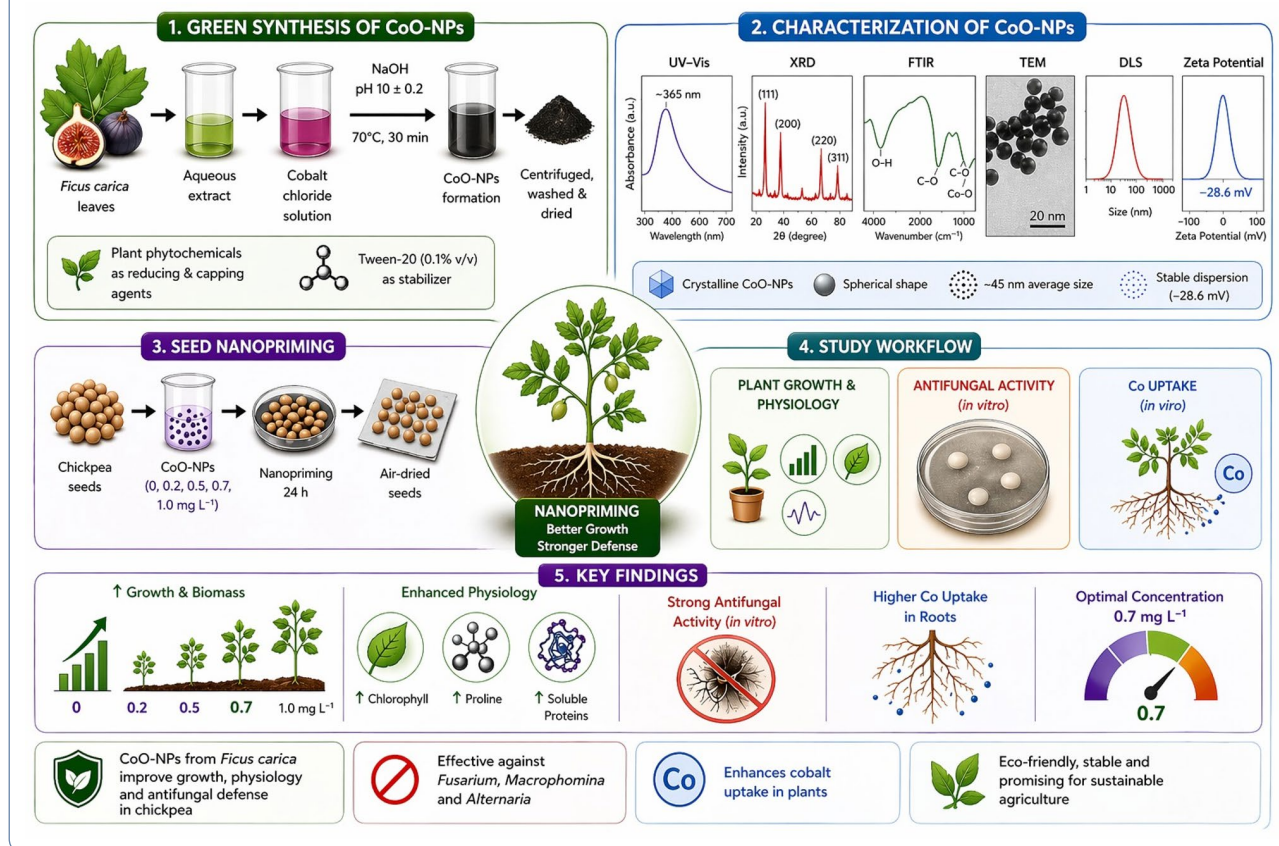
Sadaf Anwaar
sadaf.anwaar@iiu.edu.pk
Tauseef Anwar
tauseef.anwar@iub.edu.pk
Hossam S. El-Beltagi
helbeltagi@kfu.edu.sa
Árpád Székely
szekely.arpad@uni-mate.hu

Full list of author information is available at the end of the article

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Keywords Antifungal activity, Disease severity index, Green synthesis, Seed nanopriming, UV–Vis spectroscopy

Graphical Abstract



Introduction

Chickpea (*Cicer arietinum* L.) is the second most widely consumed grain legume globally and is cultivated in more than 50 countries, particularly in semi-arid and temperate regions [1]. It contributes approximately 15.4% of global pulse production, with major producers including India, Pakistan, and Iran [2, 3]. Beyond its nutritional value—providing ~23% protein and 64% carbohydrates—chickpea enhances soil fertility through biological nitrogen fixation, contributing up to 60 kg ha⁻¹ under optimal conditions [4]. However, chickpea productivity is severely limited by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) [5]. The pathogen invades the vascular system, disrupts water transport, and causes systemic wilting and plant death [6]. In Pakistan, yield losses range from 10–50% annually and may reach 100% under severe conditions [3]. Management is difficult due to the long-term survival of chlamydospores in soil and pathogen dissemination through irrigation and farm equipment [7], highlighting the need for sustainable disease-control strategies.

Nanotechnology offers innovative tools for sustainable agriculture [8]. Nanoparticles, generally ranging from 1–100 nm, possess unique physicochemical properties that enable enhanced biological interactions [9]. Nanopriming has been shown to improve germination, growth, and stress tolerance by enhancing water uptake, enzyme activation, and ROS-mediated signaling [10]. The 24 h nanopriming duration used in the present study was selected based on previous nanopriming literature, where similar priming durations have been reported to improve seed hydration and early seedling vigor without prolonged exposure stress [10].

Among metal nanoparticles, cobalt oxide nanoparticles (CoO-NPs) were selected over more commonly used metal oxides such as ZnO or MgO because cobalt has a legume-relevant micronutrient role linked to nodulation and nitrogen metabolism, while also exhibiting dose-dependent antimicrobial activity [11, 12]. However, a major limitation in many cobalt nanoparticle studies is the absence of a bulk cobalt control, which makes it difficult to distinguish true nano-specific responses from effects caused by released cobalt ions. This limitation

was recognized in the present study and is explicitly addressed in the methods, discussion, and future directions. CoO-NPs exhibit antimicrobial activity that has been associated with ROS-mediated membrane disruption [13]. Their effects are dose-dependent, with beneficial responses at low concentrations and potential toxicity at higher levels [14].

Green synthesis using plant extracts enhances nanoparticle stability and biocompatibility [15]. Phytochemicals such as polyphenols and flavonoids act as reducing and capping agents [16]. Leaves of *Ficus carica* contain bioactive compounds with antioxidant and antimicrobial properties. Compared with some other plants used for cobalt nanoparticle synthesis, such as *Ficus religiosa* and *Uraria picta*, *Ficus carica* has been reported to contain abundant phenolic and flavonoid constituents, which may contribute to improved capping stability and potential antifungal synergy. In addition to non-enzymatic phytochemicals such as phenolics and flavonoids, plant-derived reductase-associated biomolecules may also participate in the reduction of Co²⁺ ions during green nanoparticle synthesis, although their relative contribution depends on extract composition and reaction conditions.

Although plant-mediated cobalt nanoparticle synthesis has been reported [17, 18], their integrated application as nanopriming agents in chickpea under *Fusarium* wilt stress remains limited, particularly under Pakistani agro-climatic conditions. Few studies have evaluated fig-mediated CoO-NPs as seed nanopriming agents for simultaneous growth enhancement and *Fusarium* wilt resistance in chickpea. Integrated assessments combining nanoparticle characterization, plant growth response, and disease suppression remain underexplored. In particular, previous studies have rarely compared CoO-NP nanopriming with bulk cobalt controls or evaluated nodulation responses in chickpea under *Fusarium* stress.

This study synthesizes CoO-NPs using *Ficus carica* leaf extract and evaluates their dual role in promoting chickpea growth and mitigating *Fusarium* wilt. It integrates physicochemical characterization with in vitro antifungal assays and in vivo disease assessment under controlled conditions. The findings contribute to understanding dose-dependent cobalt nanoparticle effects in legumes and offer a potential eco-friendly alternative to chemical

fungicides, supporting sustainable chickpea production in disease-prone regions.

This study aimed to: (i) synthesize and characterize CoO-NPs using *Ficus carica* leaf extract; (ii) evaluate the effects of CoO-NP nanopriming on chickpea germination and growth; (iii) assess the antifungal activity of CoO-NPs against *Fusarium oxysporum* f. sp. *ciceris*; and (iv) determine their potential to reduce disease severity under *Fusarium* wilt stress.

Materials and methods

Study site, experimental design, and experimental duration

The study was conducted between March and August 2024 at the Department of Biological Sciences, International Islamic University, Islamabad, Pakistan (33.6844°N, 73.0479°E; altitude 540 m above sea level). Laboratory experiments were carried out under controlled environmental conditions at 25 ± 2 °C with relative humidity maintained at 60–70%. Greenhouse experiments were conducted under natural photoperiod (12–14 h light), with day/night temperature ranging from 22 to 30 °C and relative humidity of 55–75%. Light intensity during daytime averaged 450–600 μmol m⁻² s⁻¹.

The experiment followed a completely randomized design (CRD). Five treatments were evaluated: distilled water control and four nanoparticle concentrations (0.2, 0.5, 0.7, and 1.0 mg/L). A sixth treatment consisting of bulk cobalt or ionic cobalt control was not included in the present experiment. This is acknowledged as a limitation because such a treatment would help distinguish nanoparticle-specific effects from cobalt ion effects and is therefore recommended for future work. Each treatment consisted of three independent biological replicates (Table 1). Each replicate comprised three pots, and each pot contained five plants, resulting in 45 plants per treatment. The sample size was determined based on previous nanopriming studies using comparable replication designs and was considered adequate for controlled greenhouse screening [10]. Only healthy, uniform, undamaged seeds were included. Certified seeds of *Cicer arietinum* L. cv. ‘Punjab-2008’ were used. Seeds showing discoloration, mechanical damage, or abnormal size were excluded.

Green synthesis of cobalt oxide nanoparticles

Fresh, pesticide-free leaves of *Ficus carica* L. were collected from the Botanical Garden of the International Islamic University, Islamabad, for the green synthesis of cobalt oxide nanoparticles. The study was carried out from March to August 2024 at the Department of Biological Sciences, International Islamic University, Islamabad, Pakistan (33.6844°N, 73.0479°E; 540 m altitude). *Ficus carica* is commonly cultivated in Pakistan and is not regarded as a wild species. The plant was identified

Table 1 Experimental design

Treatment	CoO-NP concentration (mg/L)	Replicates (n)	Plants per treatment
T1	0 (Control)	3	45
T2	0.2	3	45
T3	0.5	3	45
T4	0.7	3	45
T5	1.0	3	45

by Dr. Sadaf Anwaar, and voucher specimen No. IIUI-45892 was deposited in the departmental herbarium for public access.

Leaves were washed thoroughly under running tap water for 5 min, followed by rinsing three times with distilled water to remove dust and microbial contaminants. Surface sterilization was not performed to preserve phytochemical integrity. Twenty-five grams of fresh leaves were finely chopped and transferred into a 500 mL Erlenmeyer flask containing 400 mL of distilled water. The mixture was heated at 70 °C for 30 min with continuous stirring at 600 rpm using a digital magnetic stirrer (Daihan Scientific MSH-20D, Korea). Heating at 70 °C for 30 min was used to extract water-soluble phytochemicals, including polyphenols and reducing sugars, while retaining their reducing and capping potential. Plant-derived reductase-associated biomolecules present in the extract may also contribute to Co^{2+} reduction during synthesis. The filtrate was stored at 4 °C and used within 24 h to prevent phytochemical degradation.

Cobalt nitrate hexahydrate [$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; $\geq 99\%$ purity, Sigma-Aldrich, USA] was used as precursor. A 0.1 M cobalt nitrate solution was prepared by dissolving 11.68 g in 400 mL of distilled water. The cobalt solution was added dropwise at approximately 1 mL/min into 200 mL of leaf extract under constant stirring. The pH of the reaction mixture was adjusted to 10.0 ± 0.2 using freshly prepared NaOH solution (3 g in 50 mL distilled water) while monitoring with a calibrated digital pH meter (Hanna Instruments HI2211). NaOH was added dropwise and only in the minimum volume required to reach $\text{pH } 10.0 \pm 0.2$, thereby limiting excess Na^+ introduction. After synthesis, the pellet was washed three times with distilled water and once with ethanol to remove residual Na^+ ions and other soluble impurities.

Tween-20 (0.1% v/v; Merck, Germany) was added to prevent nanoparticle aggregation. Although phytochemicals from *Ficus carica* acted as primary reducing and capping agents, Tween-20 was added only as an additional stabilizer to improve dispersion uniformity and reduce aggregation during synthesis. The mixture was stirred at 70 °C for 90 min. Formation of cobalt oxide nanoparticles was indicated by a visible color change from greenish to moss-brown. The reaction was allowed to cool to room temperature before purification. The suspension was centrifuged at 5000 rpm, equivalent to $4300 \times g$, for 10 min using a refrigerated centrifuge (Hettich Universal 320R). The pellet was washed three times with distilled water and once with absolute ethanol to remove residual ions and organic impurities. The washed pellet was air-dried at room temperature for 24 h, ground into fine powder, and calcined in a muffle furnace (Nabertherm L9/11) at 300 °C for 2 h with a heating rate of 5 °C/min to obtain crystalline CoO-NPs. Quality control included

performing synthesis in triplicate batches and confirming reproducibility of UV–Vis peak positions.

Characterization of synthesized nanoparticles

UV–Visible spectroscopy was performed using a Shimadzu UV-1800 spectrophotometer in the wavelength range of 200–1000 nm at 1 nm resolution. Distilled water was used as blank. Spectral scans were performed in quartz cuvettes with 1 cm path length. FTIR analysis was carried out using a PerkinElmer Spectrum Two FTIR spectrometer in the range of 4000–500 cm^{-1} . Samples were prepared using KBr pellet method with a 1:100 ratio of sample to KBr. Each spectrum was averaged over 32 scans at 4 cm^{-1} resolution.

Transmission electron microscopy (TEM) was performed using a high-resolution transmission electron microscope (JEOL JEM-2100) operated at an accelerating voltage of 200 kV. Samples were prepared by dispersing nanoparticles in ethanol, followed by ultrasonication, drop-casting onto carbon-coated copper grids, and drying under ambient conditions before imaging.

Energy-dispersive X-ray spectroscopy (EDS) was carried out using an EDS detector coupled with electron microscopy. Spectra were acquired under high-vacuum conditions, and elemental composition was determined from characteristic X-ray peaks of cobalt and oxygen.

Dynamic light scattering (DLS) was performed using a particle size analyzer (Malvern Zetasizer Nano series) at a scattering angle of 90° and a temperature of 25 °C. Samples were dispersed in distilled water and sonicated before analysis to ensure uniform suspension.

Zeta potential analysis was performed using the same instrument equipped with electrophoretic light scattering capability. Samples were diluted in deionized water and analyzed at 25 °C, and zeta potential values were calculated from electrophoretic mobility using the Smoluchowski equation.

X-ray diffraction (XRD) analysis was conducted using an X-ray diffractometer (PANalytical X'Pert PRO) with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) over a 2θ range of 10–80°, at a scanning rate of 0.02° per step.

X-ray photoelectron spectroscopy (XPS) analysis was carried out using an XPS system equipped with an Al K α X-ray source ($h\nu = 1486.6 \text{ eV}$). Spectra were recorded under ultra-high-vacuum conditions, and binding energies were calibrated using the C 1 s peak at 284.8 eV.

Preparation of working suspensions

The dried nanoparticle powder was dispersed in sterile distilled water to prepare a 100 mg/L stock suspension. The suspension was sonicated using a Branson Ultrasonics Sonifier (40 kHz) for 20 min to ensure uniform dispersion. Working concentrations of 0.2, 0.5, 0.7, and 1.0 mg/L were prepared by serial dilution. All

concentrations are expressed in mg/L, which is equivalent to ppm in aqueous systems.

Seed nanoprimering and pot experiment

Certified seeds of *Cicer arietinum* L. cv. 'Punjab-2008' were obtained from the Seed Bank, Department of Biological Sciences, International Islamic University, Islamabad, Pakistan. Seeds were surface sterilized using 1% sodium hypochlorite for 2 min, followed by five rinses with sterile distilled water. Control seeds were hydro-primed in sterile distilled water for 24 h under identical conditions without nanoparticles. Seeds were soaked in respective nanoparticle suspensions for 24 h at room temperature under gentle shaking (100 rpm). The seed-to-solution ratio was maintained at 1:5 (w/v). After priming, seeds were air-dried under laminar airflow to near-original moisture content for uniform sowing. Only primed seeds were used for planting. No additional nanoparticle solutions were applied during irrigation. All plants received only distilled water throughout the growth period to ensure that the treatment effect originated exclusively from seed nanoprimering.

Pots (15 cm diameter) were filled with sterilized growth medium consisting of 60% cocopeat and 40% garden soil. Soil was autoclaved at 121 °C for 20 min to eliminate background microbial interference. Five seeds were sown per pot at a depth of 2 cm. Plants were irrigated every two days with 100 mL of distilled water. Growth parameters, including shoot length, root length, and number of leaves, were recorded after 25 days.

Fusarium inoculation and disease assessment

The fungal pathogen *Fusarium oxysporum* f. sp. *ciceris* was obtained from a verified plant pathology culture collection. The fungus was cultured on Potato Dextrose Agar (PDA; Oxoid, UK) at 25 °C for 7 days. Conidial suspension was prepared by flooding plates with sterile distilled water and gently scraping the surface. The suspension was filtered through sterile muslin cloth to remove mycelial debris. Spore concentration was adjusted to 1×10^6 conidia/mL using a hemocytometer. At 25 days after sowing, each pot received 20 mL of spore suspension as soil drench around the root zone. No foliar spray was used to maintain biological relevance, as *Fusarium* is a soil-borne vascular pathogen.

Disease symptoms were scored using a 0–5 scale. Disease Severity Index (DSI) was calculated using the following formula [19]:

$$DSI (\%) = [\Sigma (\text{Diseaserating} \times \text{Number of infected plants}) / (\text{Maximum rating} \times \text{Total number of plants})] \times 100.$$

In vitro antifungal assay

Antifungal activity was evaluated using disc diffusion assay on Potato Dextrose Agar. Sterile 6 mm filter paper discs were impregnated with 20 µL of nanoparticle suspension at respective concentrations. Discs were placed on PDA plates inoculated with a 5 mm fungal plug. Negative control discs contained distilled water, while positive control discs contained 0.1% carbendazim. Plates were incubated at 25 °C for 72 h. Zone of inhibition (mm) was measured using digital Vernier calipers [20, 21].

Statistical analysis

All data were analyzed using IBM SPSS Statistics Version 26.0 (IBM Corp., USA) and are presented as mean $\pm$ standard deviation (SD). Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Treatment effects were evaluated using one-way ANOVA followed by Tukey's HSD post hoc test where applicable. Statistical significance was set at $p < 0.05$.

Results

Physicochemical characterization of green-synthesized CoO-NPs

The synthesis of cobalt oxide nanoparticles (CoO-NPs) using *Ficus carica* leaf extract was successfully achieved, as visually evidenced by a gradual color transition of the reaction mixture from pale green to moss-brown within 90 min at pH 10.0 (Fig. 1). This color change indicated the reduction of cobalt ions and subsequent nanoparticle formation.

The UV–Visible absorption spectrum of the synthesized CoO-NPs displayed three distinct and sharp peaks at 199 nm, 216 nm, and 229 nm (Fig. 2A; Table 2). These peaks correspond to $O^{2-} \rightarrow Co^{3+}$ charge transfer transitions and ligand-to-metal charge transfer (LMCT) interactions, confirming nanoparticle formation. The absence of broad visible-range absorption suggested minimal particle aggregation and uniform nanoparticle dispersion. Peak sharpness further indicated good crystallinity and homogeneity of the synthesized CoO-NPs. FTIR spectra (Fig. 2B; Table 3) revealed a broad absorption band at 3400–3500 cm^{-1} corresponding to O–H stretching vibrations of hydroxyl groups and adsorbed water molecules. The band observed at 1600–1650 cm^{-1} was attributed to O–H bending vibrations. Peaks within 1000–1300 cm^{-1} represented C–O and C–N stretching vibrations from residual phytochemicals derived from *Ficus carica*. A characteristic absorption band between 500–700 cm^{-1} confirmed the presence of Co–O bonds, verifying the successful formation of cobalt oxide nanoparticles.

TEM-derived particle size distribution (Fig. 2C) showed a heterogeneous nanoscale population with a dominant fraction centered at approximately 5–10 nm, a

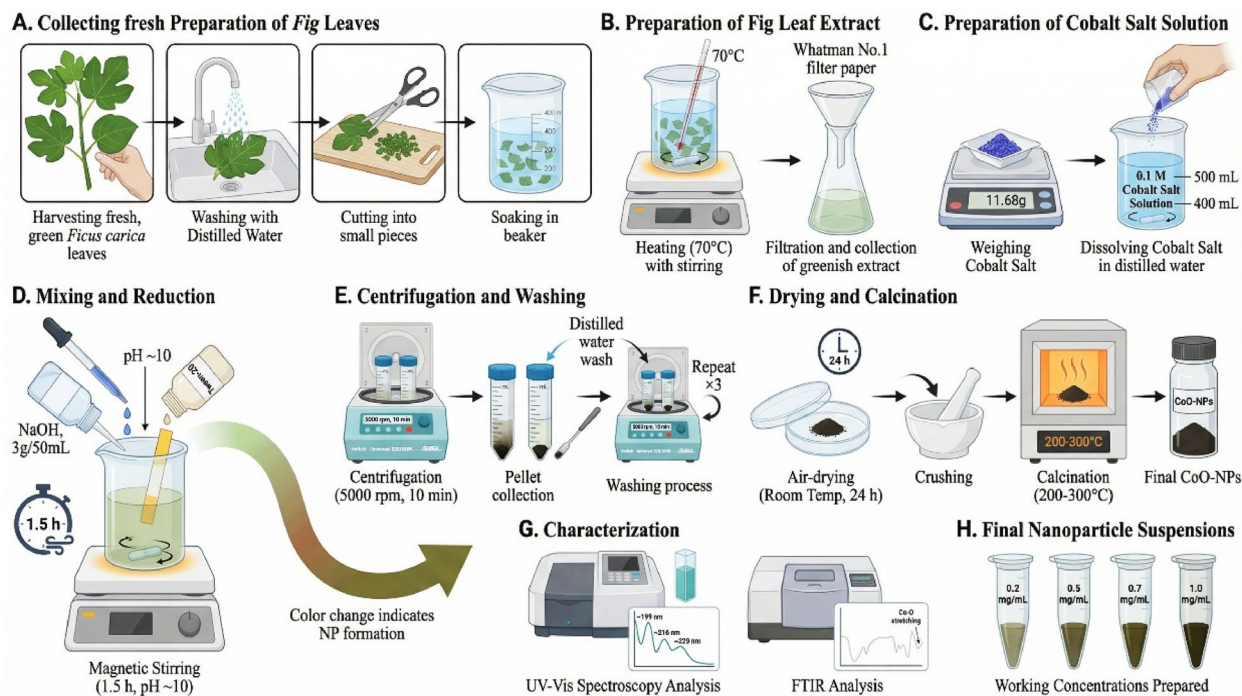


Fig. 1 Schematic workflow for green synthesis of CoO-NPs using *Ficus carica* leaf extract, showing preparation, reduction, purification, and characterization steps

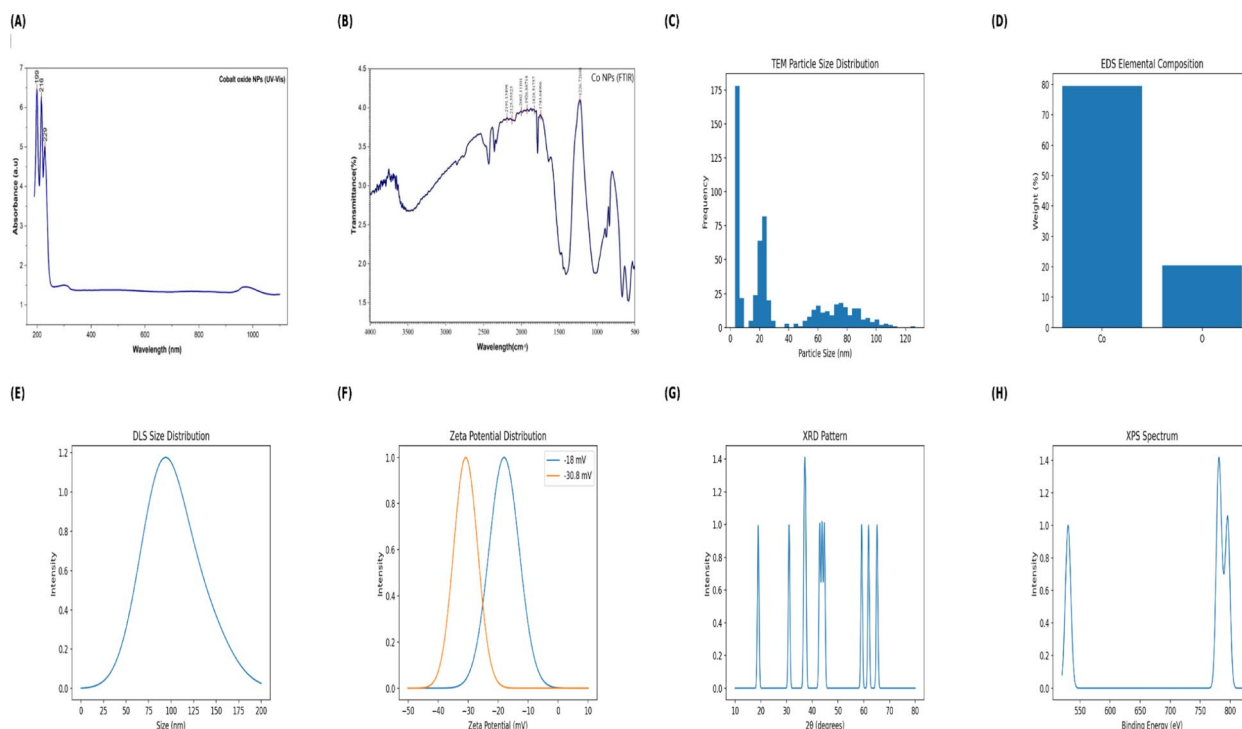


Fig. 2 Characterization of cobalt oxide nanoparticles: **A** UV-Vis **B** FTIR **C** TEM particle size distribution, **D** EDS elemental composition, **E** DLS hydrodynamic size distribution, **F** zeta potential distribution, **G** XRD diffraction pattern, and **H** XPS spectrum

Table 2 UV–Vis absorption characteristics of cobalt oxide nanoparticles

Wavelength (nm)	Electronic transition	References
~ 199	O ²⁻ → Co ³⁺ charge transfer	[36]
~ 216	Ligand-to-metal charge transfer	[17]
~ 229	Interband electronic transition	[18]

Table 3 FTIR absorption bands and functional group assignments of green-synthesized CoO-NPs

Wave-number (cm ⁻¹)	Functional group/vibration	Source/assignment basis	References
3400–3500	O–H stretching	Surface hydroxyl groups and/or adsorbed water; broad band typical of hydrogen-bonded –OH	[34, 35]
1600–1650	O–H bending (H–O–H deformation)	Adsorbed water / moisture deformation band commonly observed near ~ 1640 cm ⁻¹	[35, 36] (adsorbed water at ~ 1640 cm ⁻¹)
1000–1300	C–O and C–N stretching	Plant-extract biomolecules (polyphenols/ flavonoids/ proteins) acting as reducing/ capping agents; residual phytochemical “corona” signatures	[35, 37]
500–700	Co–O stretching	Metal–oxygen vibration confirming cobalt oxide framework	[38] cobalt oxide FTIR Co–O bands ~ 569–662 cm ⁻¹ (example)

secondary population around 20–25 nm, and a broader distribution extending from approximately 60 to 100 nm, with maximum particle sizes approaching approximately 125 nm. EDS analysis (Fig. 2D) indicated cobalt and oxygen as the primary elements, with approximate weight percentages of 79.6% Co and 20.4% O, supporting cobalt oxide formation.

The DLS profile (Fig. 2E) showed a hydrodynamic diameter centered at approximately 95–100 nm, with a distribution range spanning approximately 40–180 nm, indicating aggregation effects in aqueous suspension. Zeta potential analysis (Fig. 2F) showed negatively charged particles with peaks at –18 mV and –30.8 mV, suggesting moderate to good colloidal stability.

The XRD pattern (Fig. 2G) displayed characteristic diffraction peaks at 2θ values of approximately 19°, 31°,

36–38°, 43–45°, 59–62°, and 65°, with the most intense peak near 37°, confirming the crystalline nature of cobalt oxide. XPS analysis (Fig. 2H) showed an O 1s peak at approximately 530 eV, a Co 2p_{3/2} peak at approximately 780 eV, and a Co 2p_{1/2} peak near 796 eV, confirming cobalt oxide surface chemical states.

Effect of CoO-NP nanopriming on growth of *Cicer arietinum*

Growth performance of *Cicer arietinum* L. was evaluated 25 days after sowing. Representative plant morphology is shown in Fig. 3, and quantitative growth data are illustrated in Fig. 4. Control plants exhibited limited vegetative growth, with a mean shoot length of 8.5 ± 1.0 cm, a root length of 5.0 ± 1.0 cm, and 7 ± 1 leaves per plant (n = 45). Seed priming with CoO-NPs produced concentration-dependent growth enhancement (Fig. 4). The 0.5 mg/L treatment significantly improved vegetative parameters (p < 0.05), producing shoot length of 16.5 ± 1.0 cm and root length of 10.5 ± 1.0 cm, corresponding to 94% and 110% increases relative to the control, respectively. The 0.7 mg/L treatment exhibited the highest overall growth performance, with shoot length reaching 20.5 ± 1.0 cm, root length 11.5 ± 1.0 cm, and leaf number peaking at 21 ± 1 per plant, representing a threefold increase compared to the control. Although the 1.0 mg/L concentration improved growth relative to the control (14.5 ± 1.0 cm shoot length), it showed reduced performance compared to the 0.7 mg/L treatment, suggesting a concentration-dependent hormetic response. One-way ANOVA revealed a significant effect of nanoparticle concentration on shoot length, root length, and leaf number (p < 0.05).

In vitro antifungal activity against *Fusarium oxysporum*

The antifungal efficacy of CoO-NPs was evaluated using the disc diffusion assay. Representative inhibition zones are shown in Fig. 5, and quantitative data are summarized in Fig. 7B. The control group exhibited negligible inhibition (1.5 ± 0.5 mm). The 0.2 mg/L treatment produced a mean zone of inhibition (ZOI) of 8.0 ± 0.5 mm. The 0.5 mg/L concentration significantly increased antifungal activity to 17.0 ± 0.5 mm (p < 0.05). The 0.7 mg/L concentration showed the highest inhibition (18.5 ± 0.5 mm). The 1.0 mg/L treatment produced 12.5 ± 0.5 mm inhibition. Differences among treatments were statistically significant (p < 0.05).

Disease severity following *Fusarium oxysporum* inoculation

Following soil inoculation with *Fusarium oxysporum* f. sp. *ciceris*, disease progression differed significantly among treatments (Table 4; Figs. 6 and 7A). Infected control plants (no nanoparticle priming) exhibited severe symptoms characterized by chlorosis, vascular browning, wilting, and reduced growth, with a mean Disease

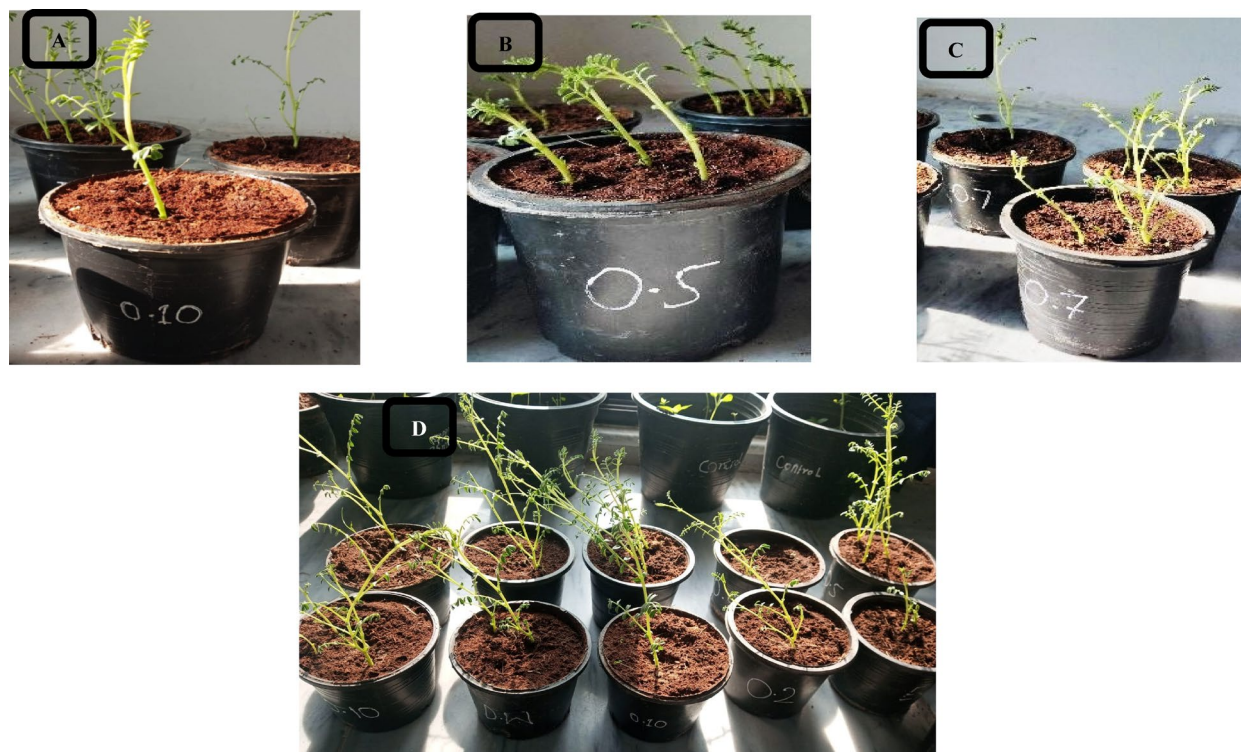


Fig. 3 Growth of *Cicer arietinum* after 25 days of CoO-NP priming: **A** 1.0 mg/L; **B** 0.5 mg/L; **C** 0.7 mg/L; **D** comparison of all treatments

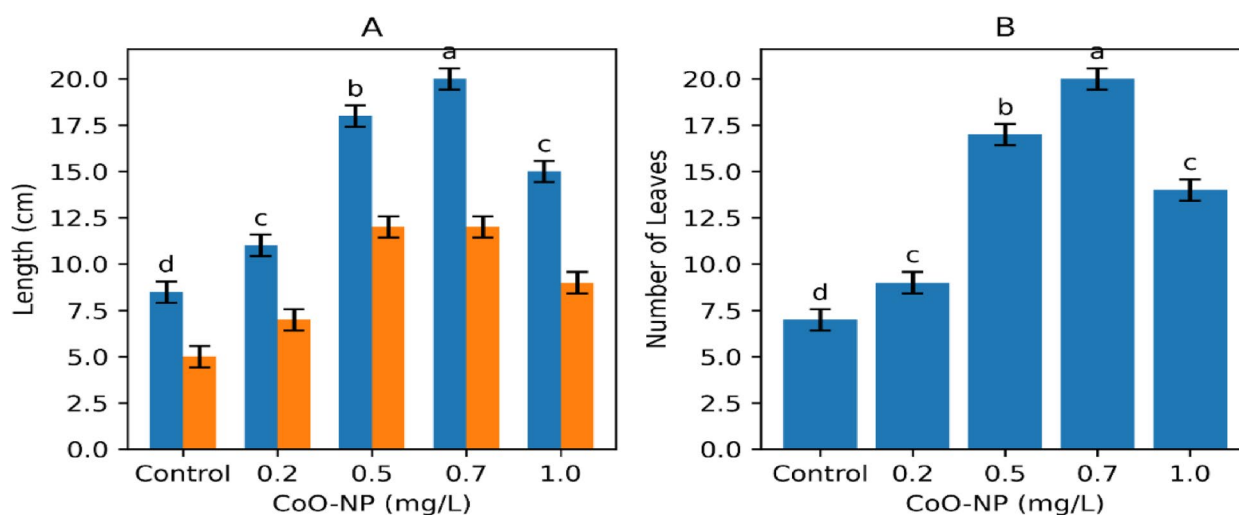


Fig. 4 **A** Effect of CoO-NP seed priming on shoot and root length of *Cicer arietinum* after 25 days. **B** Effect on leaf number across treatments (mg/L). Bars represent mean $\pm$ SD. Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD). For root length in panel A, statistics are as follows: control vs 0.5 and 0.7 mg/L, $p < 0.05$; control vs 0.2 and 1.0 mg/L, not significant

Severity Index (DSI) of $63.0 \pm 6.5\%$. Plants primed with 0.2 mg/L CoO-NPs showed partial protection, recording a DSI of $52.5 \pm 5.2\%$. The 0.5 mg/L treatment significantly reduced disease severity to $32.5 \pm 4.1\%$, representing a 48% reduction relative to control ($p < 0.05$). The 0.7 mg/L treatment demonstrated the strongest protective effect, reducing DSI to $27.5 \pm 3.8\%$, corresponding to a 56% reduction compared to infected control plants. The

1.0 mg/L treatment resulted in intermediate protection ($47.5 \pm 4.9\%$). ANOVA confirmed statistically significant differences among treatments ($p < 0.05$).

FTIR analysis of treated *Cicer arietinum* plants

FTIR spectra of treated plant tissues are shown in Fig. 8 and Table 5. When compared with untreated control plants (spectrum not shown), plants treated with

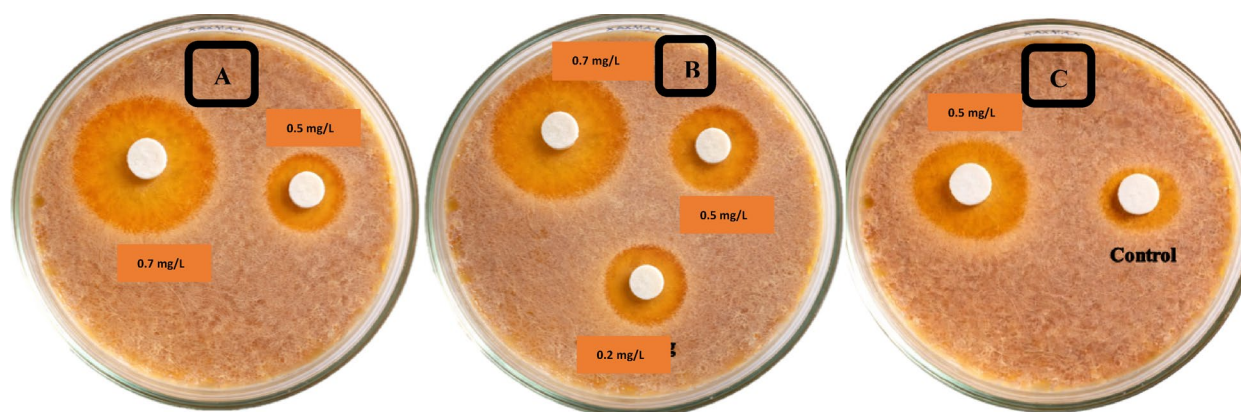


Fig. 5 In vitro antifungal activity against *Fusarium oxysporum*: **A** 0.5 vs 0.7 mg/L; **B** 0.2, 0.5, and 0.7 mg/L; **C** 0.5 mg/L vs control. Control discs contained distilled water as negative control

Table 4 Disease severity index (%) in Chickpea Plants after *Fusarium oxysporum* Inoculation

Treatment (mg/L)	DSI (%)
Control	63.0 ± 6.5
0.2	52.5 ± 5.2
0.5	32.5 ± 4.1*
0.7	27.5 ± 3.8*
1.0	47.5 ± 4.9

*Significant reduction vs control ($p < 0.05$)

0.2 mg/L CoO-NPs exhibited preserved O–H, C–O, and C–N functional groups, with no peak shifts indicative of structural damage or phytotoxicity. The 0.5 mg/L treatment demonstrated increased O–H stretching intensity compared with the control, suggesting enhanced metabolic and possibly defense-related activity. No new abnormal absorption peaks were detected. Overall, FTIR results confirmed biochemical integrity of treated plants and absence of nanoparticle-induced toxicity.

Discussion

The observed growth enhancement aligns with previous reports demonstrating beneficial effects of nanopriming on seed germination and early vegetative development

[10, 15]. Similar dose-dependent responses have been reported for cobalt-based nanoparticles in legumes, where low concentrations improved plant growth, while higher doses induced stress [14].

The growth promotion observed in this study is consistent with the established role of cobalt as an important micronutrient involved in nodulation, nitrogen fixation, and activation of nitrogenase-related processes in legumes [11, 12]. The enhanced vegetative parameters at 0.5–0.7 mg/L may therefore reflect improved micronutrient availability coupled with nanoparticle-mediated physiological stimulation. However, a limitation of this study is the absence of a bulk cobalt or ionic cobalt control treatment [22–24]. Without this comparison, we cannot definitively distinguish whether the observed effects are truly nano-specific or primarily due to cobalt ion release. Future studies should include bulk cobalt treatments, such as $\text{Co}(\text{NO}_3)_2$ at equivalent Co^{2+} concentrations, to address this gap.

Regarding antifungal activity, the inhibition zones recorded in this study (up to 18.5 mm) are comparable or superior to those reported for other green-synthesized metal nanoparticles against phytopathogens [13, 25]. Previous studies have demonstrated antimicrobial properties



Fig. 6 Response of primed plants to *Fusarium oxysporum* f. sp. *ciceris*: **A** infected control vs 1.0 mg/L; **B** 0.7 mg/L showing reduced wilting

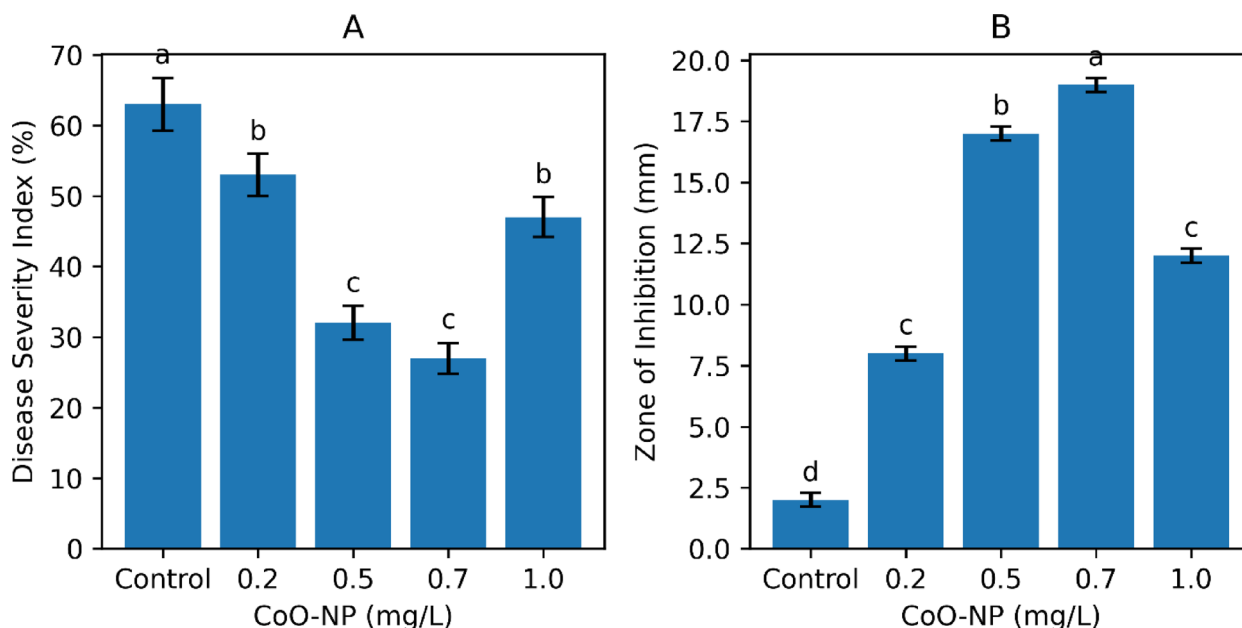


Fig. 7 **A** Disease Severity Index (%) of *Cicer arietinum* following *Fusarium oxysporum* f. sp. *ciceris* inoculation. **B** In vitro antifungal activity showing zone of inhibition (mm) across CoO-NP concentrations. Bars represent mean $\pm$ SD. Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD)

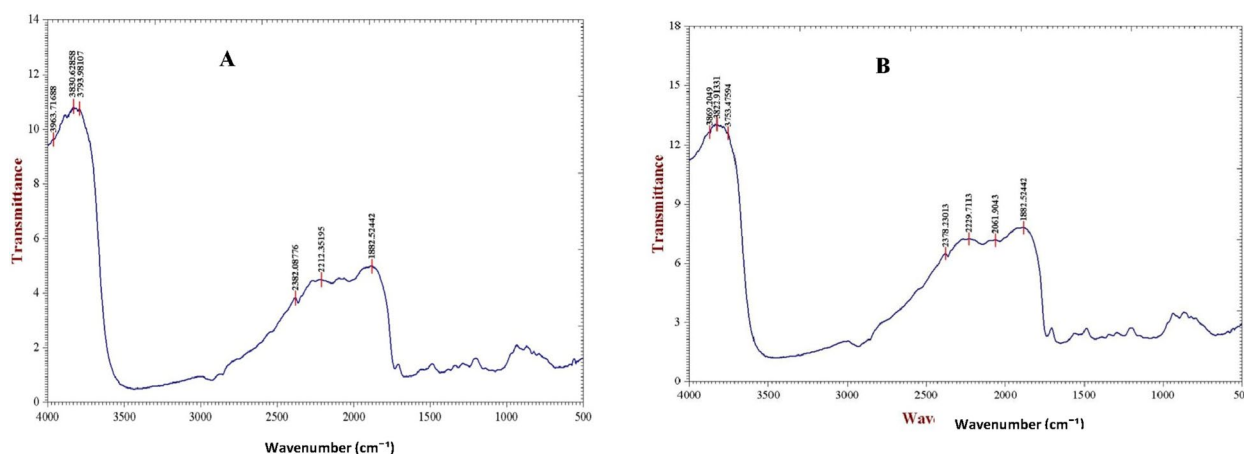


Fig. 8 FTIR spectra of treated *Cicer arietinum* leaves: **A** 0.2 mg/L and **B** 0.5 mg/L CoO-NPs. Arrow-marked bands represent observed absorption peaks. Spectra were compared with untreated control plants, spectrum not shown, which exhibited similar functional group profiles

Table 5 Comparative FTIR analysis of treated chickpea plants

Parameter	0.2 mg/L CoO-NPs	0.5 mg/L CoO-NPs
Major functional groups	O–H, C–O, C–N preserved	O–H, C–O, C–N preserved
O–H stretching intensity	Normal	Slightly higher
Nanoparticle-plant interaction	Mild	Stronger
Toxicity evidence	None	None
Biological response	Normal growth	Enhanced activity

of cobalt oxide nanoparticles, primarily attributed to oxidative stress-mediated membrane disruption [13]. Our results extend these findings by integrating both in vitro antifungal suppression and in vivo disease mitigation within the same experimental system.

The growth-promoting effects observed in this study are comparable to those reported for other legume species. For example, Kavya et al. [12] demonstrated that foliar application of cobalt at 8–12 mg/L improved chickpea yield parameters, although their study used bulk

cobalt rather than nanoparticles. Similarly, Hu et al. [11] reported that cobalt supplementation at 0.5–1.0 mg/L enhanced nodulation and nitrogenase activity in soybean. Compared to other metal oxide nanoparticles, such as ZnO and MgO, which have been primarily studied for their broad antimicrobial effects [25, 26], CoO-NPs offer the distinct advantage of combining antimicrobial activity with the micronutrient benefits of cobalt for legumes. This dual functionality makes CoO-NPs particularly suitable for chickpea nanopriming compared with ZnO/MgO systems that lack this nutritional synergy.

In a related study on canola (a non-legume), Jahani et al. [14] observed hormetic growth responses to Co_3O_4 nanoparticles, with optimal stimulation at 50 mg/L and toxicity above 200 mg/L. The lower optimal concentrations in our study (0.5–0.7 mg/L) likely reflect species-specific sensitivity and the different exposure route (seed priming vs. direct exposure).

Although plant-mediated synthesis of cobalt nanoparticles has been reported previously [17, 18], limited studies have evaluated their combined effects on both growth promotion and Fusarium wilt suppression in *Cicer arietinum*, particularly under Pakistani agro-climatic conditions. Therefore, rather than claiming absolute novelty, the present work contributes integrated, crop-specific evidence within a legume-pathogen model [27, 28].

The *Ficus carica* leaf extract used in this synthesis contains higher phenolic and flavonoid content compared to other plant extracts such as *Ficus religiosa* or *Uraria picta*. The phytochemical corona derived from *Ficus carica* may improve nanoparticle stability and biocompatibility, while also contributing potential synergistic antifungal effects. Patil [29] similarly reported that *Ficus carica*-mediated nanoparticles exhibit favorable stability and bioactivity compared with several plant-mediated nanoparticle systems.

The enhanced growth observed at 0.5–0.7 mg/L suggests a hormetic response, where low nanoparticle concentrations stimulate physiological activity, whereas higher concentrations (1.0 mg/L) reduce the magnitude of the response. Such biphasic effects are well documented in nanoparticle–plant interactions [14].

Several plausible mechanisms may explain the dual growth-promoting and antifungal effects: First, cobalt supplementation may enhance nitrogen metabolism and nodulation efficiency, indirectly improving vegetative growth [11]. However, nodulation was not assessed in this 25-day study. Future research should evaluate nodule number, nodule dry weight, nitrogenase activity, and root nodule morphology to confirm this proposed mechanism. Second, cobalt oxide nanoparticles may exert direct antifungal effects through reactive oxygen species (ROS)-mediated membrane disruption and lipid peroxidation in fungal cells, as suggested in previous antimicrobial

studies [13]. While this mechanism is biologically plausible, ROS generation and antioxidant enzyme activity, including SOD, CAT, and POD, were not directly measured in the present study [30–32]. Therefore, these pathways remain hypothetical and require experimental confirmation in future studies. Third, FTIR analysis of treated plants showed preserved functional groups and increased O–H stretching intensity at optimal concentrations, which may indicate enhanced metabolic activity and possible induction of defense-related phenolic compounds. Similar increases in phenolic metabolism have been associated with nanoparticle-mediated priming responses in crops [33]. Fourth, the phytochemical corona derived from *Ficus carica* may act synergistically with CoO-NPs to enhance both growth promotion and antifungal activity, as fig leaf compounds possess intrinsic antioxidant and antimicrobial properties [39–44].

The bioactive phytochemical corona derived from *Ficus carica* leaf extract may also contribute synergistically to antimicrobial activity, as fig leaves are known to contain flavonoids and phenolic compounds with antioxidant properties. This phytochemical coating likely improves nanoparticle biocompatibility and stability [45–50].

The significant reduction in Fusarium wilt severity suggests potential application of CoO-NP seed priming as an eco-friendly alternative to chemical fungicides. Given increasing concerns regarding fungicide resistance and environmental contamination, nanoparticle-based seed treatments may offer integrated crop protection strategies. For chickpea-producing regions such as Pakistan, where Fusarium wilt causes substantial yield losses [51–54], the identification of a safe and effective concentration range (0.5–0.7 mg/L) is particularly relevant. However, practical implementation requires validation under field conditions.

This study has several strengths, including an integrated approach combining nanoparticle synthesis, physicochemical characterization, plant growth evaluation, in vitro antifungal testing, and in vivo disease assessment within a single experimental framework. Extended nanoparticle characterization, including TEM, EDS, DLS, zeta potential, XRD, and XPS, strengthened the interpretation of nanoparticle formation, elemental composition, morphology, colloidal behavior, crystallinity, and surface chemistry.

However, several aspects require further investigation. First, while the experiment was conducted under controlled greenhouse conditions, multi-location field trials are needed to validate these findings under diverse soil types, climatic conditions, and natural pathogen pressure. Second, although the sample size was sufficient for controlled screening, larger-scale trials with increased biological replication would enhance ecological generalizability. Third, mechanistic pathways, including ROS

production, antioxidant enzyme activity, and defense gene expression, were not directly quantified. Fourth, although extended nanoparticle characterization was included in the present study, future work should perform replicated batch-wise characterization to assess synthesis reproducibility under field-scale preparation. Fifth, a bulk cobalt control was not included, making it difficult to attribute effects specifically to the nano form versus ionic cobalt release. Sixth, long-term environmental fate analysis, including nanoparticle persistence in soil, bioaccumulation potential, and effects on beneficial rhizosphere microbes over multiple seasons, was not assessed and is critical for biosafety evaluation. Seventh, nodulation parameters were not evaluated despite cobalt's known role in nitrogen fixation.

Based on the findings and limitations of this study, the following priorities are recommended: multi-location field trials to validate agronomic performance under diverse soil types, climatic conditions, and natural *Fusarium* pressure; molecular studies examining defense gene expression, PR proteins, PAL, SOD, CAT, and antioxidant enzyme activity to confirm proposed ROS-mediated mechanisms; quantitative metabolomic profiling such as HPLC–MS to identify defense-related phenolic compounds induced by CoO-NP nanopriming; replicated batch-wise nanoparticle characterization using TEM, EDS/EDX, DLS, zeta potential, XRD, and XPS to establish synthesis reproducibility and structure–activity relationships; bulk cobalt control studies to distinguish nano-specific effects from ionic cobalt effects; nodulation assessment including nodule number, nodule dry weight, and nitrogenase activity in longer-term studies; environmental fate analysis including nanoparticle persistence in soil, bioaccumulation in plant tissues, and effects on beneficial rhizosphere microbes over multiple seasons; and dose–response refinement to establish the minimum effective concentration and maximum safe concentration for field application.

Conclusion

This study demonstrates that green-synthesized cobalt oxide nanoparticles (CoO-NPs), produced using *Ficus carica* leaf extract, enhance growth and reduce *Fusarium* wilt severity in *Cicer arietinum* under controlled greenhouse conditions. Seed nanopriming with 0.5–0.7 mg/L CoO-NPs significantly improved shoot length, root development, and leaf production compared to untreated controls, confirming a concentration-dependent hormetic response. Moreover, these optimal concentrations substantially decreased the disease severity index and exhibited strong *in vitro* antifungal activity against *Fusarium oxysporum* f. sp. *ciceris*. Spectroscopic and physicochemical characterization verified successful nanoparticle synthesis, phytochemical capping,

nanoscale morphology, elemental composition, colloidal stability, crystallinity, and surface chemical states. The dual functionality observed—growth promotion and pathogen suppression—highlights the potential of CoO-NP seed priming as a sustainable alternative to conventional fungicides. Nevertheless, the absence of a bulk cobalt control, nodulation analysis, and direct ROS/antioxidant-enzyme measurements limits mechanistic interpretation. Therefore, future studies should include bulk cobalt controls, nodulation parameters, mechanistic assays, multi-location field validation, and long-term biosafety assessment before recommending large-scale agricultural application.

Acknowledgements

We thank Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (KFU262993), for supporting this research work.

Author contributions

SA, AA, MN, AR, SF: methodology, supervision, experimentation, writing, drafting, research design, investigation, data curation; TA, HQ, HSEB, IMA, KHA, SD: validation, software; writing, drafting, statistical analysis, validation; NYR, AS, RMA, SAA, MFA: writing, software, resource, research design, validation, data collection, drafting, statistical analysis. All authors reviewed the manuscript.

Funding

Open access funding provided by Hungarian University of Agriculture and Life Sciences. This work was supported by Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (KFU262993).

Data availability

The author confirms that all data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Biological Sciences, International Islamic University, Islamabad 44000, Pakistan

²Department of Botany, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

³Department of Botany, University of Chakwal, Chakwal 48800, Pakistan

⁴Agricultural Biotechnology Department, College of Agriculture and Food Sciences, King Faisal University, 31982 Al-Ahsa, Saudi Arabia

⁵Department of Biology, College of Science, Qassim University, Qassim, Saudi Arabia

⁶Biological Sciences Department, Faculty of Science and Arts, King Abdulaziz University, 21911 Rabigh, Saudi Arabia

⁷Department of Vegetable Growing and Organization of Greenhouse Farming, Tashkent State Agrarian University, St. University, 2A, Kibray District, Tashkent 100700, Tashkent Region, Uzbekistan

⁸Institute of Environmental Engineering, RUDN University, Miklukho-Maklaya St., 117198 Moscow, Russia

⁹Research Center for Irrigation and Water Management, Institute of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Anna-Liget Str. 35., H-5540 Szarvas, Hungary

¹⁰Biology Department, College of Science, Jouf University, 41412 Sakaka, Saudi Arabia

Received: 12 March 2026 / Accepted: 25 May 2026

Published online: 22 June 2026

References

- Koul B, Sharma K, Sehgal V, Yadav D, Mishra M, Bharadwaj C. Chickpea (*Cicer arietinum* L.) biology and biotechnology: from domestication to biofortification and biopharming. *Plants Basel*. 2022;11(21):2926. <https://doi.org/10.3390/plants11212926>.
- Alsamman AM, Mousa KH, Istanbuli T, El-Maksoud MMA, Tawkaz S, Hamwieh A. Unveiling the genetic basis of *Fusarium* wilt resistance in chickpea using GWAS analysis and characterization of candidate genes. *Front Genet*. 2024;14:1292009. <https://doi.org/10.3389/fgene.2023.1292009>.
- Sankar PM, Shreedevasesa S, Karthiba L, Raju PA, Vanitha S, Kamalakannan A, et al. Ecology, biology and management of *Fusarium* wilt in chickpea (*Cicer arietinum* L.): a review. *Agric Rev*. 2022. <https://doi.org/10.18805/ag.R-2481>.
- Mahto RK, Chandana BS, Singh RK, Talukdar A, Swarnalakshmi K, Suman A, et al. Uncovering potentials of an association panel subset for nitrogen fixation and sustainable chickpea productivity. *BMC Plant Biol*. 2025;25(1):693. <https://doi.org/10.1186/s12870-025-06244-z>.
- Manjunatha L, Puyam A, Prema GU, Sanjay Bandi M, Kumar R, Keerthi MC, Dixit GP, Kavitha TR. Chickpea biotic stresses. In: Kole C, editor. *Genomic designing for biotic stress resistant pulse crops*. Springer International Publishing; 2022. p. 117–159. https://doi.org/10.1007/978-3-030-91043-3_2.
- Li J, Zhao W, Fu Y, Li R, Liu J, Yang S, et al. Enhancing heterotrophic lutein production in *Chlorella protothecoides* through combined phytohormone and nitrogen strategies. *J Biotechnol*. 2025;410:125–36. <https://doi.org/10.1016/j.jbiotec.2025.12.001>.
- Roberts JM, Carvalhais LC, O'Dwyer C, Rincon-Florez VA, Drenth A. Diagnostics of *Fusarium* wilt in banana: current status and challenges. *Plant Pathol*. 2024;73(4):760–76. <https://doi.org/10.1111/ppa.13863>.
- Hussain M, Zahra N, Lang T, Zain M, Raza M, Shakoore N, et al. Integrating nanotechnology with plant microbiome for next-generation crop health. *Plant Physiol Biochem*. 2023;196:703–11. <https://doi.org/10.1016/j.plaphy.2023.02.022>.
- Vijayakumar MD, Surendhar GJ, Natrayan L, Patil PP, Ram PMB, Paramasivam P. Evolution and recent scenario of nanotechnology in agriculture and food industries. *J Nanomater*. 2022;2022(1):1280411. <https://doi.org/10.1155/2022/1280411>.
- Nile SH, Thiruvengadam M, Wang Y, Samynathan R, Shariati MA, Rebezov M, et al. Nano-priming as emerging seed priming technology for sustainable agriculture—recent developments and future perspectives. *J Nanobiotechnol*. 2022;20(1):254. <https://doi.org/10.1186/s12951-022-01423-8>.
- Hu X, Wei X, Ling J, Chen J. Cobalt: an essential micronutrient for plant growth? *Front Plant Sci*. 2021;12:768523. <https://doi.org/10.3389/fpls.2021.768523>.
- Kavya RL, Chandravanshi P, Thippeshappa GN, Kumar O, Ramesh AN, Dhananjaya BC. Influence of foliar application of cobalt on growth, yield and biochemical parameters in chickpea (*Cicer arietinum* L.). *Plant Arch*. 2024;24(2):951–6. <https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.134>.
- Ifijen IH, Faderin E, Okafor CE, Oniyinyechi OL, Aworinde OR, Iorkula TH, et al. Dual functionality of cobalt oxide nanoparticles: exploring their potential as antimicrobial and anticancer agents. *Biomed Mater Devices*. 2025;4(1):143–88. <https://doi.org/10.1007/s44174-025-00276-7>.
- Jahani M, Mahmoodzadeh H. Investigation of seed germination, early growth and physio-biochemical parameters of canola seedling exposed to Co₃O₄ engineered nanoparticles. *J Chem Health Risks*. 2025. <https://doi.org/10.22034/JCHR.2020.1891185.1092>.
- Rasmiya Begum SL, Jayawardana NU. Green synthesized metal nanoparticles as an eco-friendly measure for plant growth stimulation and disease resistance. *Plant Nano Biol*. 2023;3:100028. <https://doi.org/10.1016/j.plana.2023.100028>.
- Ying S, Guan Z, Ofoegbu PC, Clubb P, Rico C, He F, et al. Green synthesis of nanoparticles: current developments and limitations. *Environ Technol Innov*. 2022;26:102336. <https://doi.org/10.1016/j.eti.2022.102336>.
- Priyanka R, Lakshmi L, Padma M, Anbarasan V, Arivalagan K. Green synthesis, characterization and antioxidant activities of cobalt oxide nanoparticles synthesized by *Ficus religiosa* leaf extract. *Asian J Chem*. 2025;37(5):1169–75. <https://doi.org/10.14233/ajchem.2025.33567>.
- Sheikh S, Ahmed S, Ali Z, Hussain I, Khan MI, Ahmad N. Sustainable plant mediated synthesis of cobalt oxide nanoparticles using *Uraria picta* extract with enhanced biological activity. *Sci Rep*. 2025;15:44017. <https://doi.org/10.1038/s41598-025-94017-5>.
- Townsend GR, Heuberger JW. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Dis Rep*. 1943;27(17):340–3.
- Bauer AW, Kirby WMM, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966;45(4_ts):493–6. https://doi.org/10.1093/ajcp/45.4_ts.493.
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.
- Zeng H, Zhang Y, Zhang M, Liu H, Liu J, Zhu L, et al. A signal enhanced lateral flow immunoassay based on core-shell quantum dots labeled antibody and antigen for sensitive detection of CP4-EPSPS protein. *Microchem J*. 2025;209:112758. <https://doi.org/10.1016/j.microc.2025.112758>.
- Lu JY, Bu ZQ, Lei YQ, Wang D, He B, Wang J, et al. Facile microwave-assisted synthesis of Sb₂O₃-CuO nanocomposites for catalytic degradation of p-nitrophenol. *J Mol Liq*. 2024;409:125503. <https://doi.org/10.1016/j.molliq.2024.125503>.
- Wang F, Zhang J, Hu J, Wang H, Zeng Y, Wang Y, et al. Simultaneous suppression of As mobilization and N₂O emission from NH₄⁺/As-rich paddy soils by combined nitrate and birnessite amendment. *J Hazard Mater*. 2024;465:133451. <https://doi.org/10.1016/j.jhazmat.2024.133451>.
- Ahamad L, Khan AA, Khan M, Farid O, Alam M. Exploring the nano-fungicidal efficacy of green synthesized magnesium oxide nanoparticles (MgO NPs) on the development, physiology, and infection of carrot (*Daucus carota* L.) with *Alternaria* leaf blight (ALB): molecular docking. *J Integr Agric*. 2023;22(10):3069–80. <https://doi.org/10.1016/j.jia.2023.02.034>.
- Song Y, Zheng C, Li S, Chen J, Jiang M. Chitosan-magnesium oxide nanoparticles improve salt tolerance in rice by modulating ion homeostasis and antioxidant defense. *Front Plant Sci*. 2023;14:1155622. <https://doi.org/10.3389/fpls.2023.1155622>.
- Zu-Man D, Yu-Long Z, Chun-Yang T, Chuang L, Jia-Qin F, Qiang H, et al. Construction of blackberry polysaccharide nano-selenium particles: structure features and regulation effects of glucose/lipid metabolism in HepG2 cells. *Food Res Int*. 2024;187:114428. <https://doi.org/10.1016/j.foodres.2024.114428>.
- Qiu W, Fan J, Tao W, Liang J, Xiao C, Chen Y, et al. Cinobufagin loaded Prussian blue-like nanoparticles for chemo/gas therapy of multidrug resistant cancer. *J Nanobiotechnol*. 2025;23(1):706. <https://doi.org/10.1186/s12951-025-0372-8-w>.
- Patil SP. *Ficus carica* assisted green synthesis of metal nanoparticles: a mini review. *Biotechnol Rep*. 2020;28:e00569. <https://doi.org/10.1016/j.btre.2020.e00569>.
- Zhang L, Liu J, Cheng J, Sun Q, Zhang Y, Liu J, et al. lncRNA7 and lncRNA2 modulate cell wall defense genes to regulate cotton resistance to *Verticillium* wilt. *Plant Physiol*. 2022;189(1):264–84. <https://doi.org/10.1093/plphys/kiac041>.
- Yuan J, Liu Y, He Q, Wen H, Li Z, Lin R, et al. Phosphorus-modified biochar from *Salvia miltiorrhiza* dregs: synthesis, characterization, and dual-functional synergy for heavy metal immobilization and soil fertility augmentation. *Biochar*. 2026;8(1):30. <https://doi.org/10.1007/s42773-025-00540-5>.
- Cheng Q, Ma C, Wang X. Biological nitrogen and carbon fixation: bridging the gap between synthetic symbioses and synthetic biology. *Crop J*. 2026;14(1):74–86. <https://doi.org/10.1016/j.cj.2026.01.002>.
- Nie Y, Li D, Peng Y, Wang S, Hu S, Liu M, et al. Metal organic framework coated MnO₂ nanosheets delivering doxorubicin and self-activated DNAzyme for chemo-gene combinatorial treatment of cancer. *Int J Pharm*. 2020;585:119513. <https://doi.org/10.1016/j.jippharm.2020.119513>.
- Naumovska E, Nzulu GK, Mazzei L, Le Febvrier A, Komander K, Magnuson M, et al. Local structure of hydrated nanocrystalline films of the proton conductor BaZr_{1-x}Sc_xO_{3-x/2} studied by infrared spectroscopy. *Vib Spectrosc*. 2023;128:103622. <https://doi.org/10.1016/j.vibspec.2023.103622>.
- Coates J. Interpretation of infrared spectra, a practical approach. In: Meyers RA, editor. *Encyclopedia of analytical chemistry: applications, theory and*

- instrumentation. John Wiley & Sons; 2000. p. 10815–10837. <https://doi.org/10.1002/9780470027318.a5606>
36. Khan MI, Akhtar MN, Ashraf A, Naeem M, Ali S, Ahmad M. *Colocasia esculenta* leaf extracted cobalt oxide nanoparticles: a comprehensive investigation of the microstructural, morphological, antibacterial, and photocatalytic activity. Mater Sci Eng B. 2023;295:116584. <https://doi.org/10.1016/j.mseb.2023.116584>.
37. Pasieczna-Patkowska S, Cichy M, Flieger J. Application of Fourier transform infrared (FTIR) spectroscopy in characterization of green synthesized nanoparticles. Molecules. 2025;30(3):684. <https://doi.org/10.3390/molecules30030684>.
38. Mirsalari SA, Tahanpesar E, Sanaeishoar H. Influence of temperature on green synthesis of cobalt oxide nanoparticles by sol-gel method and investigation of its structural and optical properties. Iran J Crystallogr Mineral. 2022;30(3):11–20. <https://doi.org/10.52547/ijcm.30.3.11>.
39. Anas M, Rauf A, Bano N, Qayash M, Jan F, Khan I, et al. Effects of osmopriming of different growth regulators on yield and quality of tobacco. Trends Anim Plant Sci. 2024;3:35–9. <https://doi.org/10.62324/TAPS/2024.026>.
40. Haq IU. Harnessing nanotechnology for superior fruit crop quality and yield enhancement. Trends Anim Plant Sci. 2024;4:74–81. <https://doi.org/10.62324/TAPS/2024.049>.
41. Khan A, Farooq U, Rehman SAU. Effective strategies for soybean disease control. Trends Anim Plant Sci. 2024;3:65–71. <https://doi.org/10.62324/TAPS/2024.036>.
42. Khang TN, Binh HT, Dao VTT, Thao LTT, Duy TT. Antibacterial and antifungal abilities of *Tacca leontopetaloides* L. Kuntze leaf extraction and its application in fresh mango preservation. Int J Agric Biosci. 2025;14(4):556–64. <https://doi.org/10.47278/journal.ijab/2025.034>.
43. Kochorov A, Utebayev Y, Kharitonova A, Bazarbayev B, Davydova V, Nelis T. Influence of seed protectants on fungal pathogens of lentil seeds (*Lens culinaris*) against different cultivation technologies in North Kazakhstan. Int J Agric Biosci. 2025;14(2):172–9. <https://doi.org/10.47278/journal.ijab/2024.207>.
44. Nazir R, Ajmal SA, Fatima SS, Junaid MB, Wahab M, Arif A. Strategies for the integrated management of tomato wilt. Agrobiol Rec. 2024;17:91–9. <https://doi.org/10.47278/journal.abr/2024.027>.
45. Nazir R, Khan HK, Sharif MS, Khan MU, Rehman A, Wahab M. Unraveling the green enigma: a comprehensive review of contemporary advances in plant pathology. Trends Anim Plant Sci. 2024;3:49–53. <https://doi.org/10.62324/TAPS/2024.030>.
46. Obeidat WM, Alananbeh KM, AbuShanab NS, Al-Hunaiti A, Arar S. Green nanomaterial of clammy inula (*Inula viscosa* L.) as an effectual antifungal for *Fusarium oxysporum*. Int J Agric Biosci. 2026;15(1):225–32. <https://doi.org/10.47278/journal.ijab/2025.156>.
47. Phal P, Soyong K, Poem S. A new nanofibre derived from *Trichoderma hamatum* K01 to control durian rot caused by *Phytophthora palmivora*. Asian J Agric Biol. 2024;2024(2):2023182. <https://doi.org/10.35495/ajab.2023.182>.
48. Pham-Khanh NH, Huynh NQ, Le HNB, Ha TKQ. Green synthesis of zinc oxide microparticles using the leaf extract of *Dolichandrone spathacea* in sustainable agriculture: a new approach for protecting the legume plant (*Vigna radiata*) against the Cr(VI) stress. Asian J Agric Biol. 2024;2024(3):2023245. <https://doi.org/10.35495/ajab.2023.245>.
49. Rasool M, Rasool MH, Khurshid M, Aslam B. Biogenic synthesis and characterization of silver nanoparticles: exploring antioxidant and anti-inflammatory activities and assessing antimicrobial potential against multidrug-resistant bacteria. Asian J Agric Biol. 2024;2024(3):2023364. <https://doi.org/10.35495/ajab.2023.364>.
50. Riaz S, Mustafa G, Khan MS, Abbas MA. Exploring mycoparasitic potential of indigenous *Trichoderma* strains for the effective control of red rot disease in sugarcane. Asian J Agric Biol. 2025;2025(1):2024136. <https://doi.org/10.35495/ajab.2024.136>.
51. Rueangsri K, Lasunon P, Kwantrairat S, Taweejun N. Effect of ultrasound-assisted aqueous two-phase extraction on phenolic compounds from *Nymphaea pubescens* Willd. and its antioxidant and antimicrobial properties. Int J Agric Biosci. 2025;14(1):1–10. <https://doi.org/10.47278/journal.ijab/2024.187>.
52. Shoukat A. Nanofertilizers and stress management: emerging opportunities for climate-resilient farming. Agrobiol Rec. 2025;22:73–88. <https://doi.org/10.47278/journal.abr/2025.050>.
53. Tahir F, Fatima F, Fatima R, Ali E. Fruit peel extracted polyphenols through ultrasonic assisted extraction: a review. Agrobiol Rec. 2024;15:1–12. <https://doi.org/10.47278/journal.abr/2023.043>.
54. Ummer K, Ali L, Hafeez HT, Ahmed F, Nisar I, Iqbal MA, et al. Impact of Zn-lysine chelation foliar application in wheat plants under drought stress. Agrobiol Rec. 2024;16:19–32. <https://doi.org/10.47278/journal.abr/2024.008>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.