



The Effect of Foliar Spraying of Biosynthesized Copper Nanoparticles from Aqueous Extract of *Ziziphus spina-christi* Leaves on The Morphological Characteristics and Active Materials of Fenugreek Plant *Trigonella foenum-graecum*

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ABSTRACT

This work examines the effects of foliar spraying of copper nanoparticles (CuNPs) made from an aqueous extract of *Ziziphus spina-christi* leaves on the morphological and physiological traits of *Trigonella foenum-graecum*. UV-Vis spectra showed an absorption peak of CuNPs at 236 nm. The atomic force microscopy (AFM) images of extract exhibited an irregular, uneven surface with mixed particle sizes, randomly distributed clusters, and high surface roughness (average 5.7 nm). In contrast, AFM images of CuNPs revealed spherical to semi-spherical NPs with an average diameter of 53.91 nm and a surface roughness of 1.22 nm. The FTIR spectrum of the plant extract showed phenolic O–H, carbonyl C=O, and C–O functional groups. In the CuNPs, noticeable peak shifts and reduced intensity indicate that hydroxyl and carbonyl groups are involved in the reduction of Cu ions and the capping of nanoparticles, thereby enhancing their stability during synthesis. All parameters decreased at the highest concentration, whereas lower concentrations promoted increased flowering and improved seed development.

A significant increase in the content of valuable secondary metabolites in fenugreek seeds was recorded after treatment with CuNPs, with the effect increasing in a concentration-dependent manner up to the highest dose tested (200 ppm).

Keywords: Copper nanoparticles; *Ziziphus spina-christi*; *Trigonella foenum-graecum*; Secondary metabolites.

1. INTRODUCTION

1.1 Agriculture and Nanotechnology

Materials at the nanoscale (1-100 nm) have distinct physical, chemical, optical, and biological characteristics when compared with their bulk equivalents. This fact is what makes nanotechnology so fascinating (Sabir *et al.* 2022). Recently, nanoparticles have emerged as a transformative instrument in agriculture, primarily owing to their distinctive physicochemical characteristics, such as a high surface-area-to-volume ratio, enhanced reactivity, and efficacy as nutrient delivery systems (Gupta *et al.* 2025). Nanotechnology offers multiple agricultural applications, such as nano-fertilizers for enhanced nutrient use efficiency, nanosensors for precise monitoring of soil and plant health, and nano-pesticides for targeted pest management (Shang *et al.* 2019; Kah *et al.* 2018). Nanomaterials used as fertilizers, growth stimulants and delivery agents for bioactive compounds enable higher efficiency and targeted agricultural action

(Verma *et al.* 2022). They enhance root development, seed germination, nutrient use and photosynthesis, leading to higher crop productivity. They make plants more resistant to salt, drought, and extreme temperatures and support sustainable agriculture. Nanomaterials modulate stress genes and signaling pathways, boost antioxidant systems, and control reactive oxygen species, helping plants endure stress (Zaman *et al.* 2025). They make plants more resistant to salt, drought, and extreme temperatures and support sustainable agriculture. Nanomaterials modulate stress genes and signaling pathways, boost antioxidant systems, and control reactive oxygen species, helping plants endure stress. (Mittal *et al.* 2013).

1.2 Bio Synthesis of NPs

The Chemical techniques for fabrication nanoparticles use hazardous chemicals and environmentally unfriendly, while green or biosynthesis is safe, environmentally friendly, and sustainable in the long term. Plant extracts contain phytochemicals such as

terpenoids, alkaloids, flavonoids, and polyphenols that reduce the size of nanoparticles, encapsulate them, and stabilize them to make them biocompatible (Baral *et al.* 2025; Velsankar *et al.* 2020; Jadoun *et al.* 2021; Halawani, 2017). Green synthesis of NPs with plant extracts like *Z. spina-christi* (Christ's thorn) is greener than chemical synthesis. The abundant glycosides, tannins, and polyphenols in *Z. spina-christi* leaf extract reduce, capping and stabilize in NPs synthesis. (Murthy *et al.* 2020).

1.3 Phytostimulatory Effects of Bio-synthesized CuNPs

Modern agriculture has made great use of green-synthesized NPs, especially CuNPs, which boost agricultural yield and increase medicinal plants' bioactive compound synthesis (Srinivasan, 2006). their dual function as micronutrients and stimulators of secondary metabolite formation, CuNPs have become very important. Their tiny size and strong reactivity allow them to modulate physiological processes like photosynthesis, enzyme activity, and stress tolerance, and to be efficiently absorbed through foliar spray. (Memon *et al.* 2020; Song *et al.* 2017). Foliar sprays of green-synthesized NPs boost stress tolerance, chlorophyll content, and seed germination in various crops, according to several studies. (Atia and Oraibi, 2024). Copper nanoparticles boost biomass, root, and shoot length. CuNPs could boost plant growth and productivity by incorporating compounds into plant cells. Copper nanoparticles may have nutritional value as an ingredient in nano-biofertilizers. (Shende *et al.* 2017).

1.4 Fenugreek (*Trigonella foenum-graecum*)

Fenugreek is an herbaceous annual plant belonging to Fabaceae cultivated widely for its seeds and leaves, which are rich in bioactive compounds. The plant exhibits trifoliate leaves, hollow stems, yellowish-white flowers, and long slender pods containing 10–20 seeds. Morphologically, it is known for its short height (20–50 cm), moderate branching, and high seed yield. Its leaves and seeds have high content of proteins, dietary fiber, natural compounds, trigonelline, flavonoids, diosgenin, and saponins, which make it valuable as both a food and a medicinal plant (Wani and Kumar, 2018). Seeds contain saponins (6.2%), volatile oils (2.45%), and alkaloids (0.93%), which are crucial for medicinal applications (Mohammed and Abdul-Elah, 2009). They contain pharmacological constituents like diosgenin, trigonelline, galactomannan, flavonoids, and alkaloids, which exhibit antidiabetic, antioxidant, and anti-inflammatory properties (Almatroodi *et al.* 2021). The bioactive chemicals found in fenugreek can be affected by nutritional and environmental conditions and increasing their levels using stimulators such as NPs can be an effective and promising approach (Doaa *et al.* 2023). However, growing fenugreek comes with

challenges like environmental stress and low production of valuable bioactive compounds. Recent progress in nanotechnology especially in eco-friendly green synthesis of NPs has opened new ways to enhance secondary metabolite synthesis and plant growth (Qasim and Atia, 2024). Among these, CuNPs have drawn significant interest because they are affordable, have strong catalytic activity, and play key roles in plant functions such as enzyme activation and photosynthesis (Murthy *et al.* 2020).

1.5 The Christ's Thorn (*Ziziphus spina-christi* L.)

Ziziphus spina-christi L. is a multi-use tree native to tropical and subtropical regions. It is a member of the Rhamnaceae family and goes by the name Sidr. Various *Ziziphus* species have long been used in traditional medical treatment such as bruises, fractures, chest pain, headaches, oral disorders and dandruff. The leaves of *Z. Spina-christi* are especially valued for their anti-inflammatory properties and used in treating wounds, skin diseases and eye problems (Ghafoor *et al.* 2012; Ghazanfar, 1994). *Z. Spina-christi* leaves are a rich source of phytochemicals like alkaloids, saponins, tannins, flavonoids, and phenolic acids (Sarjito *et al.* 2021; Alhassan *et al.* 2019). Flavonoids, phenolic compounds, triterpenic acids, and polysaccharides have been reported as the major phytoconstituents of *Zizyphus* species. The screening tests of Sidr leaf extract phytochemical revealed that it contained secondary metabolite compounds, including alkaloids, saponins, tannins, steroids, and flavonoids (Soni and Malik, 2021). *Z. spina-christi* has emerged as a promising source of reducing and stabilizing agents for biosynthesized NPs due to its rich phytochemical composition. Aqueous leaf extracts of it utilized for biosynthesis of CuNPs producing stable NPs with significant biological activities. They exhibited potent antibacterial and antifungal activities against several pathogenic microorganisms and plant pathogens. Furthermore, copper nanoparticles are an important agricultural nanomaterial that can enhance plant growth and development by stimulating physiological processes, improving nutrient availability, and increasing plant tolerance to biotic stresses. (El-Abeid *et al.* 2024; Khani *et al.* 2018).

In this context, the present study aims to investigate the impact of foliar spray of CuNPs biosynthesized using an aqueous leaf extract of *Z. Spina-christi* on the morphological characteristics (e.g., Number of leaves/branch, flowers/plant, legumes/experimental unit and weight of one legume, ten grains, legumes/ experimental unit and number of grains/legume) and biochemical compounds (e.g., diosgenin, trigonelline and Total : alkaloid, glycoside, saponin) in *Trigonella foenum-graecum* seeds.

2. MATERIALS AND METHODS

2.1 Preparation of Leaves Aqueous Extract *Z. Spina-christi*

To prepare the *Z. Spina-christi* extract, the leaves were thoroughly rinsed with running water to remove any contaminants. After soaking for 30 minutes, the leaves were dried thoroughly with dry air. Using sterile scissors, they were cut into small pieces. Then, 50 g of the chopped leaves was mixed with 250 mL of distilled water. The mixture was shaken overnight at 45 °C, and the resulting extract was stored at 4 °C (Atia *et al.* 2020).

2.2 Green Synthesis of CuNPs

The green synthesis of CuNPs was carried out by adding 100 mL of aqueous leaf extract to 400 mL of a 0.2 M $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ solution in a ratio of 1:4, while stirring continuously. After 24 hours of incubation, the mixture was brought to room temperature. The solution was centrifuged for 15 minutes at 100,000 rpm, and a noticeable shift in color from blue to a light brownish hue was noted. To ensure the precipitate was free of contaminants, it was rinsed with deionized water. Then, the Cu NPs were dried, crushed, and subjected to various analyses (Murthy *et al.* 2020).

2.3 Characterization of Biosynthesized CuNPs

The absorbance and reflectance spectra of the samples were recorded in the 200 to 800 nm range using a UV-visible spectrophotometer, which detects collective oscillations of conduction band electrons and plasmon resonance. Fourier transform infrared spectroscopy (FTIR) was employed to identify the functional groups present in the biomolecules. In addition, the size and diameter of the synthesized CuNPs, along with their surface morphology and roughness, were analyzed using atomic force microscopy (AFM).

Different concentrations of CuNPs solution were prepared (50, 100, 150 and 200) ppm.

To determine the seed germination percentage, 25 fenugreek seeds were seeded onto filter papers, that were later placed in Petri dishes under sterile conditions in a growth chamber. The germination percentage was calculated using the equation:

$$\text{Seed Germination Percentage (GP\%)} = \frac{\text{Number of Germinated Seeds}}{\text{Number of Tested Seeds}} \times 100,$$

where: GP% is seed germination percentage, GN is Number of germinated seeds, SN is Number of tested seeds. $(\text{GP\%}) = 21/25 \times 100 = 84\%$

2.4 Field Experiment

The experiment was conducted in the greenhouse of the Plant Technology Department/College of Biotechnology during the 2022–2023 growing season. The fenugreek plant seeds were planted in containers with soil, with two replicates for each CuNPs treatment: (0, 50, 100, 150 and 200) ppm. Distilled water served as the control for comparison at the concentration of 0 ppm. The seeds and seedlings that germinated were carefully watched, nurtured, and watered. Following 25 days of cultivation, the initial foliar spraying technique (4-6 leaves) was followed by a second spraying, a week later. Following 77 days of planting, samples of the vegetation were collected for study, and the seeds were collected 152 days later.

The Morphological and physiological characteristics were studied, including: plant height (cm), number of leaves/branch, flowers/plant, legumes, weight of legumes /experimental unit (pot) (g), one legume and ten grains (g), and number of grains/legumes. Saponin content, trigonelline content (ppm), diosgenin content (ppm), and total glycoside and alkaloid content in the seeds were estimated according to the methods of (Ezeabara *et al.* 2014; Shailajan *et al.* 2011; Li *et al.* 2012; Tofighi *et al.* 2016; and Ajanal *et al.* 2018), respectively.

2.5 The Experiment Design

Eight experimental units were used in this study, as the design followed a Randomized Complete Block Design (RCBD) with one factor, namely the plant. This factor consisted of four levels, each representing a different concentration of CuNPs.

2.6 Data Analysis

For both field experiments, the data were statistically analyzed using a Randomized Complete Block Design (RCBD) to evaluate the effects of different factors on the studied traits. Treatment means were compared using the Least Significant Difference (LSD) test at a significance level of 0.05 to determine statistically significant differences (SAS, 2012).

3. RESULT AND DISCUSSION

3.1 Green Synthesis and Characterization of CuNPs

Biosynthesis of metallic nanoparticles (MtNPs) using aqueous plant extracts, such as those from *Z. spina-christi*, represents an eco-friendly and sustainable approach. Biosynthesis of CuNPs was successfully achieved by using the *Z. Spina-christi* leaves extract as a reducing and stabilizing agent. A primary indication of CuNPs formation was the visible color change in the

reaction mixture after the addition of copper salt ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$). The Color of plant extract changed from green to dark turquoise (Fig. 1A-D). It happened due to the bioreduction of Cu^{2+} ions to Cu^0 by phytochemicals in the plant extract, such as polyphenols, flavonoids and terpenoids. They act as reducing, capping and stabilizing factors in green synthesis of CuNPs. The synthesis of CuNPs is attributed to Surface Plasmon Resonance (SPR) excitation in metallic nanostructures.

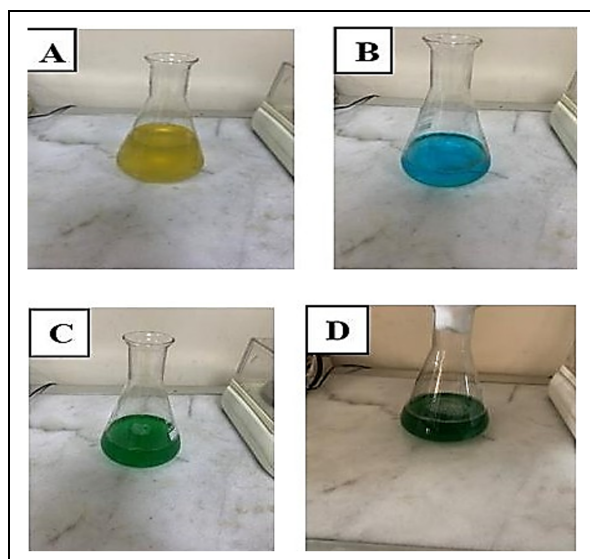


Fig. 1: Changing color of *Z. spina-christi* leaves extract within synthesis of CuNPs

3.2 UV-Vis Spectra

Broad absorption bands in the range 250–400 nm, with prominent peaks around 270–280 nm and an extending shoulder up to 320–350 nm were exhibited at the aqueous extract of *Z. spina-christi* leaves, highest absorption peak is observed at 269 and 235 nm (Fig. 2A). They confirm the presence of polyphenols, flavonoids and aromatic acids. The carbonyl ($\text{C}=\text{O}$) hydroxyl and ($-\text{OH}$) functional groups in these phytochemicals act as reducing capping and stabilizing agents in CuNPs synthesis polyphenols, and aromatic acids. The hydroxyl ($-\text{OH}$) and carbonyl ($\text{C}=\text{O}$) functional groups in these phytochemicals act as reducing and stabilizing agents during nanoparticle biosynthesis (Baral *et al.* 2025; Bisso *et al.* 2022). Figure 2b shows a distinct absorption band at 236 nm and a strong surface plasmon resonance band at 560–580 nm in the UV-Vis spectrum, while this band is absent in the plant extract. This surface plasmon resonance peak confirms the formation of CuNPs and indicates the reduction of Cu^{2+} ions to Cu^0 according to the oscillations of the collective electrons within the NPs.

3.3 FTIR Spectroscopy

The FTIR consider an important for identifying the functional groups of biomolecules in the aqueous

extract of of *Z. spina-christi* leaves. FTIR spectrum reveals characteristic peaks corresponding to different phytochemicals in the extract, including O–H stretching vibrations ($\sim 3200\text{--}3400\text{ cm}^{-1}$) associated with phenols and alcohols, $\text{C}=\text{O}$ stretching ($\sim 1630\text{--}1650\text{ cm}^{-1}$) related to flavonoids and terpenoids, and C–O vibrations ($\sim 1050\text{--}1100\text{ cm}^{-1}$) (Fig. 3A). In the spectrum of the CuNPs, observed shifts, reductions in intensity, and broadening of these peaks emphasize the active involvement of these functional groups. particularly, carbonyl and hydroxyl groups from polyphenols and terpenoids are responsible for reducing Cu^{+2} ions to Cu^0 and capping NPs to prevent their aggregation.

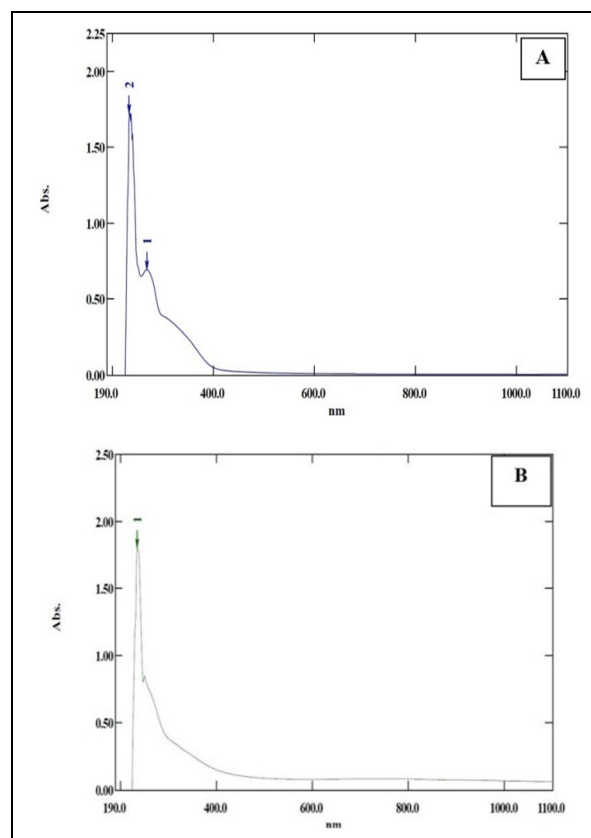


Fig. 2: UV visible spectrum of: (A) *Z. spina-christi* extract (B) CuNPs

3.4 Atomic Force Microscopy

High-resolution topography data from AFM revealed the size, shape, and surface features of green-synthesized CuNPs. The 2D and 3D AFM images, along with particle size distribution histograms, revealed predominantly spherical to semi-spherical NPs. The sizes are between 20 to 90 nm with an average diameter of 53.91 nm. AFM images of the aqueous *Z. spina-christi* extract showed an irregular, uneven surface with mixed particle sizes, randomly distributed clusters, and high surface roughness (average 5.7 nm), attributed to different plant constituents such as polyphenols, polysaccharides, proteins, and flavonoids. While, the

biosynthesized CuNPs showed well-defined, separated nanoparticles with smoother surfaces, improved homogeneity, and significantly reduced roughness (average 1.22 nm). height distribution and Granularity analyses demonstrated the reduction in particle size following nanoparticle formation, indicating effective

reduction of Cu^{2+} ions and stabilization of CuNPs by plant biomolecules. The 3D AFM topography confirmed reduced surface roughness and better dispersion, suggesting strong interactions between functional groups and CuNPs ($-\text{OH}$, $-\text{COOH}$, and NH_2) present in the *Z. Spina-christi* extract (Fig. 4 A, B, C and D).

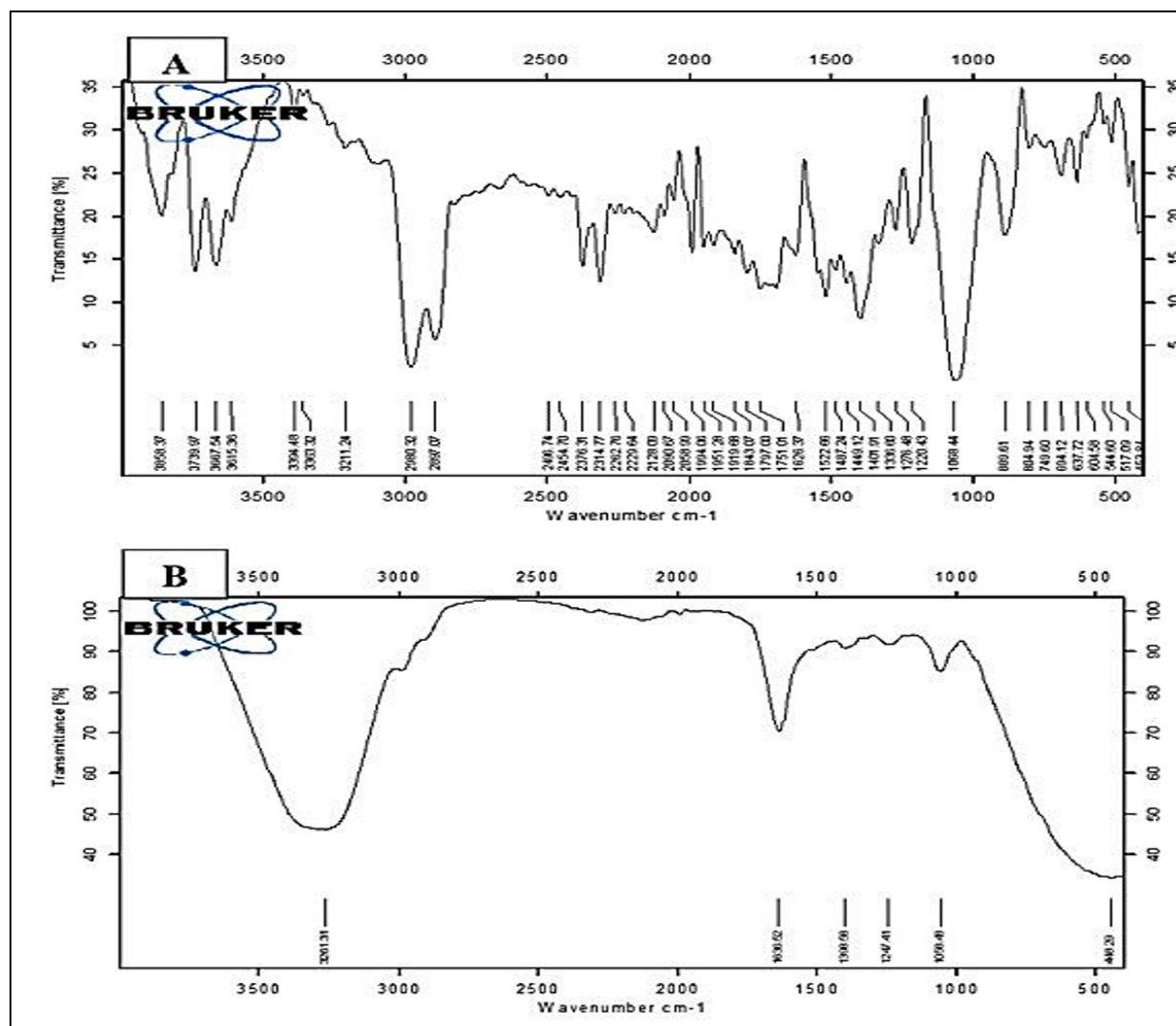


Fig. 3: FTIR spectroscopy of (A) *Z. Spina-christi* extract and CuNP3.4 atomic force microscopy

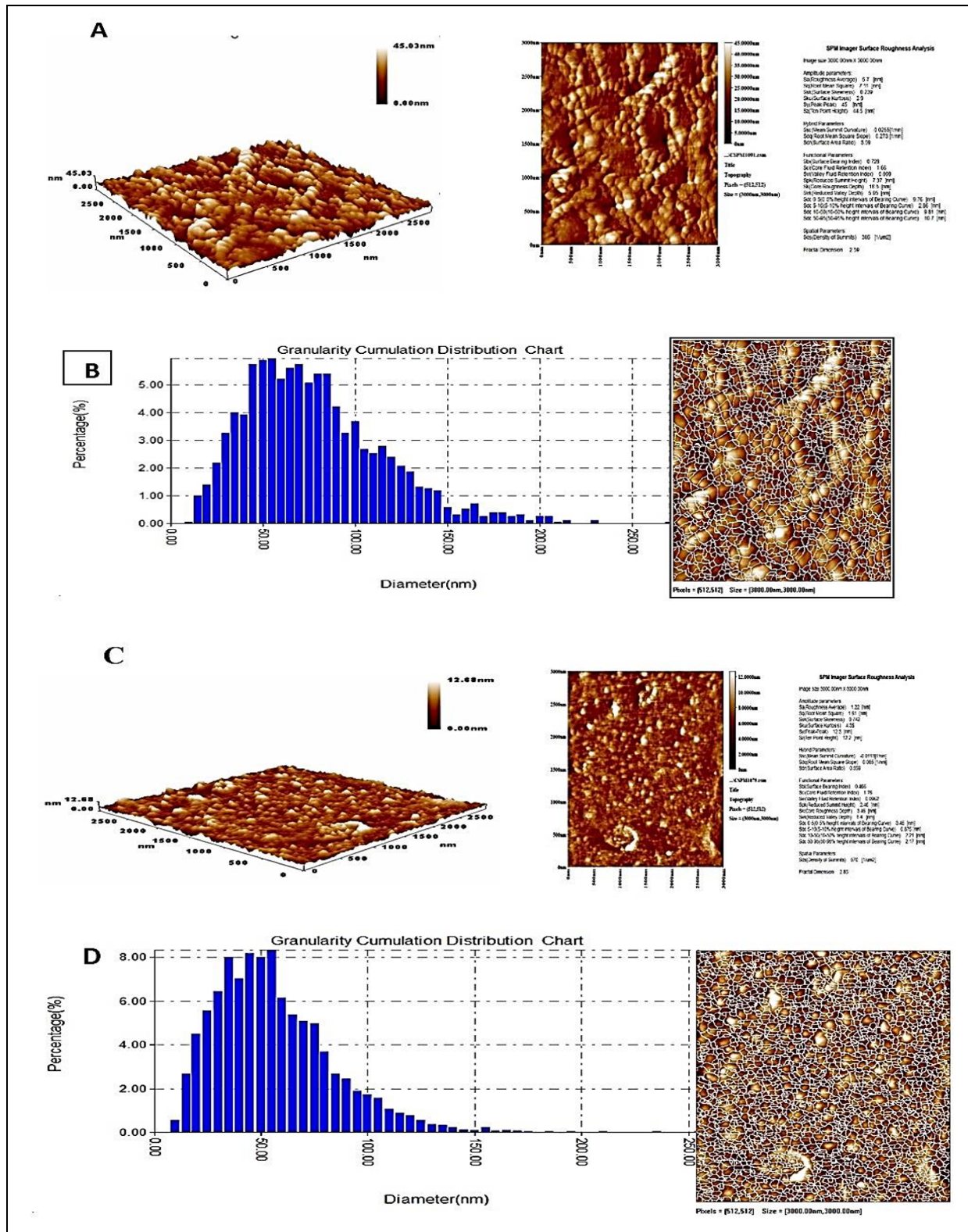


Fig. 4: AFM images and granularity cumulation distribution of: A& B: *Z. Spina-christi* leaf extract. C & D: CuNPs

3.5 Effects of Biosynthesized CuNPs on Fenugreek Growth and Yield Parameters

Foliar spray application of biosynthesized CuNPs had a significant, concentration-dependent effect on the growth, yield, and productivity of *Trigonella foenum-graecum*, as shown in Tables 1-3.

3.6 Morphological and Physiological Characteristics

Treatment with CuNPs at concentrations of 50–150 ppm significantly enhanced growth parameters compared with the control (0 ppm). Plant height increased from 27 cm in the control to a maximum of 40 cm at 150 ppm. Similarly, the number of leaves per branch, flowers per plant, and legumes per pot showed significant improvement, with the highest values recorded at 150 ppm (23 leaves per branch, 7 flowers per plant, and 106 legumes per pot) (Table 1). The nano-form of copper likely enhances its bioavailability and uptake efficiency compared to conventional salts. In legumes, CuNPs application has been linked to enhanced nodulation, nitrogen fixation, and higher yields. Foliar CuNPs treatments also boost biomass and photosynthetic efficiency by enhancing enzyme activity and uptake of nutrient (Murthy *et al.* 2020; Jasim *et al.* 2017). However, notable decrease in all parameters was observed at the highest concentration (200 ppm). According to Adrees *et al.* (2015), legume yield per pot decreased sharply from 106 at 100 ppm to 45 at 200 ppm. It appears that plants benefit from lower CuNPs concentrations but are severely damaged by higher ones. Excess copper ions may produce oxidative stress, which damages cells and inhibits growth, explaining the observed toxicity.

3.7 Legume and Grain Quality

The weight of a single legume increased nearly threefold from 0.28 g (control) to 0.84 g (150 ppm), and the grains number per legume increased from 8 to 21. Total weight of legumes per pot also significantly increased (39.63 g at 150 ppm) (Table 2). These improvements show that CuNPs not only helped the plants grow better but also improved flower and seed development, likely by helping the plants use nutrients more effectively and increasing photosynthesis. MetNPs such as CuNPs exhibit unique physicochemical properties that play an important role in improving nutrient uptake, resulting in increased the growth of plant and secondary metabolites synthesis (Mittal *et al.* 2013).

3.8 Enhancement of Secondary Metabolites

T. foenum-graecum (fenugreek) is especially notable for its abundance of active compounds like flavonoids, saponins and alkaloids, which provide antioxidant, antimicrobial activities (Khalofah *et al.*

2021). One of the most important results recorded in the present work was the increase in the content of valuable secondary metabolites in fenugreek seeds after treatment with CuNPs (Table 3). A significant increase was recorded, dependent on concentration, up to the highest dose used (200 ppm). The total alkaloids increased from 35.8 to 44.4 mg/100g, total saponins increased from 0.84% to 1.25%, trigonelline (key bioactive alkaloid) increased from 51.5 ppm to 75.6 ppm and diosgenin (important steroidal sapogenin) increased from 66.9 ppm to 99.9 ppm. This enhancement of secondary metabolism is a well-established response to nanoparticle-induced stress. The NPs act as abiotic promoters and cause a mild oxidative signal that activates the plant's defense pathways, including the shikimic acid and phenylpropanoid pathways, leading to higher production of alkaloids and saponins (Marslin *et al.* 2017). These finding agree well with studies on other medicinal plants where metal NPs boosted the production of phytochemicals. The growth-enhancement and stimulating effects of CuNPs on fenugreek are well reported by previous studies. For example, studies using CuNPs synthesized from *Ziziphus spina-christi* leaf extract have reported similar results. (Baral *et al.* 2025) showed that CuNPs synthesized from *Z. Spina-christi*, with sizes of 30–60 nm and SPR peak at 570 nm, greatly improved wheat seedlings growth, such as shoot length and biomass, under both normal and drought stress conditions. A study by Sadak (2019) on fenugreek reported that foliar application of chemically synthesized CuNPs enhanced plant growth, yield, and the levels of diosgenin and trigonelline. These findings are consistent with the present results obtained using biosynthesized CuNPs, indicating that the beneficial effects on plant performance are similar regardless of the nanoparticle synthesis method. Biosynthesized nanoparticles are often better than chemically synthesized ones because they are coated with natural plant compounds, which can improve their compatibility with plants and increase their positive effects. The growth and yield results indicate that different physiological processes respond optimally to different CuNP concentrations. For example, the highest number of legumes was recorded at 100 ppm, whereas the highest pod weight was observed at 150 ppm, suggesting a concentration-dependent variation in plant response. Foliar application of CuNPs, also helps plants adapt to environmental stress more efficiently (Murthy *et al.* 2020). CuNPs are therefore gaining significant attention for their ability to function both as essential nutrients and as elicitors that boost plant metabolic activity, largely due to their easy absorption and influence on photosynthesis, enzyme function, and stress resilience (Memon *et al.* 2020; Song *et al.* 2017). In fenugreek (*Trigonella foenum-graecum*), CuNPs treatment stimulated root growth, increased phenolic and flavonoid production, and enhanced overall biomass by modulating nutrient uptake and stress-response mechanisms (Doaa *et al.* 2023).

Table 1. Effect of CuNPs on morphological and physiological characteristics of fenugreek plant parts

Concentration (ppm)	Mean Height of Plant (cm)	Mean of Leaves Number / Branch	Mean of Flowers Number / Plant	Mean of Legumes Number / Experimental Unit (Pot)
0.0 (control)	27	9	4	71
50	39	13	5	85
100	39	17	6	106
150	40	23	7	90
200	34	14	5	45
LSD 0.05				
CuNPs concentrations	7.07	3.31	1.77	22.25

Table 2. Effect of CuNPs on weight and number of fenugreek grains/ legume

Concentration (ppm)	Weight of One Legume (gm)	Number of Grains / Legumes	Weight of Ten Grains	Weight of Legumes/ Experimental Unit (pot) gm
0.0 (control)	0.28	8	0.14	26.47
50	0.81	13	0.18	29.19
100	0.81	16	0.17	35.63
150	0.84	21	0.12	39.63
200	0.57	15	0.10	13.60
LSD 0.05				
CuNPs concentrations	0.20	3.31	0.04	4.43

Table 3. Effect of CuNPs on secondary metabolites in fenugreek seeds

Concentration ppm	Mean of Total Alkaloid Content (mg/100 gm)	Total Glycoside Content %	Total Saponin Content %	Trigonelline (ppm)	Diosgenin (ppm)
0.0 (control)	35.8	1.85	0.84	51.5	66.9
50	36.9	1.92	0.92	59.8	76.9
100	39.0	1.98	1.01	64.8	84.7
150	41.5	2.04	1.11	70.5	93.9
200	44.4	2.10	1.25	75.6	99.9
LSD 0.05					
CuNPs concentrations	1.65	0.48	0.16	7.24	4.01

4. CONCLUSION

Foliar application of biosynthesized copper nanoparticles induces a hormetic response in fenugreek. At optimal concentrations, CuNPs act as active nano-fertilizer, significantly improving growth, yield, and yield components. At the same time, they function as effective nano-promoters, enhancing the synthesis of pharmaceutically active secondary metabolites such as diosgenin and trigonelline. These findings, supported by similar results from studies using *Ziziphus spina-christi*-derived CuNPs, highlight the transformative potential of plant-based nanotechnology in developing more valuable and productive agricultural systems.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCE

- Adrees, P. M., Ali, S., Rizwan, M., Zia-ur-Rehman, M., Ibrahim, M., Abbas, F., Farid, M., Qayyum, M. F. and Irshad, M. K., Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: A review, *Ecotoxicol. Environ. Safe.*, 119, 186-197(2015).
<https://doi.org/10.1016/j.ecoenv.2015.05.011>
- Ajanal, M., Gundkalle, M. B. and Nayak, S. U., Estimation of Total alkaloid in Chitravati by UV-Spectrophotometer, *Ancient Sci. Life*, 31(4), 198-201(2018).
<https://doi.org/10.4103/0257-7941.107361>

- Alhassan, K. A., Indabawa, A. S. and Shah, M. M., Phytochemical analysis, proximate composition and antibacterial activities of *Ziziphus* species (*Z. jujube* and *Z. spina christi*), *J. Appl. Adv. Res.*, 4(1), 42-46 (2019).
<https://doi.org/10.21839/jaar.2019.v4i1.262>
- Almatroodi, S. A., Almatroudi, A., Alsahli, M. A. and Rahmani, A. H., Fenugreek (*Trigonella foenum-graecum*) and its Active Compounds: A Review of its Effects on Human Health through Modulating Biological Activities, *Pharmacogn J.*, 13(3), 813-821 (2021).
<https://doi.org/10.5530/pj.2021.13.103>
- Atia, W. J. and Oraibi, A. G., Silver nanoparticles and NPK fertilizer effects on the proline, peroxidase, and catalase enzymes in wheat, *SABRAO J. Breed. Genetics*, 56(6), 2405-2415 (2024).
<https://doi.org/10.54910/sabrao2024.56.6.22>
- Atia, W. J., AL-Kaisy, W. A. and Oraibi, A. G., Investigation the effects of biosynthesized silver nanoparticles on catalyase and peroxidase enzymes activity of two wheat (*Triticum Aestivum* L) cultivars *in vitro*, *Eur. Asian J. Bio Sci.*, 14, 5203-5213 (2020).
- Baral, J., Pokharel, N., Dhungana, S., Tiwari, L., Khadka, D., Pokhrel, M., R. and Poudel, B. R., Green Synthesis of Copper Oxide Nanoparticles Using *Mentha* (Mint) Leaves Characterization and Its Antimicrobial Properties with Phytochemicals Screening, *J. Nepal Chem. Soc.*, 45(1), 111-121 (2025).
<https://doi.org/10.3126/jncs.v45i1.74491>
- Bisso, B. N., Roland, N. E. N., Roland, T. T. and Paul, D. J., Phytochemical Screening, Antioxidant, and Antimicrobial Activities of Seven Under investigated Medicinal Plants against Microbial Pathogens, *Adv. Pharma. Sci.*, 1-8 (2022), (2022).
<https://doi.org/10.1155/2022/1998808>
- Doaa, E., Elsherif, D., Abd-ElShafy, E. and Khalifa, A. M., Impacts of ZnO as a nanofertilizer on fenugreek: some biochemical parameters and SCoT analysis, *J. Gene. Eng. Biotechnol.*, 21(1), 52 (2023).
<https://doi.org/10.1186/s43141-023-00501-0>
- El-Abeid, S. E., Mosa, M. A., El-Tabakh, M. A. M., Saleh, A. M., El-Khateeb, M. A. and Haridy, M. S. A., Antifungal activity of copper oxide nanoparticles derived from *Zizyphus spina* leaf extract against Fusarium root rot disease in tomato plants, *J. Nanobiotechnol.*, 22(1), 28 (2024).
<https://doi.org/10.1186/s12951-023-02281-8>
- Ezeabara, C. A., Okeke, C. U., Aziagba, B. O., Iiodibia, C. V. and Emeka, A. N., Detrmination of saponin continent of various parts of six *Citrus* species, *Int. Res. pure Appl. Chem.*, 4(1), 137-143 (2014).
<https://doi.org/10.9734/IRJPAC/2014/5831>
- Ghafoor, A. O., Qadir, H. K. and Fakhri, N. A., Analysis of phenolic compounds in extracts of *Ziziphus spina-christi* using RPHPLC methods, *J. Chem. Pharm. Res.*, 2012(46), 3158-3163 (2012).
- Ghazanfar, S. A., Handbook of Arabian medicinal plants, *CRC Press*, 272 (1994).
<https://doi.org/10.1201/b14834>
- Gupta, C., Gupta, M. K. and Tripathi, S., Nanotechnology: a promising technology in sustainable agriculture, *Discover Biotechnol.*, 2(1), 12 (2025).
<https://doi.org/10.1007/s44340-025-00022-1>
- Halawani, E. M., Rapid Biosynthesis Method and Characterization of Silver Nanoparticles Using *Zizyphus spina christi* Leaf Extract and Their Antibacterial Efficacy in Therapeutic Application, *J. Biomater. Nanobiotechnol.*, 8(1), 22-35 (2017).
<https://doi.org/10.4236/jbmb.2017.81002>
- Jadoun, S., Arif, R.; Jangid, N. K. and Meena, R. K., Green synthesis of nanoparticles using plant extracts: A review, *Environ. Chem. Lett.*, 19(1), 355-374 (2021).
<https://doi.org/10.1007/s10311-020-01074-x>
- Jasim, B., Thomas, R., Mathew, J., and Radhakrishnan, E. K., Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonella foenum-graecum* L.), *Saudi Pharma. J.*, 25(3), 443-447 (2017).
<https://doi.org/10.1016/j.jsps.2016.09.012>
- Kah, M., Kookana, R. S., Gogos, A. and Bucheli, T. D., A critical evaluation of nanopesticides and nanofertilizers against conventional analogues, *Nat. Nanotechnol.*, 13(8), 677-684 (2018).
<https://doi.org/10.1038/s41565-018-0131-1>
- Khalofah, A., Kilany, M. and Migdadi, H., Assessment of Morpho-Physiological and Biochemical Responses of Mercury-Stressed *Trigonella foenum-graecum* L. to Silver Nanoparticles and *Sphingobacterium ginsenosidiumtrans* Applications, *Plants*, 10(7), 1349 (2021).
<https://doi.org/10.3390/plants10071349>
- Khani, R., Roostaei, B., Bagherzade, G. and Moudi, M., Green synthesis of copper nanoparticles by fruit extract of *Ziziphus spina-christi* (L.) willd.: application for adsorption of triphenylmethane dye and antibacterial assay, *J. Mol. Liq.*, 255, 541-549 (2018).
<https://doi.org/10.1016/j.molliq.2018.02.010>
- Li, P., Mou Y., Lu, S., Sun, W., Lou, J., Yin, C. and Zhou, L., Quantitative determination of diosgenin in *Dioscorea zingiberensis* cell cultures by microplate-spectrophotometry and high-performance liquid chromatography, *Afr. J. Pharma. Pharmacol.*, 6(15), 1186-1193 (2012).
- Marslin, B. G., Sheeba, C. J. and Franklin, G., Nanoparticles Alter Secondary Metabolism in Plants via ROS Burst, *Front. Plant Sci.*, 8, 832 (2017).
<https://doi.org/10.3389/fpls.2017.00832>

- Memon, R., Memon, A. A., Sherazi, S. T. H., Sirajuddin, B. A., Raza, S. M., Mahesar, S. A., Rajar, K. and Agheem, M. H., Application of synthesized copper nanoparticles using aqueous extract of *Ziziphus mauritiana* L. leaves as a colorimetric sensor for the detection of Ag⁺, *Turk. J. Chem.*, 44(5), 1376-1385 (2020).
<https://doi.org/10.3906/kim-2001-51>
- Mittal, A. K., Chisti, Y., and Banerjee, U. C., Synthesis of metallic nanoparticles using plant extracts, *Biotechnol. Adv.*, 31(2), 346-356 (2013).
<https://doi.org/10.1016/j.biotechadv.2013.01.003>
- Mohammed, W. Y. and Abdul-Elah, S. M., Isolation of Some Active Materials from *Trigonella foenum-graecum* Seeds and Study of Their Biological Activity, *J. Univers. Anbar Pure Sci.*, 3(3), 136-145 (2009).
<https://doi.org/10.37652/juaps.2009.15536>
- Murthy, H. C. A., Desalegn, T., Kassa, M., Abebe, B. and Assefa, T., Synthesis of Green Copper Nanoparticles Using Medicinal Plant *Hagenia abyssinica* (Brace) JF. Gmel. Leaf Extract: Antimicrobial Properties, *J. Nanomater.*, 2020, 1-12 (2020).
<https://doi.org/10.1155/2020/3924081>
- Qasim, H. S. and Atia, W. J., The role of silver nanoparticles biosynthesized from *Citrus aurantium* Leaves in some Vegetative and physiological characteristics of fenugreek plant (*Trigonella foenum-graecum* L.), *Int. J. Sci. Res. Archive*, 2024, 11(02), 247-259 (2024).
<https://doi.org/10.30574/ijrsra.2024.11.2.0394>
- Sabir, S., Arshad, M., Ilyas, N., Naz, F., Amjad, M. S., Malik, N. Z. and Chaudhari, S. K., Protective role of foliar application of green-synthesized silver nanoparticles against wheat stripe rust disease caused by *Puccinia striiformis*, *Green Proce. Syn.*, 11(1), 29-43(2022).
<https://doi.org/10.1515/gps-2022-0004>
- Sadak, M. S., Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum*), *J. Nanobiotechnol.*, 43(1), 38 (2019).
<https://doi.org/10.1186/s42269-019-0077-y>
- Sarjito, Lukita, P. L., Riyadi, P. H., Desrina and Prayitno, S. B., Phytochemical Analysis and Antibacterial Activities of Sidr Leaf Extract (*Ziziphus spinachristi*) against Pathogenic Bacteria in Aquaculture, *Pertanika J. Trop. Agric. Sci.*, 44 (4), 845 - 864 (2021). <https://doi.org/10.47836/pjtas.44.4.08>
- SAS Institute Inc. Statistical Analysis System (SAS) User's Guide: Statistics, Version 9.1. Cary, North Carolina, USA: SAS Institute Inc. (2012).
- Shailajan, S., Menon, S., Singh, A., Mhatre, M. and Sayed, N., A validated RP-HPLC method for quantitation of trigonelline from herbal formulations containing *Trigonella foenum-graecum* (L.) seeds, *Pharma. Meth.*, 2(3), 157-160(2011).
<https://doi.org/10.4103/2229-4708.90354>
- Shang, Y., Hasan, M. K., Ahammed, G. J., Li, M., Yin, H. and Zhou, J., Applications of nanotechnology in plant growth and crop protection: A review, *Mol.* 2019, 24(14), 2558 (2019).
<https://doi.org/10.3390/molecules24142558>
- Shende, S., Rathod, D., Gade, A. and Rai, M., Biogenic copper nanoparticles promote the growth of pigeon pea (*Cajanus cajan* L.), *IET Nanobiotechnol.*, 2017, 11(7), 773-781(2025).
<https://doi.org/10.1049/iet-nbt.2016.0179>
- Song, Z., Kennedy, P. G., Liew, F. J. and Schilling, J. S., Fungal endophytes as priority colonizers initiating wood decomposition, *Func. Eco.*, 31(2), 407-418 (2017).
<https://doi.org/10.1111/1365-2435.12735>
- Soni, H. and Malik, J. K., Phyto-pharmacological potential of *Zizyphus jujube*: A review, *Scholars Int. J. Biochem.*, 4(1), 1-5 (2021).
<https://doi.org/10.36348/sijb.2021.v04i01.001>
- Srinivasan, K., Fenugreek (*Trigonella foenum-graecum*): A Review of Health Beneficial Physiological Effects, *Food Rev. Int.*, 22(2), 203-224 (2006).
<https://doi.org/10.1080/87559120600586315>
- Tofighi Z. and saeidi, G., Determination of cardiac glycosides and total phenols in different generations of *Securigera securidaca* suspension culture, *Res. J. Pharma.*, 3(2), 2016: 25-31(2016).
- Velsankar, K., Kumar, R. M. A., Preethi, R; Muthulakshmi, V. and Sudhahar, S., Green synthesis of CuO nanoparticles via *Allium sativum* extract and its characterizations for antimicrobial, antioxidant, antilarvicidal activities, *J. Environ. Chem. Eng.*, 8(5), 104123(2020).
<https://doi.org/10.1016/j.jece.2020.104123>
- Verma, K. K., Song, X. P; Joshi, A., Tian, D. D., Rajput, V. D., Singh, M., Arora, J., Minkina, T. and Li, Y. R., Recent Trends in Nano-Fertilizers for Sustainable Agriculture under Climate Change for Global Food Security, *Rev. Nanomater.*, 12(1), 173(2022).
<https://doi.org/10.3390/nano12010173>
- Wani, S. A. and Kumar, P., Fenugreek: A review on its nutraceutical properties and utilization in various food products, *J. Saudi Soc. Agri. Sci.*, 17(2), 97-106(2018).
<https://doi.org/10.1016/j.jssas.2016.01.007>
- Zaman, W., Ayaz, A. and Park, S., Nanomaterials in Agriculture: A Pathway to Enhanced Plant Growth and Abiotic Stress Resistance, *Plants*, 14(5), 716(2025).
<https://doi.org/10.3390/plants14050716>