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Silver Nanoparticles Influence on Growth, Biochemistry, and Yield of Fenugreek (Trigonella foenum-graecum)

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Short Report

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Abstract

The present study investigates the effects of foliar application of Rhizopus-mediated silver nanoparticles (RAgNPs), chemically synthesized AgNPs, and Rhizopus extract on growth, physiological traits, secondary metabolite accumulation, and antioxidant activity in fenugreek (*Trigonella foenum-graecum* L.) plants. Treatments at various concentrations (20–100 mg/L) were evaluated, with the 60 mg/L RAgNP application producing the most pronounced responses. RAgNPs significantly increased shoot and root growth, total biomass, yield components, and marked enhancements in photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, and total pigments). Secondary metabolite analysis revealed substantial increases in total phenolics (121%) and flavonoids (87%) in RAgNP-treated plants, correlating with elevated total antioxidant capacity and ferric reducing antioxidant power (FRAP) activity. Moreover, diosgenin content was enhanced, suggesting activation of the phenylpropanoid pathway and related metabolic routes. The superior performance of RAgNPs over chemically synthesized AgNPs and Rhizopus extract alone is attributed to synergistic effects between the silver nanoparticulate core and fungal bioactive compounds, which act as potent nanoelicitors.

These findings highlight biologically synthesized silver nanoparticles as effective tools for improving crop growth, photosynthetic efficiency, antioxidant defense, and producing pharmaceutically valuable metabolites, offering promising applications in sustainable agriculture and medicinal plant biotechnology.

Highlights

Rhizopus-mediated AgNPs significantly enhanced fenugreek growth, biomass, and yield traits.

RAgNP treatment markedly increased chlorophyll a, chlorophyll b, carotenoids, and total photosynthetic pigments.

Total phenolics and flavonoids improved substantially as well as antioxidant capacity and FRAP activity were strongly elevated.

RAgNPs enhanced diosgenin accumulation, indicating activation of phenylpropanoid and related metabolic pathways.

Biogenic AgNPs outperformed chemically synthesized AgNPs and Rhizopus extract, acting as potent nanoelicitors for sustainable crop improvement.

1 Introduction

Nanotechnology has emerged as one of the most rapidly advancing scientific and industrial domains of the 21st century. Nano-compounds possess distinctive physicochemical characteristics compared to their bulk counterparts, including ultra-small particle size (1–100 nm), exceptionally high specific surface

area, elevated surface energy, abundant active sites, and superior catalytic and adsorptive capabilities[1,2]. These attributes underpin their wide-ranging applications in electronics, medicine, agriculture, environmental remediation, and energy systems. The global market demand for nanoparticles (NPs) is projected to reach USD 51 billion with an estimated annual production of over 10,000 tonnes by 2026 [3], reflecting their pervasive role in modern technology and industry.

However, the growing use of engineered nanomaterials (ENMs) has raised concerns about their unintended environmental release during production, application, and disposal. ENMs can enter terrestrial ecosystems via wastewater, atmospheric deposition, agricultural inputs, or direct industrial discharge, eventually accumulating in soils where they interact with plants—the primary producers in the food web[4.5,6]. Prolonged exposure may lead to changes in plant physiology, growth, and genetic stability, with potential implications for food security and ecosystem health.

Among ENMs, nano-metal oxides such as zinc oxide (ZnO) and aluminum oxide (Al_2O_3) have attracted particular attention due to their widespread usage in cement manufacturing, chemical industries, sensors, textiles, cosmetics, nanomedicine, and electronics[7]. ZnO nanoparticles are increasingly explored as nanofertilizers owing to their slow-release properties, improved bioavailability, and potential to enhance nutrient uptake efficiency[8]. Conversely, numerous studies have reported that ZnO NPs, depending on concentration and particle characteristics, can inhibit seed germination, reduce root elongation, alter biomass accumulation, and cause oxidative stress in various plant species such as corn, cucumber, soybean, ryegrass, wheat, squash, and cowpea[9,10].

Similarly, various crop species have documented the phytotoxicity of silver nanoparticles (AgNPs) and biogenic silver nanoparticles (bio-AgNPs). At elevated concentrations, AgNPs can inhibit seed germination, suppress root elongation, and impair seedling growth in crops such as wheat, maize, rice, and soybean[11,12]. Bio-AgNPs, though often reported to be less toxic due to their biocompatible capping agents, can still induce growth retardation and oxidative stress at high doses[13]. Reported effects range from negligible growth inhibition—as observed in Arabidopsis thaliana at moderate concentrations[14]—to significant reductions in root biomass, chlorophyll content, and cellular integrity in cucumber and lettuce[15,16]. These contrasting outcomes indicate that the phytotoxic response to AgNPs is influenced by factors such as plant species, developmental stage, nanoparticle concentration, surface chemistry, and exposure duration, with bio-AgNPs generally showing a more moderated toxicity profile than their chemically synthesized counterparts.

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume widely cultivated in Egypt, India, and other parts of Asia and the Middle East, valued for its culinary, nutritional, and medicinal properties. Belonging to the family Leguminosae, fenugreek seeds and leaves are rich in proteins (notably lysine and L-tryptophan), vitamins, minerals (including calcium and iron), saponins, alkaloids (trigonelline), flavonoids, and mucilaginous fibers[17]. These bioactive compounds contribute to its antioxidant, hypocholesterolemic, antidiabetic, and hematinic effects. Due to its high phenolic and flavonoid content,

fenugreek extracts have demonstrated significant antioxidant activity, which adds to its pharmacological value. Additionally, agronomic benefits have been noted, such as reduced Orobanche crenata infection when intercropped with faba bean[18].

Recent advances in agricultural nanotechnology suggest that nanoparticles can act as targeted nutrient carriers, controlled-release fertilizers, and growth stimulators. Nano-enabled agro-inputs offer advantages such as minimizing nutrient leaching and volatilization, improving nutrient use efficiency, and enhancing stress tolerance[19]. In particular, nanoencapsulation techniques can protect active ingredients from premature degradation and facilitate site-specific delivery to plants. However, despite these potential benefits, knowledge gaps remain regarding nanoparticles' physiological, biochemical, and genetic effects on leguminous crops like fenugreek, especially under realistic soil conditions.

In addition to growth and yield parameters, under nanoparticle exposure not known. The findings will contribute to a deeper understanding of nanoparticle–plant interactions, inform safe and effective applications of nanotechnology in sustainable agriculture, and provide insights into the ENM exposure in legume crops.

2 Materials and methods

The seeds of Fenugreek (*Trigonella foenum-graecum* L.) were obtained from the agency in Bangalore, India. After collecting, the seeds were carefully rinsed with sterilized distilled water, surface-sterilized for 10 minutes with 0.5% sodium hypochlorite (NaOCl), and used.

2.1 Chemicals

hemicals include gallic acid, 3,4,5-trihydroxy benzoic acid, and silver nitrate ($AgNO_3$). Ferric chloride ($FeCl_3$), potassium ferricyanide ($K_3[Fe(CN)_6]$), ascorbic acid, quercetin (2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one), methanol acetonitrile, acetone, ethanol, ethyl acetate, 3,5 dinitro salicylic acid, sodium hydroxide, phenol, Rochelle Na-K tartrate, sodium hydroxide, sodium sulfite, glucose, ammonium molybdate, sulfuric acid, sodium phosphate, potassium phosphate dibasic anhydrous, potassium dihydrogen phosphate, sodium phosphate, sodium phosphate, potassium phosphate, potassium phosphate, sodium phosphate, potassium phosphate, potassium phosphate, potassium phosphate, potassium dihydrogen phosphate, and trichloroacetic acid. Every compound was purchased commercially and used without further processing (Sigma-Aldrich).

2.2 Preparation of Fungal Extract

The fungus Rhizopus spp. was cultured in 250 cc of potato dextrose broth for 15 days at 25 ± 2°C after being received from the MFT Department stock culture collection at CSIR-CFTRI, Mysore, India. The mycelial mat was collected, cleaned with sterile distilled water, and dried in an oven at 40 ± 2°C. The fungal mat was homogenized with a pestle and mortar, and 10g of dry cell powder was suspended in 100 mL of double-distilled water. For hydrolysis, it was autoclaved at 15 psig and 121°C for 20 minutes.

The hydrolysate was then centrifuged for ten minutes at 5000 rpm, with the supernatant collected and kept at 4°C for later use.

2.3 Synthesis and Characterization of nanoparticles

Silver nanoparticles were produced and characterized as previously described[20,21] and utilized for application to Fenugreek (*Trigonella foenum-graecum* L.).

2.4 Germination of seeds

The Fenugreek seeds were treated with 0.5% sodium hypochlorite (v/v) for 3 minutes and 70% ethanol (w/v) for 60 seconds. The different concentrations of nanosuspensions (Ag, R-Ag NPs dispersed in water (nano priming), as well as bulk AgNO3, deionized water alone (hydropriming), were created by scattering the particles in deionized water for 30 minutes with ultrasonic vibration (100 w, 40 kHz). Seeds were soaked in varying NP concentrations over 24 hours at 25°C. The seedlings were grown in greenhouses, and physiological and biochemical differences between treatment groups were measured in 30-day-old seedlings.

2.5 Plant material and growth parameter

The Fenugreek experiment was conducted in the greenhouse. Blocks that were completely randomized were used as the experimental design. Samples were taken 30 days after sowing (DAS) to evaluate the crop's performance in terms of growth parameters. Each treatment had four repetitions, each containing fifty plants. For healthy plants, measurements were made of the weight of each pot, the lengths of the shoots and roots, and the germination of the seeds.

2.6 Determination of seedlings growth and biomass

Distilled water was used to properly wash the Ag NP and control seedlings to remove the nutrients and nanoparticles. Growth studies were then performed on the seedlings. After the plants were harvested, their fresh weight (FW) was immediately measured using an electronic balance. The FW was then separated into leaves and stems and stored in an ultradeep freezer at − 80°C for further analysis.

2.7Analysis of chlorophyll and carotenoid content

Fresh leaf samples were extracted using a mortar and pestle with 90% (v/v) acetone (1:10 w/v). After centrifuging the resultant mixture for 10 minutes at 7000 g, the clear supernatant was collected, and a double-beam spectrophotometer (Thermo Scientific, Genesys 150-UV-Visible Spectrophotometer, Waltham, MA, USA) was used to measure the absorbance (A) at 661.5, 663, 645, and 450 nm. The formula created by Lichtenthaler[22]was used to determine the concentrations of chlorophyll a (Chl.a), chlorophyll b (Chl.b), and total chlorophyll (Chl.t).

$$\text{Chl.a } (\mu g\ mL^{-1}) = 11.24A661.5 - 2.04A645$$

$$\text{Chl.b } (\mu g\ mL^{-1}) = 20.13A645 - 4.19A661.5$$

Chl.t (μg mL^{-1}) = 7.05A661.5 + 18.09A645

$$\text{Total carotenoids} = \frac{1000\times A450 - [1{:}9\times \text{chl(a)} + 63{:}14\times \text{chl(b)}]}{214}$$

2.8 Sample collection

One gram of leaf material was weighed and put into a sanitized container using a mortar and pestle. To guarantee complete extraction, 1 mL of an 80% alcohol solvent was then added to the container. The container was firmly closed and agitated in a gyratory shaker at 100 g for 30 minutes to speed up the extraction process. The container was then centrifuged for 10 minutes at 10,000 g in order to separate the liquid and solid components. The resultant pellet containing the extracted substance was meticulously gathered and put through the same extraction procedure again to guarantee optimal extraction effectiveness.

The integrity of the extracted material was maintained by carefully combining the two supernatants from the first extraction and re-extraction processes and transferring them into an amber tube to protect the contents from light interference. Each extraction and re-extraction phase was carried out three times to guarantee thorough extraction and optimize the production of the intended components.

2.9 Total phenolic content determination

After carefully pipetting 0.1 mL of the known extract volume into a test tube, 3 mL of distilled water was added. The mixture was then incubated for three minutes with the addition of 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 20% (w/v) Na_2CO_3 solution. After that, the tube was held in hot water for a minute while being gently vortexed. The absorbance at 650 nm was measured once the contents of the tube had cooled. The amount of phenolics in each sample was recorded using a gallic acid reference graph. In 1965, Sngleton and Joseph Rossi[23] calculated TPC as GAE/100g leaf weight (FW).

2.10 Total flavonoid content determination

To get the proper concentration, 100% ethanol was combined with the TFC of each crude extract. The diluted sample was then mixed with 1 mL of a 2% (w/v) methanolic AlCl3 solution. A double-beam spectrophotometer was used to assess the reaction mixture's absorbance at 430 nm following a 15-minute room temperature waiting period. The total milligrams of gallic acid equivalents (GE) per 100 grams of leaf material were determined using this method[24].

2.11 Antioxidant activity

2.11.1 Total Antioxidant activity

The experiment treated a 0.3 mL extract containing 2 mg mL^{-1} with 4 mmol L^{-1} of ammonium molybdate and 28 mmol L^{-1} of sodium phosphate. After that, the combination was heated to 950C and given 90 minutes to react. Following the solution's cooling to room temperature, a Thermo Scientific Genesys 150-UV-Visible double-beam spectrophotometer in Waltham, Massachusetts, USA, was used to measure the absorbance at 695 nm. Grams of ascorbic acid equivalent (AAE) per 100g of fresh weight (FW) was the unit of measurement for total antioxidant activity (TAA)[25].

2.11.2 Assay for ferric-reducing power antioxidants (FRAP)

For every extract, the following process was used: For 30 minutes, 2.5 mL of 1% (w/v) $K_3Fe(CN)_6$ and 2.5 mL of 0.2 mol L^{-1} phosphate buffer (pH 6.6) were incubated at 50°C. Following incubation, 2.5 mL of 10% (v/v) TCA was added, and the mixture was centrifuged for 10 minutes at 1000 g. After that, 2.5 mL of distilled water, 2.5 mL of upper-layer solution, and 0.1% (w/v) $FeCl_3$ were added. The absorbance at 700 nm was measured following each of the three repetitions of this process. Grams of AAE per 100 grams of leaf material were then calculated using the observed absorbance[26].

2.12 Diosgenin quantification

Diosgenin was extracted using the Paramesha et al. (2021)[27] technique of acid hydrolysis with ethanolic sulfuric acid. 15 cc of 2.5 M ethanolic sulfuric acid was added to 1 g of oven-dried (45°C) material after it had been finely macerated. After four hours of refluxing at 73–74°C, the mixture was allowed to settle to ambient temperature before being filtered through Whatman No. 1 paper. After using 10 M NaOH to neutralize the filtrate to pH 7.0, it was extracted three times using 15 ml of n-hexane each. With the aid of a rotary evaporator (Hei-VAP Advantage, Heidolph Instrument GmbH & Co. KG, Schwabach, Germany), the combined n-hexane fractions were evaporated at a lower pressure. After dissolving the residue in 1 milliliter of acetonitrile:water (9:1, v/v), it was filtered through a 0.45 µm membrane and put through HPLC analysis. Chromatographic separation was carried out using acetonitrile:water (90:10, v/v) at a flow rate of 1 ml/min, maintained at 35°C, on a C18 Luna column (250 × 4.6 mm, 12 nm, 5 µm) under isocratic conditions. The wavelength of detection was 194 nm. By comparing diosgenin to a standard reference substance (Sigma-Aldrich, Bangalore, India), it was possible to identify and quantify it.

2.13 Statistical analysis

The experiment was run in triplicate, and the results are shown in the tables and figures. The t-test for estimated statistically significant differences at ($p < 0.05$) and the mean value SD ($n = 4$) are the findings.

3 Results

3.1 Nanoparticle Characterization

3.1.1 NP UV-visible spectroscopy

The extracts' observed colour changes from yellow to brown, caused by the harmonic conversion of silver nitrate to biogenic silver nanoparticles, provided a visual cue for the NPs synthesis. The

nanoparticles demonstrate the implication of an Ag + ion being converted to an Ag0. The biogenic silver nanoparticle peaks are located at R-Ag-408 (Fig. 1).

3.1.2 FTIR analysis

Biogenic AgNPs' FTIR examination revealed strong peaks at 3286, 2191, 1630, 1102, 927, and 586 cm^{-1}. These peaks corresponded to the following: The main amine of the protein is stretched N-H; alkanes are stretched C-H; conjugated alkanes are stretched C = C; the protein's methylene tails (CH3-R); polyphenolic aliphatic amines are stretched C-N; and stretches O-H. The silver nitrate FTIR investigation showed strong peaks at 3318, 2102, 1634, 1351, 1165, 1120, 980, 948, 925, and 566 cm^{-1}. The related peaks were as follows: C = O stretching of carboxylic acid, N-H stretching of amines, C-C aromatic stretching, C-H rocking vibration of alkanes, ring C-C-C symmetric bending, and alkane C-H stretching (Fig. 1).

3.1.3 TEM analysis.

Transmission electron microscopy (TEM) was used to further investigate the size and morphology of the blended silver nanoparticles. The TEM pictures showed that the integrated silver nanoparticles had a spherical, polydisperse form with a diameter between 25 and 45 nm (Fig. 1)

3.2 Plant growth

The data in Table 1 reveal that treating fenugreek seeds with nanopaticles had varying effects on seedling growth during an 8-week period in pots. The effect of various AgNP concentrations on shoot length, root length, shoot weight, and root weight in fenugreek plants. Foliar spraying of fenugreek plants with varying AgNP concentrations improved all these growth parameters compared to untreated plants. The data demonstrated a gradual rise in shoot length, number of leaves per plant, and shoot weight as AgNP concentrations climbed from 0 to 80 mg/L. At 80 mg/L, the reaction reduced but remained higher than the control. The maximum response in all growth criteria was observed with 60mg/L AgNPs.

Table 1
Effect of NPs on physiological attributes of Fenugreek seedling after 30day.

NP Treatment	Shoot length (cm)	Root length(cm)	Seedling length(cm)	Shoot weight (gm)	Root weight(gm)	Seedling Weight(gm)
Control	15.7 ± 1.58f	8 ± 1.49f	23.7 ± 2.03f	0.45 ± 0.07de	0.044 ± 0.01cde	0.494 ± 0.08c
Rh5	15.9 ± 2.11e	8.9 ± 1.19d	24.8 ± 2.6d	0.49 ± 0.20b	0.10 ± 0.02a	0.59 ± 0.22b
Rh10	19.6 ± 1.07a	9.5 ± 1.84a	29.1 ± 1.72a	0.65 ± 0.07a	0.05 ± 0.01cd	0.7 ± 0.08a
Rh15	18.32 ± 0.91b	9.31 ± 0.62b	27.63 ± 1.38b	0.47 ± 0.10c	0.11 ± 0.04a	0.58 ± 0.14b
Rh20	12.6 ± 1.58l	8 ± 2.62f	20.6 ± 1.84k	0.26 ± 0.07i	0.06 ± 0.01b	0.32 ± 0.08g
Ag 5	13.77 ± 0.8j	7.11 ± 1.45h	20.88 ± 1.18j	0.38 ± 0.1g	0.06 ± 0.015cd	0.44 ± 0.115ef
Ag10	12.66 ± 1.5k	7.44 ± 2.96g	20.1 ± 1.76m	0.26 ± 0.05i	0.05 ± 0.01cd	0.31 ± 0.06g
Ag15	16.51 ± 1.44c	9.13 ± 1.05c	25.64 ± 1.89c	0.45 ± 0.05e	011 ± 0.06a	11.45 ± 0.06e
Ag20	14 ± 1.88i	8.9 ± 1.79d	22.9 ± 2.388h	0.508 ± 0.09b	0.08 ± 0.02b	0.588 ± 0.02b
RAg 5	15.09 ± 0.83h	5.2 ± 1.39j	20.29 ± 1.29l	0.46 ± 0.09cde	0.03 ± 0.01e	0.49 ± 0.1d
RAg 10	15.33 ± 1.5g	6.66 ± 2.29i	21.99 ± 1.86i	0.36 ± 0.09h	0.06 ± 0.015c	0.42 ± 0.105f
RAg 15	15.7 ± 1.63f	8.8 ± 2.14e	24.5 ± 2.026e	0.396 ± 0.10f	0.044 ± 0.012de	0.44 ± 0.112ef
RAg 20	16.4 ± 1.42d	6.7 ± 1.76i	23.1 ± 1.887g	0.467 ± 0.13cd	0.062 ± 0.02c	0.529 ± 0.15c

The maximum shoot length for Rhizopus extract foliar spray was 19.6 ± 1.07 cm at a dosage of 40mg/L. Chemically produced AgNPs had a maximum shoot length of 60mg/L, while RAgNPs show a maximum response of 16.4 ± 1.42 cm at 80mg/L, compared to 15.7 ± 1.58 cm in the control group.

The Rhizopus extract improved root length by 9.5 ± 1.84 cm at 40mg/L, AgNPs by 9.13 ± 1.05 cm at 60mg/L, and RAgNPs by 8.8 ± 2.14 cm at 60mg/L when compared to control treated seedlings 8 ± 1.49 cm.

The average shoot and root weights followed a similar pattern for the relevant nanoparticle treatment as the shoot and root lengths. Seedlings treated with Rhizopus extract at 40mg/L had a maximum shoot weight of 0.65 ± 0.07gm, compared to the control group's 0.45 ± 0.07gm. Seedlings treated with Rhizopus extract at 40mg/L showed larger average root weights (0.11 ± 0.04 gm) than the control (0.044 ± 0.01 gm).

AgNP-treated seedlings had the same shoot weight as the control group (0.45 ± 0.07gm). Seedlings treated with 60mg/L AgNPs had larger average root weights (0.11 ± 0.06 gm) than the control (0.44 ± 0.01 gm).

Seedlings treated with 80mg/L RAgNPs produced a maximum shoot weight of 0.062 ± 0.02 gm, compared to 0.45 ± 0.07 gm in the control group. Seedlings treated with Rhizopus extract at 60mg/L had larger average root weights (0.529 ± 0.15 gm) than the control (0.044 ± 0.01 gm) (Table 1).

3.3 Photosynthetic pigments

Photosynthetic pigments of fenugreek leaves sprayed with different concentrations of Rhizopus extract, AgNPs and RAgNPs. The results revealed the significant increases in all photosynthetic pigment contents (chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll) in response to treatment with different concentrations. Increased AgNP concentrations resulted in a significant increase in photosynthetic pigments gradually up to 80mg/L. If nanoparticles foliar spray caused toxicity in developing seedlings, photosynthetic pigments decreased; however, if NPs increased seedling vigour, photosynthetic pigments increased relative to the control. The most effective treatment was 60 mg/L RAgNPs, which gave the highest increases in all photosynthetic pigments. Biogenic RAgNPs seedlings increased chlorophyll content by 34%, AgNPs shows 30% and Rhizopus extract shows 19% increase in chlorophyll content at 60 mg/L, 80mg/L, and 40mg/L respectively, and Carotenoids rose by 60%, 74%, and 3% at 40mg/L, 60mg/L, 40mg/L for RAgNPs, AgNPs, and Rhizopus Extract treatments (Fig. 2).

3.4 Influence of NPs on total phenolics, flavonoid contents

The Phenolic and flavonoid content varied significantly among the treatments, with the most pronounced changes observed at the 60 mg/L foliar application level. Total phenolic content increased by approximately 121% in plants treated with Rhizopus-mediated silver nanoparticles (RAgNPs), 51% in those treated with chemically synthesized AgNPs, and 92% in plants receiving Rhizopus extract alone, compared to the untreated control. A similar pattern was recorded for flavonoid accumulation, where RAgNPs, AgNPs, and Rhizopus extract treatments enhanced levels by 87%, 74%, and 47%, respectively, over control values.

The higher accumulation of phenolics and flavonoids in RAgNP-treated plants suggests that these biologically synthesized nanoparticles act as stronger secondary metabolism elicitors than chemically synthesized nanoparticles or the fungal extract alone. This enhanced response may be attributed to the synergistic effect of silver’s nanoparticulate form—capable of generating localized stress signals—and

bioactive metabolites from Rhizopus sp., which can trigger phenylpropanoid pathway activation together. The resulting upregulation of key enzymes such as phenylalanine ammonia-lyase (PAL) likely drives the increased phenolic and flavonoid biosynthesis, enhancing the plant's antioxidant defense potential (Fig. 3).

3.5 Effect of nanoparticle on TAA and FRAP

Foliar application of nanoparticles at an 80 mg/L concentration led to a marked enhancement in the total antioxidant activity of Fenugreek seedlings, indicating an adaptive physiological response to nanoparticle-induced stress. Compared with the untreated control, total antioxidant activity increased by approximately 30% in plants treated with Rhizopus-mediated silver nanoparticles (RAgNPs), 60% in those treated with chemically synthesized AgNPs, and 30% in plants receiving Rhizopus extract alone. This suggests that while all treatments activated antioxidant defense systems, chemically synthesized AgNPs elicited the strongest overall antioxidant response at this concentration, potentially due to a higher oxidative stress load that required greater detoxification.

In parallel, the ferric reducing antioxidant power (FRAP) assay revealed a significant improvement in the reducing potential of plant extracts under 60 mg/L nanoparticle foliar treatment. Relative to the control, FRAP activity was elevated by 82% for RAgNP-treated plants, 57% for AgNP-treated plants, and 48% for plants treated with Rhizopus extract. The stronger FRAP enhancement in RAgNP-treated plants at this concentration indicates that biologically synthesized nanoparticles may more efficiently stimulate the biosynthesis or activation of non-enzymatic antioxidants (e.g., phenolics, flavonoids, ascorbate) that contribute to ferric ion reduction capacity. Together, these results suggest that while nanoparticle treatments enhance both antioxidant activity and reducing power, the optimal concentration and nanoparticle type determine whether the effect is predominantly a stress-mitigation response or a metabolic activation phenomenon (Fig. 4).

3.6 Diosgenin quantification

To investigate the impact of Ag-NPs on diosgenin biosynthesis, the diosgenin content of the treated and control seedlings was measured. Hexane was employed to extract diosgenin from the seedlings. After dissolving the obtained extract in methanol, diosgenin was quantified using the HPLC Method and UV Spectroscopy. Using UV spectroscopy, the concentration of diosgenin in the treated seedlings increased by 5.64 ± 0.23 mg/100gm (36%), 11.24 ± 0.26 mg/100gm (172%), and 11.32 ± 0.21 mg/100gm (174%) for Rhizopus extract, AgNPs, and RAgNPs at 80, 80, and 60 mg/L nanoparticle concentrations, respectively. It was 4.13 ± 0.20 mg/100gm for the untreated (control) group. With Rhizopus extract, AgNPs, and RagNPs at 60, 80, and 60 mg/L nanoparticle concentrations, respectively, the concentration of diosgenin in the treated seedlings increased by 6.49 ± 0.85 mg/100gm (33%), 11.81 ± 0.26 mg/100gm (143%), and 12.49 ± 0.75 mg/100gm (157%). In contrast, the untreated (control) group had a concentration of 4.85 ± 0.52 mg/100gm (Fig. 5).

4 Discussion

The results of this study clearly demonstrate that foliar application of Rhizopus-mediated silver nanoparticles (RAgNPs), chemically synthesized AgNPs, and Rhizopus extract positively influenced multiple growth and biochemical traits in fenugreek plants. All tested concentrations, particularly 60 mg/L, significantly enhanced shoot length, root length, total seedling length, and both fresh and dry weights of shoots and roots compared with untreated controls. RAgNPs consistently induced the greatest improvements among the treatments, indicating a superior stimulatory effect on plant development.

Comparable growth-promoting responses to AgNPs have been reported in several plant species. Sadak (2019)[28] observed that low concentrations of AgNPs enhanced growth in common bean and corn, while increased growth parameters in wheat following AgNP foliar sprays. The mechanism behind this stimulation may involve modulation of ethylene signaling, reducing stress-induced ethylene levels that typically inhibit growth[29]. The physiological impact of AgNPs also depends on particle size and morphology. Syu et al. (2014)[30] demonstrated that decahedral AgNPs induced the highest root growth promotion in Arabidopsis thaliana, while spherical AgNPs had no such effect but instead triggered higher anthocyanin accumulation—evidence that nanoparticle shape can influence the direction of metabolic responses.

Beyond vegetative growth, all photosynthetic pigments—chlorophyll a, chlorophyll b, carotenoids, and total pigments—were significantly increased by nanoparticle treatments. Enhanced pigment content likely improves light capture efficiency and photosynthetic rate, resulting in greater carbohydrate production and biomass accumulation. These observations align with previous reports that AgNPs promote photosynthesis through effects on nitrogen metabolism and pigment biosynthesis[28,31]. Racuciu and Creange (2007)[32] further highlighted a dose-dependent effect: pigment stimulation at low AgNP concentrations but inhibition at high doses. The enhancement observed in our study may also be partially explained by the capacity of metal nanoparticles to improve energy conversion efficiency in photosynthetic systems[33] .

Yield parameters also improved significantly, with the highest values recorded for RAgNP treatment at 60 mg/L. Similar yield-enhancing effects of AgNPs have been observed in mung bean[34] and wheat[35], supporting the notion that yield gains may be a downstream outcome of improved growth, photosynthetic performance, and biochemical defense status.

Importantly, the study revealed substantial phenolic and flavonoid content increases, particularly in RAgNP-treated plants, where total phenolics rose by 121% and flavonoids by 87% at 60 mg/L. The enhanced accumulation of these metabolites suggests that RAgNPs act as stronger secondary metabolism elicitors than chemically synthesized AgNPs or Rhizopus extract alone. This effect is likely due to a synergistic interaction between the nanoscale silver core—capable of generating localized oxidative or stress signals—and bioactive fungal metabolites from Rhizopus sp. both can activate the phenylpropanoid pathway. The activation of phenylalanine ammonia-lyase (PAL) and related biosynthetic

enzymes likely underpins the observed increases in phenolics and flavonoids, compounds that play key roles in plant antioxidant defense[20,36,37].

These secondary metabolite responses were closely linked with improvements in antioxidant activity. Total antioxidant capacity increased by 30%, 60%, and 30% for RAgNP, AgNP, and Rhizopus extract treatments, respectively, at 80 mg/L. Furthermore, ferric reducing antioxidant power (FRAP) activity rose sharply, with the highest increase (82%) recorded in RAgNP-treated plants at 60 mg/L, followed by AgNPs (57%) and Rhizopus extract (48%). The stronger FRAP response in RAgNP treatments points to enhanced biosynthesis of non-enzymatic antioxidants (phenolics, flavonoids, ascorbate), reinforcing the connection between secondary metabolite induction and antioxidant defense.

The elicitation effect observed here mirrors findings from[38], who reported that oligosaccharides from Fusarium oxysporum enhanced diosgenin biosynthesis in Dioscorea zingiberensis. Diosgenin, a sapogenin with diverse biological functions, is known to contribute to disease resistance and overall plant vigor. Similarly, Syu et al. (2014)[30] found that AgNP exposure induced anthocyanin accumulation in Arabidopsis, while Sharna et al. (2012)[39] demonstrated dose-dependent stimulation of growth, antioxidant properties, and photosynthesis in *Brassica juncea*. Given that the role of AgNPs as nanoelicitors of medicinal and defense-related secondary metabolites is still relatively unexplored, the current findings are both novel and significant.

The enhanced diosgenin content observed in our fenugreek plants may result from activation of multiple signaling pathways, including those regulating 1-aminocyclopropane-1-carboxylic acid (ACC) metabolism and phytohormone production, as previously suggested for AgNP-treated plants[30]. Supporting literature also indicates that biosynthesized AgNPs can promote seed germination, protein and carbohydrate synthesis, and antioxidant enzyme activities[40].Taken together, our results support a multifaceted role of AgNPs—particularly RAgNPs—in promoting growth, improving photosynthetic efficiency, enhancing secondary metabolite accumulation, strengthening antioxidant defenses, and ultimately boosting yield.

5 Conclusion

This study demonstrates that foliar application of Rhizopus-mediated silver nanoparticles (RAgNPs) exerts a pronounced positive effect on fenugreek plants' growth, physiological performance, and biochemical profile. At the optimal concentration of 60 mg/L, RAgNPs significantly enhanced vegetative growth, photosynthetic pigment content, yield components, and the accumulation of phenolics, flavonoids, and diosgenin, alongside marked improvements in antioxidant capacity. These effects surpassed those achieved with chemically synthesized AgNPs or Rhizopus extract alone, indicating a synergistic advantage of the biological synthesis approach. The observed benefits are likely mediated through nanoparticle-induced activation of the phenylpropanoid pathway, modulation of ethylene signaling, and stimulation of both enzymatic and non-enzymatic antioxidant systems.

Overall, the findings highlight RAgNPs as potent nanoelicitors capable of improving crop productivity and enhancing the biosynthesis of pharmaceutically valuable metabolites. This work provides novel insights into the application of biologically synthesized nanoparticles in sustainable agriculture and medicinal plant biotechnology, paving the way for their potential use in precision crop management and value-added metabolite production.

Declarations

Author contribution

APV & KSM: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Validation, Writing original draft & editing (Equal). UK: Formal analysis, Writing review & editing. PG: Conceptualization, Supervision, Resources, Investigation, Data curation, Visualization, Validation, Writing review & editing.

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Ethics approval and consent to participate

Ethical declaration is not applicable for this article.

Conflict of Interest

The authors declare that they have no competing financial interests.

Data availability

The data supporting the results of this study are available upon request from the corresponding author Dr. Giridhar Parvatam (giridharp.cftri@csir.res.in).

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Figures

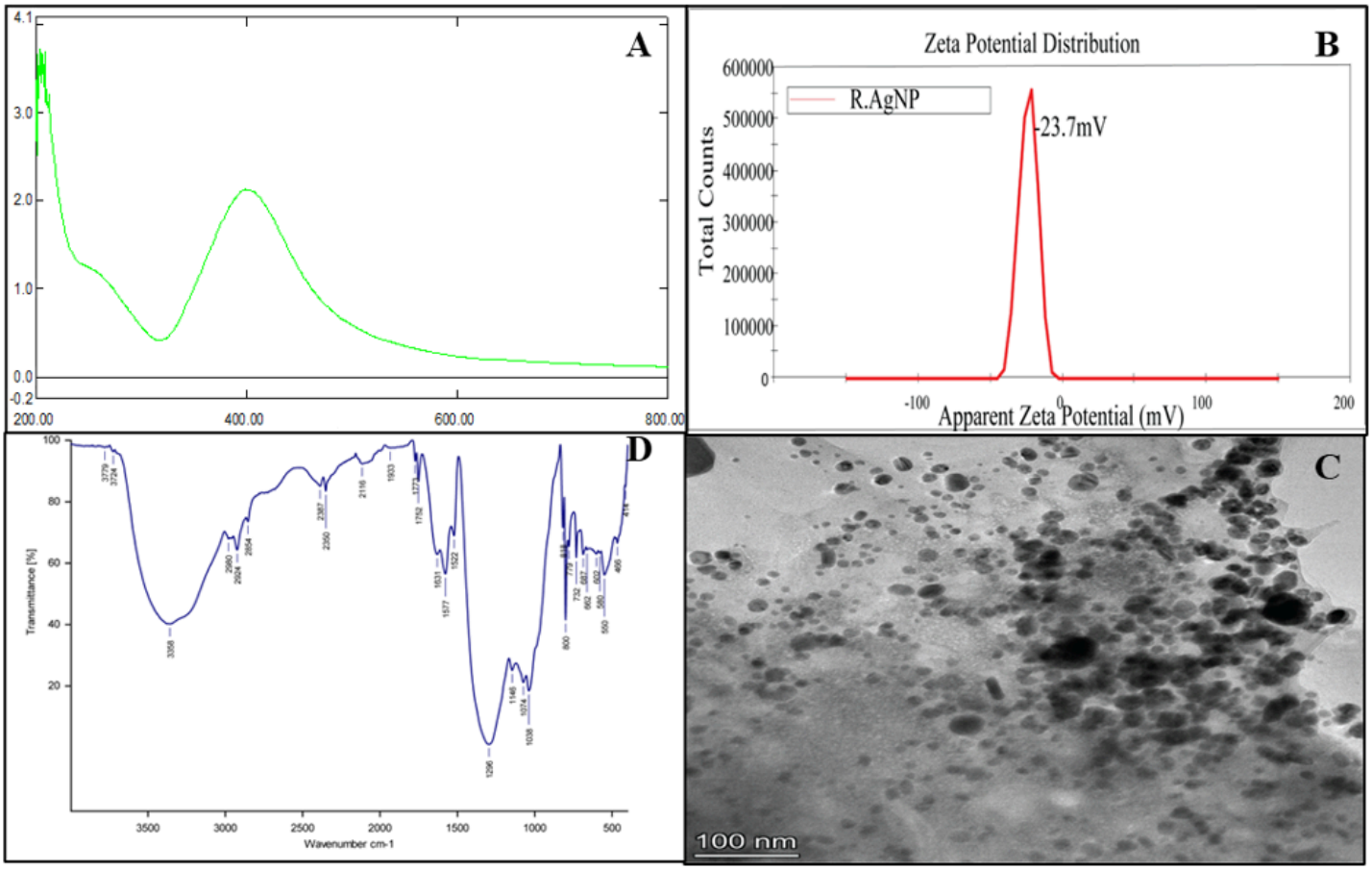


Figure 1

Characterization of nanoparticles. (A) UV Spectroscopy of R-Ag NPs (Rhizopus- Silver nitrate NPs); (B)Zeta Potential of Nanoparticle (C)R-AgNPs Fourier transform infrared spectroscopy (FTIR) (D) R-Ag NPs Transmission electron microscopy (TEM).

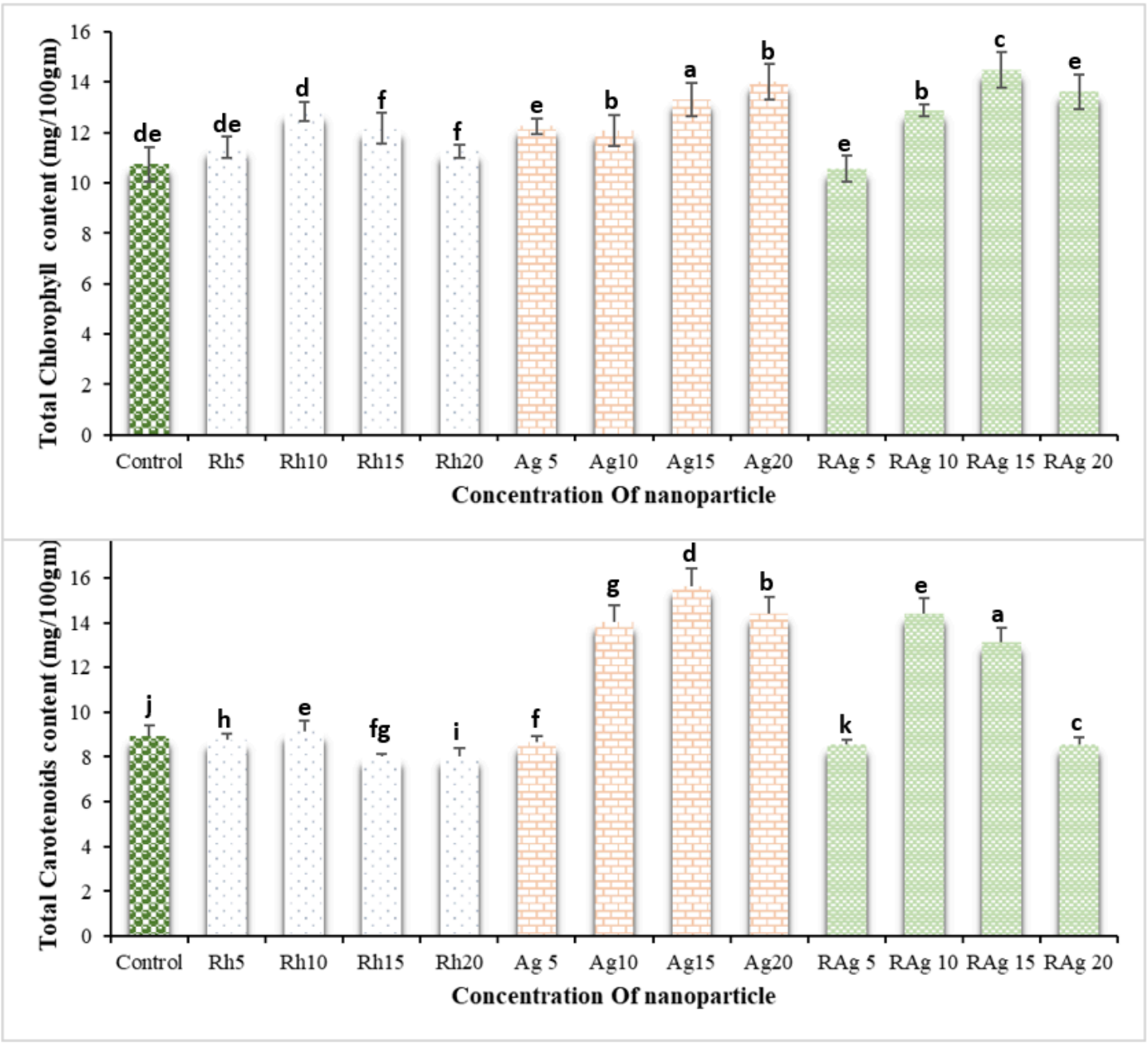


Figure 2

Foliar nanoparticles' impact on fenugreek seedlings' levels of carotenoids and chlorophyll. The averages of three separate replicates are shown by the standard deviation, values ± (n=4). At $P<0.05$, based on Tukey's Multiple Range Test.

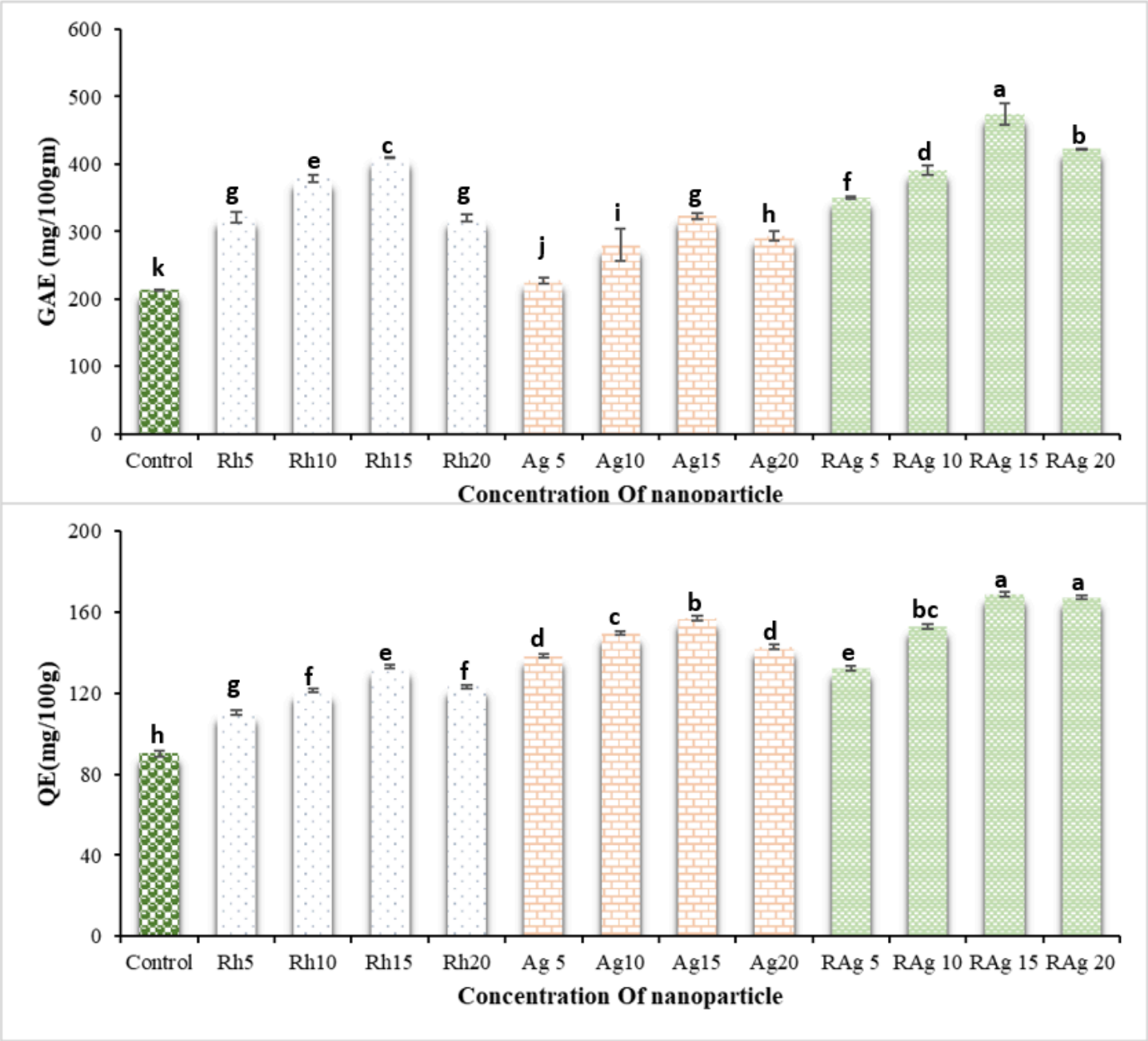


Figure 3

Foliar nanoparticle's impact on the fenugreek seedlings' total phenolic and flavonoid contents. The averages of three separate replicates are shown by the standard deviation, values ± (n=4). At P<0.05, based on Tukey's Multiple Range Test.

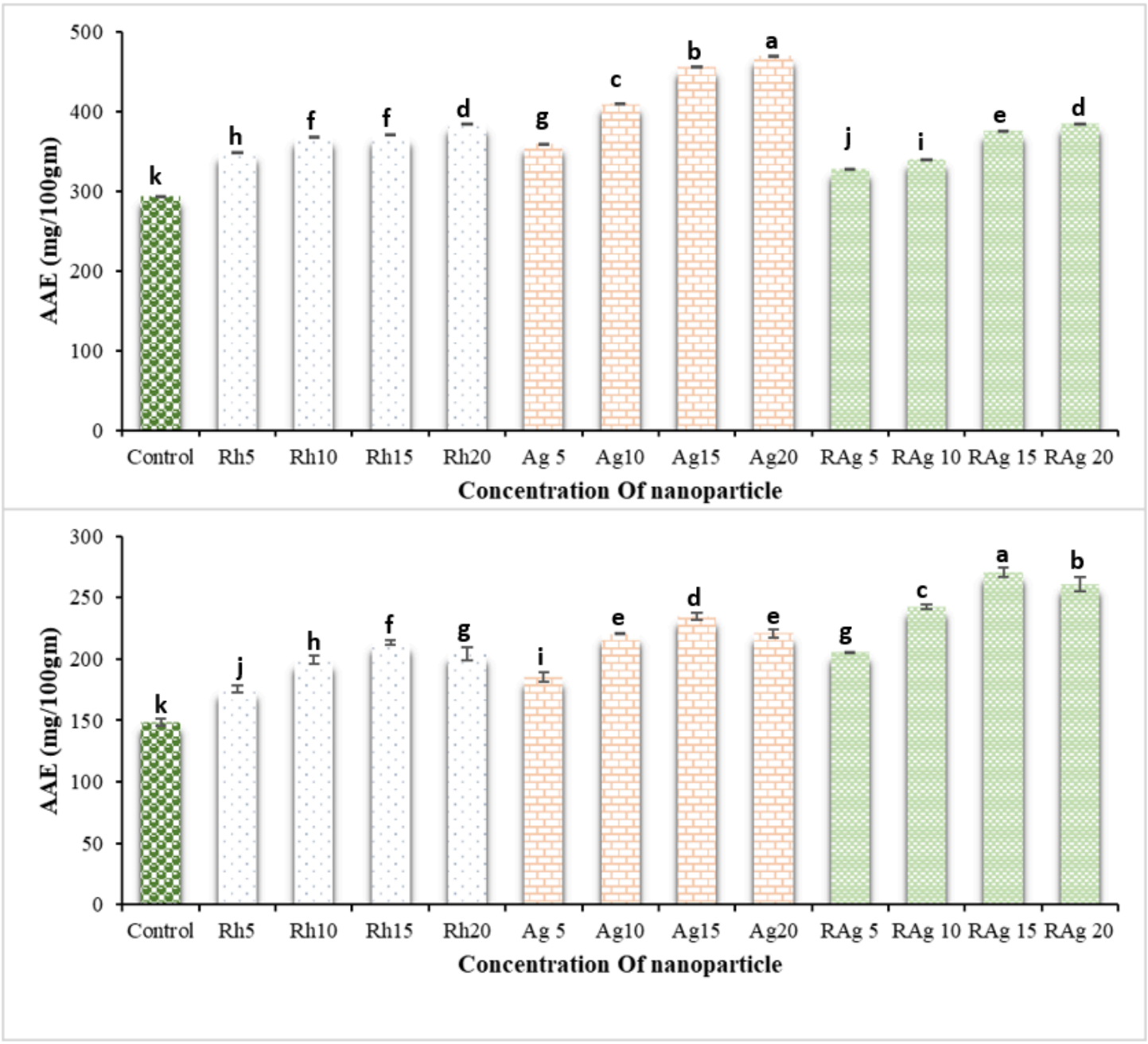


Figure 4

Foliar nanoparticle's impact on fenugreek seedlings' levels of FRAP and total antioxidants. The averages of three separate replicates are shown by the standard deviation, values ± (n=4). At $P<0.05$, based on Tukey's Multiple Range Test.

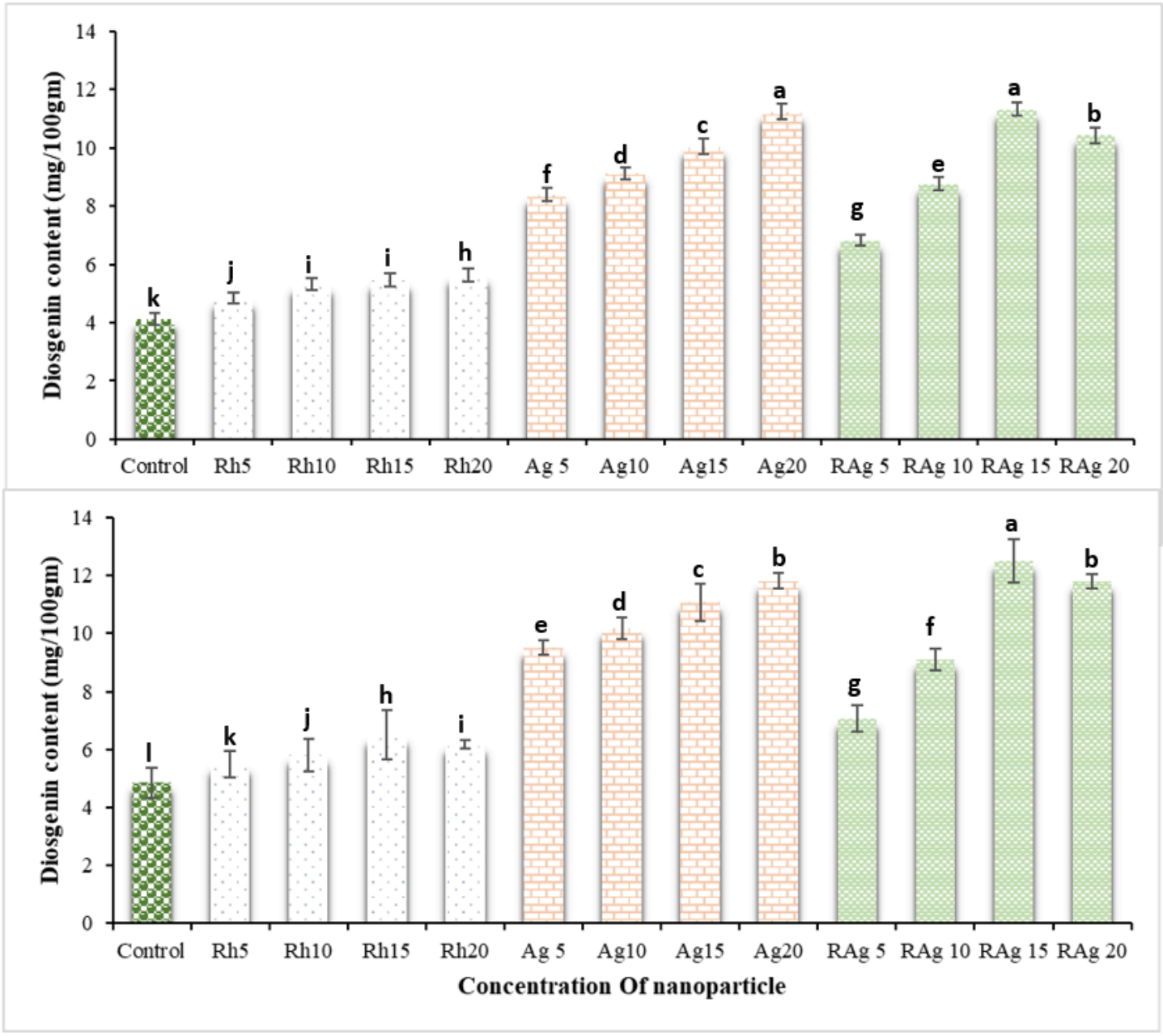


Figure 5

Foliar nanoparticle's impact on fenugreek seedlings' diosgenin content. The averages of three separate replicates are shown by the standard deviation, values ± (n=4). At P<0.05, based on Tukey's Multiple Range Test.



Figure 6

Fenugreek Seedling Treatment with foliar nanoparticles: seedlings 10 and 30 days old

Supplementary Files

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