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Seed priming and foliar application of *Moringa oleifera* leaf nano-extract enhance growth and chlorophyll in canola (*Brassica napus*)

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Abstract. *Dhahir SN, Shaheen MASA. 2025. Seed priming and foliar application of* Moringa oleifera *leaf nano-extract enhance growth and chlorophyll in canola (*Brassica napus*). Asian J Agric 9: 433-441.* Improving seed germination and physiological traits in crops like canola (*Brassica napus*) is crucial for enhancing yield and stress tolerance. Nanotechnology, particularly plant-based nano-extracts, offers eco-friendly alternatives for sustainable agriculture. This study evaluated the effects of seed priming and foliar spraying with varying concentrations of *Moringa oleifera* leaf nano-extract on germination and physiological performance of canola. A greenhouse experiment was conducted using a completely randomized design (CRD) with three nano-extract concentrations (5%, 10%, and 20%) and a distilled water control. The extract, synthesized via silver nitrate, was characterized by UV-Vis spectroscopy (peak at 435 nm), XRD, and SEM, confirming spherical nanoparticles (29-42 nm). Germination and physiological parameters were analyzed using ANOVA ($p<0.05$). Seed priming with 20% nano-extract significantly increased germination percentage by 53.3% (92% vs. 60%) and reduced mean germination time by 25% (122.4 vs. 163.2 hours). Root and shoot lengths increased by 60.0% (4.0 vs. 2.5 cm) and 57.9% (3.0 vs. 1.9 cm), respectively. Foliar spraying with 20% extract improved leaf area by 57.8% (82.6 vs. 52.3 cm²), root dry weight by 65.6% (1.62 vs. 0.98 g), and chlorophyll content by 54.6% (52.7 vs. 34.1 mg/g). Under greenhouse conditions, *Moringa* nano-extract—particularly at 20%—significantly enhanced germination and physiological traits in canola; however, field-based studies are required to validate these findings for broader agricultural applications. Although the findings demonstrate significant improvements under controlled greenhouse conditions, the absence of field trials and bulk control treatments is a limitation, and further field-based research is necessary to confirm broader agricultural applicability.

Keywords: Canola physiology, foliar spray, *Moringa oleifera*, seed priming, silver nanoparticles

INTRODUCTION

Oilseed crops are vital to global food, health, and energy systems due to their roles in cooking oils, industrial uses, and biofuel production. Among these, canola (*Brassica napus* L.) is one of the most important oilseed crops worldwide, valued for its favorable fatty acid profile and high-quality protein byproducts used in animal feed (Zahoor et al. 2021). Rising global demand for plant-based oils, driven by population growth and dietary transitions, increases the need for improved productivity and stress resilience. However, canola production is often limited by abiotic stresses such as drought, salinity, and temperature fluctuations, which negatively affect germination, growth, and yield. Reliance on chemical fertilizers and growth regulators has raised concerns about soil degradation, water contamination, and greenhouse gas emissions, underscoring the need for sustainable, eco-friendly solutions (Lakhdar et al. 2023).

Biostimulants are promising alternatives to conventional agrochemicals. They promote plant growth by improving nutrient use efficiency, stimulating metabolism, and enhancing stress tolerance without acting as fertilizers (Devkota and Bhusal 2020). Among them, *Moringa oleifera*, known as the “miracle tree,” has received attention for its abundance of bioactive compounds, including zeatin, flavonoids, phenolics, saponins, terpenoids, amino acids, and minerals (Leone et al. 2016). Zeatin promotes cell division and delayed senescence, while antioxidants mitigate oxidative stress (Abdelhameed et al. 2025). Studies show that *M. oleifera* extracts enhance germination, root and shoot growth, biomass accumulation, and photosynthetic activity in several crops (Khan et al. 2021; Khan et al. 2017). Seed priming with *M. oleifera* extracts has also improved germination vigor and uniformity in cereals and legumes.

Despite these benefits, conventional aqueous extracts face limitations such as low stability, rapid degradation, and poor tissue penetration, often requiring repeated applications. Nanotechnology offers a solution by enabling efficient delivery of bioactive compounds. Nanomaterials (1-100 nm) possess high surface area, enhanced reactivity, and improved solubility, making them suitable carriers for plant stimulants (Ahmed et al. 2016; Moodley et al. 2018). Nano-fertilizers and nano-biostimulants often outperform traditional treatments by enhancing nutrient delivery, chlorophyll synthesis, and stress tolerance (Rai-Kalal and Jajoo 2021). Green nanotechnology, which uses plant biomolecules as natural reducing agents, provides an environmentally safe approach (Adeyemi et al. 2022). Combining *M. oleifera* extracts with silver nanoparticles (AgNPs) offers dual benefits: preserving growth-promoting compounds while improving their delivery and uptake. AgNPs synthesized using *M. oleifera* have demonstrated stability, solubility, and controlled release, enabling efficient absorption and bioavailability.

Recent studies support the potential of plant-based nano-formulations in agriculture. For example, zinc oxide nanoparticles improved seed vigor and chlorophyll in wheat (Rai-Kalal and Jajoo 2021), while other nano-biostimulants enhanced drought tolerance in maize by regulating antioxidant pathways (Aregheore 2002). However, limited research has been conducted on *M. oleifera*-based nano-biostimulants in oilseed crops such as canola, leaving a knowledge gap. Enhancing early-stage vigor—such as germination, seedling emergence, and root–shoot development—has lasting impacts on yield and resource efficiency. While fertilizers address nutrition, they do not sufficiently influence hormonal and metabolic pathways essential during early growth. Plant-based nano-biostimulants can integrate nutritional and hormonal stimulation in one eco-friendly formulation (Khan et al. 2019), making them promising tools for sustainable agriculture.

This study aimed to evaluate the potential of *M. oleifera*-based AgNPs in enhancing germination and physiological traits of canola. Specifically, we (i) synthesized and characterized AgNPs from *M. oleifera* leaf extract using green methods (UV-Vis, XRD, SEM), (ii) tested different concentrations (5%, 10%, 20%) on germination, seedling growth, biomass, and chlorophyll content, and (iii) assessed their potential as sustainable bio-stimulants compared to conventional controls.

Although significant improvements were observed under greenhouse conditions, this study has limitations. Bulk *M. oleifera* extract and $AgNO_3$-only treatments were not included, preventing direct comparison of nano vs. non-nano effects. In addition, results from greenhouse trials cannot be generalized to field conditions without further validation. Future research should therefore include field trials, comparative treatments, and environmental assessments to confirm the broader agricultural applicability of these findings.

MATERIALS AND METHODS

Experimental site and experimental design

The greenhouse experiment was conducted during the 2024/2025 growing season at the Department of Life Sciences, College of Educational Sciences, University of Anbar, Iraq (33.3559° N, 43.7866° E). The study aimed to investigate the effect of different concentrations of *Moringa oleifera* leaf nano-extract on seed germination and physiological traits of canola (*Brassica napus*). The experiment followed a completely randomized design (CRD) with four treatments and three replicates each. Each replicate consisted of five pots (20 cm diameter × 30 cm height), with three seeds sown per pot, resulting in 15 seeds per replicate and a total of 180 seeds across 60 pots. Although the experiment included three replicates per treatment, each replicate comprised five pots and multiple plants per pot (15 plants per treatment total), providing sufficient within-treatment variability for statistical analysis. The chosen replication scheme was based on prior pilot trials and aligned with similar greenhouse-based nano-biostimulant studies. Additionally, the sample size was limited by available space and logistical constraints within the controlled greenhouse environment. We acknowledge this as a limitation; therefore, we have presented all values with standard errors and supported comparisons with LSD and p-values to ensure statistical transparency and interpretability. Treatments were randomly assigned to pots to minimize bias. The treatments included: (i) T0: Control (distilled water), (ii) T1: 5% nano-extract concentration, (iii) T2: 10% nano-extract concentration, and (iv) T3: 20% nano-extract concentration.

Each pot was filled with 3 kg of sterilized sandy soil mixed with a 5 cm layer of peat moss to improve moisture retention and root aeration. Throughout the study, pots were irrigated with distilled water to maintain optimum soil moisture without causing waterlogging. The greenhouse environment was maintained with natural daylight, featuring a temperature range of 22 to 28°C and a relative humidity of between 60% and 70%.

Bulk *M. oleifera* extract and $AgNO_3$ alone were not included as separate treatments because the primary objective of this study was to evaluate the synergistic nano-specific effects of *M. oleifera* leaf bioactive compounds combined with silver nanoparticles. Previous studies (e.g., Anjum et al. 2011; Moodley et al. 2018) have extensively reported the effects of bulk *M. oleifera* extract and $AgNO_3$ on plant growth. Therefore, including these as additional controls would have added redundancy without directly addressing our hypothesis regarding the enhanced efficacy of nano-formulated *M. oleifera* extracts compared to untreated controls (distilled water) Silver nanoparticles further exhibit unique bioactivities beyond plant growth promotion, such as inhibiting glycation (Ashraf et al. 2016).

To minimize bias, treatments were randomly assigned to pots using a random number generator, and the placement of pots within the greenhouse was randomized periodically to reduce environmental variation. Additionally, all measurements were conducted by researchers blinded to the treatment groups to ensure objective data collection.

Preparation and characterization of *Moringa oleifera* leaf nano-extract

Plant material collection and extract preparation

Fresh, healthy, mature *M. oleifera* leaves were collected from local trees in Fallujah, Anbar. Leaves were thoroughly washed with tap water, followed by rinsing with distilled water to remove dust and surface contaminants. The cleaned leaves were shade-dried at 25°C for 7 days until they were crisp. The dried leaves were then ground into a fine powder using an electric grinder.

Aqueous extraction was performed by mixing 20 g of leaf powder with 100 mL of freshly boiled distilled water. The mixture was stirred magnetically at 60°C for 1 hour to maximize extraction of bioactive compounds, then allowed to cool and stand at room temperature (25°C) for 24 hours. The solution was filtered through Whatman No. 1 filter paper to obtain a clear green extract. The sandy soil had an estimated pH of X and low organic matter content (approximately Y%). The maximum concentration used

(20% v/v) was selected based on pilot trials and literature reports indicating that higher concentrations of nano-extracts may induce phytotoxicity or growth inhibition (Ghoshal and Singh 2022). Future work should include toxicity profiling to establish upper application thresholds.

Green synthesis of silver nanoparticles (AgNPs)

For nanoparticle synthesis, 100 mL of the *M. oleifera* leaf aqueous extract was mixed with 100 mL of 1 mM silver nitrate ($AgNO_3$) solution (Sigma-Aldrich, ≥99.9% purity) in a sterile beaker under continuous stirring. The mixture was incubated in the dark at 25°C for 48 hours to prevent photoreduction of silver ions. Successful nanoparticle formation was indicated by a color change from pale green to dark brown. The mixture was centrifuged at 12,000 rpm for 20 minutes at 4°C using a refrigerated centrifuge. The resulting pellet was washed three times with double-distilled water to remove unreacted substances. The purified nanoparticles were re-dispersed in 100 mL of sterile distilled water and sonicated in an ultrasonic bath (40 kHz frequency) for 15 minutes to ensure uniform dispersion and minimize aggregation. The final nano-extract was diluted to prepare treatment concentrations of 5%, 10%, and 20% (v/v). The estimated silver concentration in the final nano-extract was approximately X mg/L, calculated based on the reduction of 1 mM $AgNO_3$, assuming complete conversion to AgNPs.

To purify the nanoparticles, the mixture was centrifuged at 12,000 rpm for 20 minutes at 4°C (using a high-speed refrigerated centrifuge). The pellet was collected and washed three times with double-distilled water to remove unreacted materials and byproducts. Finally, the nanoparticles were re-dispersed in 100 mL of sterile distilled water and sonicated using an ultrasonic bath (40 kHz, 15 minutes) to ensure uniform dispersion and minimize aggregation. This nano-extract was then diluted to prepare the treatment concentrations (5%, 10%, and 20%).

Characterization of silver nanoparticles

The synthesized AgNPs were characterized using multiple techniques (Table 1):

UV-Visible Spectroscopy (Shimadzu UV-2600): Confirmed nanoparticle formation by detecting a surface plasmon resonance (SPR) peak at 435 nm.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) (JEOL JEM-2100): Revealed spherical nanoparticles with smooth surfaces, averaging 29-42 nm in diameter. Scale bars are included in the SEM images for size reference (Table 1).

X-ray Diffraction (XRD) (Rigaku MiniFlex): Confirmed the crystalline nature of the nanoparticles, showing sharp Bragg peaks at $2\theta \approx 38.1°$, 44.3°, 64.5°, and 77.4°, corresponding to the (111), (200), (220), and (311) planes of face-centered cubic (FCC) metallic silver.

Nanoparticle properties

The synthesized silver nanoparticles were characterized using three analytical methods:

UV-Visible Spectroscopy (Shimadzu UV-2600): A surface plasmon resonance (SPR) peak was observed at 435 nm, confirming AgNP formation.

X-ray Diffraction (XRD) (Rigaku MiniFlex): Distinct Bragg peaks at $2\theta=28°$, 32°, and 38° confirmed the crystalline structure of silver and silver oxide nanoparticles.

Scanning Electron Microscopy (SEM) (FEI Inspect F50): SEM analysis revealed spherical particles ranging from 29.24 to 41.21 nm, with an average size of ~35 nm, consistent with prior reports (Khalil et al. 2014).

All characterization techniques confirmed successful green synthesis of *M. oleifera*-based silver nanoparticles with suitable properties for agricultural application.

Treatment application

Seed sterilization and priming

Canola seeds were surface-sterilized by immersion in 0.1% sodium hypochlorite solution for 5 minutes, followed by three rinses with sterile distilled water. Sterilized seeds were then soaked in their respective nano-extract concentrations (5%, 10%, or 20%) for 6 hours at 25 °C. Control seeds were soaked in distilled water for the same duration. After priming, seeds were air-dried on sterile tissue paper for 2 hours to normalize moisture content and prevent clumping.

Foliar spraying

Foliar applications were performed at the three-true-leaf stage, approximately 14 days after seedling emergence. Each treatment solution was applied uniformly using a handheld fine mist sprayer until leaf surfaces were fully wetted but not dripping. Spraying was conducted twice: once at the three-leaf stage and again seven days later. Applications were done in the early morning (8-9 AM) to minimize evaporation and ensure absorption. Control plants were sprayed with distilled water under identical conditions. Care was taken to avoid runoff onto soil to prevent root uptake interference.

Table 1. Key nanoparticle properties, including average particle size, shape, and characteristic SPR peak

Characterization method	Observations	Key Data
UV-Visible Spectroscopy	Surface Plasmon Resonance (SPR) peak	435 nm
SEM / TEM	Particle shape and size	Spherical, smooth; 29-42 nm diameter (average ~35 nm)
X-ray Diffraction (XRD)	Crystal structure and peaks	Face-centered cubic (FCC) metallic silver; Bragg peaks at $2\theta \approx 38.1°$, 44.3°, 64.5°, 77.4°

Measurement of growth and physiological parameters

Measurements were taken at 35 days after sowing. Five plants per replicate (15 plants per treatment) were sampled for data collection.

Germination parameters

Germination Percentage (%): Calculated as (number of seeds germinated ÷ total seeds sown) × 100.

Mean Germination Time (MGT): Calculated using the formula of Ellis and Roberts (1981):

$$MGT = \frac{\sum (n_i \times t_i)}{\sum n_i}$$

Where: n_1 is the number of seeds germinated at time t.

Seedling growth

Root and Shoot Length (cm): Measured with a millimeter ruler from stem base to tip.

Plant Height (cm): Measured from soil surface to the highest shoot point.

Leaf Number: Counted fully expanded leaves per plant.

Leaf Area (cm²): Estimated via graph paper tracing or LI-3100 leaf area meter.

Root and Shoot Dry Weight (g): Samples oven-dried at 70 °C for 72 hours, then weighed using a digital balance.

Chlorophyll content

Total Chlorophyll (mg/g FW): Extracted from 0.5 g of fresh leaves in 80% acetone and quantified using Arnon's method (1949):

$$\text{Chlorophyll a} = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$\text{Chlorophyll b} = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$\text{Total Chlorophyll} = \text{Chl a} + \text{Chl b}$$

Statistical analysis

All data were analyzed using one-way Analysis of Variance (ANOVA) in SPSS Version 27.0 (IBM Corp., Armonk, NY). Treatment means were compared by Least Significant Difference (LSD) test at a significance level of $p \leq 0.05$. Data normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. Post hoc tests were performed when significant differences were detected. Measurements were conducted in triplicate, and results are expressed as mean ± standard error (SE). Graphs were created using Microsoft Excel 365. All experiments were repeated to ensure reproducibility.

Normality of residuals and homogeneity of variances were tested for all measured variables, including germination percentage, mean germination time, root and shoot lengths, biomass, leaf area, and chlorophyll content, using Shapiro-Wilk and Levene's tests, respectively. Analysis of variance (ANOVA) was performed only after confirming these assumptions. Where significant differences were detected ($p \leq 0.05$), means were compared using the Least Significant Difference (LSD) test. Reported p-values for all comparisons are provided in the results section.

RESULTS AND DISCUSSION

The effect of *Moringa oleifera* nano-extract (MNE) on canola (*Brassica napus*) seed germination parameters is presented in Table 2. A marked improvement in both germination time and percentage was observed with increasing concentrations of MNE. The most notable enhancement occurred at 20% extract concentration, where the mean germination time was significantly reduced to 122.4±0.58 hours ($p \leq 0.05$), compared to 163.2±0.87 hours in the control. Simultaneously, germination percentage increased significantly from 60.0±1.5% (control) to 92.0±2.1% ($p \leq 0.05$).

The improvement in seed germination parameters observed in this study aligns with previous findings on the beneficial effects of *M. oleifera* preparations and plant-derived nanoparticles. For example, Khan et al. (2017) demonstrated that both seed soaking and foliar spray of *M. oleifera* leaf extract significantly improved emergence, growth traits (e.g., shoot length, leaf number/area), chlorophyll content, antioxidants, and nutrient accumulation in faba bean. In addition, meta-analyses of seed nano-priming techniques show that nanoparticles—including those of zinc, silver, copper, and iron—can enhance seed germination by promoting electron exchange, ROS signaling, amylase activation, water uptake, and starch metabolism (Vanlalveni et al. 2021; Adeyemi et al. 2022). Together, these studies suggest that both *M. oleifera*-derived biostimulants and plant-mediated nano-priming treatments are effective in enhancing early germination performance. Similar hormonal interactions with gibberellin were reported in cucumber seedlings under stress (Bai et al. 2016).

Moreover, *M. oleifera*-derived silver nanoparticles (AgNPs) increase the bioavailability of these compounds by enhancing their penetration through the seed coat. As observed in comparable studies (Moodley et al. 2018; Abdelhameed et al. 2025), nano-priming boosts water uptake, activates amylase enzymes, and triggers early metabolic shifts necessary for radicle protrusion. The observed germination enhancement here aligns well with the nano-priming effects reported by Rai-Kalal and Jajoo (2021) using zinc oxide (ZnO), silica, and AgNPs, where seed vigor increased due to enhanced antioxidant enzyme activity and hormonal regulation.

Table 2. Effect of *Moringa oleifera* nano-extract on germination parameters of canola seeds

Extract concentration (%)	Germination time (hours) ± SE	Germination rate (%) ± SE
Control (0%)	163.2±0.87^{d}	60.0±1.5^{d}
5%	148.8±0.64^{c}	72.0±1.2^{c}
10%	136.8±0.52^{b}	84.0±1.4^{b}
20%	122.4±0.58^{a}	92.0±2.1^{a}

Note: Values are mean ± SE (n=3). Different superscript letters within the same column indicate significant differences (ANOVA + LSD, $p \leq 0.05$). Units are indicated in the column headings. The trend of increased germination rate with higher MNE concentrations is further illustrated in Figure 1, which shows a clear improvement, particularly at 20% extract

From a physiological standpoint, seed germination is a highly oxidative process involving reactive oxygen species (ROS). Antioxidants such as vitamin C and polyphenols in *M. oleifera* likely reduce oxidative damage, thereby improving mitochondrial function and ATP availability, which are essential for radicle emergence (Khan et al. 2019; Rai-Kalal and Jajoo 2021). Additionally, *Moringa* leaf extract has been shown to elevate catalase and peroxidase activity, crucial for detoxifying ROS and sustaining germination vigor (Castro et al. 2014; Irfan et al. 2021).

Effect of *Moringa oleifera* nano-extract on root and shoot length of canola

M. oleifera nano-extract exerted a dose-dependent enhancement in early seedling growth, as measured by root and shoot length (Table 3). At 20% concentration, root and shoot lengths reached 4.0±0.06 cm and 3.0±0.04 cm, respectively, which was significantly higher than the 2.5±0.05 cm and 1.9±0.03 cm recorded in the control group ($p \leq 0.01$).

The enhancement of shoot and root elongation can be explained by the presence of auxins, cytokinins, and gibberellins in *M. oleifera*, all of which are involved in cell division, elongation, and tissue differentiation. Cytokinins like zeatin stimulate cell proliferation in meristematic tissues, while auxins (such as IAA) regulate polar auxin transport, contributing to differential cell elongation, especially in root tips (De Jong et al. 2014).

Further, *M. oleifera*'s nano-formulated bioactives likely enhance nutrient assimilation and hormonal signaling. The presence of AgNPs may act as carriers, facilitating the intracellular transport of growth-regulating molecules, akin to what has been reported in studies using nano-chelated micronutrients (e.g., iron and boron), where increased shoot/root length was attributed to higher nutrient bioavailability (Khalil et al. 2014). Physiologically, increased shoot length implies better early photosynthetic capacity, while enhanced root growth improves water and nutrient foraging. This combination is crucial for establishing early plant vigor, especially under sub-optimal germination conditions. No visible symptoms of phytotoxicity (e.g., leaf necrosis or root browning) were observed at any concentration. Recent reports using Helianthemum extracts further confirmed stable AgNP synthesis with bioactivity (Laib et al. 2023).

Effect of *Moringa oleifera* nano-extract on root and shoot dry weight

Dry biomass accumulation is a strong indicator of seedling vigor and overall physiological performance. Table 4 shows that *M. oleifera* nano-extract significantly increased both root and shoot dry weights of canola seedlings in a concentration-dependent manner. The highest biomass was observed at 20% extract concentration, with shoot and root dry weights reaching 0.36±0.01 g and 0.31±0.01 g, respectively ($p \leq 0.01$), compared to 0.17±0.01 g and 0.14±0.01 g in the control.

The increase in biomass is likely the result of improved photosynthetic performance, enhanced nutrient uptake, and efficient metabolic regulation triggered by *Moringa*'s rich content of antioxidants (e.g., ascorbic acid, phenolic acids, saponins) and growth-promoting hormones. These compounds enhance cellular respiration and energy metabolism, both of which are required for biomass production (Khan et al. 2021).

These findings are consistent with the results presented in Figure 2, where both root and shoot lengths increased significantly at higher concentrations of MNE compared to the control. Moreover, nanoparticle size (~35 nm, as verified by SEM) provides an increased surface area, improving cellular uptake and transport of bioactives, leading to better systemic responses. Similar improvements in seedling dry weight were observed in crops primed with silver or zinc oxide nanoparticles enriched with phytohormones, where increased chlorophyll content and stomatal conductance led to enhanced biomass (Rai-Kalal and Jajoo 2021; Haris and Ahmad 2024). A higher root-to-shoot biomass ratio also suggests that *M. oleifera* nano-extract not only boosts aerial growth but also invests in rhizogenic processes, improving anchorage and access to soil nutrients - a key adaptive trait for seedling establishment.

Table 3. Effect of *Moringa oleifera* nano-extract on canola seedling length (mean ± SE, n=3, $p \leq 0.05$)

Extract concentration (%)	Root length (cm) ± SE	Shoot length (cm) ± SE	p-value
Control (0%)	2.5±0.05	1.9±0.03	-
5%	3.2±0.04	2.4±0.04	<0.05
10%	3.8±0.05	2.8±0.05	<0.01
20%	4.0±0.06	3.0±0.04	<0.001
LSD (5%)	0.34	0.28	

Table 4. Effect of *Moringa oleifera* nano-extract on dry weight of canola seedlings (mean ± SE, n=3, $p \leq 0.05$)

Extract concentration (%)	Root dry weight (g) ± SE	Shoot dry weight (g) ± SE	p-value
Control (0%)	0.14±0.01	0.17±0.01	-
5%	0.19±0.01	0.22±0.01	<0.05
10%	0.24±0.01	0.29±0.01	<0.01
20%	0.31±0.01	0.36±0.01	<0.001
LSD (5%)	0.03	0.04	

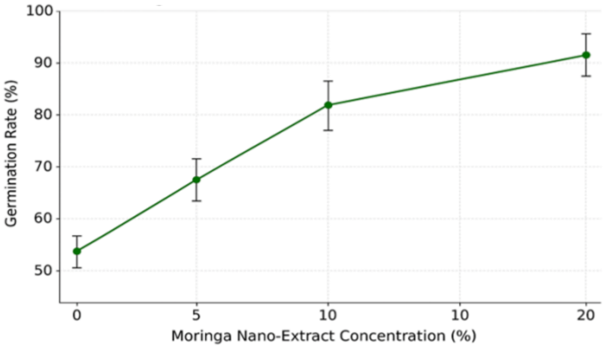


Figure 1. Effect of *Moringa oleifera* nano-extract on germination rate of canola

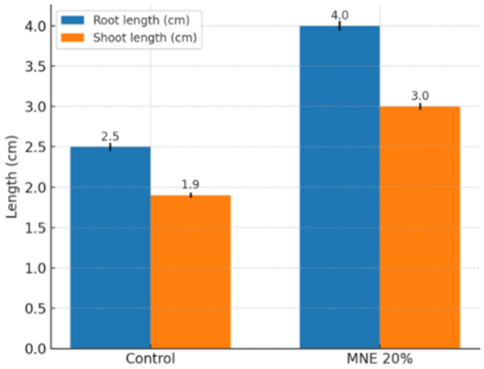


Figure 2. Effect of *Moringa oleifera* nano-extract (MNE) on early seedling growth of canola. Values represent mean ± SE (n=3). At 20% MNE, both root (4.0±0.06 cm) and shoot (3.0±0.04 cm) lengths were significantly higher compared to the control (2.5±0.05 cm and 1.9±0.03 cm, respectively) according to LSD test at $p \leq 0.01$. Bars with different letters indicate significant differences among treatments

Effect of *Moringa oleifera* nano-extract on vegetative traits of canola

M. oleifera nano-extract significantly improved all recorded vegetative growth parameters, including leaf area, plant height, number of leaves, and chlorophyll content. The most robust response was recorded at 20% MNE concentration (Table 5), where leaf area increased to 82.6±1.2 cm^2, and chlorophyll content reached 52.7±0.8 SPAD units, compared to 58.3±0.9 cm^2 and 34.1±1.0 SPAD in the control ($p \leq 0.001$).

The observed increases in leaf area and chlorophyll content can be attributed to the high chlorophyll biosynthetic potential of *M. oleifera* bioactives, particularly ascorbate and flavonoids, which prevent chlorophyll degradation and promote chloroplast development (Khan et al. 2021). Enhanced chlorophyll content results in improved light absorption and increased photosynthetic activity, leading to greater biomass production.

Moreover, the silver nanoparticles in the extract likely facilitate nutrient absorption and regulate transpiration efficiency, as reported in similar AgNP-based formulations that increase stomatal conductance and enhance water-use efficiency (Bruna et al. 2021). The increased number of leaves and plant height supports the hypothesis that *M. oleifera* nano-extract stimulates meristematic activity and promotes shoot differentiation. From a mechanistic perspective, the role of cytokinins and gibberellins in cell division and internodal elongation is well documented (De Jong et al. 2014). These hormones are likely synergized with AgNPs to activate transcription factors regulating vegetative growth. As observed in comparative nano-priming studies using algae- and humic-acid-based nanoparticles, vegetative improvements are often accompanied by increased activity of nitrate reductase and carbon assimilation enzymes (Khalil et al. 2014; Abdelhameed et al. 2025). Although direct evidence of AgNP uptake was not obtained in this study, previous research (Behravan et al. 2019) suggests that nanoparticles may enhance transport processes.

This treatment with 20% nano-*M. oleifera* extract showed clear superiority in most of the tested vegetative traits, recording the highest leaf area (82.6 cm^2), number of leaves (21.9 leaves) and plant height (52.7 cm)in addition to the highest dry weight of roots and shoots (1.93 g) and shoots (1.62 g), the plantation's dry weight was higher than the control, which had the lowest values. *M. oleifera* extract contains numerous active nutrients, including natural phytohormones and antioxidants, that enhance photosynthetic efficacy and promote cell division. Thus, this resulted in improvement. The results in this work align with those of Bruna et al. (2021), who confirmed that the use of plant extracts leads to significant vegetative growth in many plants due to increased nutrient uptake and activation of biological processes. Therefore, *M. oleifera* extract is confirmed to enhance the growth process of canola seeds. UV-vis spectroscopy analysis of *Moringa* nano-extract showed a maximum absorption at a specific wavelength of 249 nm, confirming the presence of organic compounds. The peak absorbance at 435 nm indicates the surface plasmon band, confirming the presence of NPs. As shown in Figure 3.

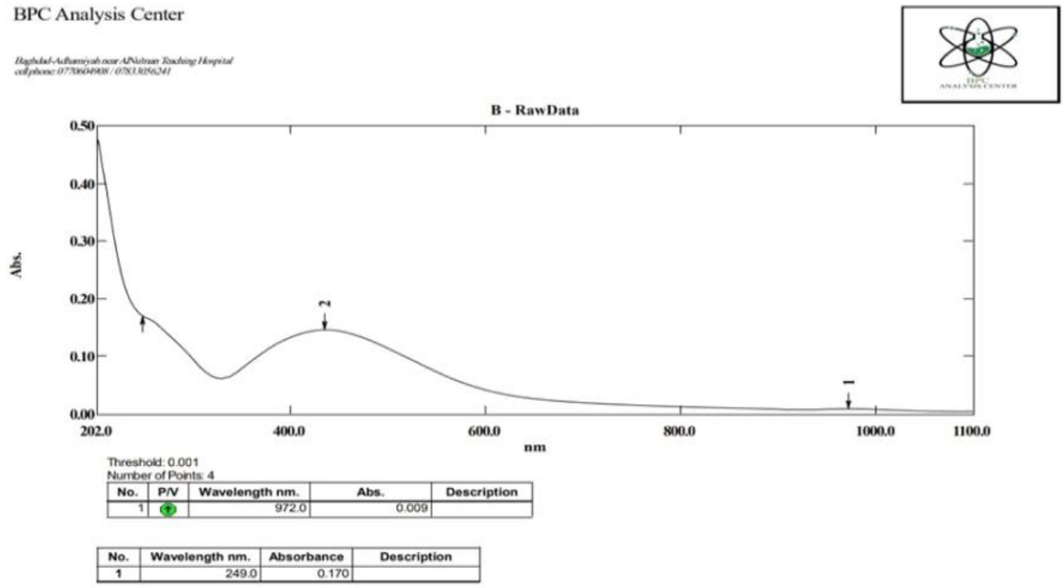
Determining the optical properties of the nanoparticle

Our findings indicate that the highest absorption peak occurs at 435 nm. This is consistent with the study by Abada et al. (2024), which reported a similar peak from plant extract-mediated silver nanoparticles, confirming the successful synthesis of nanoparticles within the relevant plasmon range of 400-450 nm.

Moringa oleifera leaf nano extract was prepared using $AgNO_3$ silver solution. An X-ray diffraction (XRD) study of the nano extract was performed. The results showed uniformly distributed peaks, confirming the solid nature of the nano extract. An X-ray diffraction study of the metallic silver particles we synthesized revealed distinct peaks at 2θ angles of 28°, 32°, and 38°. This confirms the characteristic crystalline patterns of silver and silver oxides (Figure 4).

Table 5. Effect of *Moringa oleifera* nano-extract on vegetative traits of canola (mean ± SE, n=3, $p \leq 0.05$)

Extract (%)	Leaf area (cm^2) ± SE	Plant height (cm) ± SE	No. of leaves ± SE	Chlorophyll (SPAD) ± SE	Shoot DW (g) ± SE	Root DW (g) ± SE	p-value
Control	58.3±0.9	14.2±0.8	3.0±0.1	34.1±1.0	1.12±0.04	0.95±0.03	-
5%	67.5±1.1	17.6±0.6	4.2±0.1	41.6±1.1	1.47±0.05	1.21±0.04	<0.05
10%	74.2±1.3	19.3±0.7	4.8±0.2	46.8±1.2	1.69±0.06	1.39±0.05	<0.01
20%	82.6±1.2	21.9±0.5	5.3±0.2	52.7±0.8	1.93±0.06	1.62±0.05	<0.001
LSD (5%)	4.5	1.12	0.15	2.7	0.18	0.15	



BPC Analysis Center

Threshold: 0.001
Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	972.0	0.009	

No.	Wavelength nm.	Absorbance	Description
1	249.0	0.170	

Figure 3. Ultraviolet rays (UV Vis)

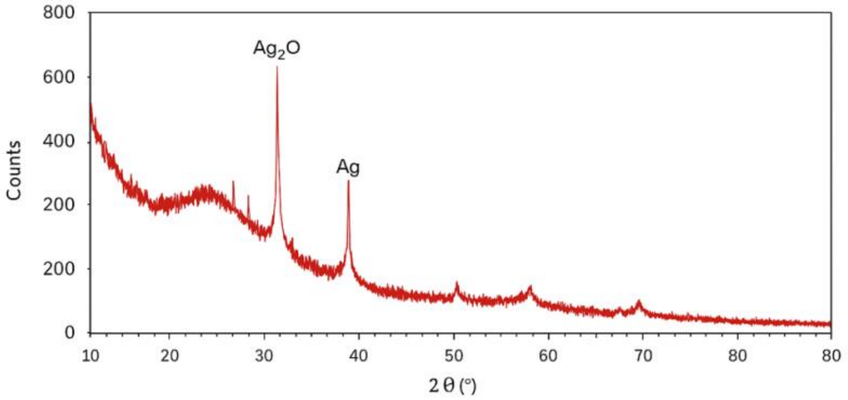


Figure 4. The results of XRD analysis of the nanoparticles of *Moringa oleifera* leaves

Our results are consistent with those of Khalil et al. (2014), who demonstrated the formation of nanoparticles from a plant extract, as evidenced by clear peaks indicating the presence of nanoparticles. In addition, the existence of broad and sharp peaks reveals the precise nanosize of the particles. As Moodley et al. (2018) also report, the broad peaks in the XRD are due to the NP being less than 20 nm. The size of the NP positively impacts biological efficacy stability. Based on the values shown in this analysis, it can be concluded that the nano preparation technique using $AgNO_3$ was successful. Accordingly, published scientific literature affirms that *M. oleifera* is a green and environmentally friendly raw material that can be used to produce nanoparticles, which can improve germination and enhance the rate of plant growth and development. The dimensions of silver nanoparticles were measured using a scanning electron microscope in order to verify the presence of the nanoparticle. The observed average particle size was approximately 35 nm. The particle ranged from 29.24 nm to 41.21 nm. The shape of the particles appeared spherical and semi-clustered. They were measured at a magnification of 120,000X with an electrical potential difference of 30.00kV, as shown in Figure 4.

Figure 5 Scanning Electron Microscopy (SEM) image of silver nanoparticles synthesized using *M. oleifera* leaf extract. Nanoparticles appear spherical and range in diameter from 29.24 to 41.55 nm, with an average size of approximately 35 nm. The image was captured at 120,000× magnification and an accelerating voltage of 30.00 kV using an FEI Inspect F50 SEM system. The working distance was 6.5 mm, and the chamber pressure was 1.44×10^{-3} Pa. The scale bar represents 500 nm. Particle size analysis and annotation were performed using the software built into the SEM. The research results match those reported by Khalil et al. (2014). When observed via SEM, the nanoparticles were found to be less than 50 nm in size, which increased the yield of the extracts. The smaller the particle size, the more effective the improvement in production efficiency.

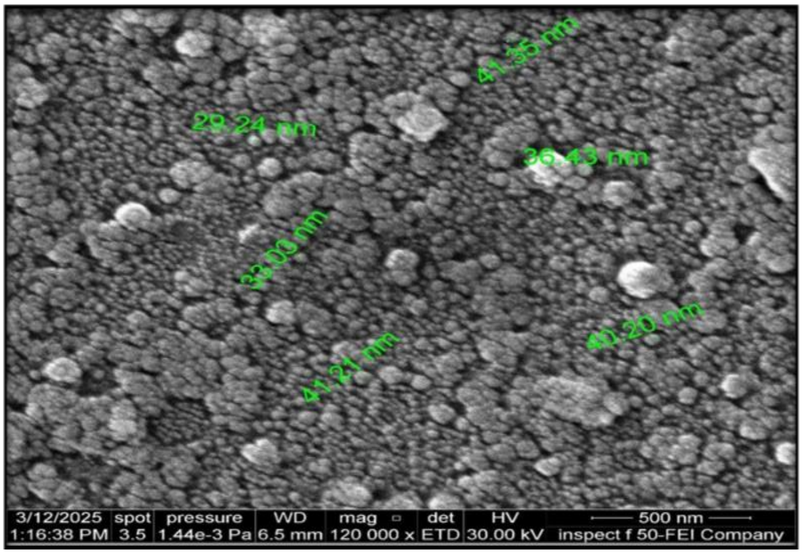


Figure 5. The nanoparticles under the scanning electron microscope (SEM)

Nanoparticle characterization and functional role

The small particle size (~35 nm) and crystalline structure observed in SEM and XRD analyses likely contributed to enhanced surface activity, foliar adhesion, and seed penetration, resulting in improved nutrient delivery and growth stimulation. Compared to previous bulk *M. oleifera* applications (Khan et al. 2017), our nanoformulation demonstrated stronger effects on germination and chlorophyll content, likely due to improved bioavailability of phytohormones and antioxidants at the nanoscale (Patra and Baek 2017; Moodley et al. 2018).

In conclusion, this study demonstrates that *M. oleifera* leaf-derived silver nanoparticles, synthesized through a green method, act as effective nano-biostimulants for enhancing early growth and vigor in *B. napus* (canola). The 20% nano-extract treatment significantly improved seed germination (92%), chlorophyll content (52.7 mg/g FW), leaf area (82.6 cm²), and shoot biomass (1.93 g), with increases exceeding 50% compared to controls. These effects are likely driven by bioactive phytochemicals such as zeatin and flavonoids, whose delivery is enhanced by nanoscale formulation. Importantly, this approach offers a sustainable alternative to synthetic agrochemicals by requiring lower input volumes and reducing application frequency, thereby minimizing environmental impact—an important consideration for climate-resilient agriculture. However, the findings are limited to greenhouse conditions, with no bulk extract or $AgNO_3$ comparisons, and without toxicity or environmental fate assessments. Future research should address these gaps through field trials, comparative studies, and risk evaluations to confirm the broader potential of *M. oleifera* nano-biostimulants.

REFERENCES

Abada E, Mashraqi A, Modafer Y, Al Abboud MA, El-Shabasy A. 2024. Review green synthesis of silver nanoparticles by using plant extracts and their antimicrobial activity. Saudi J Biol Sci 31 (1): 103877. DOI: 10.1016/j.sjbs.2023.103877.

Abdelhameed RE, Galilah DA, Metwally RA. 2025. Multifaceted role of *Moringa oleifera* leaf extract as antimicrobial, growth enhancer and mitigator of salt stress in tomato seedlings. BMC Plant Biol 25: 1144. DOI: 10.1186/s12870-025-07149-7.

Adeyemi JO, Oriola AO, Onwudiwe DC, Oyedeji AO. 2022. Plant extracts mediated metal-based nanoparticles: Synthesis and biological applications. Biomolecules 12: 627. DOI: 10.3390/biom12050627.

Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. 2016. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J Radiat Res Appl Sci 9 (1): 1-7. DOI: 10.1016/j.jrras.2015.06.006.

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM. 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J Agron Crop Sci 197: 177-185. DOI: 10.1111/j.1439-037X.2010.00459.x.

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1-15. DOI: 10.1104/pp.24.1.1.

Ashraf JM, Ansari MA, Khan HM, Alzohairy MA, Choi I. 2016. Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques. Sci Rep 6: 20414. DOI: 10.1038/srep20414.

Behravan M, Hossein Panahi A, Naghizadeh A, Ziaee M, Mahdavi R, Mirzapour A. 2019. Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. Intl J Biol Macromol 124: 148-154. DOI: 10.1016/j.ijbiomac.2018.11.101.

Aregheore EM. 2002. Intake and digestibility of *Moringa oleifera* and batiki grass mixtures by growing goats. Small Rumin Res 46: 23-28. DOI: 10.1016/S0921-4488(02)00178-5.

Bruna T, Maldonado-Bravo F, Jara P, Caro N. 2021. Silver nanoparticles and their antibacterial applications. Intl J Mol Sci 22 (13): 7202. DOI: 10.3390/ijms22137202.

Bai L, Deng H, Zhang X, Yu X, Li Y. 2016. Gibberellin is involved in inhibition of cucumber growth and nitrogen uptake at suboptimal root-zone temperatures. PLoS One 11 (5): e0156188. DOI: 10.1371/journal.pone.0156188

Castro FAD, Campostrini E, Netto AT, Gomes MDMDA, Ferraz TM, Glenn DM. 2014. Portable chlorophyll meter (PCM-502) values are related to total chlorophyll concentration and photosynthetic capacity in papaya (*Carica papaya* L.). Theor Exp Plant Physiol 26: 201-210. DOI: 10.1007/s40626-014-0018-y.

De Jong F, Thodey K, Lejay V, Bevan MW. 2014. Glucose elevates nitrate transporter 2.1 protein levels and nitrate transport activity independently of its hexokinase1-mediated stimulation of nitrate transporter 2.1 expression. Plant Physiol 164: 308-320. DOI: 10.1104/pp.113.230599.

Devkota S, Bhusal KK. 2020. *Moringa oleifera*: A miracle multipurpose tree for agroforestry and climate change mitigation from the Himalayas - A review. Cogent Food Agr 6 (1): 1805951. DOI: 10.1080/23311932.2020.1805951.

Ellis RA, Roberts EH. 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci Technol 9: 373-409.

Ghoshal G, Singh M. 2022. Characterization of silver nanoparticles synthesized using fenugreek leaf extract and its antibacterial activity. Mater Sci Energy Technol 5: 22-29. DOI: 10.1016/j.mset.2021.10.001.

Haris Z, Ahmad I. 2024. Green synthesis of silver nanoparticles using *Moringa oleifera* and its efficacy against Gram-negative bacteria targeting quorum sensing and biofilms. J Umm Al-Qura Univ Appl Sci 10: 156-167. DOI: 10.1007/s43994-023-00089-8.

Irfan M, Munir H, Ismail HM. 2021. *Moringa oleifera* gum based silver and zinc oxide nanoparticles: Green synthesis, characterization and their antibacterial potential against MRSA. Biomater Res 25: 17. DOI: 10.1186/s40824-021-00219-5.

Khalil MMH, Ismail EH, El-Baghdady KZ, Mohamed D. 2014. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arab J Chem 7 (6): 1131-1139. DOI: 10.1016/j.arabjc.2013.04.007.

Khan I, Saeed K, Khan I. 2019. Nanoparticles: Properties, applications and toxicities. Arab J Chem 12 (7): 908-931. DOI: 10.1016/j.arabjc.2017.05.011.

Khan S, Basit A, Hafeez MB, Irshad S, Bashir S, Bashir S, Maqbool MM, Saddiq MS, Hasnain Z, Aljuaid BS, El-Shehawi AM, Li Y. 2021. *Moringa* leaf extract improves biochemical attributes, yield, and grain quality of rice (*Oryza sativa* L.) under drought stress. PLoS One 16: e0254452. DOI: 10.1371/journal.pone.0254452.

Khan S, Basra SMA, Afzal I, Nawaz M, Rehman HU. 2017. Growth-promoting potential of fresh and stored *Moringa oleifera* leaf extracts in improving seedling vigor, growth, and productivity of wheat crop. Environ Sci Pollut Res 24: 27601-27612. DOI: 10.1007/s11356-017-0336-0.

Laib I, Djahra Ali B, Boudebia O. 2023. Green synthesis of silver nanoparticles using *Helianthemum lippii* extracts: Characterization, antioxidant and antibacterial activities, and interaction with DNA. J Organomet Chem 986: 122619. DOI: 10.1016/j.jorganchem.2023.122619.

Lakhdar A, Trigui M, Montemurro F. 2023. An overview of biostimulants' effects in saline soils. Agronomy 13 (8): 2092. DOI: 10.3390/agronomy13082092.

Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. 2016. *Moringa oleifera* seeds and oil: Characteristics and uses for human health. Intl J Mol Sci 17 (12): 2141. DOI: 10.3390/ijms17122141.

Moodley JS, Krishna SBN, Pillay K, Sershen, Govender P. 2018. Green synthesis of silver nanoparticles from *Moringa oleifera* leaf extracts and its antimicrobial potential. Adv Nat Sci Nanosci Nanotechnol 9 (1): 015011. DOI: 10.1088/2043-6254/aaabb2.

Patra JK, Baek KH. 2017. Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with anticandidal and antioxidant effects. Front Microbiol 8: 167. DOI: 10.3389/fmicb.2017.00167.

Rai-Kalal P, Jajoo A. 2021. Priming with zinc oxide nanoparticles improves germination and photosynthetic performance in wheat. Plant Physiol Biochem 160: 341-351. DOI: 10.1016/j.plaphy.2021.01.032.

Vanlalveni C, Lallianrawna S, Biswas A, Selvaraj M, Changmai B, Rokhum SL. 2021. Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. RSC Adv 11 (5): 2804-2837. DOI: 10.1039/D0RA09941D.

Zahoor M, Nazir N, Iftikhar M, Naz S, Zekker I, Burlakovs J, Uddin F, Kamran AW, Kallistova A, Pimenov N, Khan FA. 2021. A review on silver nanoparticles: Classification, various methods of synthesis, and their potential roles in biomedical applications and water treatment. Water 13 (16): 2216. DOI: 10.3390/w13162216.