




## Research Article

# Phytotoxicity and Antimicrobial Activity of Green Synthesized Silver Nanoparticles Using *Nigella sativa* Seeds on Wheat Seedlings

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Recently, the green synthesis of nanomaterials has grown in popularity and has become one of the most used approaches. Plant extracts are safe for the environment and could be cost-effective for nanoparticle preparation. Silver nanoparticles (AgNPs) have been synthesized using aqueous extracts of *Nigella sativa* (*N. sativa*) seeds. The formation of AgNPs was confirmed by using an X-ray diffractometer, a UV-visible spectrometer, and a transmission electron microscope. The phytotoxicity and genotoxicity of different AgNP concentrations (12.5, 25, 50, 75, and 100  $\mu\text{g}\cdot\text{L}^{-1}$ ) were evaluated by wheat (*Triticum aestivum* L.) seed germination. The results showed that AgNPs did not significantly affect germination, while root and coleoptile lengths decreased considerably. On the contrary, the biomass of seedlings markedly increased in response to AgNP treatments. Moreover, genotoxicity was detected, especially at high concentrations of AgNPs. DNA, RNA, and total soluble proteins of wheat seedlings significantly decreased. In addition, antimicrobial activities of biosynthesized AgNPs were detected.

## 1. Introduction

Silver nanoparticles (AgNPs) have attracted wide attention due to their multiple applications in antibacterial [1–4], photocatalytic [5], medical [6], optical [7–9], and electrical fields [10–12], leading to commercialized and industrial applications. AgNPs have been synthesized by various chemical methods, which are expensive and use toxic chemical reagents [13]. The green synthesis of AgNPs has received significant attention due to their environmental friendliness and can be further processed for industrial use [14–17]. Microorganisms (actinomycetes, bacteria, fungi, viruses, and yeast) and plant-mediated synthesis are primary

sources of NP synthesis by biological methods [18]. Compared to intracellular synthesis using microorganisms, using plant extracts to synthesize NPs has advantages as it does not require complex and specialized processes such as isolation, culture maintenance, and multiple purification steps. For the above reasons, researchers have mainly focused on developing green methods that utilize different parts of plants, e.g., from leaf [19–22], peel [23, 24], flower [23], fruit [25], and root [26]. Many compounds in plant extracts (such as polyphenols, flavonoids, ascorbic acid, terpenoids, and proteins) play essential roles in metal ion absorption, precursor salt reduction, and capping agents' inherent antibacterial properties [18, 22, 27–30]. With increasing

antibiotic resistance and the development of new antibiotics, research has begun to use these antibacterial nanoparticles as new medical devices.

Drug-resistant pathogens are considered a global threat of growing concern to human, animal, and environmental health, so there is a crucial demand for new and active metabolites [31]. AgNPs exhibit a strong antimicrobial activity and thus might be developed as a new type of antimicrobial agent for treating bacterial infections, including multidrug-resistant bacterial infections [32].

Silver is often used as nitrate for antibacterial effects, but when AgNPs are used, the surface area exposed to microorganisms greatly increases. The bactericidal effect of AgNPs on *Staphylococcus aureus*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Bacillus cereus*, *Listeria innocua*, *Salmonella*, and other bacteria has been confirmed [33, 34]. Cholerae suis nanoparticles [35–37] can lead to a wide variety of toxicological effects on human [38] environment [39], bacteria [40], and aquatic organisms [41]. Only a few studies on vascular plants showed that AgNPs have detrimental effects on plant growth [42, 43]. Plants are a significant trophic level. There are, however, only a few reports on the impact of nanomaterials on plants. There are, however, only a few studies on the impact of nanomaterials on plants reported the nanomaterials' uptake, accumulation, and transport within the plant, as well as the subsequent interactions with biomolecules such as nucleic acids, including proteins, enzymes, and cell structures [44, 45]. In addition, the presence of AgNPs significantly decreased wheat grains' amino acid and protein contents [46]. Moreover, in the case of *Arachis hypogaea* L., AgNPs were found to exhibit a dose-response relationship in peanut tissues [47].

Using silver nitrate solution, this investigation used *N. sativa* seeds' aqueous liquid extract to convert silver ions to AgNPs [48]. AgNPs were tested for antibacterial activity against *E. coli*, *P. aeruginosa*, *Klebsiella* spp, and *Proteus* spp. In addition, some of the phytotoxicity and genotoxicity of wheat seedlings exposed to Ag NPs generated biologically are studied and evaluated in this work.

## 2. Materials and Methods

**2.1. Materials.** All chemicals and reagents used were of analytical grade. Ethanol HPLC grade of 99.9% purity and silver nitrate ( $\text{AgNO}_3$ , molecular mass of  $169.87 \text{ g}\cdot\text{mol}^{-1}$ , and purity  $\geq 99.0\%$ ) were purchased from Scharlau, Spain, and Sigma-Aldrich, UK, respectively. Dry *N. sativa* seeds were purchased from the local market (Kheir Zaman market, Assuit, Egypt). Mueller–Hinton agar (MHA) was purchased from Oxoid, UK.

**2.2. Preparation of *N. sativa* Seed Aqueous Liquid Extract.** Deionized water was used to wash *N. sativa* seeds many times. Twenty grams of coarsely powdered *N. sativa* seeds was cooked in 100 milliliters of double-distilled water for 3 minutes before being filtered using Whatman No. 1 filter

paper. For later usage, the filtrate was collected and kept at  $4^\circ\text{C}$ .

**2.3. Biosynthesis of AgNPs.** AgNPs were synthesized using  $1 \times 10^{-3} \text{ M}$  of  $\text{AgNO}_3$  and aqueous *N. sativa*, where four mL of aqueous liquid extract was added to 96 mL  $\text{AgNO}_3$  that was exposed to sunshine [49, 50]. The seed extract's color changed from yellow to dark brown, indicating that AgNPs were produced. The solution's color change was monitored regularly.

## 2.4. Characterization of AgNPs

**2.4.1. UV-Visible Analysis.** To monitor the complete biological reduction of  $\text{AgNO}_3$  to AgNPs, 1 mL of the sample suspension was diluted with 2 mL of distilled water, and a UV-visible spectrophotometer (Shimadzu UV probe 1800) was used to record the spectrum of the sample within a scan range of 200 to 700 nm.

**2.4.2. X-Ray Analysis.** X-ray diffraction (XRD) with a scan rate of  $20 \text{ min}^{-1}$ , a working voltage of 40 kV, and a monochromatic filter with a  $2\theta$  range of  $10\text{--}80$  was used to investigate the phase and purity of the prepared AgNPs. It is used to check the phase recognition and characterization of the crystal structure of nanoparticles. The average size of AgNPs was estimated by applying Scherer's formula [51]:

$$D = \frac{k\lambda}{\beta \cos \theta}, \quad (1)$$

where  $D$  is the average grain size,  $k$  is a constant equal to 0.89,  $\lambda$  is the X-ray wavelength (0.1542 nm), and  $\beta$  is the full width at half maximum (rad) of the considered diffraction peak, and  $\theta$  is the Bragg angle, obtained with the (1 1 1) line of purest silicon as the standard.

**2.4.3. TEM Analysis.** The morphology and size of the obtained AgNPs are examined using transmission electron microscopy (TEM). The study was conducted using a 200 kV ultrahigh-resolution transmission electron microscope (JEOL, JEM 2100 h with EELS). The TEM grid was prepared by placing  $5 \mu\text{L}$  of AgNP solution onto a carbon-coated copper grid and drying it under a lamp.

**2.5. Antibacterial Activity Assay Using the Well Diffusion Method.** Four clinical bacterial strains (*E. coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp, and *Proteus* spp) were used in this study. The agar well diffusion method by Mueller–Hinton agar (MHA) (Oxoid, UK) ( $2.0 \text{ g}\cdot\text{L}^{-1}$  beef infusion solids,  $1.5 \text{ g}\cdot\text{L}^{-1}$  starch, and  $17.5 \text{ g}\cdot\text{L}^{-1}$  casein hydrolysate in 1000 mL distilled water,  $\text{pH } 7.4 \pm 0.2$ ) was used to evaluate the antibacterial activity of the Ag NP stock solution ( $170 \mu\text{g}\cdot\text{mL}^{-1}$ ). The agar surface was inoculated by spreading a bacterial suspension containing approximately  $1.5 \times 10^7 \text{ CFU}\cdot\text{mL}^{-1}$  onto the entire surface using a sterile spreader. Five different AgNP stock solution concentrations were investigated, including 12.5, 25, 50, 75, and

$100\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ . The stock concentrations were chosen to be below the toxicity level of Ag, where Tiwari et al. [52] have proven that a dose of  $10\text{ mg}\cdot\text{kg}^{-1}$  is safe and that of  $20\text{ mg}\cdot\text{kg}^{-1}$  is toxic. AgNP solution ( $100\text{ }\mu\text{L}$ ) was added to the well with a micropipette via a 10 mm diameter hole punched aseptically with a sterile cork borer. Agar plates were incubated at  $37^\circ\text{C}$  for 24 h, and inhibition zones were measured. Saline (0.85%) solution was used as a negative control, while tetracycline antibiotic ( $100\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ ) was used as a positive control, where it is used to treat a wide variety of infections (ranked as one of the top three antibiotics in the clinical prescriptions in the USA) and has broad-spectrum activity, better tissue permeability, and low price [53]. Minimum inhibitory concentration (MIC) values were visually determined and calculated according to the National Committee for Clinical Laboratory Standards (NCCLS 2003). The determination of minimum bactericidal concentration (MBC) was performed using the method proposed by Ozturk and Ercisli [54].

**2.6. Wheat Treatment.** Wheat seeds (*Triticum aestivum* L.) were immersed in 70% ethanol for 2 min and then treated with 10% bleach for 15 min. After that, they were soaked in distilled water for 2 h and then soaked in a series of prepared AgNP concentration ( $12.5, 25, 50, 75$ , and  $100\text{ }\mu\text{g}\cdot\text{L}^{-1}$ ) suspensions for about 2 h after being rinsed 4 times with distilled water. One piece of filter paper was put into each Petri dish, and 5 mL of the test solution was added. The seeds were transferred into the filter paper, with 10 seeds per dish and 1 cm distance between them [55]. The dishes were covered and sealed with tape, incubated at room temperature, and then allowed to grow for 2 weeks. After 10 days of growth, the germination rate, coleoptile, root lengths, and biomass were calculated [56].

## 2.7. Molecular Analysis

**2.7.1. Nucleic Acid Determination.** DNA extraction was performed according to standard protocols [57–59], and briefly, nucleic acids (DNA and RNA) were extracted, in which 0.5 gm of powder samples of plant seedlings was homogenized in 10% perchloric acid and extracted in a series of perchloric acid pH gradients. The quantity of powder needed for extraction of DNA and RNA was transferred to a new tube using a sterile spatula, and the estimated weight was determined based on preliminary volume/weight determination. DNA and RNA were extracted using a phenol/chloroform-free method. The integrity of isolated DNA and RNA was checked by electrophoresis on 0.8% agarose gel, followed by visualization of the bands under gel documentation. The quantitative measurement of nucleic acid samples was carried out by measuring the absorbance at 595 nm. The nucleic acid samples were stored at  $-80^\circ\text{C}$  for further use. Burton [59] estimated total DNA using di-phenylamine reaction, and the optical density was measured at 595 nm. In addition, orcinol reaction was used to determine total RNA, according to the method proposed by Schneider [58], with an optical density of 660 nm.

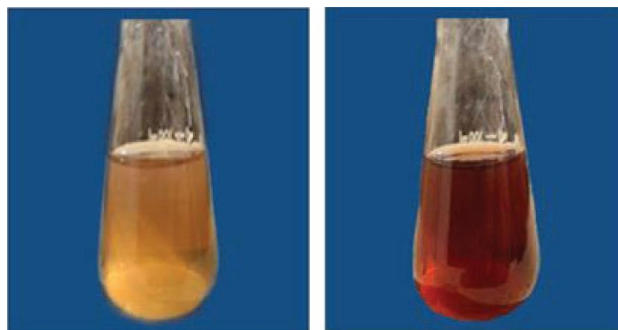


FIGURE 1: The change in the color of the solution from yellow to dark brown.

**2.7.2. Protein Determination.** According to Bradford [60], the total soluble protein was estimated, and 0.1 g of fresh root tissue was crushed with liquid nitrogen, extracted with 1 mL of 80% ethanol, precipitated at 4000 rpm for 15 minutes, and dissolved in 1 mL of phosphate buffer (pH = 7.0). The optical density was read at 595 nm.

**2.7.3. Statistical Analysis.** The statistical analysis of data comes from the mean and standard deviation ( $\pm$ ) of five replicate samples in each experimental group according to statistical validation guidelines [61–65]. To determine the significance of the difference between the means, a test was performed only within the two significance levels (0.05 and 0.1) for cytological observation.

## 3. Results and Discussion

**3.1. Ultraviolet-Visible Spectroscopy.** The UV-vis spectrum analysis of biosynthetic AgNPs revealed a peak at 424.1 nm wavelength (Figure 1), within the specified range of AgNPs, and indicated their presence in the reaction mixture. The green synthesis of AgNPs using *N. sativa* seed extract was successful, as the color of the reaction medium gradually changed from yellow to dark brown (Figure 1), indicating that silver nitrate in aqueous solution was reduced to surface plasmon vibrations in AgNPs. The position of the surface plasmon resonance was checked over three weeks, showing the exact position of suspended AgNPs at the prominent peak at 424.1 nm, as shown in our previous report, confirming the stability of the suspension of AgNPs over the tested period. Qualitative phytochemical analysis of *N. sativa* seed extract has been widely investigated in many studies by standard protocols and found that it contains carbohydrates, oil contents, different types of amino acids and proteins, flavonoids, steroids, and alkaloids [66–68]. The Ag ions could be reduced by the action of flavonoids, alkaloids, and polyphenols in the *N. sativa* seed extract [66].

**3.2. X-Ray Diffraction Analysis.** XRD analysis showed four distinct diffraction peaks at  $2\theta$  of 37.9, 44.1, 64.2, and 77.2. They could be indexed according to Miller indices at hkl as (111), (200), (220), and (312) crystalline planes, respectively, of spherical Ag according to JCPDS file No. 04–0783

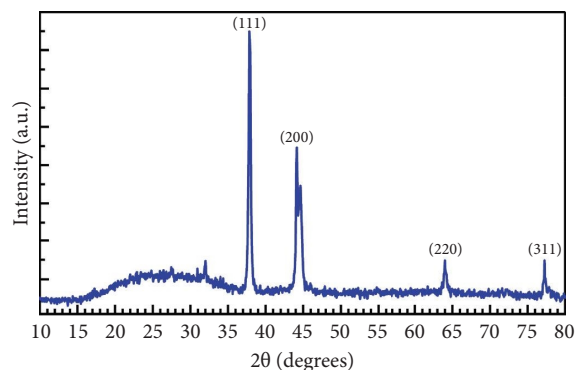


FIGURE 2: XRD pattern of AgNPs using *N. sativa*.

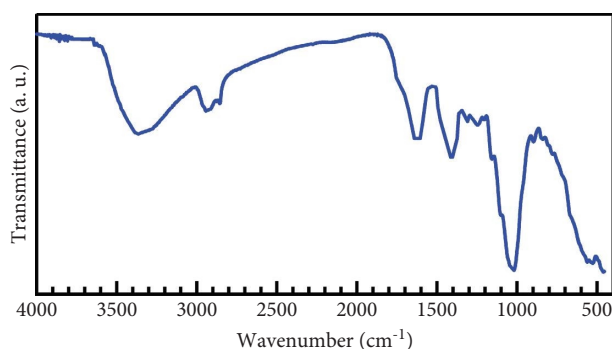


FIGURE 3: FTIR spectra of AgNPs using *N. sativa* extract.

[69–72]. This analysis revealed that nanoparticles are orthorhombic crystals. The high intensities of the peaks indicate the active silver composition with indexing (Figure 2). Thus, XRD confirms the crystalline nature of silver nanoparticles, and from the angle value, it is clear that the compound is stable [27, 28]. The calculated average crystallite size of AgNPs by the XRD line broadening method was  $\sim 7.5$  nm.

**3.3. FTIR Analysis.** The FTIR spectra of control dried *N. sativa* seed extract and synthesized AgNPs are shown in Figure 3. The results indicate absorption bands at  $3345\text{ cm}^{-1}$  (O–H stretching, H-bonding of alcohols, phenols, and N–H stretching of primary and secondary amines of plant protein),  $2924$  and  $2849\text{ cm}^{-1}$  (C–H stretching),  $1627\text{ cm}^{-1}$  (C=C stretching and N–H bend),  $1440\text{ cm}^{-1}$  (C–C stretching) and  $1034\text{ cm}^{-1}$  (C–O stretching of alcohols, carboxylic acids, esters and ethers, and C–N stretching) [73–75]. There is a shift of the absorption bands of  $3345$  to  $3360$ ,  $2924$  to  $2915$ ,  $1627$  to  $1637$ , and  $1440$  to  $1010\text{ cm}^{-1}$  after bioreduction for the bonds C=C and C=O which derived from the compounds such as flavonoids and alkaloids in *N. sativa* seeds. So, it is assumed that these biomolecules and some proteins are responsible for capping, stabilizing, and reducing  $\text{Ag}^+$  to AgNPs [74, 75]. FTIR analysis indicated the involvement of amides, alkanes, carboxyl, alcohols, and phenols in synthesized AgNPs.

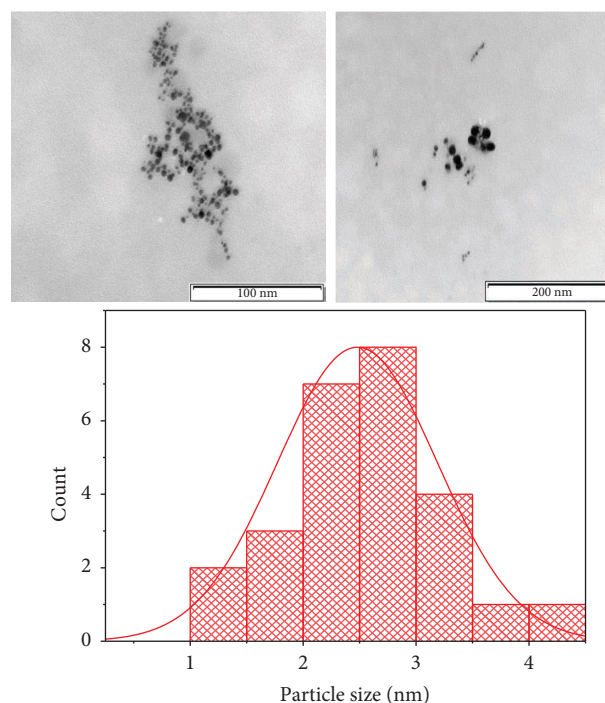


FIGURE 4: TEM images and particle size distribution of AgNPs synthesized by *N. sativa*.

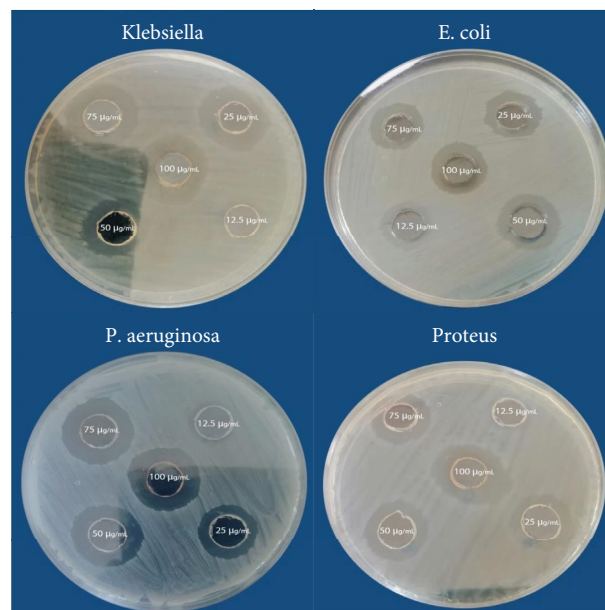


FIGURE 5: Bacterial growth inhibition zone achieved in the agar well diffusion method by the concentration of AgNPs at  $12.5$ ,  $25$ ,  $50$ ,  $75$ , and  $100\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ .

**3.4. Transmission Electron Microscopy.** The composition and size distribution of nanoparticles might be studied using a transmission electron microscope. The TEM images and particle size distribution images of AgNPs obtained in this investigation are presented in (Figure 4), with particle sizes ranging from  $1$ – $5$  nm, an average particle diameter of  $3$  nm [76], and a standard deviation of  $0.73$ . The findings agree

TABLE 1: The diameter of the inhibition zone (mm) of AgNPs against the selected bacterial strains.

Bacterial strains	Control		AgNP concentration ( $\mu\text{g}\cdot\text{mL}^{-1}$ )*				
	Pos.	Neg.	100	75	50	25	12.5
<i>E. coli</i>	18 $\pm$ 0.18 <sup>a</sup>	10 $\pm$ 0.0 <sup>b</sup>	19 $\pm$ 0.22 <sup>a</sup>	18 $\pm$ 0.20 <sup>a</sup>	18 $\pm$ 0.19 <sup>a</sup>	17 $\pm$ 0.16 <sup>a</sup>	10 $\pm$ 0.00 <sup>b</sup>
<i>P. aeruginosa</i>	10 $\pm$ 0.0 <sup>c</sup>	10 $\pm$ 0.0 <sup>c</sup>	28 $\pm$ 0.29 <sup>a</sup>	27 $\pm$ 0.26 <sup>a</sup>	27 $\pm$ 0.23 <sup>a</sup>	24 $\pm$ 0.23 <sup>a</sup>	13 $\pm$ 0.11 <sup>b</sup>
<i>Klebsiella</i> spp	10 $\pm$ 0.0 <sup>c</sup>	10 $\pm$ 0.0 <sup>c</sup>	25 $\pm$ 0.44 <sup>a</sup>	24 $\pm$ 0.41 <sup>a</sup>	21 $\pm$ 0.25 <sup>b</sup>	17 $\pm$ 0.25 <sup>b</sup>	10 $\pm$ 0.00 <sup>c</sup>
<i>Proteus</i> spp	12 $\pm$ 0.10 <sup>b</sup>	10 $\pm$ 0.0 <sup>c</sup>	26 $\pm$ 0.34 <sup>a</sup>	19 $\pm$ 0.31 <sup>b</sup>	17 $\pm$ 0.25 <sup>b</sup>	16 $\pm$ 0.14 <sup>b</sup>	10 $\pm$ 0.00 <sup>c</sup>

Values followed by the same letter in the row do not differ by the Tukey test at 5% probability. \* Average of three replicates. Tetracycline: positive control. Saline: negative control. a = nonsignificant; b = significant; c = highly significant at the 0.05 level.

TABLE 2: MIC ( $\mu\text{g}\cdot\text{mL}^{-1}$ ) and MBC ( $\mu\text{g}\cdot\text{mL}^{-1}$ ) of AgNPs against the selected bacterial strains.

Clinical bacterial strains	Concentration ( $\mu\text{g}\cdot\text{mL}^{-1}$ )*	
	MIC	MBC
<i>E. coli</i>	10.23 $\pm$ 0.2	56.26 $\pm$ 1.2
<i>P. aeruginosa</i>	9.03 $\pm$ 0.1	67.72 $\pm$ 1.0
<i>Klebsiella</i> spp	11.80 $\pm$ 0.3	76.71 $\pm$ 1.3
<i>Proteus</i> spp	9.59 $\pm$ 0.2	72.88 $\pm$ 1.3

Values followed by the same letter in the same column do not differ by the Tukey test at 5% probability. \* Average of three replicates.

with the XRD results, which confirmed the formation of NPs. Furthermore, AgNPs were found to have spherical shapes with a narrow size distribution.

**3.5. Evaluation of the Antibacterial Activity of AgNPs (In Vitro).** The antibacterial activity of AgNPs was determined against four clinical bacterial strains (*E. coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp, and *Proteus* spp). The results showed the following: the negative control does not show any clear zone, the positive control affects only *E. coli* and *Proteus* spp., and AgNPs affect four bacterial strains. The inhibition zones of AgNPs for the four bacterial strains are shown in Figure 5 and summarized in Table 1. Although tetracyclines are broad-spectrum antibiotics that affect both Gram-positive and Gram-negative bacteria, in addition to rickettsiae, chlamydia, mycoplasma, and protozoan parasites [77], the prepared AgNPs show higher antibacterial activity than tetracyclines.

MIC is defined as the lowest concentration of the antibacterial agent that inhibits bacterial growth by serial dilution [32]. According to the results acquired from the in vitro antimicrobial assays, the MIC for the synthesized Ag NP against a wide range of Gram-negative bacteria ranged from 9.03 to 11.8  $\mu\text{g}\cdot\text{mL}^{-1}$ , while MBC ranged from 56.26 to 76.71  $\mu\text{g}\cdot\text{mL}^{-1}$ . MBC was defined as the lowest active concentration, killing approximately 99.9% of the bacterial inocula after 24 h incubation at 37°C [78]. Data (Table 2) showed that both MBC and MIC showed nonsignificant different values ( $p < 0.05$ ) among microorganisms. In this study, MBC of green AgNPs for *E. coli* and *P. aeruginosa* (56.26 and 67.72  $\mu\text{g}\cdot\text{mL}^{-1}$ , respectively) were higher than those found elsewhere [79], whereas MBC for *E. coli* and *P. aeruginosa* (8.0 and 1.0  $\mu\text{g}\cdot\text{mL}^{-1}$ , respectively) and lower than those found by Lima et al. [80] where MBC for *E. coli* (125  $\mu\text{g}\cdot\text{mL}^{-1}$ ) and by Burgaz et al. [81] where MBC for

*P. aeruginosa* (>200  $\mu\text{g}\cdot\text{mL}^{-1}$ ). This variation (from our study to other studies) might be due to the methodology used to prepare silver nanoparticles and the size of the silver nanoparticles used. Thus, it is demonstrated that *N. sativa* plants could be promising in producing AgNPs, which could act as antimicrobial agents.

**3.6. Effect of Silver Nanoparticles on the Germination and Seedling Growth of Wheat.** Compared to the control, the results (Table 3) clearly show that varying concentrations of AgNPs did not affect grain germination rates (untreated). There were no visible variations in the appearance of grains germinated with or without AgNPs, showing that nanoparticles have no toxicological impact on grains during germination. This is in line with the findings of the previous research study, which found that nanoparticles (Ag, Zn, and Al) do not affect seed germination. This could be explained by the grain coatings' protective action, which can have selective permeability. AgNPs can combine or form complexes with ligands, reducing toxicity and exposure to grains and seedlings.

The seed coat of tobacco seeds was most likely not permeable to Al oxide NPs; hence, the germination rate was unaffected. AgNPs had a statistically significant decrease in both radicle and coleoptile lengths regarding the influence of nanoparticles on grain growth. Overall, the average inhibition rose as the concentration increased (Table 3 & Figure 6). On the contrary, exposure to AgNPs resulted in a considerable increase in fresh and dry grain weights, particularly at 75  $\mu\text{g}\cdot\text{L}^{-1}$ . The same results were observed by the author in [82], who studied the effect of AgNPs on common beans and corn. The author found that freshness and dryness increased at lower concentrations (20, 40, and 60 ppm) and decreased in response to high concentrations (80 and 100 ppm). The obtained results also indicated that high concentrations (100  $\mu\text{g}\cdot\text{L}^{-1}$ ) of AgNPs had marked decreases in the biomass of wheat seedlings. The reduced growth of seedlings treated with AgNPs may be due to inhibiting metabolic reactions. When treated with biosynthetic AgNPs, the effects found in wheat grain biomass are negligible. A plant bioassay is an important test to detect genetic toxicity, toxic substances, and environmental pollution [83, 84].

**3.7. Effect of AgNPs on the Contents of DNA, RNA, and Soluble Protein.** The obtained results (Table 4) show that small concentrations of AgNPs (25, 50  $\mu\text{g}\cdot\text{L}^{-1}$ ) had a slightly inhibitory effect on the contents of DNA, RNA, and soluble protein, while higher concentrations (75 and 100  $\mu\text{g}\cdot\text{L}^{-1}$ )



TABLE 3: The effect of the phytotoxicity of different concentrations (25, 50, 75, and 100  $\mu\text{g}\cdot\text{L}^{-1}$ ) of AgNPs on wheat germination and seedling growth.

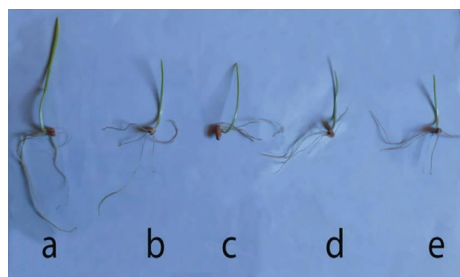
Concentration ( $\mu\text{g}\cdot\text{L}^{-1}$ )	Germination (%)	Radical length (cm)	Coleoptile length (cm)	Fresh weight (mg)	Dry weight (mg)
Control 0.0	98.0 $\pm$ 2.34	7.3 $\pm$ 0.76	5.50 $\pm$ 0.22	1.50 $\pm$ 0.31	0.49 $\pm$ 0.07
25	97.5 $\pm$ 2.30 <sup>a</sup>	5.8 $\pm$ 0.32 <sup>b</sup>	4.24 $\pm$ 0.31 <sup>a</sup>	1.61 $\pm$ 0.10 <sup>b</sup>	0.55 $\pm$ 0.05 <sup>b</sup>
50	98.0 $\pm$ 1.87 <sup>a</sup>	4.1 $\pm$ 0.60 <sup>b</sup>	4.28 $\pm$ 0.17 <sup>b</sup>	1.56 $\pm$ 0.11 <sup>a</sup>	0.54 $\pm$ 0.12 <sup>b</sup>
75	97.0 $\pm$ 1.45 <sup>a</sup>	5.3 $\pm$ 0.35 <sup>b</sup>	3.45 $\pm$ 0.19 <sup>c</sup>	1.92 $\pm$ 0.09 <sup>c</sup>	0.69 $\pm$ 0.07 <sup>c</sup>
100	96.7 $\pm$ 2.39 <sup>a</sup>	4.3 $\pm$ 0.44 <sup>b</sup>	3.32 $\pm$ 0.07 <sup>c</sup>	1.74 $\pm$ 0.86 <sup>b</sup>	0.59 $\pm$ 0.08 <sup>c</sup>

a = nonsignificant; b = significant; c = highly significant at the 0.05 level.

TABLE 4: Effect of different concentrations of AgNPs on DNA, RNA, and total soluble protein contents of wheat seedlings after 10 days.

Concentration ( $\mu\text{g}\cdot\text{L}^{-1}$ )	DNA (mg·g <sup>-1</sup> )	RNA (mg·g <sup>-1</sup> )	Total soluble protein (mg·g <sup>-1</sup> ·DW)
0	1.89 $\pm$ 0.28	2.36 $\pm$ 0.50	28.65 $\pm$ 0.68
25	1.88 $\pm$ 0.31 <sup>a</sup>	2.38 $\pm$ 0.65 <sup>a</sup>	27.90 $\pm$ 0.76 <sup>a</sup>
50	1.69 $\pm$ 0.43 <sup>a</sup>	2.07 $\pm$ 0.39 <sup>a</sup>	25.43 $\pm$ 1.20 <sup>a</sup>
75	1.26 $\pm$ 0.51 <sup>b</sup>	1.86 $\pm$ 0.51 <sup>b</sup>	20.65 $\pm$ 1.18 <sup>b</sup>
100	1.23 $\pm$ 0.44 <sup>b</sup>	1.56 $\pm$ 0.29 <sup>c</sup>	17.82 $\pm$ 1.05 <sup>c</sup>

a = nonsignificant; b = significant; c = highly significant at the 0.05 level.

FIGURE 6: Effect of AgNPs on wheat growth and development after 10 days: (a) control, (b–e) exposure to AgNPs (25, 50, 75, and 100  $\mu\text{g}\cdot\text{L}^{-1}$ ).

significantly induced an inhibitory effect. The results showed that small concentrations of AgNPs had a stimulating effect on the growth of plantlets, while enhanced concentrations induced an inhibitory effect. Conversely, the decrease in protein concentration can be attributed to the breakdown of existing proteins and the decrease in de novo synthesis. This decrease in protein content can be used as a biological indicator of plant metal stress [85]. In addition, it was found that TiO<sub>2</sub> nanoparticles negatively impact tobacco growth and development and miRNA expression [86].

Moreover, variations in the protein pattern and DNA content were observed by Eweis et al. [42] on the callus of *Solanum nigrum* L. exposed to biologically synthesized AgNPs. They reported variations in the protein pattern and DNA content between callus control (no AgNPs) and callus exposure to AgNPs. Genetic stability between callus control and callus under AgNP exposure was interrelated.

#### 4. Conclusions

Due to the many applications of silver nanoparticles in different fields, their migration and penetration into the ecosystem have become inevitable. Therefore, potential risks such as the destruction of ecosystems by nanoparticles, declining food quality and output, and even harm to human

health have attracted people's attention. First, among the different biological tools used to produce nanoparticles, plant products are given priority. Active materials produced by the green synthetic route of various plant parts have more potential for Gram-positive and Gram-negative microorganisms and different cancer cell lines. They have higher resistance than those produced by chemical and thermal physical methods. Among various plant products, plant seeds have attracted much attention. The synthesis of biomedically important NPs (nanoparticles) using *N. sativa* extracts showed several advantages according to antimicrobial activity because of their unique chemical properties and diverse secondary compounds that function as precursor molecules involved in forming NPs. Second, this study focused on the impacts of AgNPs on plants at the morphological and molecular levels. The results showed that AgNPs did not significantly affect germination and morphological characters, whereas the genotoxicity of AgNPs at the molecular level was detected, especially at a high dose of AgNPs. Further research is needed to evaluate the phytotoxicity and the tolerance mechanism of AgNPs. At the same time, a standardized method for standardized AgNP exposure needs to be established to allow comparison between different species.

#### Abbreviations

DNA:	Deoxyribonucleic acid
<i>E. coli</i> :	<i>Escherichia coli</i>
FTIR:	Fourier-transform infrared spectroscopy
MBC:	Minimum bactericidal concentration
MHA:	Mueller–Hinton agar
MIC:	Minimum inhibitory concentration
NPs:	Nanoparticles
<i>P. aeruginosa</i> :	<i>Pseudomonas aeruginosa</i>
RNA:	Ribonucleic acid
TEM:	Transmission electron microscopy
XRD:	X-ray diffraction.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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