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Plant Mediated Synthesis of Silver Nanoparticle From *Helianthus annus* L. Seeds and Evaluation of their Antioxidant Potential and Effect of Phytochemicals on *Capsicum frutescens* L.

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Abstract: Nanotechnology is a scientific field that provides endless prospects for growth in industries, including agriculture, pharmaceuticals, the industrial sector, the environment, and stress tolerance. AgNPs were green-synthesized from *Helianthus annuus* L. seeds. UV, SEM, and EDX analyses were used to characterize the samples. The nanoparticles were validated using UV-Vis absorption spectroscopy at wavelengths ranging from 300 to 800 nm. SEM observations reveal that the silver nanoparticles exhibit a homogeneous distribution and spherical shape. EDX analysis revealed exposure, and the structure confirmed the presence of pure silver in the synthesized silver nanoparticles. To validate the crystalline nature of the AgNPs, the dried powdered sample was subjected to the emission peaks of 20.0 kV = 34.39, which corresponded to the silver crystal planes. Green-synthesized AgNPs were evaluated for their *in vitro* antioxidant and biostimulant effects on *Capsicum frutescens* L. The highest percentages of inhibition were observed in DPPH* (41.79%), ABTS+ (78.65%), H_2O_2 (48.87%), hydroxyl radical scavenging (25.98%), and total antioxidant activity (1.458±0.010 mg/g). The effects of AgNPs at different concentrations (0, 1, 2, 3, 4, and 5 mg/mL) on seed germination, growth, and phytochemicals in *Capsicum frutescens* L. were studied. Lower concentrations of AgNPs (1 mg/mL and 2 mg/mL) had a greater effect on *Capsicum frutescens* L. growth and increased biochemical and Phytochemical content.

Keywords: plant-mediated synthesis; silver nanoparticle; *Helianthus annuus* L. seeds; antioxidant activity; bio-stimulant efficiency.

1. Introduction

Nanotechnology is a branch of science concerned with the manipulation, manufacture, and application of materials at the nanoscale. Nanotechnology advances their scientific understanding by allowing them to explore and expand their field of expertise in herbal plant biology [1]. Metal nanoparticles, in particular, have migrated outside the region and become more desirable, allowing for faster reactions and higher manufacturing yields. Metallic nanoparticles are inexpensive, environmentally friendly, and non-toxic, which reduces the accumulation of hazardous waste. Green metallic nanoparticle production is safer for biological and environmental applications [2,3].

The main objective of implementing green nanotechnology in agriculture is to reduce negative environmental implications and high fertilizer prices, whereas green nanoparticles (GNPs) derived from various plants reduce harmful emissions of carbon dioxide, nitrous oxide, and methane. Green nanoparticles are also used to increase agricultural output while reducing farmers' health concerns [4]. The synthesized silver nanoparticles have different sizes, shapes, and structures; they have been broadly explored in the range of 1–100 nm. The shape and size of plant-derived NPs are more stable, and they produce more than other methods [5]. They possess alternative approaches to improving various applications, such as biomedical applications for drug delivery, wound healing, tissue scaffolding, and protective coatings. Synthesized nanosilver has a notable accessible surface area that allows the binding of any ligands [6]. SEM, XRD, UV-Vis spectra, TEM, EDAX, FTIR, and EDS/EDX are some general techniques used to characterize nanoparticles [7].

Aside from plants, microorganisms such as bacteria [8], yeast [9], and fungi [10] are used to generate nanoparticles in an environmentally friendly manner. However, other challenges arose during the synthesis process, including the need for numerous purification steps and the complexity and difficulty of maintaining microbial cultures. Plants, on the other hand, contain a large number of phytoconstituents; thus, synthesizing nanoparticles from plants yields a diverse range that can be used in a variety of applications. Sunflower seeds include tocopherols and polyphenols, two important bioactive components that contribute to their antioxidant properties [11]. AgNPs have well-established antibacterial capabilities [12], and they are used in agriculture to treat fungal illnesses [13,14].

Crop plant growth and development are assisted by antiphytogenic properties. Silver nanoparticles enhanced seed germination and growth in maize (*Zea mays* L.), watermelon (*Citrullus lanatus* Thunb.), and zucchini (*Cucurbita pepo* L.). However, maize root elongation decreased [15]. Other crops, including *Brassica juncea*, *Panicum virgatum*, *Phytolacca americana*, *Phaseolus vulgaris*, and *Zea mays*, have shown promising results [16-18]. It promotes seed germination in *Boswellia ovalifoliolata* and *Pennisetum glaucum* [19,20]. Its inclination causes resistance to abiotic stress. *Triticum aestivum* L. may tolerate salt stress [21]. The treatment of AgNPs to wheat (*Triticum aestivum* L.) and tomato (*Solanum lycopersicum* L.) reduces the negative effects of freezing and salt [22,23]. It makes maize more resistant to heat stress [24].

Green nanoparticles can boost plant growth, leaf health and greenness, pigmentation, antioxidant enzymes, and flower vase longevity [13]. Several studies have demonstrated that appropriate AgNP concentrations promote seed germination [25,26], plant development [27,28], photosynthetic quantum efficiency, and chlorophyll content [29,30]. AgNPs have been utilized as insecticides to alleviate the problem of plant pests. This lowers chemical fertilizer consumption and conversion in traditional agriculture systems. It is applied as a foliar spray to prevent mold, rot, fungal, and other microbial-related plant problems [31]. *Capsicum frutescens* L., also known as chili, is one of the world's most economically and agriculturally important vegetable crops. The pepper is an autogamous plant from tropical America that belongs to the Solanaceae family. It is utilized as a traditional medicine because it contains phytochemicals that can be used to treat cough, toothache, sore throat, parasite infections, rheumatism, and wound healing [32]. The chili contains plenty of water-soluble vitamin C and fat-soluble vitamin A [28].

The present study focused on the synthesis of AgNPs using *Helianthus annus* L. seeds. We explored and analyzed the *in vitro* antioxidant activity of synthesized silver nanoparticles. The efficiency of green-synthesized silver nanoparticles in primary and secondary metabolite production in the agricultural crop (*Capsicum frutescens* L.) has been investigated.

2. Materials and Methods

2.1. Green synthesis of silver nanoparticles from Helianthus annuus L. seeds.

Helianthus annuus L. is commonly called a sunflower. They are coming under the Asteraceae family. The collected *Helianthus annuus* L. seeds are washed thoroughly with tap water to remove unwanted debris and contaminant impurities from the seeds. Afterward, they rinsed with distilled water to remove the seed coat. Then the seeds are air-dried to remove excess moisture content at room temperature. In this, the dried seeds were ground into fine powder with a motor and a pestle. Next, seed powder at 1:10 W/V was taken and mixed with 100 mL of distilled water, and it was left overnight with magnetic stirring at 350 rpm at room temperature. The aqueous mixture was filtered with Whatman No. 1 filter paper. The clear filtrate was collected and used for further synthesis of silver nanoparticles. For the synthesis of silver nanoparticles, about 50 mL of aqueous seed extract was mixed with an equal volume of 0.07 M silver nitrate, and the mixture was continuously stirred. The appearance of the silver color indicated the formation of AgNPs. After that, the synthesized nanoparticles were stored for further analysis. The synthesized nanoparticles' physical properties, such as solubility and purity, were analyzed using UV spectrometry in the range of 300–800 nm [33].

2.2. Morphological characterization of green-synthesized silver nanoparticles.

The absorption spectra of the produced nanoparticle powder were examined in the 200–800 nm range (UV1800). The nanoparticles' size and shape were investigated using scanning electron microscopy (SEM) (Zeiss, Gemini Sem, and Sigma FE-SEM). The high voltage is 30.00 kV, and the magnification range is 5000x (10 µm) to 20000x (3 µm). Atomic force microscopy (Bruker) was used to study the three-dimensional topography and surface measurement of silver nanoparticles. The silver nanoparticles' XRD was performed at 20 kV with a pulse rate of 8.18 kcps [34, 35].

2.3. In vitro antioxidant activity of synthesized silver nanoparticles.

2.3.1. DPPH* radical scavenging activity.

DPPH* radicals were scavenged by antioxidants through the donation of protons, forming reduced DPPH* according to the method [36]. The various concentrations of the aqueous extract of the sample (4.0 mL) were mixed with 1.0 mL of a methanolic solution containing DPPH* radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. BHA was used as a standard. The percentage of DPPH decolorization in the sample was calculated according to the equation:

$$\% \text{ decolorization} = 1 - \frac{\text{Sample ABS}}{\text{Contol ABS}} \times 100 \quad (1)$$

2.3.2. ABTS+ radical scavenging activity.

The scavenging activity of the AgNPs on $ABTS^+$ radical action was measured at 734 nm, followed by the method [37]. Samples were diluted to produce 0.2 to 1.0 mg/mL. The reaction was initiated by the addition of 1.0 mL of diluted $ABTS^+$ to 10 µl of different concentrations of aqueous extracts from the sample or 10 µl of methanol as a control. The absorbance was read at 734 nm, and the percentage inhibition was calculated. The inhibition was calculated according to the equation

Where A0 is the absorbance of the control reaction, and A1 is the absorbance of the test compound.

$$\% \text{ inhibition } I = \frac{A1}{A0} \times 100 \qquad (2)$$

2.3.3. Hydrogen peroxide scavenging activity.

The samples were taken at different concentrations (0.4–2 mg/mL) and added to 2.4 mL of phosphate buffer containing hydrogen peroxide. The identical reaction mixture without the sample was taken as a negative control. Then, the reaction mixer was incubated at room temperature for 10 minutes against the blank (phosphate buffer). The absorbance of the reaction mixture was measured at 230 nm in spectrophotometry [38]. The scavenging activity (%) was calculated as:

$$\text{Scavenging activity } (\%) = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100 \qquad (3)$$

2.3.4. Hydroxyl radical-scavenging activity.

The scavenger of H_2O_2 is an important antioxidant defense mechanism. The reaction mixture of 2.0 mL contained 1.0 mL of 1.5 mM $FeSO_4$, 0.7 mL of 6 mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate, and varying concentrations of the extract. After incubation for 1 hour at 37°C, the absence of the hydroxylate salicylate complex was measured at 562 nm [39]. The percentage scavenging effect was calculated as

$$\text{Scavenging activity } (\%) = 1 - \frac{A1 - A2}{A0} \times 100 \qquad (4)$$

Where A0 was the absorbance of the control (without extract), and A1 was the absorbance in the presence of the extract, A2 was the absorbance without sodium salicylate.

2.3.5. Determination of total antioxidant activity.

The total antioxidant activity was determined by the phosphomolybdenum method [40]. The absorbance is measured at 695 nm using UV/Vis spectrophotometry. The antioxidant capacity was expressed as the ascorbic acid equivalent (AAE) by using the standard ascorbic acid. Prepare a concentration of 1 mg/mL of standard and extract solution; from that, take 1 mL of each sample, respectively. To all the tubes, add 2.0 mL of phosphomolybdenum reagent. The 2.0 mL of reagent alone serves as a blank. All the tubes are incubated at 97°C for 90 minutes. It was cooled, and the absorbance was measured at 695 nm using UV/Vis spectrophotometry against the blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE) by using the standard ascorbic acid.

2.4. Biostimulant activity on biosynthesized silver nanoparticles from Helianthus annuus L. seeds.

2.4.1. Seed germination test.

A chili (*Capsicum frutescens* L.) seed was taken and disinfected with 0.1% sodium hypochlorite and 70% ethanol, followed by rinsing with double-distilled water several times for the prevention of surface fungal or bacterial contamination. Afterward, the healthy and uniform seeds were selected and treated with 0, 1, 2, 3, 4, and 5 mg/mL of AgNPs. Then, the percentage of germination was recorded on the basis of seed germination.

$$Total\ seed\ germination\ (\%) = \frac{Total\ number\ of\ germinated\ seeds}{Total\ seeds} \times 100 \quad (5)$$

2.4.2. Morphological characteristics.

The germinated seeds were analyzed for various growth parameters, including total length, shoot length, root length, and the fresh and dry weights of the whole plant. The plants were selected randomly, the lengths were measured, and the mean values are expressed in cm. The plants were kept in aluminum foil, weighed on an electronic balance, and the fresh weight was recorded. After that, the plants are placed in a hot air oven to dry at 70°C for 48 hours, and then the dry weight is measured using a weighing balance. The values are recorded in mg/g dry weight.

2.5. Preliminary phytochemical screening analysis of AgNPs-treated Capsicum frutescens L. seeds.

2.5.1. Qualitative analysis.

The preliminary phytochemical constituents present in the sample were tested by various methods: Mayer’s test for alkaloids, the ninhydrin test for amino acids, Benedict's test for carbohydrates, phenolic compounds by the ferric chloride test, the presence of tannins by the alkaline reagent test, and Millon’s test for proteins.

2.5.2. Quantitative analysis.

The biochemical composition was quantified using different methods, including the following: chlorophyll content in the sample was estimated at [41]. Carbohydrate content was estimated by the method [42], and glucose was used as a standard. Proteins were quantified using Lowry’s method by using bovine serum albumin as a standard. The total amino acid content was estimated using the ninhydrin method. Ninhydrin, a powerful oxidizing agent, decarboxylates alpha-amino acids and yields an intensely colored bluish-purple product. Leucine has been used as a standard, which is calorimetrically measured at 570 nm. The total phenol content in plant tissues was estimated using the method described [43], in which gallic acid was used as the standard.

2.6. Statistical analysis.

Statistical analyses have been done using Microsoft Excel (2007). Mean values for each treatment were determined, and the treatment means were compared using an analysis of variance (T3 test) and Duncan post hoc. All values were acquired from three independent analyses. The results are provided as the mean ± standard deviation and were computed in Excel. Statistical data were analyzed using a one-way analysis of variance (ANOVA) in SPSS version 16.

3. Results and Discussion

3.1. Silver nanoparticles synthesized from Helianthus annuus L. and its characterization analysis.

The current study focused on silver nanoparticles green-synthesized from *Helianthus annuus* L. seeds. UV, EDX, and SEM techniques were used for the characterization assessment. The *in vitro* antioxidant activity has been analyzed for green-synthesized silver nanoparticles. *Capsicum frutescens* L. was investigated for the potential effects and biostimulant activity of green-synthesized AgNPs. Biological techniques that use less expensive sources are used as AgNP precursors. The green synthesis of nanoparticles has attracted significant attention because it uses non-toxic phytochemicals and avoids hazardous substances that would otherwise be used in chemical synthesis [44]. Silver nanoparticles were synthesized from *Helianthus annuus* L. seeds. The seeds were extracted with distilled water, and then the filtrate containing 0.07 M silver nitrate was added and mixed. In this work, the nanoparticles transformed from light to dark brown (Figure 1). The color varies depending on the extraction technique. The morphological structure and size are depicted (Figures 2 and 3). AgNP is synthesized in an aqueous extract to add silver nitrate metal salts to the plant extract; silver ions bind to proteins and water-soluble compounds via -OH and -COOH groups, causing conformational changes in the protein molecule that contribute to the captured metal ion transformation into a silver nanoparticle [45]. Silver nanoparticles were synthesized in water with *Helianthus annuus* L. seeds and 0.07 M silver nitrate. In this study, the nanoparticles changed color from pale to dark brown (Figure 1). Protein amino acids and cysteine residues also help to reduce silver and generate AgNPs [46,47]. The bio-reduction of Ag was accomplished by trapping Ag^+ ions on protein surfaces via electrostatic interactions between silver ions and proteins in plant extract. Proteins decrease Ag^+ ions, causing secondary structural changes and the creation of silver nuclei. Silver nuclei form and expand sequentially as Ag^+ ions are reduced and accumulate at the nuclei, resulting in AgNPs [48]. As in previous investigations, silver nanoparticles have been produced from the *A. millefolium* plant's aqueous and methanol extracts. The color varies based on the extraction procedure. Silver nanoparticles synthesized from water, ethanol, and methanol extracts were spherical, which was according to our results [49]. Several reaction parameters influence the size and shape of silver nanoparticles. The reaction parameters include the concentration of silver nitrate, the volume or concentration of plant extract, the reaction temperature, the reaction time, and the pH of the reaction mixture. The characteristics of biosynthesized AgNPs are highly dependent on reaction parameter optimization [13].

The pH of 7 often reduces the proportion of Ag^+ to AgO during AgNPs synthesis, with the maximum concentration of AgNPs found at pH 7-9. Many studies have shown that increasing the pH level in alkalinity increases the rate of AgNP synthesis. The spherical shape of AgNPs was found to have a higher pH value, increasing the reaction rate [50]. The simplest method for monitoring AgNP formation is to observe the color change from yellow to brown visually. A spectrophotometer was further used to confirm the tracking process and detect nanoparticle peaks in the visible area of the UV–Vis spectrum at a wavelength between 400 and 450 nm [51]. Our studies revealed that the nanoparticles were confirmed using UV-Vis absorption spectroscopy and analyzed at wavelengths that extend from 300 to 800 nm. The peaks were observed at 400-450 nm (Figure 2a).

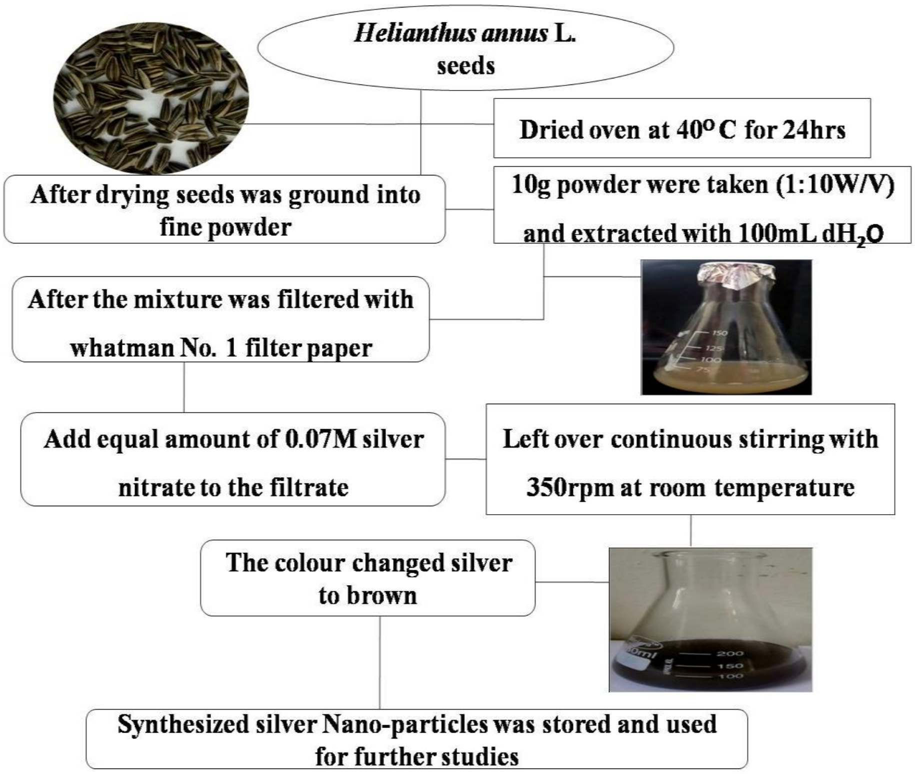

Figure 1. Schematic representation of silver nanoparticles' biological synthesis from *Helianthus annuus* L. seeds.

The investigation contrasts the intensity of light transmitted through the material with a reference measurement of the incident light source. Nanoparticles are usually detected at wavelengths ranging from 400 to 800 nm. Surface plasmon resonance (SPR) has been demonstrated to occur in the presence of AgNPs throughout a certain wavelength range. According to [52], scanning electron microscopy is used to investigate the chemical structure of the synthesized silver nanoparticles. The exterior characteristics and size of the synthesized silver nanoparticles were investigated using FE-SEM. The FE-SEM measurements demonstrate that the silver nanoparticles have a homogeneous distribution, and the lower and higher maximum wavelengths (λmax) suggest a smaller average size and larger concentrations of AgNPs. Similar to our findings, spherical shape AgNPs were detected under 10µm and 5µm (Figure 3). Silver nanoparticles have surface plasmon resonance (SPR), as indicated by a strong EDAX signal at about 3 keV. SPR occurs when conduction electrons on the nanoparticle surface resonate with incident X-ray photons, resulting in a strong emission signal. A minor silicon peak was also observed, most likely due to contamination during sample processing rather than bacterial production [53]. The EDAX was exposed, and the structure revealed the presence of pure silver material in the synthesized silver nanoparticles (Figure 2b). The EDX instruments also confirmed the presence of the silver element in the synthesized AgNPs. Silver ions are produced through oxidation, moisture, the balancing process, and the breakdown of capping and stabilizing chemicals on AgNPs [54]. Scanning electron microscopy is used to investigate the chemical structure of the silver nanoparticles. The surface characteristics and size of the produced silver nanoparticles were examined using SEM, which demonstrated that the silver nanoparticles have a homogeneous distribution and a spherical shape (Figure 3). The surface morphology and topography of the AgNPs showed both individual, well-defined AgNPs and many aggregates, with an average particle count of 0.5 in the selected image area (nm). The EDAX was exposed, and the structure revealed that the synthesized silver nanoparticles contained pure silver (Figure 2b). The dried powdered sample was EDX analyzed to confirm the crystalline nature of the AgNPs based on the emission peaks of 20.0 kV = 34.39, which corresponded to the silver crystal planes in Figure 2b. The EDX instruments also confirmed the presence of silver in the synthesized AgNPs.

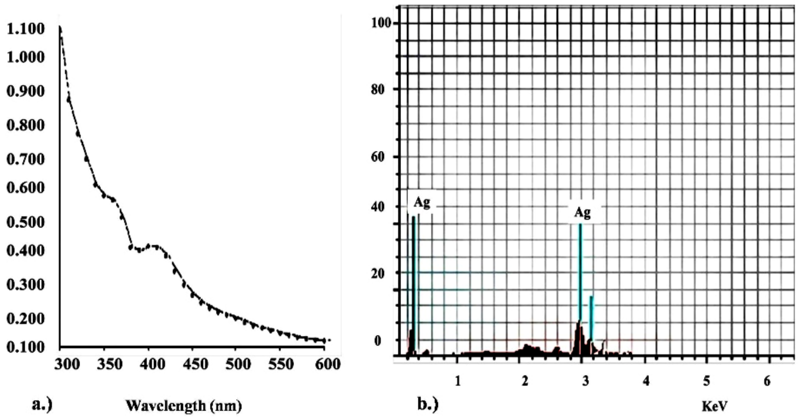

Figure 2. **(a)** Characterization Analysis of green synthesized Ag NPs by UV spectroscopy; **(b)** EDX (Energy Dispersive X-Ray) characterization Analysis of green synthesized Ag NPs.

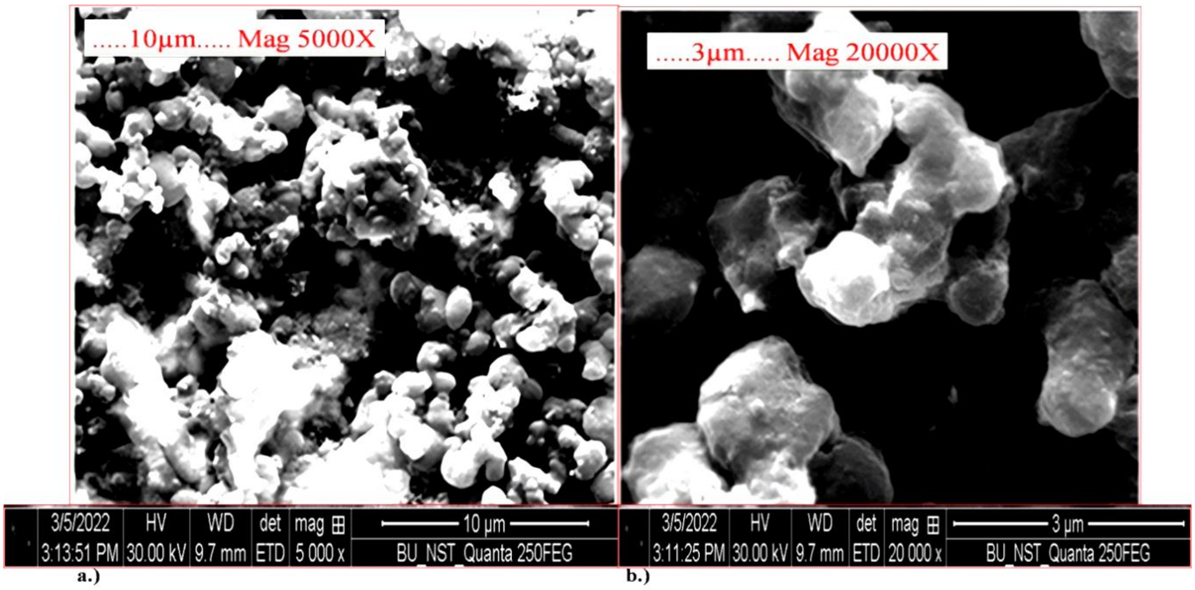

Figure 3. SEM characterization analysis of green-synthesized Ag NPs.

3.2. In vitro antioxidant activity of synthesized silver nanoparticles.

Antioxidants are a type of antioxidative agent. Antioxidants are natural or synthetic substances that can prevent or delay oxidative damage to cells (ROS, RNS, free radicals, and other reactive species). 2,2-diphenyl-1-picrylhydrazyl is a stable purple free radical that reacts with a hydrogen donor. The existence of a delocalized spare electron across the molecule prevents dimerization and also gives the molecule color [55]. The compounds' antioxidant ability may be boosted by binding to the AgNPs' larger surface area. The attraction created by electrostatic interactions between negatively charged phytochemicals and positively or neutrally charged AgNPs boosted bioactivity [56-58]. The green-synthesized AgNPs were tested for potential *in vitro* antioxidant properties. We observed the highest amount of radical scavenging activity at an AgNP concentration of 1 mg/mL (Table 1). The existing studies' DPPH* results suggest a good source of antioxidant effects. The free radical scavenging capacity of silver nanoparticles towards DPPH* radicals increased with concentration. Similar to our investigations, DPPH* results indicate a good source of antioxidant activity. The maximum suppression of scavenged antioxidants by AgNPs (41.79%) (Figure 4a). Antioxidant activity can reduce the fraction of autoxidation by lowering initiation events or by interacting with chain-carrying radicals [59]. Different processes contribute to nanomaterials' antioxidant potential; however, in the case of biogenic silver nanoparticles (AgNPs) or other metallic nanoparticles, catalase mimic (CAT-mimetic) is the primary mechanism [60]. According to prior references, biogenic AgNPs can degrade hydrogen peroxide to water and oxygen (H_2O_2 to H_2O and O_2) on their surface and have antioxidant properties [61]. The lowest concentration detected was 0.2 mg/mL (22.02%), as shown in Figure 4b. Figure 4c shows that the hydrogen peroxide radical scavenging activity has the highest inhibition level (48.87%). This is the primary reason why the majority of plant-based biosynthesized silver nanoparticles (AgNPs) exhibit antioxidant activity. The ABTS+ decolorization test generates the ABTS+ chromophore by oxidizing ABTS with ammonium persulfate. It applies to both hydrophilic and lipophilic substances [62]. Our studies showed that the maximum amount of ABTS+ at 1 mg/mL (78.65%) was recorded (Figure 4b). When compared with the control, the highest inhibition was found.

Table 1. Effect of green-synthesized AgNPs on *in vitro* antioxidant activity.

Concentrations mg/mL	**DPPH* (%)**		**ABTS+ (%)**		**H_2O_2 (%)**		**Hydroxyl radical scavenging (%)**	
	AgNPs	BHA	AgNPs	Trolox	AgNPs	AA	AgNPs	AA
0.2 mg/mL	2.355	41.870	22.023	2.210	15.726	20.927	9.635	29.531
0.4 mg/mL	4.817	79.566	28.401	5.102	20.551	24.248	14.895	39.843
0.6 mg/mL	7.533	87.836	59.183	7.057	34.147	31.578	20.833	54.062
0.8 mg/mL	10.276	89.241	73.554	19.132	42.293	33.771	24.479	69.739
1 mg/mL	41.790	90.967	78.656	44.132	48.872	37.969	25.989	79.322

Concentrations mg/mL	**Total antioxidant activity (mg/g)**	
	AgNPs	**Ascorbic acid**
0.2 mg/mL	0.818±0.011	0.320±0.027
0.4 mg/mL	1.035±0.030	0.518±0.020
0.6 mg/mL	1.086±0.017	0.605±0.018
0.8 mg/mL	1.205±0.005	0.765±0.005
1 mg/mL	1.458±0.010	0.983±0.013

A one-way ANOVA found a significant difference in the means of dependent variables among $p<0.001$. The p-value is less than the alpha level of 0.005, indicating that the means of the groups differ significantly.

The maximum ABTS+ radical scavenging activity was observed at 1 mg/mL (78.65%), as measured at 734 nm. The hydroxyl radical scavenging activity increases the scavenging impact while increasing the concentration; at 1 mg/mL, it has 25.98% activity (Figure 4d). Similar to our findings, the results showed that AgNPs exhibit 20% hydroxyl radical scavenging activity [63]. The silver nanoparticles' total antioxidant activity was assessed using phosphomolybdenum reduction activity, resulting in (1.458±0.010 mg/g dry weight) ascorbic acid equivalents at 1 mg/mL concentration. The quantity was reported as (0.818±0.011 mg/g) dry weight at 0.2 mg/mL (Figure 4e) (Table 1). The total antioxidant activity was evaluated using the phosphomolybdenum technique, which is based on the sample reducing Mo(VI) to Mo(V) and forming a green phosphate/Mo(V) complex at an acidic pH.

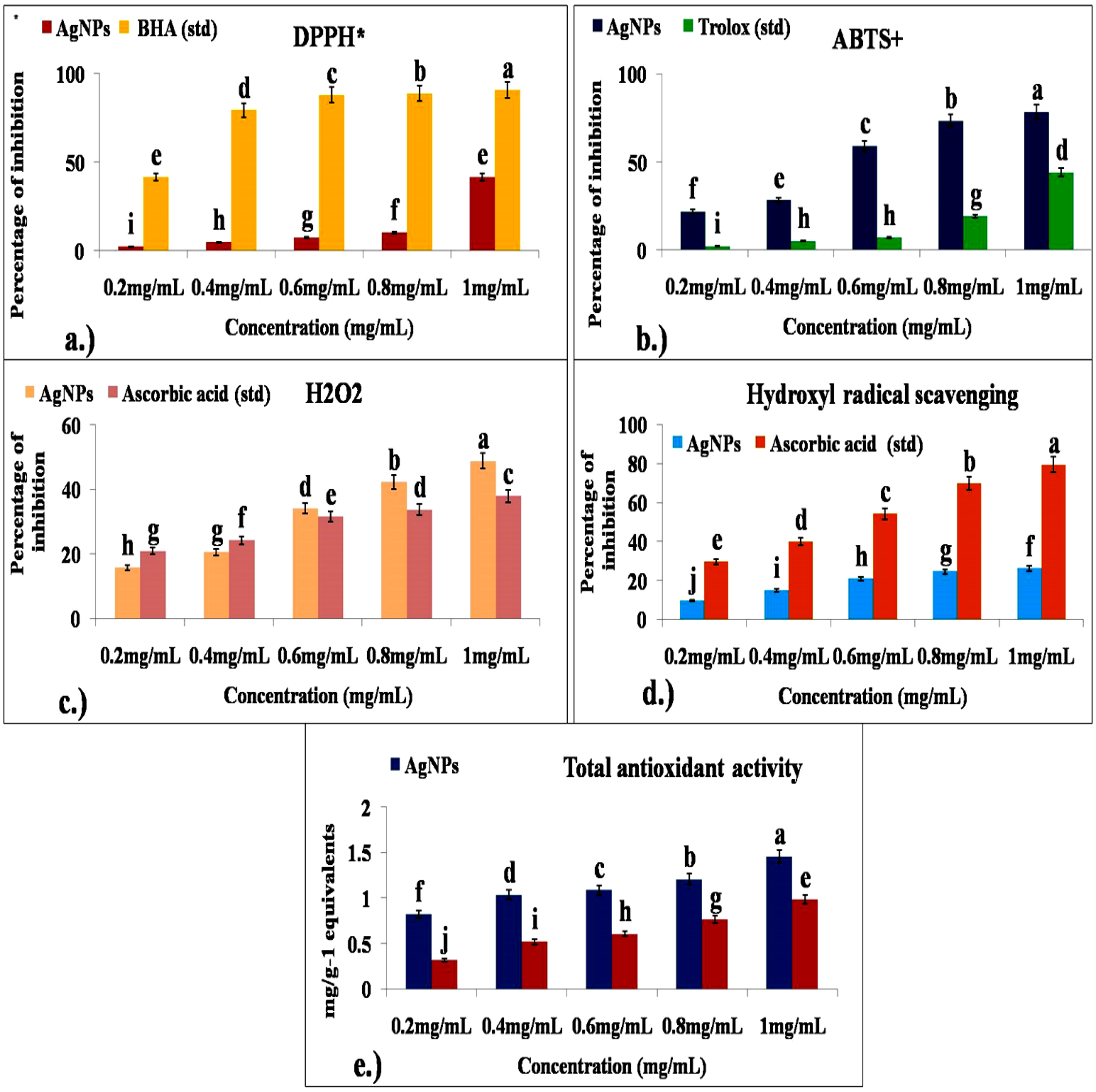

Figure 4. *In vitro* antioxidant efficiency of green-synthesized silver nanoparticles.

3.3. Biostimulant effect of synthesized silver nanoparticles on Capsicum frutescens L.

NPs, particularly metallic ones, have received significant attention in agriculture for their ability to stimulate plant growth and regulate metabolism. AgNPs, among other forms of NPs, have attracted significant interest due to their unique physicochemical properties, which can influence a range of physiological and biochemical processes in plants. AgNPs can affect metabolic processes like glucose metabolism, protein synthesis, and secondary metabolite generation. For example, AgNPs may induce the production of phenolic chemicals that aid in plant disease defense. AgNPs have emerged as a highly effective approach for enhancing plant growth and regulating metabolism. Their antibacterial qualities, ability to boost photosynthesis, and influence on hormonal and ROS signals make them a promising agricultural choice [64,65]. *Capsicum frutescens* L. plants respond strongly to inorganic fertilizers in the early phases of vegetative growth, but this response decreases as the plant matures. The farmers continue to use inorganic fertilizer on their crops because it provides quick nutrition, but also raises production costs. Inorganic fertilizers are manufactured materials that, when applied in excess, cause the soil to become toxic. Thus, the primary objective of this study is to avoid excess application of chemical fertilizer to the soil. Furthermore, farmers will have effective nutrient management for the *Capsicum frutescens* L. life cycle [66]. Nanoparticles are more soluble, have a larger surface area, and are more reactive than bulk materials. As a result, nanoparticles have the potential to contribute to global sustainable agriculture, particularly by enhancing plant growth and development. AgNPs with a 100–250 nm range are more soluble in water, which increases their activity [67]. Nanoparticles have had a significant impact on numerous cellular processes in plants, including sensing, signaling, transcription, transcript processing, translation, and post-translational protein modifications [68]. Our studies showed that the data in Figure 5 demonstrate the seed germination percentage of *Capsicum frutescens* L. with varied AgNPs concentrations. A significant effect of seed germination was observed. When compared to the control, the 2 mg/mL concentration enhanced the largest number of seed germinations (97%). As the concentration of AgNPs increased, the germination percentage decreased significantly. The seeds treated with 5 mg/mL AgNPs showed the lowest germination rate (Figure 5a). Similar to our experiments, green-synthesized nanoparticles were treated with varying concentrations (5, 10, 15, 20, 25, and 50 ppm) during tomato seed germination. Silver nanoparticles showed a significant impact on the germination percentage, rate, duration, and vigor index at 5, 10, 15, and 20 ppm. Although there was no significant influence on germination time, seeds treated with 5 ppm AgNPs germinated faster than other treatments. Nadar and Naqeeb seeds achieved maximum germination rates of 82.33% and 86.67%, respectively, when exposed to 10 ppm AgNPs for 8 days [69]. As the concentration of AgNPs increased, the germination rate reduced substantially. The seeds treated with 5 mg/mL AgNPs exhibited the lowest germination rate (Figure 5a). The biostimulant effect of synthesized silver nanoparticles was shown at lower concentrations, which improved seed germination rates of *C. frutescens* (L.) at 2 mg/mL (97%) and 1 mg/mL (87%). Growth and germination rate were decreased when dosages were increased to 3, 4, and 5 mg/mL (72%, 63%, and 37%), compared to the control (85%) (Figure 5a). At low concentrations, AgNPs can promote plant growth by increasing root length, seed germination, and overall biomass production. Higher quantities of AgNPs, on the other hand, have been proven to inhibit plant growth by causing cellular toxicity, nutritional imbalances, and disrupting normal physiological processes. AgNPs can activate several stress-signaling pathways in plants, leading to the production of secondary metabolites, the synthesis of stress hormones such as abscisic acid (ABA), and the activation of stress-responsive genes. This may boost the plant's ability to endure environmental stresses (such as drought and salinity) while diverting resources away from growth. The impact of AgNPs on plant physiology is complex and concentration-dependent. While modest doses can promote growth and give antimicrobial benefits, higher concentrations can cause toxicity, oxidative stress, and damage to plant components [70]. The water content of the roots and shoots of faba bean seedlings ranged between reduction and rise in the AgNPs and $AgNO_3$ treatments as compared to the control; however, the concentration of 10 ppm bio-AgNPs had the greatest benefit. The change in root and shoot water content in faba bean seedlings after Nano-material priming could be attributed to several variables [71]. The present investigation showed that after 14 days, chili seedlings were measured for total plant length, root length, fresh weight, and dry weight. Table 2 shows that the highest plant length, shoot and root length, and fresh and dry weight of *Capsicum frutescens* L. were reported at 2 mg/mL and 1 mg/mL concentrations, respectively. The lowest concentration of AgNPs had a significant impact on plant development. Silver nanoparticle concentrations of 3, 4, and 5 mg/ml result in the lowest shoot length, root length, fresh weight, and dry weight (Figure 5b, c). After 14 days, chili seedlings were assessed for overall plant length, root length, fresh weight, and dry weight. Table 2 demonstrates that the greatest plant length, shoot and root length, and fresh and dry weight of *Capsicum frutescens* L. were found at doses of 2 mg/mL and 1 mg/mL, respectively. The lowest concentration of AgNPs had a noticeable effect on plant growth. Silver nanoparticle concentrations of 3, 4, and 5 mg/mL produce the shortest shoot length, root length, fresh weight, and dry weight (Figures 5b, c). In the present study, root and shoot length were increased at lower dosages (1 and 2 mg/mL) of Ag NPs-treated *C. frutescens* (L). (Figure 6) (Table 2). The accumulation of nanoparticles on plant leaves acts as a heat source for the canopy, affecting gas and moisture exchange through stomatal blockage and eventually disrupting various physiological and cellular processes [72]. Nanoparticles can increase the production of stress-responsive genes, activate defense mechanisms against pathogens, and promote food intake. As a result, these effects improve overall plant health and performance [73]. Similar to our findings, the application of a higher concentration of 10 mg/L Ag NPs lowered the height by 20.74% in this variable, which was 17.34% lower than the control. The presence of 20 mg/L Ag NPs reduced height by more than 20% in the tomato cultivars tested as compared to the control [74]. Similarly to our studies, the basil plant's growth-related parameters, such as root and shoot length and weight, decreased with the amount of silver particles and silver nanoparticles. Silver nanoparticles at 80 ppm caused higher growth parameters than silver nitrate at the same concentration. Root length increased by 52% and 68%, respectively, as compared to the control group [75]. Furthermore, nanomaterials have been shown to alter root function and hydraulic conductivity by expressing aquaporins and enhancing root osmoregulatory capacity, thereby improving plant water absorption and transport [76]. Nanoparticles have a considerable impact on several physiological aspects of plant growth, including seed germination, germination rate, seed vigor index, and osmolyte accumulation (soluble sugars, proline, glycine betaine, and free amino acids). These alterations, along with changes in photosynthetic pigments, transpiration, and stomatal conductance, allow plants to withstand the deleterious impacts of abiotic stress [72,74]. Metallic nanoparticles significantly impact the ultrastructure of plant chloroplasts. The concentration of the nanoparticle solution clearly correlates with the amplitude of these changes. For instance, Arabidopsis chloroplasts become spherical rather than lens-shaped when exposed to 0.5–3 mg/L of silver nanoparticles. Concurrently, the quantity and size of plastoglobules increase, thylakoids with inflated lumens form, and the chloroplast stroma becomes more opaque, making it harder to distinguish thylakoids within it. There are electron-dense inclusions, commonly referred to as nanoparticle deposits [77]. Metal nanoparticles can improve the efficiency of chemical energy production in photosynthetic systems. A larger concentration of photosynthetic pigments was induced during seed germination. Similar to our research, the synthesized Ag, Cu, and Cu-Ag NPs were used for seed priming and foliar spray on three *Capsicum annuum* L. cultivars, Arka Sweta (AS), Arka Meghana (AM), and Arka Harita (AH), which were grown in greenhouses. Seed priming with varying NP concentrations (1, 10, and 20 ppm). Plant tissues had considerably higher levels of chlorophyll (51–142%), carotenoids (23–94.2%), total phenolic content (73%), and total flavonoid content (57%), compared to the control ($p < 0.05$). A foliar spray of NPs (20–100 ppm) protects chili plants from thrips infestation (30–76%). The foliar spray boosted chlorophyll (15–62%), carotenoids (15–50%), total phenolic content (20–62%), total flavonoid content (64–99%), and reducing sugars (15–97%) [78]. In our studies, seedlings treated with lower concentrations developed more pigment. Similar to our findings, organically synthesized AgNPs improved seed germination and seedling growth in *Boswellia ovalifoliolata* [79]. AgNPs-treated seedlings contain substantially more primary and secondary phytochemical components than control seedlings, including alkaloids, amino acids, carbohydrates, phenolic compounds, tannins, and proteins (Table 3). In our studies, seedlings treated with lower amounts produced more pigment. AgNP-treated seeds have higher levels of chlorophyll a, chlorophyll b, and total chlorophyll than sprouted *Capsicum frutescens* L seedlings. Plants treated with 2 mg/mL Ag NPs showed the greatest total chlorophyll levels (5.096±0.043 mg/g) (Table 4, Figure 7a). At larger dosages of 3 mg/mL Ag NPs, chlorophyll levels steadily decreased in *Capsicum frutescens* L. The study discovered that *Capsicum frutescens* L. treated with AgNPs had a significant amount of carbohydrate at 2 mg/mL (0.0522±0.012 mg/g). The lowest concentration was found at 5 mg/mL (0.0394±0.012 mg/g). The study revealed that *Capsicum frutescens* L. treated with Ag NPs had the maximum protein concentration at 2 mg/mL (0.0445±0.001 mg/g). The control protein level is (0.021 ± 0.001 mg/g). Plants treated with 2 mg/ml (0.182±0.0004 mg/g) AgNPs exhibited higher amino acid content. The control plant had lower amino acid content (0.112±0.001 mg/g) than the other treatment plants. Plants treated with 2 mg/mL (1.256±0.049 mg/g) with Ag NPs showed increased total phenolic content. The control plant had lower phenol content (0.846±0.011 mg/g) than the other Ag NP-treated plants (Table 5; Figure 7). AgNPs improved the morphological and biochemical properties of *Brassica juncea*, common bean, and corn [13,16, 51]. Similarly, foliar treatment of AgNP-treated fenugreek plants increased total carbohydrate and protein levels compared to control plants [10]. The study found that *Capsicum frutescens* L. treated with AgNPs contained a substantial amount of carbohydrate at 2 mg/ml (0.0522±0.012 mg/g). Because of their free hydroxyl groups, phenols are thought to be antioxidants. The free hydroxyl groups in phenolics are responsible for free radical scavenging action [80]. The study found that *Capsicum frutescens* L. treated with Ag NPs had the highest protein content at 2 mg/mL (0.0445±0.001 mg/g). The control protein level is 0.021±0.001 mg/g. Plants treated with 2 mg/ml (0.182±0.0004 mg/g) AgNPs showed increased amino acid content. Similar to our findings, we produced silver nanoparticles from *Cola nitida* pod extract and studied their effects on *Amaranthus caudatus* L. at concentrations of 25, 50, 75, and 100 ppm of AgNPs, compared with the control. Silver affects the proteins involved in the oxidation and reduction reactions, which has a deleterious effect on homeostasis regulation [25]. Plants treated with 2 mg/mL (1.256±0.049 mg/g) of Ag NPs exhibited a higher total phenolic content. The antioxidant activity of *A. caudatus* cultured with AgNPs decreased as their concentration increased. This study demonstrated that concentration-dependent AgNPs can be employed to increase the antioxidant activity and phytochemical content of plants [81]. Furthermore, the lack of established methodologies and the variety of plant extracts significantly reduce repeatability, necessitating additional research to improve procedures for large-scale synthesis. This shows that difficulties with NP quality, insufficient control over particle stability, and variability in plant extracts caused by growth and seasonal factors should be addressed to improve. Addressing these problems is critical to reducing the risk of increased energy consumption, higher production costs, and lower particle quality in green synthesis applications [82]. Farmers increase revenue while also improving the quality and nutritional value of their product by minimizing losses. In contrast to traditional synthetic fertilizers and pesticides, which pose environmental and health risks and are prohibitively expensive, the new technology of nanopesticide and nanofertilizer formulations offers increased efficiency, target specificity, and safety [83,84]. This technique has multiple benefits: it is simple, inexpensive, ecologically friendly, and eliminates the need for hazardous chemicals [85]. In the future, nanoparticles showed promise for regulating agricultural stressors, acting as nanofertilizers to resist abiotic stresses such as heavy metals, drought, salt, and temperature extremes (both cold and heat stress) [86,87]. They can also minimize soil nutrient deficits, increase crop nutritional value, and raise agricultural output [88].

Table 2. Effect of green-synthesized AgNPs on growth characteristics of *Capsicum frutescens L*.

Treatment (mg/mL)	Shoot length (cm^{-1})	Root length (cm^{-1})	Fresh weight (mg/g^{-1})	Dry weight (mg/g^{-1})
Control	3.033±0.057	6.566±0.737	0.036±0.002	0.0038±0.0002
1mg/mL	4.600±0.100	7.466±0.503	0.042±0.001	0.0042±0.0003
2mg/mL	5.067±0.057	8.566±0.115	0.046±0.001	0.0054±0.0002
3mg/mL	3.033±0.057	2.200±0.100	0.030±0.004	0.0033±0.0002
4mg/mL	2.067±0.057	1.366±0.057	0.026±0.005	0.0030±0.0004
5mg/mL	1.900±0.854	1.066±0.057	0.023±0.003	0.0230±0.0002

A one-way ANOVA found a significant difference in the plant height, $F(5, 12)=43.243$, and weight across means of dependent variables among groups, $F(11, 24)=181.490$, $p<0.001$. The p-value is less than the alpha level of 0.005, indicating that the means of the groups differ significantly.

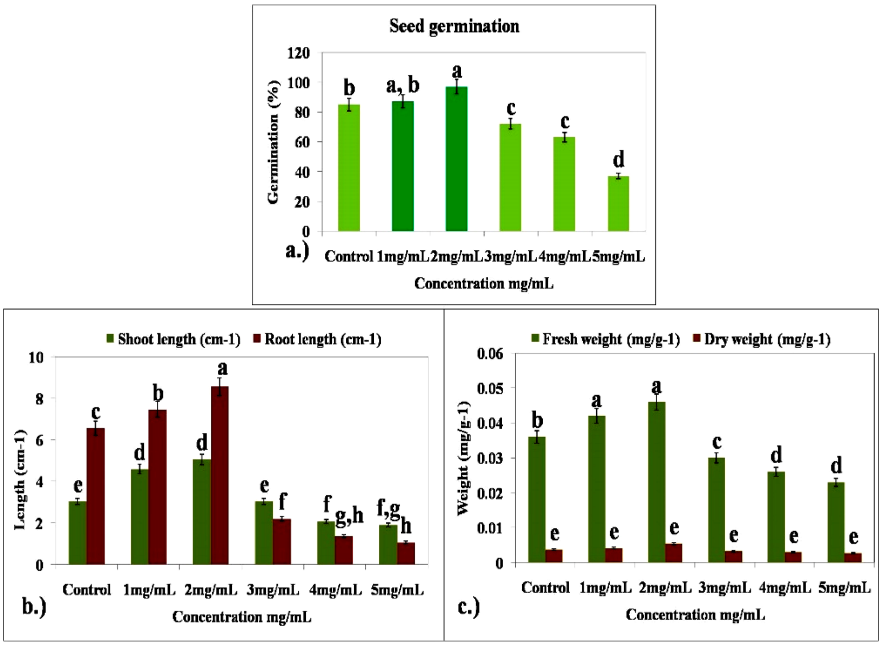

Figure 5. Biostimulant effect of green-synthesized silver nanoparticles on *Capsicum frutescens L*.

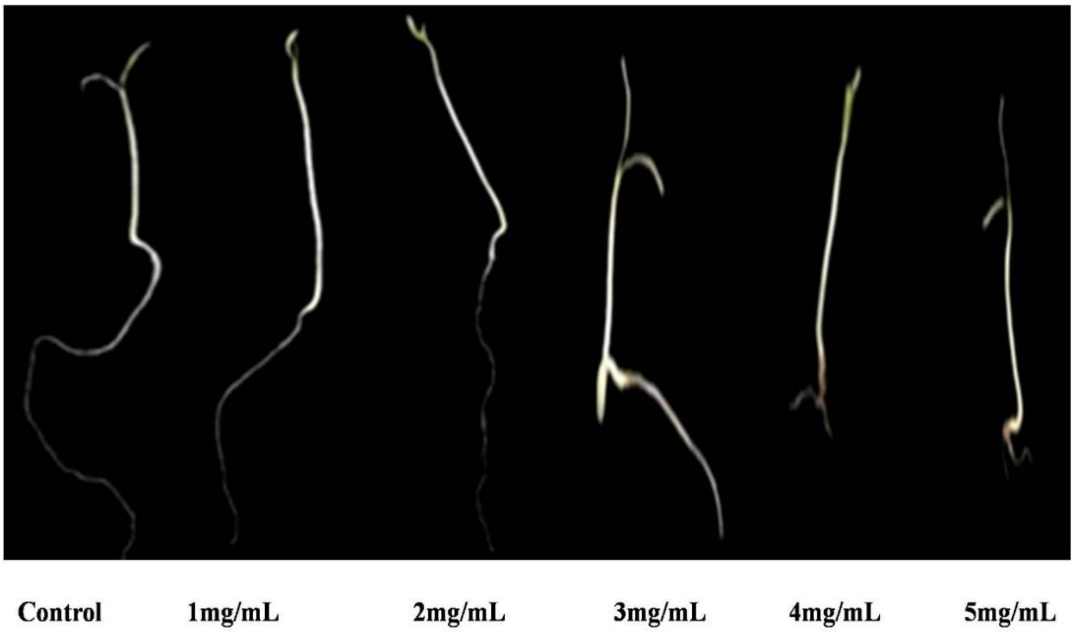

Figure 6. Morphological characteristics of green-synthesized silver nanoparticles on *Capsicum frutescens L*.

Table 3. Effect of various concentrations of synthesized silver nanoparticles on *Capsicum frutescens* L. and their qualitative analysis.

S. NO	Preliminary test	Control	1 mg/mL	2 mg/mL	3 mg/mL	4 mg/mL	5 mg/mL
1	Test for Alkaloids; (a) Mayer's test	++	+++	+++	+	+	+
2	Test for Amino acids; (a) Ninhydrin test	++	+++	+++	++	+	+
3	Test for Carbohydrates; (a) Benedict's test	++	+++	+++	+	+	+
4	Test for Phenolic Compounds and Tannins; a. Ferric Chloride test	+++	+++	+++	++	++	+
	b. Alkaline reagent test	++	++	++	+	+	+
5	Test for Proteins; Millon's test	++	++	++	+	+	+

(+++) Indicates the preliminary phytochemical compounds strongly present; **(++)** indicates the medium level phytochemical compounds present; **(+)** indicates the less amount of phytochemical compounds present.

Table 4. Effect of various concentrations of synthesized silver nanoparticles on the chlorophyll content of *Capsicum frutescens* L.

S. NO	AgNPs Treatments	Chlorophyll A	Chlorophyll B	Total chlorophyll mg/g^{-1}
1	Control	1.808±0.001	1.480±0.007	3.288±0.008
2	1mg/mL	1.563±0.066	2.257±0.008	3.820±0.074
3	2mg/mL	3.338±0.024	1.758±0.019	5.096±0.043
4	3mg/mL	2.285±0.012	1.040±0.021	3.325±0.033
5	4mg/mL	1.733±0.101	0.818±0.008	2.551±0.109
6	5mg/mL	1.730±0.010	0.066±0.002	1.796±0.012

A one-way ANOVA found a significant difference in the means of dependent variables among groups, $F(17, 36)=460.250$, $p<0.001$. The p-value is less than the alpha level of 0.005, indicating that the means of the groups differ significantly

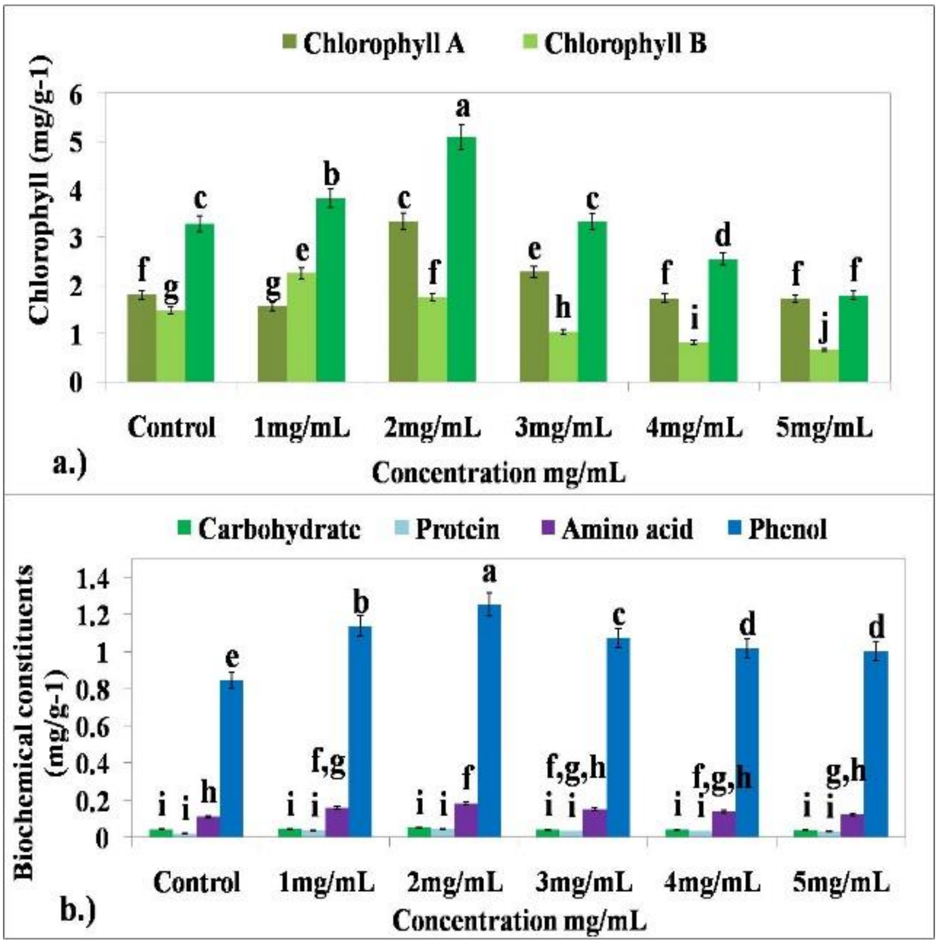

Figure 7. Phytochemical constituents' quantification of green-synthesized silver nanoparticles on *Capsicum frutescens L.*

Table 5. Effect of green-synthesized AgNPs on phytochemical constituents of *Capsicum frutescens* L.

S.NO	Biochemical composition (mg/g^{-1} dry weight)				
	Treatment	**Carbohydrate**	**Protein**	**Amino acid**	**Phenol**
1	Control	0.0435±0.004	0.021±0.001	0.112±0.001	0.846±0.010
2	1mg/mL	0.0443±0.0004	0.037±0.0003	0.158±0.002	1.139±0.039
3	2mg/mL	0.0522±0.011	0.045±0.0009	0.182±0.0004	1.256±0.048
4	3mg/mL	0.0418±0.003	0.035±0.001	0.152±0.002	1.074±0.020
5	4mg/mL	0.0415±0.005	0.035±0.0001	0.139±0.004	1.019±0.090
6	5mg/mL	0.0394±0.012	0.032±0.001	0.123±0.0004	1.001±0.037

A one-way ANOVA found a significant difference in the means of dependent variables among groups, $F(23, 48)=976.838$, $p<0.001$. The p-value is less than the alpha level of 0.005, indicating that the means of the groups differ significantly.

4. Conclusions

Nanotechnology is a promising field with the potential to transform or create new opportunities in the biomedical and agricultural sectors. It is non-toxic, inexpensive, and has a lower concentration, making it more useful in the biomedical field. The current study revealed the green-synthesized AgNPs' effective antioxidant potential at 1 mg/mL concentration. Morphological and biochemical alterations were observed in Capsicum frutescens L. treated with various concentrations of green-synthesized AgNPs. The morphological features (total length, root length, shoot length, fresh weight, and dry weight) were measured after 14 days. Preliminary qualitative and quantitative testing was recorded. The preliminary phytochemicals discovered included alkaloids, amino acids, carbohydrates, proteins, phenols, and tannins. The biological substance was measured using estimation methods. The biological constituents chlorophyll, phenol, carbohydrate, protein, and amino acid were shown to be more concentrated at 2 mg/mL. The control-treated plant contained fewer phytochemical compounds. Green-synthesized silver nanoparticles (AgNPs) hold enormous promise for agriculture in disease prevention, pest control, and plant growth promotion. They offer a promising, ecologically friendly alternative to existing chemical treatments. By minimizing toxic byproducts, natural reducing agents promote environmental safety. Even if they appear promising, careful consideration must be given to the long-term effects on ecosystems, soil health, and non-target creatures. To ensure sustainable use without compromising environmental integrity, practical applications should be balanced with careful risk assessments. To maximize their efficacy and reduce any hazards, more research is necessary. It is also becoming increasingly significant in agriculture. In this approach, green-synthesized AgNPs are used to boost crop yields while also protecting against various illnesses caused by bacteria, fungi, and pests. It is advised to utilize lower amounts of AgNPs in agricultural settings.

Author Contributions

Conceptualization, C.K.; formal analysis, N.R. and S.S.; writing—original draft preparation, N.R.; writing—review and editing, N.R.; supervision, C.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no competing interests.

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