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Impact of Spirulina-Derived Silver Nanoparticles on Bioactive Compound Accumulation in *Zantedeschia aethiopica* Cell Suspension Cultures

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Abstract

This study explains how phytochemicals and their activities in *Zantedeschia aethiopica* cell suspension cultures (CSC) are affected by biosynthesized silver nanoparticles (Ag-NPs) by spirulina aqueous extract (SAE). At 30 days of CSC, the medium containing Murashige and Skoog (MS) salts, sucrose (30 g/L), in addition to 1.5 g/L of SAE, and green-synthesized Spirulina silver nanoparticles (SP-AgNPs) was suitable for the accumulation of biomass and bioactive compounds. SP-AgNPs (5 mg/L) induced CSC extracts to greatly increase the production of total phenolic content (TPC) (4.27 mg/g-FW), total flavonoid content (TFC) (3.88 mg/g FW), and DPPH radical scavenging assay (RSA) (80.03%) than in the control CSC extracts for TPC, TFC, and DPPH (0.61 and 0.42 mg/g, and 48.80%), respectively. The contents of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were highly elevated in SP-AgNPs (5 mg/L)-elicited CSC (4.5, 3.3, and 2.5 U/mg) respectively when compared with non-elicited CSC. SP-AgNPs-elicited CSC showed more phenolic compounds (1422.28µg/g) than the control CSC (306.31 µg/g, respectively). The SP-AgNPs-elicited CSC extracts in *Zantedeschia aethiopica* had elevated levels of secondary metabolites due to these metabolic modifications. According to the study, the elicitation procedure is helpful in increasing the accumulation of flavonoid and phenolic compounds as well as antioxidant properties.

Keywords: Cell suspension cultures; Secondary metabolites; *Spirulina platensis*; Spirulina silver nanoparticles; antioxidant activity.

1. Introduction

Ornamental plants like *Zantedeschia aethiopica* (Calla lily), are crucial for the international horticulture market due to their attractive state and decorative leaves [1]. It is indigenous to the African continent's tropical regions [2]. However, large-scale cultivation of calla lilies faces challenges such as lack of quality planting material and resistance to biotic and abiotic stress [3]. *In vitro* culture is an efficient and fast method for producing high-quality plants, with micropropagation being a suitable method [1].

Plant biotechnology has enhanced disease and pest resistance, as well as physical and chemical stress tolerance [4]. *In vitro* tissue culture provides a large amount of homogeneous plant material with minimal space requirements and year-round availability, allowing for sterile experiments independent of weather or outside influence [5]. Tissue culture techniques have proven beneficial for producing high-quality planting material for farmers [6]. Using growth regulators in the culture media to control differentiation and dedifferentiation is a common practice among these methods [7].

Plant cell suspension cultures are produced using friable callus and are mostly utilized for the large-scale synthesis of secondary metabolites for medicinal purposes [6]. These secondary metabolites have various uses, including drugs, flavorings, bio-pesticides, pigments, fragrances, and food additives [8]. Cell suspension culture is the most effective in vitro plant tissue culture technology for fulfilling the increasing industrial need for more secondary metabolite (SM) production [9]. Elicitation is the most commonly used technique for successfully generating SMs by stimulating their biosynthesis route [10]. The media for callus were re-optimized induction to produce more friable callus, and external application of elicitors (biotic or abiotic) in conjunction with a plant membrane receptor activates specific genes, promoting the targeted SMs to accumulate [11].

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Spirulina platensis (Arthrospira) is a member of the cyanobacteria [12]. For plants, spirulina is a valuable source of minerals, proteins, vitamins, fatty acids, vital amino acids, polypeptides, and phytohormones phycocyanin, carotenoids, antioxidant compounds, and other beneficial compounds [13]. Spirulina extract (SE) is used as a biotic elicitor, while plant growth regulators like 2,4-dichlorophenoxyacetic acid (2.4D) and naphthyl acetic acid (NAA) are used as abiotic elicitors. Nanotechnology is a rapidly growing field in modern materials science, with recent studies showing its beneficial effects on plant tissue culture processes such as somatic embryogenesis, callus induction, cell dedifferentiation, genetic modification, and secondary metabolite production [2].

Abiotic elicitor-potent nanoparticles have emerged as a novel strategy to phytochemical production in addition to tissue culture [14]. This technology allows you to receive chemicals that would normally be expensive or difficult to obtain in an affordable and accessible way. Modern technological approaches like tissue culture and nanoparticle-based techniques allow researchers to provide a consistent and sustainable supply of bioactive substances [15]. Nanoparticles (NPs) have been shown to improve biochemical profile, seed germination, root/shoot length, and seedling vigor index in medicinally significant plants [16]. Silver nanoparticles (Ag-NPs) are critical in the medical industry for their ability to inhibit bacteria and microorganisms. According to Nindawat and Agrawal [17], the synthesis of green nanoparticles is a straightforward, scalable, economical, sustainable, and energy-efficient substitute for physical and chemical processes. In *Artemisia absinthium* plantlets subjected to various nanoparticles (NPs), an increase in enzymatic components such as superoxide dismutase, peroxidase, and catalase was observed [18]. Additionally, non-enzymatic components including total phenolics and total flavonoids, were also elevated [18]. Ag-NPs produced by algae are especially interesting because of their ability to capture metals and decrease metal ions, which makes them useful bioagents for heavy metal contamination [19]. In addition to the capping and reducing qualities of proteins, lipids, carbohydrates, vitamins, carotenoids, and secondary metabolites, green algae can additionally synthesize silver nanoparticles both inside and outside of cells [20,21]. However, limited studies have been conducted on the green synthesis of Ag-NPs by spirulina biomass and their biological activity.

The purpose of this research is to investigate the impact of bio-fabricated silver Ag-NPs, either in the presence or absence of spirulina aqueous extract (SAE), on the generation of secondary metabolites, including enzymatic and non-enzymatic products, proliferation, and morphogenic changes in cell suspension cultures.

2. Materials and methods

2.1. Chemicals and plant material

Gallic acid, silver nitrate and quercetin standard were obtained from Sigma-Aldrich, St. Louis, MO, USA. Folin-Ciocalteu's reagent was obtained from Merck, Schnell Dorf, Germany. Na_2CO_3 was obtained from VWR Chemicals, Darmstadt, Germany. Growth hormones (naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA)) and MS media ingredients were provided by Hi Media, Mumbai, India, PTC grade. Agar was obtained from Oxoid, England. Aluminium chloride ($AlCl_3$) was obtained from Carl-Roth, Carlsruhe, Germany. The tissue culture technique has been performed at the tissue culture laboratory, Botany Dept., Faculty of Agriculture, Cairo University. The leaf explants were taken from terminal cuttings of mother plants, and Fresh plants of calla lily (*Zantedeschia aethiopica)* (Lamiaceae), were collected from the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

2.2. Preparation of Spirulina microalgae

Stocks of *spirulina platensis* algae were kept in a lab at 29 °C for 16 hours in the day and 8 hours in the dark. *Spirulina platensis* microalgae were cultivated using Zarrouk culture media. Given the sensitivity of algae cultivation, carrying out the process in clean and isolated conditions is essential. Therefore, a separate and isolated room was designated for algae cultivation. The microalgae had a seven-day initial germination time. The microalgae were moved to a bigger container, and a fresh culture medium was added once they had entered the logarithmic growth phase. The biomass was extracted after two weeks of growth. To prevent the effects of salt, the algae mass was rinsed five times using purified water before being dried at 40 °C and utilized in the experiment's subsequent phases [22].

2.3. Preparation of Spirulina Aqueous Extract (SAE)

According to Amin *et al.* [23], This Spirulina aqueous extract (SAE) was prepared. Sterilized distilled water was used to grind five grams of air-dried *Spirulina platensis* cells into a powder while they cooled. After passing through a 50-mesh polyester rope, the slurry was centrifuged for 10 minutes at 5000 rpm. After making 100 mL of the clear supernatant, it was sterilized using a 0.25 µ Millipore membrane.

2.4. Synthesis of silver nanoparticles

The SP-AgNPs were obtained by mixing 5 g of powdered Spirulina platensis from an exponential growth phase with 100 mL of a 1 mM aqueous silver nitrate ($AgNO_3$) solution (pH7) in a 250 milliliter Erlenmeyer flask for 48 hours. At 25 °C, metal ions are reduced to nanoparticles, according to Mahdieh *et al.*, [24] .

2.5. Characterization of green-synthesized Spirulina silver nanoparticles (SP-AgNPs)

In the central lab of the Biochemistry Department at Cairo University of Faculty of Agriculture, Egypt, a T80 UV-visible spectrophotometer (UV-VIS spectrophotometer) (Loughborough, UK) was used to observe the optical properties of SP-AgNPs–VIS absorption spectroscopy. UV-VIS spectra were captured between 300 and 700 nm in wavelength [25]. The JEM-1400, JEOL type, transmission electron microscope (TEM) was used to screen SPAg-NPs morphologically. Imaging was carried out at Cairo University Research Park's (CURP) electronic microscope lab. After freshly synthesized Ag-NPs were deposited onto a glow-discharged carbon grid, they were given a few minutes to air-dry. The TEM-operated equipment was then used to examine the nanocomposite specimens' form and surface roughness [26]. Laser Doppler intensity measurements and photon correlation spectroscopy were utilized to evaluate the SP-AgNPs' zeta potential and particle size, respectively, on a Zetasizer 3000 particulate size description analyzer (Malvern Instruments) at Cairo University's Faculty of Pharmacy, a

supplementary scientific search institute with analytical services. At a scattering angle of 25°/90°C, size correction was performed three times for a total of three minutes each. Using progressive analysis, the mean hydrodynamic diameter was determined. An automatic water dip cell mode was utilized to evaluate the zeta potential adjustment [27]. Fourier transform-infrared spectroscopy (FT-IR) for detecting the functional groups was performed within a scanning range of 400–4000 cm^{-1} using a spectroscopic analyzer (JASCO FTIR-6100), at Cairo University, Egypt [21].

2.6. Culture initiation and Callus culture establishment

2.5–3 cm in diameter For 20 minutes, *Zantedeschia aethiopica* tubers had been sterilized in water at (40 °C) with a few drops of detergent, and then they were rinsed multiple times under rushing water. Following a 30-second soak in 70% (v/v) ethanol, cleaned tubers were immersed in 0.5% $HgCl_2$ for 15 minutes, then washed three times with autoclaved, deionized water. At least one tuber eye was present in the first explants, 1 cm^2 tuber fragments. The explants were soaked in a 100 mg dm^{-3} solution of L-ascorbic acid for one minute before being put on the media [28].

These explants of *Zantedeschia aethiopica* were used in a growth chamber to develop callus cultures. In actuality, naphthaleneacetic acid (NAA) (2.0 mg L^{-1}) and 6-benzylaminopurine (BA) (2.0 mg L^{-1}) were first added before they were inoculated into the solid surface of Murashige and Skoog (MS) medium starting culture comprising macro- and microelements. Furthermore, as a carbon source, 30 g L^{-1} and 8 grams per liter agar (Oxoid, England) were added to the MS media. Subsequently, the MS medium containing PGRs (pH 510, Eutech Instruments, Singapore) was lowered to 5.7 pH. The media vials were autoclaved for 20 minutes at 121 °C in a pressure vessel (Systec, Germany). After that, the cultures were kept at 25 °C in a growth room with light intensity of 40–50 mmol/s. For the callus culture to be produced, the chamber's photoperiod was optimized at 16/8 hours [29].

2.7. Establishment of Cell Suspension Culture

A friable callus weighing 0.5 g per flask produced cell suspension cultures during the stationary phase. Each fraction of friable callus was transferred to 50 mL MS into 250 mL conical flasks. liquid medium for promoting cell cultures (growth hormones and MS media ingredients were provided by Hi Media, Mumbai, India, PTC grade). Different concentrations of each individual elicitor were added to the medium, along with MS salts and a hormone combination [30]. Various elicitors were utilized alongside a control set. The tested elicitors were under sterile conditions as follows:

T0: MS media supplemented with 2 mg L^{-1} NAA and 2 mg L^{-1} BA (control).
T1: MS media prepared with 1 g L^{-1} SAE of concentration.
T2: MS media prepared with varying SAE 1.5 g L^{-1}.
T3: MS media prepared with different 1 g L^{-1} SAE and 5 mg L^{-1} SP-AgNPs.
T4: MS media prepared with a variation of 1.5 g L^{-1} SAE and concentrations of 5 mg L^{-1} SP-AgNPs.
T5: MS medium prepared with different 5 mg L^{-1} SP-AgNPs.

Cell suspension cultures were kept at 25±2 ºC and incubated at 120 rpm in the dark in a rotary shaker. According to Amin *et al.*, [23] subcultures were carried out every two weeks.

2.8. Establishing the System for Callus Suspension Culture

The loose, tender yellow callus of *Zantedeschia aethiopica* was previously obtained loose and tender. In a 100-milliliter flask with a pH of 5.8, it was inoculated with 35 mL of a suspension (MS, various conc *spirulina platensis*, and 30 g L^{-1} sucrose) at 1.0 g callus per flask (fresh weight). Following inoculation, the samples were put on a rotary shaker set to 120 rpm and 25°C in a dark culture environment.

2.9. Determining dry weight, fresh weight, and the growth curve

According to the previously established culture conditions, fresh and dry weights were recorded every five days following inoculation for a maximum of forty days, which was regarded as one cycle. During the study, the flask with the cell suspension culture was taken off the shaker, gently shaken, and a sterile graduated centrifuge tube (15 mL) containing 10 mL of cell suspension was centrifuged for 5 minutes at 6000 rpm. Once the fresh weight was recorded, it was dried at 50°C in an oven until the weight didn't change. The dry weight was also evaluated. From every sampling location, three samples were taken, and each measurement was made three times. After recording the fresh and dried weights, the callus growth curve was plotted against the culture period to depict the various stages of cell growth [31].

2.10. Preparation of *in vitro*-grown cultures of *Zantedeschia aethiopica* callus extraction for phytochemical screening

Zantedeschia aethiopica callus cultures grown *in vitro* were utilized for phytochemical analysis to evaluate the impact of the elicitors on the accumulation of bioactive secondary metabolites. Every produced culture, whether or not Ag-NPs were applied, was used. To extract phytochemicals from samples, the suggested methodology of Ali *et al.,* [15] was adhered to, with a few minor modifications. About 300 mg of powder was weighed out of each sample, dissolved in 10 mL of 50% methanol, shaken for 24 hours at 110 rpm and 25±1°C, sonicated for 30 min, vortexed for 5 min, then forcefully agitated for 15 minutes. Subsequently, the samples were centrifuged for ten min at room temperature at 6,500 rpm. A syringe was used to properly extract the supernatant, which was then transferred to new Eppendorf tubes. To ensure an accurate analysis, the plant sample was diluted until it reached a final concentration of 10 mg/mL. For additional research, the resultant solution was kept at 4°C.

2.11. Quantification of total phenolics, total flavonoids, and DPPH free radical scavenging activity

The total phenolic content of callus and cell cultures was determined using the Folin-Ciocalteu assay. A standard curve was created using standard solutions of 10, 20, 30, 40, 50, 100, 150, 200, and 300 mg/L of gallic acid (Sigma-Aldrich, St. Louis, MO,USA).

Dissolve 200 µL of gallic acid extract or standard solution and 1.8 mL of distilled deionized water in a 5 mL Eppendorf tube. 200 µL of Folin-Ciocalteu's reagent (Merck, Schnell Dorf, Germany) was added to the mixture, thoroughly mixed, and allowed to sit at room temperature for 5 min. Two mL of a 20% Na_2CO_3 solution (VWR Chemicals, Darmstadt, Germany) was well mixed the sixth minute. After diluting the mixture with 5 mL of deionized distilled water, after mixing, it was left in

the dark for 90 min at room temperature. An Analytic Jena Specord® 250 Plus UV-VIS spectrophotometer was used to measure the absorbance at 750 nm in comparison to the reagent blank. Fresh samples' total phenolic content was measured in milligrams per gram, which is equivalent to gallic acid [32].

The flavonoids in the methanolic extract of calla callus were estimated using the aluminium chloride colorimetric approach [33]. 200 µL of each extract or quercetin standard (Sigma–Aldrich, St. Louis, MO, USA) solution (10, 20, 30, 40, 50, and 100 mg/L) were taken, and the volume was increased to 1.8 mL using methanol, 0.1 mL of 10% aluminium chloride ($AlCl_3$) (Carl-Roth, Carlsruhe, Germany), and 2.8 mL of distilled water to reach the final volume of 5 mL. The reaction mixture's absorbance at 415 nm was measured after the solution had been allowed to stand at room temperature for 30 minutes. Total flavonoid content was quantified as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE/g dry weight) and calculated by the equation: T = CV/M, where T is the total amount of flavonoids, C is the concentration of quercetin estimated in mg mL^{-1}, M is the extract's weight in grams, and V is the extract solution's volume in millilitres.

The DPPH solution was initially prepared by dissolving DPPH in 100% CH_3OH to create a 0.004% solution, which remained stable for 48 hours at +4 °C in the dark. The stock solution was used to develop a range of extract dilutions. DPPH test for radical scavenging. 1 mL of each dilution and 1 mL of a 0.004% DPPH methanolic solution were added to dry tubes, and the tubes were then vortexed. For half an hour, the tubes were left at room temperature in the dark. A spectrophotometer was used to measure the absorbance at 517 nm. 1 mL of CH_3OH and 1 mL of DPPH (0.004%) were combined to create a blank or negative control. The experiment was conducted three times for every dilution. The proportion of DPPH discoloration in methanol solution is used to evaluate antioxidant activity, which represents the ability to capture free radicals: (A0-A /A0) × 100 = AA (%). A0 is the optical density of the antioxidant-free control, A0 is the optical density of the extract diluted with DPPH, and AA is the antioxidant activity percentage. The extract concentration curve will be shown using the data, which were expressed as the average of three measurements (AA = f) [34].

2.12. Determination of antioxidant enzymatic activity.

To determine the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), 100 milligrams of samples were homogenized in 1 mL of ice-cold One hundred millilitres phosphate buffer (pH 7.0) containing 2% PVP (polyvinylpyrrolidone), 0.1 mM EDTA (ethylenediaminetetraacetic acid), and 0.2% Triton X-100. After that, the samples were centrifuged for 20 minutes at 4 °C at 12,000 g. The resulting supernatant was used as the enzyme extract for all enzymatic experiments, which were conducted at 25 °C.

As explained by Beyer and Fridovich, [35], The activity of SOD was evaluated by inhibiting nitro blue tetrazolium chloride from being reduced. The reaction mixture contained 13 mM L-methionine, 2 µM riboflavin, 22.5 µM NBT, 0.1 mM EDTA, and 50–200 µl aliquots of enzyme extract. To start the reaction, the reaction mixture was exposed to light, and its absorbance was detected at 560 nm. After five minutes, the mixture's absorbance was measured once more. The negative control, or reaction combination with no enzyme extract, showed no suppression of NBT reduction. The quantity of enzyme needed for 50% inhibition in NBT reduction was determined to be one unit of SOD activity. Units of SOD activity per milligram of protein were used to express the activity.

Aebi, [36]'s approach was used to evaluate CAT activity. The reaction consisted of 50 mM phosphate buffer (pH 7), 10 mM H_2O_2, and 50 µL of enzyme. The decrease in absorbance at 240 nm was observed for three minutes after the reaction mixture was prepared. The reaction mixture did not include the enzyme extract was used as a negative control. H_2O_2 decomposition at 1µmol/min per milligram of protein was treated as one unit of enzyme activity, which was determined using the extinction coefficient of H_2O_2 ($\mathcal{E}$ = 0.036 mM^{-1} cm^{-1}) [36].

POD was determined using the [37,38] technique. Five milliliters of the test mixture for peroxidase activity contained: The peroxidase activity assay combination consisted of 125 µmoles of phosphate buffer (pH 6.8), 50 µmoles of pyrogallol, 50 µmoles of Hydrogen peroxide and 1 mL of a 20-fold diluted enzyme extract. After 5 minutes of incubation at 25°C, the reaction was terminated by adding 0.5 mL of 5% (v/v) H_2SO_4. The amount of purpurogallin generated was measured using absorbance at 420 nm.

2.13. Analyze phenolic compounds using high-performance liquid chromatography (HPLC)

HPLC analysis was performed by Sultana *et al.,* [39]. An analytical HPLC system 1260 infinite series was utilized at the National Research Centre (NRC), Dokki, Egypt, to assess the phenolic profile of callus suspension extracts. During the separation procedure, a Kromasil C_{18} column (4.6 mm x 250 mm i.d., 5 µm) was utilized. The mobile phase consisted of 0.05% trifluoroacetic acid in acetonitrile (2) and water (1), with a flow rate of 1 mL/min. Following a linear trend, the mobile phase was created in the following order: 0 min (82% A), 0-5 min (80% A), 5-8 min (60% A), 8-12 min (60% A), 12-15 min (85% A), and 15-16 min (82% A). The multi-wavelength detector was set at 280 nm. Ten microliters of the sample solutions were injected.

2.14. Statistical analysis

This study employed experimental methodologies that involved conducting all assessments four times, with the results presented as the mean value accompanied by the standard error. The statistical analysis was conducted using the Web Agri Stat Package (WASP) at the ICAR: Central Coastal Agricultural Research Institute. The research employed a one-way analysis of variance (ANOVA) to examine group differences. The least significant difference (LSD) test was utilized with significance criteria of 1% and 5% [40].

3. Results and discussion

1.1. Characterization of Synthesized SP-AgNPs

1.1.1. Ultraviolet-visible spectroscopy

In this study, the *Spirulina platensis* aqueous extract (Fig. 1 a) was applied as a source of reducing and stabilizing agents for

the biosynthesis of Ag-NPs. Sp-Ag-NPs were produced by bio-reduction of Ag^+ to Ag^0 in an aqueous extract of S. platensis, which contains bioactive compounds (Fig. 1 b and c). The biosynthesis of Ag-NPs was indicated by the color change of Spirulina extract from green to dark brown, signifying the reduction of Ag^+ and nanoparticle formation. Additionally, the color intensity of the $AgNO_3$-containing extract increased after 24 hours of incubation.

The 200–700 nm UV absorption spectra were analyzed to verify the stability and formation of silver nanoparticles. At 430 nm, (Fig. 1 d) showed that Ag-NPs had the most significant absorption, showing the surface plasmon resonance region (SPR). The SP-AgNPs produced in this work had physical and chemical characteristics that are similar to the results of Soror *et al.* [41], who found that Ag-NPs formed from *Spirulina platensis* phycocyanin showed SPR at 450 nm, indicating the synthesis of Ag-NPs.

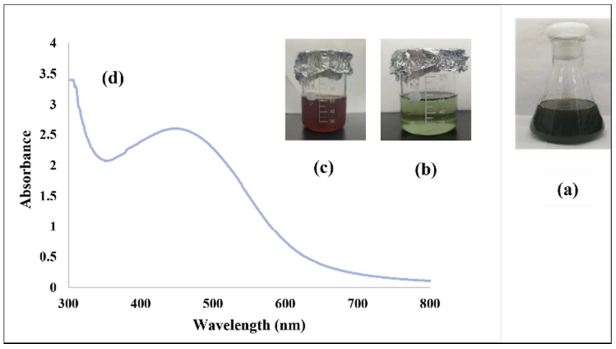

Figure 1: (a) *Spirulina platensis* aqueous extract (b) $AgNO_3$ (c) SP-AgNPs (d) UV.VIS spectrum of SPAg-NPs produced by Spirulina *platensis*

3.3.1. Transmission Electron Microscopy

TEM analysis demonstrated the size and shape of synthesized Ag-NPs, as shown in Fig. 2. It was obvious that the morphology of Ag-NPs is spherical, having a size of around 10 nm or less, and was not agglomerated. It was shown that NPs stabilized with capping agents were monodispersed. Nanoparticles exhibited better physical properties if they were produced in small sizes, as the good properties of silver nanoparticles are mostly size-dependent [42]. According to Muthusamy *et al.*, [43], the synthesized Ag-NPs were well distributed and distinguished by their spherical shape, which ranged from 5 to 50 nm. Soror *et al.*, [41] also created *Spirulina platensis* silver nanoparticles with diameters ranging from 9–55 nm and spherical shape.

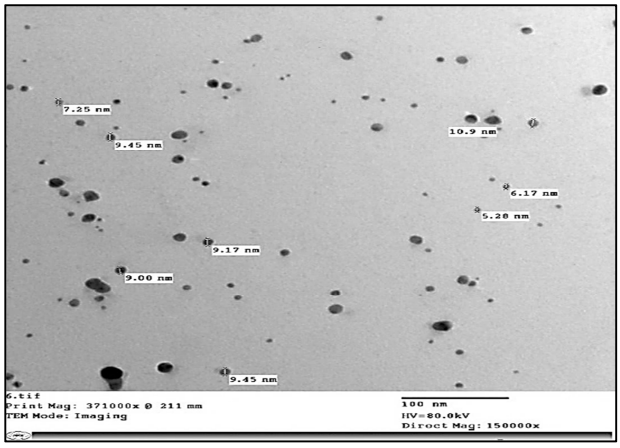

Figure 2: Transmission electron microscope (TEM) image of silver nanoparticles

3.3.2. FTIR spectrum analysis

The FTIR spectrum of Ag-NPs synthesized from *Spirulina platensis* aqueous extract (Fig. 3.a) revealed six major functional groups at 3275.82, 2932.17, 1789.01, 1632.95, 1343, and 1028.25 cm^{-1}, corresponding to monosubstituted amide, nitro, primary amide, carboxylic, and alcohol groups. The peak at 3275.82 cm^{-1} indicates Stretching of OH groups in carboxylic acids, while the band at 2932.17 cm^{-1} represents alkane amide I stretching from C to H. The band at 1632.95 cm^{-1} corresponds to -C=C- stretching in alkenes and 1789.01 cm^{-1} is typical of C=O carbonyl groups associated with proteins' amine N–H bending vibrations. The 1028.25 cm^{-1} peak signifies C-O stretching in carboxylic acid, ether, alcohol, and ester groups, aligning with findings by Ganapathy Selvam and Sivakumar [44].

The band at 1343.16 in Figure (3a) resulted from proteins' methylene scissoring vibrations, confirming their presence in Ag-NPs. FTIR spectroscopy showed that monosubstituted amides in proteins have a strong affinity for metals, suggesting that

proteins form a coating around Ag-NPs, preventing agglomeration and stabilizing them in media. These findings indicate that biological molecules contribute to the reduction and stabilization of Ag-NPs in an aqueous medium, supporting the results of Naaz *et al.* [45]. Ag-NPs interact with the -OH group of *Spirulina platensis* extract, as indicated by the peak at 526 cm^{-1}, which shows a metal–oxygen relationship. Silver ions have a great attraction for forming coordination connections with polar -OH groups, and this affinity strengthens as Ag-NPs concentration increases, supporting the results of [47]

While, the FT-IR spectrum of the crude aqueous extract of *Spirulina platensis* is shown in Fig. (3 b), and it showed that there were mainly ten bands at 3272.79, 2931.08, 1631.29, 1581.47, 1452.69, 1400.81, 1242.84, 1113.55, 1037.04, and 927.03 cm^{-1}. O–H stretching vibration was identified as the cause of the peak at 3272.79 cm^{-1} due to the presence of phenol and alcohol groups. The C–H stretching vibration of alkenes is the cause of the peaks that emerge at 2931.08 cm^{-1} . The C=O stretching and N–O asymmetric stretching vibration of nitro compounds were caused by the bands at 1631.29 and 1581.47 cm^{-1}, respectively.

The C–C stretch and methylene scissoring vibrations in the proteins are responsible for the bands at approximately 1452.69 and 1400.81 cm^{-1}, respectively, while the N–H stretching of the primary and secondary amines may be the cause of the peak at 1242.84 cm^{-1}. Similar findings were previously documented concerning these functional biochemical bonds [48]. The peaks found in 1113.55 and 1037.04 cm^{-1} were attributed to C-O stretching from carboxylic acid, ether. The peaks at 1113.55 and 1037.04 cm^{-1} corresponded to C-O stretching from alcohol, ether, ester, and carboxylic acid.

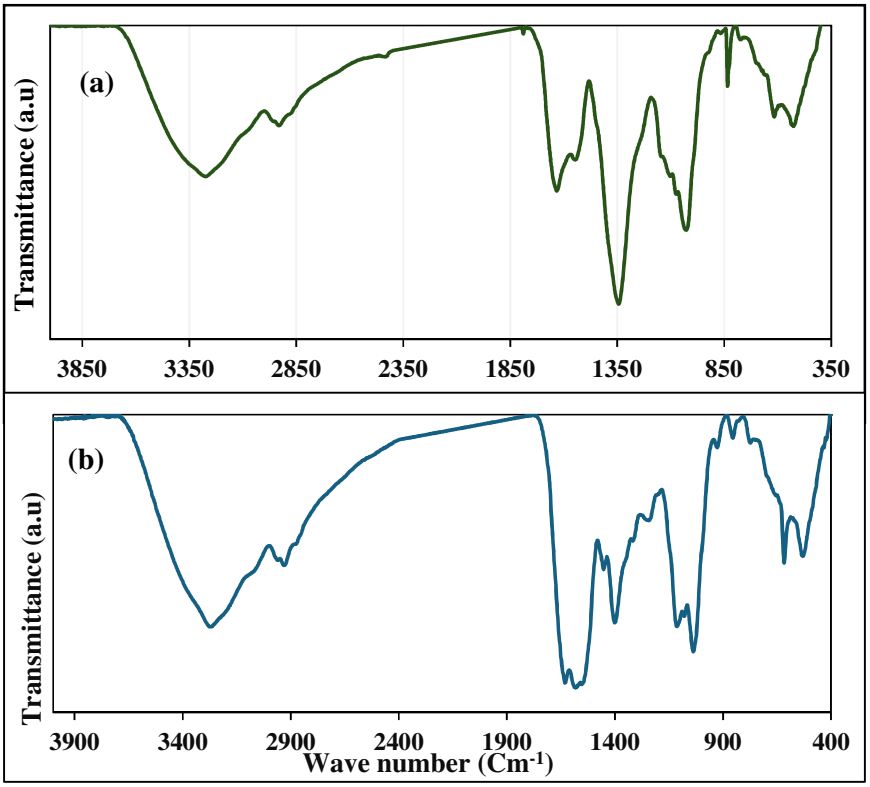

Figure 3:(a) SPAg-NPs produced using the crude aqueous extract from *Spirulina platensis*, and (b) the FT-IR spectrum of the crude aqueous extract of *Spirulina platensis*

3.1.4. Zeta potential and particle size

Figures (4 a) and (4 b) showed the dynamic light scattering (DLS) findings and the charge of Ag-NPs, respectively. The exact size was 30 nm, and the charge was −28.32 mV. Nanoparticles are stable because of their negative surface charge.

Accordingly, Kratošová *et al.* [49] discovered that sample values ranging from +30 mV to −30 mV were constant and did not aggregate. Such large negative values stop particles from aggregating because of repulsion Soror *et al,*. [41] observed that the findings of SP Ag-NPs revealed a single peak. It had a net negative charge of -26.32 mV and was precisely 30 nm in size. The stability of nanoparticles is maintained by their negative surface charge.

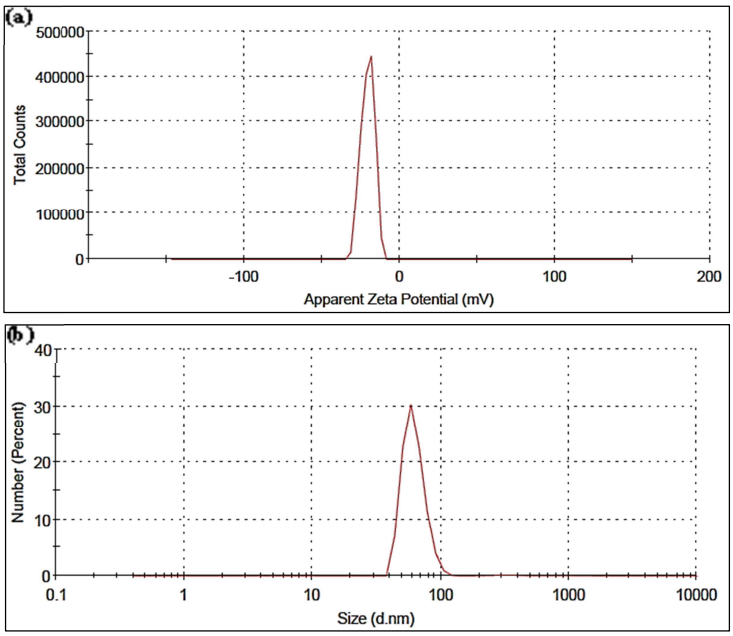

Figure 4:(a). Zeta potential and (b) Size distribution by *Spirulina platensis*-synthesised SPAg-NPs

Growth curve of suspension-cultured calluses

Using fresh and dried weights, the *Zantedeschia aethiopica* cell suspension culture growth curve was plotted, showing a sigmoidal pattern in Fig. 5(a) and 5(b). Fresh and dry weights, recorded on days 10, 20, 30, and 40, peaked at 30 days of culturing. This indicates that 30 days is the critical time for cells to enter the progressive deceleration stage.

Callus showed remarkable growth on T4 and T3 media, with the highest fresh (17.16±0.13 g) and dry weights (0.53±0.01 g) observed in T4, followed by T3 (16.46±0.11 g and 0.58±0.01 g) on day 30 (Fig. 1a, 1b). Biomass accumulation at day 30 peaked in T2 (14.79±0.05 g and 0.56±0.04 g) and T1 (12.84±0.02 g and 0.51±0.01 g) with spirulina extract and SP-AgNPs. However, T5 showed reduced weights (11.34 g and 0.45 g), while control calluses (T0) had fresh (9.12±0.29 g) and dry weights (0.61±0.09 g). After 30 days, all treatments showed a reduction, most likely associated with a lack of nutrients in the liquid MS medium, resulting in cell death (Fig. 5a, 5b).

Suspension callus culturing provides accurate environmental control, a shortened growth cycle, and effective secondary metabolite separation. It enhances metabolite production, aiding in natural compound extraction. Mathematical models are crucial for analyzing metabolite synthesis and accumulation, optimizing yield, advancing synthesis methods, and boosting plant therapeutic value [50]. The enhancing effects in cell suspension culture grown in SAE-supplemented T1 and T2 medium may be related to the composition of SAE as it consists of phytohormones, vitamins, and micronutrients, and the previous results were concomitant with the results obtained by Amin [50] who found that the *Sisymbrium irio* calli's stimulatory reactions might be connected to the fact that S. platensis is a fantastic source of vitamins and phytohormones when SAE concentrations vary (16% and 18%), the growth was increased and revealed that the growth yields of calli (FW 6.10±0.16 g and DW 0.42±0.01 g) (FW 6.95±0.19 g and DW 0.51±0.01 g) were higher than that of the control culture (untreated culture).

The impact of SP-AgNPs on plant cell development and tissue culture remains underexplored, particularly in *Zantedeschia aethiopica*. This study revealed that Ag-NPs significantly influenced callus growth, proliferation, and antioxidant biosynthesis. Biochemical effects of SP-AgNPs and spirulina extract concentrations (T3, T4, and T5) were compared to control cultures, with increased biomass observed by day 10. Contradictory findings in existing literature show that nanoparticle effects vary by species, type, size, concentration, and exposure duration. For instance, Ag-NPs at 50 mg/mL boosted callus fresh weight in *Phaseolus vulgaris* [52] and increased sugarcane biomass at 40–60 mg/L [53].

Conversely, it was shown that after being exposed to increasing concentrations of Ag-NPs for 24 hours, the fresh and dry mass of pearl millet (*Pennisetum glaucum* L.) seedlings dramatically dropped [54]. Additionally, after 14 days of exposure to Ag-NPs-Cit-L-Cys at 20 and 50 mg/L, Lemna plants displayed increased biomass for the control group [55]. In line with our results, Sanzari *et al.*, (2019) observed a reduction in growth at concentrations of Ag-NPs, which may be attributed to reduced adaptability of plant cells to Ag-NPs [56].

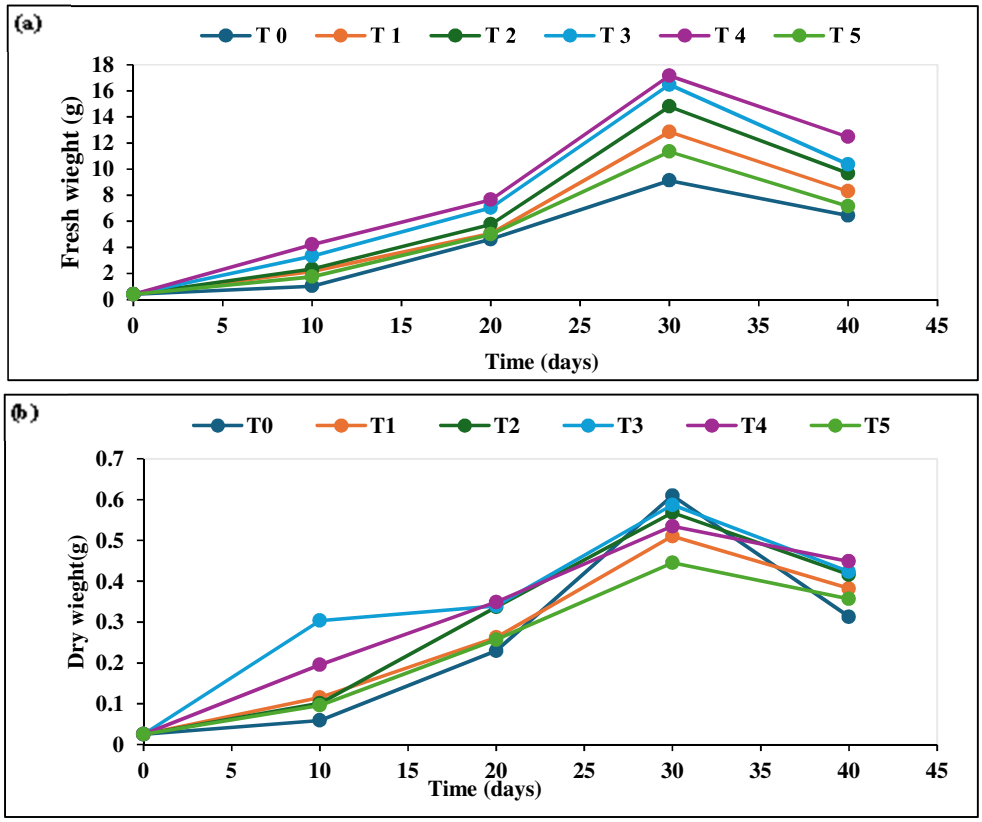

Figure 5: ***Zantedeschia aethiopica*** **cell suspension growth curve based on measurements for 40 days (a) fresh weight and (b) dried weight**

Effects of SP-AgNPs and PGRs on total phenolic content, total flavonoid content, and DPPH free radical scavenging activity in suspension cultures

The results of TPC, TFC, and DPPH are summarized in Table 1. Lower TPC values were observed in T1 and T2 (1.63±0.02 and 2.06±0.01 mg GAE/g FW) compared to T3 and T4 with spirulina silver nanoparticles and SAE (3.31±0.04 and 2.94±0.02 mg GAE/g FW). The highest TPC was recorded in T5 with SP-AgNPs (4.27±0.09 mg GAE/g FW), while the control (T0) had the lowest value (0.61±0.01 mg GAE/g FW).

The results confirmed that SP-AgNPs alone significantly enhanced TPC in callus cultures. The addition of elicitors to callus suspension culture (CSC) induces phenolic biosynthetic pathways. In this study, phenolics as non-enzymatic antioxidants significantly increased in *Zantedeschia aethiopica* under T1 and T2 treatments (Table 1), correlating with enhanced antioxidant activity. Algal extracts, such as spirulina, possess antioxidant properties that improve phenolic and flavonoid levels [57,58], consistent with T1 and T2 results.

In this study, we investigated how the polyphenol content was affected by adding SP-AgNPs to the suspension culture media of in vitro cultures. Application of SP-AgNPs in CSC stimulated the accumulation of total polyphenol and flavonoid contents in *Zantedeschia aethiopica*, and the content and distribution pattern of phenolic metabolites among the various treatments indicate a non-toxic impact of SP-AgNPs mimicking mild or no stress to the seedlings. A consistent increase in these contents was observed with Ag-NPs treatment, with CSC treated at 5 mg/L of SP-AgNPs (T5) showing significantly elevated levels. Similarly, another study reported the elicitation impact of silver and gold nanoparticles in the *Prunella vulgaris* callus culture increased the total phenolic and flavonoid contents [59]. *In vitro*-regenerated *Vanilla planifolia* shoots cultivated on MS medium, which contains 25 and 50 mg/L of Ag-NPs, showed a significant increase in total phenolic content [60].

According to earlier studies, metal nanoparticles have varying effects on the amount of polyphenols found in vegetable plants. It has been found that *Solanum tuberosum* has a higher polyphenol content when exposed to Ag-NPs [61]. Similar findings were observed in the current study, as the polyphenol content was higher than in control (T0) plants compared to T5 plants. Numerous investigations on the possible function of nanoparticles as abiotic elicitors in various plants, including *Caralluma tuberculata*, have been conducted [29] and *Lavandula angustifolia* Mill. [62], observing that the amount of phenolic

compounds increased when Ag-NP concentrations were high in line with our results. As reported by Ali *et al.* (2019) Nanoparticles may function as signaling molecules and influence the synthesis and makeup of secondary metabolites that are part of the antioxidant defence systems. Numerous studies have been published on the assessment of *Zantedeschia aethiopica*'s polyphenols, flavonoids, and antioxidant properties [32].

Table 1: The impact of various spirulina aqueous extract (SAE) and SP-AgNPs concentrations on *Zantedeschia aethiopica* cell suspension cultures' antioxidant activities, total flavonoid content (mg/g), and total phenolics

Treatments	Total phenolics (mg/g FW)	Total flavonoids (mg/g FW)	Scavenging activity%
T0	0.61±0.01^{f}	0.42±0.01^{f}	48.80±0.06^{f}
T1	1.63±0.02^{e}	1.26±0.01^{e}	63.72±0.47^{e}
T2	2.06±0.01^{d}	1.84±0.04^{d}	65.73±0.03^{d}
T3	3.31±0.04^{b}	2.29±0.03^{b}	76.18±0.21^{b}
T4	2.94±0.02^{c}	2.04±0.08^{c}	72.69±0.03^{c}
T5	4.27±0.09^{a}	3.88±0.06^{a}	80.03±0.17^{a}
LSD (0.01)	0.130	0.145	0.686
LSD (0.05)	0.093	0.103	0.489
Coefficient of variation	2.110	2.977	0.405

The values show that the mean is either plus or minus the standard error. If the values are significantly different at a significance level of $p < 0.05$, they will be indicated by superscripted letters. The different treatments within the same column are compared. LSD: Least significant difference. T0: MS media supplemented with 2 mg L^{-1} NAA and 2 mg L^{-1} BA (control). T1: MS media prepared with 1 g L^{-1} SAE of concentration. T2: MS media prepared with varying SAE 1.5 g L^{-1} . T3: MS media prepared with different 1 g L^{-1}SAE and 5 mg L^{-1} SP-AgNPs. T4: MS media prepared with a variation of 1.5 g L^{-1} SAE and concentrations of 5 mg L^{-1} SP-AgNPs. T5: MS medium prepared with different 5 mg L^{-1} SP-AgNPs. FW: fresh weight. mg: milligram.

The total flavonoid contents vary similarly for the total polyphenols (Table 1), which demonstrated notable rises across all treatments except the control. The application of SP-AgNPs in T5 resulted in the highest levels of flavonoids (3.88±0.06 mg QU/g FW), followed by a combination of SP-AgNPs and SAE in T3 (2.29±0.03 mg QU/g FW), and followed by a combination between SP-AgNPs and SAE in T4 (2.04±0.08 mg QU/g FW). In cell suspension cultures (T1 and T2) (1.26±0.01 and 1.84±0.04 mg QU/g FW), callus samples made with the same dose of silver nanoparticles and spirulina extract added did not show a discernible rise in flavonoid levels. The lowest levels were recorded for the total flavonoid contents in T0 (0.42±0.10 mg QU/g FW). Thus, it is evident from the results that silver nanoparticles alone were more supportive of enhancing TPC, and TFC activities in callus cultures.

In case of adding nanoparticles in different treatments of *Zantedeschia aethiopica* callus suspension cultures (T5, T4, and T3), an increase in flavonoids was found when silver particles only in the treatment. In line with our research results, Ali *et al.* [29] showed that the addition of silver nanoparticles in solid culture media was found to promote higher accumulation of polyphenol in the callus cultures of *Caralluma tuberculata* generated on MS media in the presence of 90 mg/L Ag-NPs. A variety of biotic and abiotic elicitors have been found to improve plant growth, multiplication, and bioactive content [63]. When spirulina extract was added to treatments T1 and T2, the concentrations were 1.26±0.01 and 1.84±0.04 mg, respectively. There was an increase in TFC compared to the control treatment (T0) in a concentration of 0.42±0.10 mg. When *Spirulina platensis* extracts were applied to *Sisynbrium irio* callus cultures, it was shown that the antioxidant activity gradually increased as the polyphenol concentration increased [51].

A significant increase in the antioxidant activity values of the extracts of treated SAE and nano compared to the control was observed in Table 1. SAE and nano treatment elicitors had stronger antioxidant potential in T3 and T4 (76.18±0.21% and 72.69±0.03%), respectively, whereas the application of nano gave the maximum antioxidant activity (80.03±0.17%) in T5. The antioxidant activity was (63.72±0.47% and 65.72±0.03%) in T1 and T2, respectively. The control (T0) had the lowest antioxidant activity, measuring 48.80±0.06%. Thus, the antioxidant power of ethanolic extracts of cell suspension culture was improved for 2 weeks with nano and SAE with 1 g/L and 1.5 g/L, respectively. Lower antioxidant potential was recorded for calluses without the treated extracts of *Zantedeschia aethiopica*.

Plant tissue's antioxidant capacity is correlated with the presence of flavonoids and phenolics. Under such conditions, the defense system in plants releases polyphenolic contents as natural antioxidants. These natural antioxidants reduce or completely inhibit the activity of free radicals, preventing the formation of ROS. As a result, they directly influence the activities of lipids, proteins, and the synthesis of DNA molecules [29]. Therefore, the polyphenolic concentration in plant-based goods is most likely what gives them their antioxidant capacity. An increase in antioxidant activity could be associated with the elevated production of secondary metabolites, such as phenols, which exhibit antioxidative properties and have the ability to directly neutralize free radicals within plant tissues [64].

Toxic free radicals and oxygen radicals are created in large enough amounts under adverse circumstances to start a chain reaction that eventually results in the death of cells or tissue [65] . Many plants emit polyphenols, an active class of natural

antioxidants, to deal with the unique environment. Antioxidants found in plants are therefore thought to scavenge harmful free radicals as well as oxygen radicals. Enhancing the synthesis of non-enzymatic components may be possible through the use of *in vitro* procedures with volatile medium additions, such light, temperature, and nanoparticles [16].

The current study reports that Ag-NPs have a beneficial effect on DPPH production in the callus of *Zantedeschia aethiopica* in T3-T5 as compared to the control (T0). Fazal *et al.* (2016) observed that the silver nanoparticles induced the highest accumulation of TPC (9.57 mg/g DW), flavonoid content (6.71 mg/g DW), and improved antioxidant activity by 87.85 %. Higher levels of several antioxidant secondary metabolites were observed to be biosynthesized when Ag-NPs were applied inductively [59]. According to Ali *et al.* (2019), who also reported that the calli established at 90 mg/L Ag-NPs exhibited the highest amount of DPPH free radical activity (90%) in line with the current findings [29].

Several physiologically active compounds, such as polysaccharides, amino acids, phytohormones, etc., can be released by spirulina to support plant growth and boost resistance to biotic and abiotic stressors [13]. This is what was noticed by adding SAE in suspension culture in T1 and T2 compared with the control (T0). SAE has been used as a biotic elicitor and was able to increase DPPH radical scavenging activity in the *Zantedeschia aethiopica* in suspension culture. Since spirulina contains vitamins and other essential components which makes it active in driving the process of metabolite synthesis and initiating plant defence responses. The use of bioengineered nanoparticles in callus suspension culture to improve *Zantedeschia aethiopica*'s non-enzymatic chemicals has not yet been reported.

SP-AgNPs and PGRs' effects on the activities of antioxidant enzymes on SOD, POD, and CAT in suspension cultures

During cell development, biotic and abiotic stressors generate harmful compounds and ROS that inhibit differentiation [66]. Plants produce enzymes like SOD, POD, and CAT to combat oxidative stress caused by ROS, providing defense against free radicals. SOD plays a key role in oxidative stress management [67]. Similarly, *Bacopa monnieri* seedlings treated with biologically synthesized Ag-NPs showed increased POD activity [68].

In this study, the activities of key antioxidant enzymes were assessed in callus suspension cultures treated with varying concentrations of SP-AgNPs, either individually or in combination with T3 or T4. Among all the treatments applied, Ag-NPs without PGRs (T5) increased the activities of SOD, POD, and CAT (4.5, 3.3, and 2.5 U/mg protein), respectively in cell suspension cultures (Figs. 6 a-c). Callus suspension cultures grown in T3 showed increased antioxidant enzyme activity (3.1, 2.8, and 1.9 U/mg, respectively) and followed by T4 values of antioxidant enzyme activities that were found to be 2.7, 2.3, and 1.4 U/mg, respectively. The values of antioxidant enzyme activities differed when adding different concentrations of spirulina extract in callus suspension culture treatments (T1 and T2), and the values were 2.1, 1.9, and 1.1 U/mg in T2 and 1.7, 1.2, and 0.6 U/mg in T1. And, when they were compared to the control treatment (T0), the results were found to be 0.5, 0.9, and 0.3 U/mg, respectively. That meant that silver nanoparticles alone at higher concentrations were more effective in producing enzymes that are antioxidants.

Gupta *et al.* [42] examined the effects of different silver nanoparticle ratios on the enzymatic activity of *Brassica juncea* callus in a similar study. Their findings revealed that higher concentrations of silver nanoparticles in the culture medium led to an enhancement in the antioxidant enzyme activities of *Brassica juncea*. Our results showed when silver nanoparticles were only used, SOD activity in callus cultures was enhanced compared to doses with the added SAE. Consistently, *Corylus avellana* cells' antioxidant defense mechanism was activated by Ag-NPs elicitation [69]. Thiruvengadam *et al.*, [69] reported that bio-synthesized Ag-NPs notably enhanced the gene expression of three key antioxidant enzymes, namely CAT, POD, and GST, in turnip [11]. Conversely, According to Ma *et al.* (2016), *Allium cepa*'s SOD activity reduced as silver nanoparticle concentration increased. In a research study, to unravel the stimulatory impact of Ag-NPs on rice *Oryza sativa L* seedling growth, Ag-NPs caused alterations in antioxidative enzyme activity and associated gene expression levels [71], and according to Gupta *et al.*, (2018), all of the AgNPs-treated seedlings exhibited increased levels of catalase and peroxidase, indicating better growth [72]. Numerous writers have observed that higher SOD activity is linked to plants' greater ability to withstand environmental stressors [71].

CAT is one of the antioxidant enzymes that may scavenge a lot of ROS. The impact of nanoparticles (NPs) on secondary metabolism in plants has been assessed in numerous studies.NP It was discovered that CeO_2 NP treatment enhanced the CAT, SOD, and POD activities in *Arabidopsis thaliana* [71]. There are not many reports on adding spirulina extract to callus suspension cultures, but most reports that used spirulina extract, whether by spraying it on the leaves or adding it to the soil, indicate that the presence of SAE protects plants from stress and helps in reducing the enzymes (CAT, POD, and COD), as occurred in our research in T1-T4.

In order to stimulate the callus of *Sisymbrium irio* L., Amin *et al.* [23] created successful suspension cultures using MS medium combined with spirulina aqueous extract (SAE) as a source of vitamins and phytohormones. The results demonstrated that the activities of CAT and POD in cell suspension cultures (0.28 and 0.05) were lower than control (4.08 and 0.20). SAE's ability to scavenge reactive oxygen species may be the cause of the earlier decreases in antioxidant enzymes. The unit of measurement for catalase activity was μ mol H_2O_2 destroyed/mg protein/min.

The antioxidant enzymes activity (SOD, CAT, and POD) in the shoot of wheat seedlings was shown to increase by 150%, 83%, 193%, and 11%, respectively, when S. platensis was applied to wheat plants [73] El-Shazoly *et al.* (2024). The effects of SAE on the antioxidant defenses of common beans grown in salty soil contaminated with heavy metals were evaluated by Rady *et al.* [74] using foliar spraying and/or soil addition. The most successful treatment was thus the integrative soil addition + foliar spraying with 40 mg SP/plant, given the notable reductions in hydrogen peroxide (42.2%) and the activities of CAT (18.1%), ascorbate peroxidase (18.3%), superoxide dismutase (SOD) (192%), and glutathione reductase (52.2%) as reinforcing mechanisms. In light of the significant decreases in hydrogen peroxide (42.2%) and the activities of catalase

(18.1%), ascorbate peroxidase (18.3%), superoxide dismutase (192%), and glutathione reductase (52.2%) as reinforcing mechanisms, the most effective treatment was thus the integrative soil addition + foliar spraying with 40 mg SP/plant.

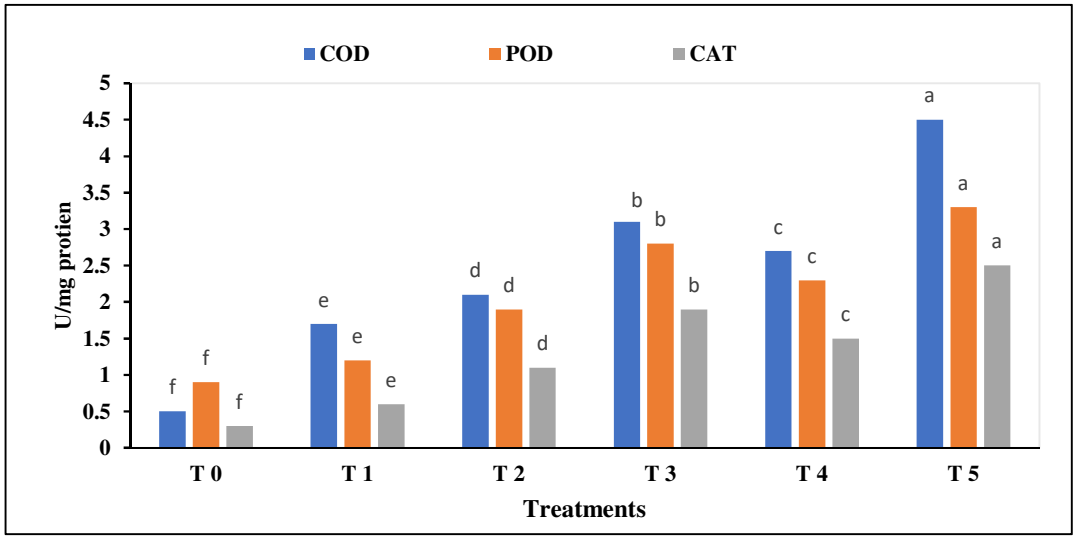

Figure 6: The antioxidant enzymes activity Assessment of enzymatic antioxidants in *Zantedeschia aethiopica* callus suspension cultures created in response to treatments with varying amounts of silver nanoparticles. activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). The data are triplicate mean values with a standard error of ±. If the values are significantly different at a significance level of $p < 0.05$, they are indicated by superscripted letters.

Phenolic compounds analysis by HPLC

The influence of SP-AgNPs and SAE on the accumulation of polyphenols in the cell suspension extract of *Zantedeschia aethiopica* was evaluated using HPLC. The quantity of phenolic compounds in *Zantedeschia aethiopica* obtained from in-cell suspension treated was compared with that of the nontreated cell suspension culture (Table 2). The induction of cell suspension cultures (T5) by using a nano elicitor considerably increased the bioactive phenolic compounds as they were 1422.28 µg/10 mL, while in the case of T3 and T4, the total phenolic compounds were 897.62 and 725.25 µg/10 mL, respectively. It was noticed that the obtained phenolic compounds from T1 and T2 were 445.51 and 503.79 µg/10 mL respectively. They (T1 and T2) were higher in concentration compared to the control treatment (T0) in a concentration of 306.31 µg/10 mL.

Table 2: HPLC study of phenolic components in SP-AgNP-elicited and non-elicited cell suspension culture (CSC) extracts of *Zantedeschia aethiopica*

Compounds to be detected	Phenolic compounds concentration (µg/g) /treatment					
	T 0	T 1	T 2	T 3	T 4	T 5
Gallic acid	28.29	46.50	58.62	50.03	43.60	264.91
Protocatechuic acid	1.90	29.36	45.05	59.04	22.09	95.07
***p*-hydroxybenzoic acid**	117.44	202.56	224.81	438.03	406.38	557.22
Syringic acid	3.26	4.86	3.74	3.31	1.08	16.19
Vanillic acid	ND	ND	1.01	0.31	0.35	9.11
Rosmarinic acid	28.85	29.87	33.67	38.43	35.67	44.87
Chlorogenic acid	ND	4.05	2.61	5.14	2.87	1.40
Caffeic acid	ND	0.29	0.27	0.96	3.59	2.27
Cinnamic acid	15.35	0.82	0.17	0.82	ND	ND
Apigenin-7-glucoside	45.7	56.3	60.23	100.29	89.4	145.6
Sinapic acid	ND	1.96	2.74	3.67	0.78	5.55
Catechin	30.93	44.87	64.41	76.94	101.56	156.18
Rutin	10.34	2.70	3.67	5.87	4.97	17.04

Quercetin	3.18	10.34	19.45	50.23	44.46	55.68
Apigenin	13.89	8.78	11.89	19.56	16.45	23.78
Kaempferol	7.18	2.25	3.45	20.37	15.96	27.41
Total	306.31	405.51	535.78	873	789.21	1422.28

T0: MS media supplemented with 2 mg L^{-1}NAA and 2 mg L^{-1} BA (control). T1: MS media prepared with 1 g L^{-1} SAE of concentration. T2: MS media prepared with varying SAE 1.5 g L^{-1}. T3: MS media prepared with different 1 g L^{-1} SAE and 5 mg L^{-1} SP-AgNPs. T4: MS media prepared with a variation of 1.5 g L^{-1} SAE and concentrations of 5 mg L^{-1} SP-AgNPs. T5: MS medium prepared with different 5 mg L^{-1} SP-AgNPs. ND: non detected.

One method used to increase the yield of bioactive chemicals in plant cell culture is elicitation [75]. To induce the ideal level of bioactive chemicals, a number of characteristics are crucial, including the elicitor's type, concentration, and exposure length [76]. Kruszka *et al.* [77] reported the elicitation of phenolic compounds and effects of treatment with silver (Ag), gold (Au), copper (Cu), and zinc oxide (ZnO) nanoparticles on plant secondary metabolism were tested using in-of *Hypericum perforatum* L suspension cultures, and variations in the accumulation of secondary metabolites were observed in cultures after exposure to different NPs. Ag-NPs significantly increased the cells' accumulation of xanthones and fusaroskyrin [77].

Phenolic compound accumulation in cell cultures can be enhanced by a variety of NP types, including Ag, Au, CuO, titanium oxide (TiO_2), and ZnO [78]. Ag-NPs treatments increased the amount of Ag in *bitter gourd* (CSC) cell suspension cultures. According to Min *et al.*, [79], Ag-NPs-elicited cell suspension cultures of *bitter gourd* have higher levels of phenolic components of flavonols, hydroxybenzoic, and hydroxycinnamic acids than non-elicited cell suspension cultures.. In the same way, our results indicated that SP-AgNPs can be considered as effective catalysts for inducing the production of phenolic in suspension culture in calla. In another study, it was observed that the secondary metabolites elicited in *Fagonia indica* varied between the types of NPs [80][79,80][79,80][79,80]. Similarly, Xing *et al.* [81] was observed that silver increased the synthesis of rosmarinic, ferulic, and caffeic acids while decreasing the amounts of cinnamic and salvianolic acids in *Salvia miltiorrhiza*.

In another study, Amer *et al.*, [82] was evaluated the effects of different *Spirulina platensis* extract on the growth and produce phenolic compounds of *Cynara cardunculus* L plants where due to spraying with spirulina extract increase resulted in the percentage of Rutin and Apigenin-7-glucoside with the values of 1.1 and 1.5 mg/plant, respectively than the control. Amin *et al.* (2009) [23] prepared successful suspension cultures that were established using MS medium mixed with spirulina aqueous extract (SAE) as a source of vitamins and phytohormones to stimulate callus of *Sisymbrium irio* L. The results showed that both flavonoids and total phenolic compounds accumulated in the medium and this was confirmed using HPLC.

In the present study, no apparent toxic effects of Spirulina platensis-derived silver nanoparticles (SP-AgNPs) were observed on *Zantedeschia aethiopica* cell suspension cultures at the applied concentrations. The cells maintained normal growth and metabolite production, indicating that the nanoparticles were well tolerated under the tested conditions. These findings are in agreement with previous studies reporting that AgNPs, when applied at optimized concentrations, can stimulate metabolite accumulation without causing significant cytotoxicity in plant cell cultures [53]. Nevertheless, nanoparticle-induced toxicity has been described in other plant systems at higher concentrations [83], suggesting that dose and exposure duration are critical factors. Therefore, further studies are needed to comprehensively evaluate the potential long-term and dose-dependent effects of SP-AgNPs on Z. aethiopica, which would provide deeper insights into their safe biotechnological application.

Conclusions

Our study demonstrated that the use of nano-elicitors, such as SP-AgNPs, effectively stimulates the production and accumulation of secondary metabolites in *Zantedeschia aethiopica* cell suspension cultures without adversely affecting growth. Green synthesis of Ag-NPs using spirulina extract proved to be an eco-friendly method, with characterization confirming the successful production of nanoparticles. The combination of 500 mg/L SP-AgNPs and 1.5 g/L spirulina extract showed the most significant impact on growth parameters, enzyme activities, and metabolite production, outperforming the control and other treatments.This study demonstrates that Spirulina platensis-derived silver nanoparticles in combination with *Zantedeschia aethiopica* cell suspension cultures can be used to improve secondary metabolite production. Such findings provide potential applications in agriculture and pharmaceutical industries and highlight the value of this approach for sustainable use of plant resources. This research highlights the potential of combining silver nanoparticles and green algae as a novel class of elicitors for enhancing secondary metabolite synthesis. However, more experimental data are needed to understand the underlying mechanisms and optimize elicitation protocols. Future studies should focus on refining bioreactor-based cultivation systems and unraveling the metabolic pathways, enzymes, and transcription factors involved in secondary metabolism. Additionally, further exploration of nanomaterials' effects on *Zantedeschia aethiopica* tissue culture could pave the way for improved propagation techniques and metabolite production strategies.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data presented in Tables and Figures

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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