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Selenium Nanoparticles Mycosynthesis Using the Endophytic Fungi Fusarium graminearum and Investigating its Antioxidants and Cytotoxicity Effect

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ABSTRACT

Mycosynthesizing Selenium nanoparticles (SeNPs) are the focus of this investigation using the endophytic fungus fusarium graminearum isolated from Salvia Rosmarinus and testing the nanoparticle's cytotoxicity and antioxidant activity. First, the fungus was isolated and purified on Potato Dextrose Agar (PDA) from Salvia Rosmarinus leaves. After that, fungal biomass was produced using a laboratory-prepared media, and the biomass was introduced to sodium selenite solution to mycosynthesised SeNPs. SeNPs were characterised using many techniques. A colour shift from pale light to deep orange is the first indicator of the SeNPs mycosynthesis. SeNPs were detected in UV–vis absorption spectra with a 273 nm band. XRD analyses prove the crystallinity phase of SeNPS with three distinctive peaks. SeNPs' FTIR spectra showed absorption peaks from 3400 to 406.98 cm1. The SEM scans showed 53–62 nm spherical particles. The particles revealed a diameter of 83.23 nm (the average) with a size range spanning from 40.22 to 74.87 nm, according to AFM pictures and charts. The synthesised SeNPs were tested for antioxidant and cytotoxic properties. Selenium nanoparticles were tested for their antioxidant capabilities by scavenging 1,1-diphenyl-2-picryl-hydrazyl. Compared to selenium salt, selenium nanoparticles' scavenging activity increased with concentration. There was a dosage relationship between SeNPs and anticancer properties.

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INTRODUCTION

Nanotechnology encompasses the study of particles within the size range of 1–100 nm, focusing on their design, production, and manipulation [1]. With different chemical, physical, high surface area-to-volume ratios, low melting point, strong photoconductivity, catalytic activity, and biological properties [2-4].

Various techniques are employed in the fabrication of nanoparticles: physical, chemical, and biological techniques [5].

Biosynthetic approaches employing either plant extracts or microorganisms have emerged as a straightforward and feasible alternative to conventional physical and chemical procedures [6]. Since the 1980s, fungi have continued to play a significant role by offering a more environmentally friendly substitute to chemically

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produced nanoparticles, known as mycosynthesis [7]. Fungi have been observed to exhibit enhanced polydispersity, structural stability, and a more comprehensive range of dimensions in the biosynthesis of diverse nanoparticles [8].

Selenium is a crucial trace nutrient that plays a vital role in supporting the human and animal immune systems [9]. The insufficiency of selenium disrupts the equilibrium between oxidant agents and antioxidants in the cell, leading to an exacerbation of hazards linked with oxidation [10]. Regrettably, over-intake of selenium supplementation can also lead to developing a range of debilitating disorders [11].

Antioxidants possess the ability to impede the process of oxidation and safeguard cells from the detrimental effects of free radicals. These free radicals are typically generated as a result of oxidative stress and are known to significantly contribute to the development of several diseases [12].

For this research, the endophytic fungus *Fusarium graminearum* was used to biosynthesis SeNPs, test their cytotoxic effects and their activity as antioxidants were analysed.

MATERIALS AND METHODS

Isolation and identification of endophytic fungus

Salvia rosmarinus leaves were washed under running water and then soaked in double-distilled water for 10 minutes. The surface of the leaves was then sterilised by soaking them in 70% ethanol for 3 minutes, 0.5% sodium hypochlorite (NaOCl) for 1 minute, and again in 70% ethanol for 30 seconds, and finally, sterilised distilled water three times. Petri dishes with PDA treated with chloramphenicol 250 mg/L were used to cultivate the sterile leaf fragments (0.5 cm). The plates were then parafilm-sealed and incubated at 250 °C until the endophytic fungus appeared. In order to obtain a pure culture, the emerging hyphal tips from the plant segments were isolated and subcultured. Fungal endophytes were identified using conventional identification guides [13, 14]. Based on macroscopic features (colony growth, shape, and colour) and microscopic features (colony surface, texture, conidia, conidiophores, and hyphal pigmentation) [15]. The potential of these endophytic fungi has been examined for employing them for the biosynthesis of SeNPs. *Fusarium graminearum* was chosen to be used for nanoparticle synthesis.

Preparation of fungal extract

The generation of the inoculum was conducted using a modified Wunder media [16]. The components of the medium included glucose (10 g/L), polypeptone (1 g/L), $(NH_4)_2SO_4$ (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), KH2PO4 (0.875 g/L), K_2HPO_4 (0.125 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), NaCl (0.1 g/L), $MnSO_4 \cdot H_2O$ (0.02 g/L), and $FeSO_4 \cdot 7H_2O$ (0.001 g/L).Each Erlenmeyer flask was supplemented with 170 ml of medium, and six mm diameter discs of the fungus, which had been cultivated on PDA medium, were introduced. The flasks that were inoculated were subjected to continuous agitation at a speed of 125 revolutions per minute (rpm) with a temperature of 28 °C for an interval of 7 days. Following the period of incubation, fungal biomass underwent filtration and was subsequently utilised in further experimental procedures.

Selenium biosynthesis

Centrifugation at 4500 rpm for 15 minutes was used to separate the harvested mycelia from the culture broth. Three times, it was washed with distilled water. After incubating for three days at 28.2 °C, 100 ml of fungal mycelia is combined with 100 ml of deionised water containing 0.4 gm of sodium selenite Na_2SeO_3 (adapted [11, 17]). The chemicals and reagents utilised in this study were procured from the American company Sigma, Ltd.

Characterisation of selenium nanoparticles

Ethanol was used prior to characterisation to separate SeNPs from fungal mycelia, then ultrasonic was used for dispersion [18].

The alteration in the hue of the mycelium solution, which was subjected to incubation with a one mM sodium selenite solution, was visually ascertained during a designated time frame. This observation serves as an indication of the bioreduction process, wherein selenium ions are converted into selenium nanoparticles. Absorption measurements were conducted with a resolution of 1 nm using a UV-visible spectrophotometer (LAMBDA 365 spectrophotometer-PerkinElmer, Waltham, MA, USA); the used wavelength is between 200 to 800 nm. X-rays at 40 KV and 30 mA were used to measure the size of nanoparticles. To examine SeNPs, an X-ray diffractometer was used after they were coated on an X-ray grid. SeNPs diameter and size were determined by measuring their peak width as well as length, The Bragg angles

used for this X-ray analysis were in a different range, U, and the analysis speed was 2U angles per minute. Assessment of the surface charge and persistence was done utilising a Zeta potential analyser (Zeta, Brookhaven, Deklab County, GA, USA). NPs were combined with potassium bromide (KBr) at a 1:100 ratio (FT-IR, PerkinElmer, Waltham, MA, USA). From 400 to 4000 cm1 of absorbance was measured. Functional classifications can be predicted based on vibrational mode. FE-SEM was used to investigate the biosynthesised SeNPs' morphology. Drop coating SeNP solutions onto a thin glass. FE-SEM (Inspect F50-FE-SEM, FEI, Eindhoven, The Netherlands) analysis was performed on SeNPs samples; the FE-SEM with an EDX attachment was used for compositional analysis and confirming the existence of elemental selenium in its natural conformation [19]. Atomic force microscopy (NaioAFM, Nanosurf AG, Liestal, Switzerland) was used to examine biosynthesized SeNPs and disclose their surface morphology, aggregation, shape, size, and distance. Using AFM image analysis software, we were able to get the most accurate reading possible [6].

Efficacy as an Antioxidant

The scavenging activities of bulk Selenium and Selenium nanoparticles were measured with concentrations of 0.0, 0.01, 0.1, 1, 10, and 100 µg/ml added to 0.5 Mm DPPH mixed with 3.3 absolute ethanol. Spectroscopy was used to determine the color change at wavelength 515 nm, meanwhile 90 minutes at 25 ^{0}C. Absolute ethanol of 3.3 ml and 0.5 ml of the sample were mixed and represented the blank one. Control was used: a tube containing absolute ethanol (3.3 ml) mixed with DPPH (0.5 ml); the removal percentage was calculated using an antioxidant activity equation [20].

$$\text{Removal percentage } = 100 - \frac{1 - \text{sample absorbency}}{\text{control absorbency}} \times 100\%$$

Chromosomal Analyses

Pre-stimulated peripheral blood lymphocytes were incubated for 72 hours at 37ºC and 5% CO2 atmospheric concentration using ten µg/mL phytoheamoglutnine (PHA). Whole blood from a healthy 25-year-old man was obtained using a sterile syringe coated with heparin. 0.5mL of blood was added to 4.5mL of completed culture medium RPMI-1640, which was supported with 10% fetal bovine serum and some antibiotics (penicillin and streptomycin). Following a 20-minute exposure to 10µg/mL Colchicine, the incubated cells were treated for an additional 20 minutes with hypotonic KCl 0.075M. Following a five-minute centrifugation at 3000 rpm, the pellet underwent three washings with a fixative solution (3:1 methanol to glacial acetic acid). The supernatant was disposed of. Giemsa stain was applied after the translucent cell suspension had been allowed to air dry for a whole night on spotless, cold slides. Chromosomal aberrations, mitotic index, and blastogenic index were measured for both exposed and non-exposed cells at varying doses of selenium nanoparticles [21]. Chromosomal aberrations are a collective of total abnormalities observed in 25 mitotic cells.

$$\text{MI} = \frac{\text{Mitotic cells}}{1000 \text{ cell}} \times 100\%$$

$$\text{BI} = \frac{\text{stimulated cells}}{1000\text{cell}} \times 100\%$$

All the experiments were performed at the cell biology laboratory of the Applied Science Department at the University of Technology in Iraq.

RESULTS AND DISCUSSION

Endophytic Fungi

The tight biological link between endophytes and their host plants produces a variety of naturally active chemicals [22]. Endophytic fungi from medicinal plants have been isolated, and most of them Ascomycota and their sexual forms [23], which can infect and live symbiotically with many herbaceous plants throughout the world [24], known as herbaceous plant fungi [25].

Biological methods use metal-absorbing microorganisms. Herbal extracts, microalgae, fungi, and bacteria are commonly used. The bottom-up approach uses fungi to reduce SeNPs, which are advantageous over other organisms. Their proteins and enzymes, such as reductases, can be used for fast and sustainable nanoparticle synthesis [26].

Although the nanoparticle synthesis mechanism is unknown, study specialists have provided some proposals. Nanoparticles are typically produced by extracellular and intracellular enzymes [27- 29]. Mehra & Winge (1991) [30] and Gezaf et al. (2022) [29] theory state that some fungi can produce extracellular metabolites and enzymes in response to environmental stressors such as metallic ions, temperature, predators, etc.

Fungi biosynthesise nanoparticles in three steps:

Step 1: collecting metal ions near fungal cells

Step 2: Cell-released enzymes decrease silver ions

Step 3: Fungal proteins and peptides stabilise metal nanoparticles [31,29].

Characterisation and analysis of mycosynthesised nanoparticles

After confirming SeNPs synthesis by observing the reaction mixture's colour shift from colourless to orange, the observed alteration in colour can be attributed to the phenomenon of surface Plasmon resonance (SPR); this phenomenon is a distinctive property displayed as a property of nanoparticles [32]. SeNPs are detected using numerous characterisation methods. The mycosynthesis of SeNPs undergoes a highly significant phase that elucidates the presence of biomolecules associated with these particles, as well as the size and shape characteristics of the resulting SeNPs [33].

Nanoparticles that mycosynthesized extracellularly are considered more advantageous, not only due to the ease of purification but also because of the enhanced rate of production [6].

Selenium nanoparticles are characterised using methods like UV-visible, XRD, FTIR, AFM, SEM, and EDX.

Analysis of SeNps UV-visible spectroscopy demonstrated vigorous SeNPs synthesis activity, as evidenced by its ability to induce a discernible change in colour and achieve the maximum surface plasmon resonance (SPR) at a wavelength of 273 nm (Fig. 1). The maximal SPR of SeNPs produced by *Penicillium corylophilum* and the fungus *P.*

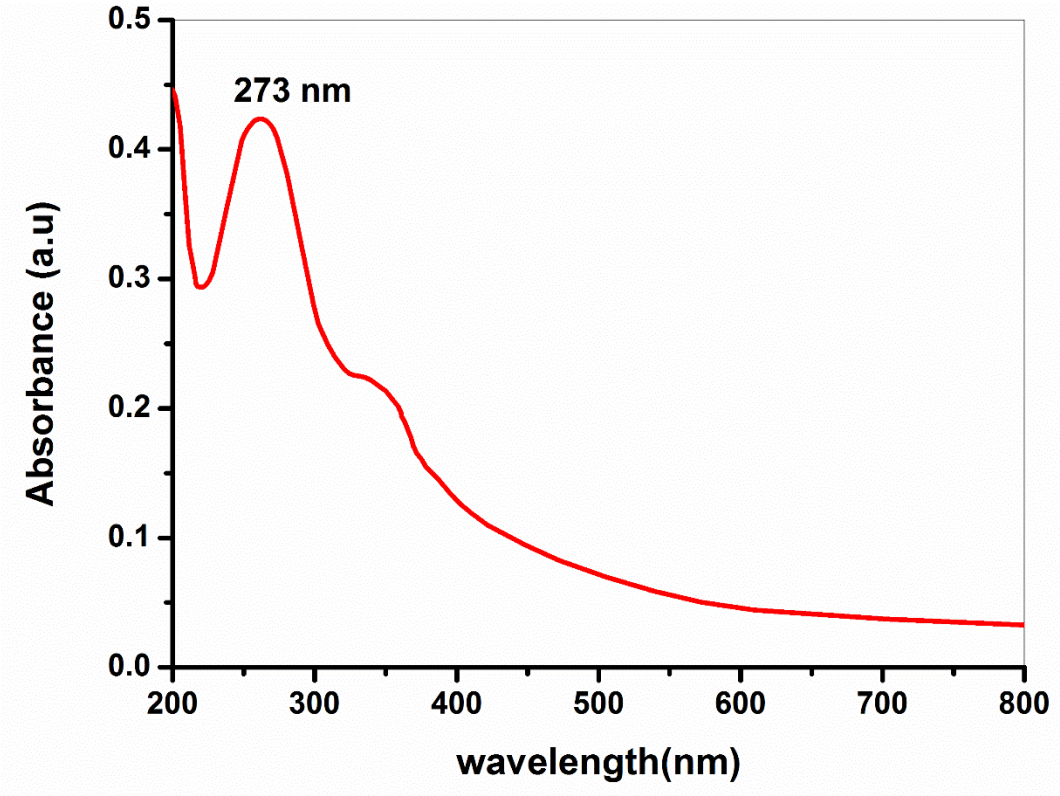

Fig. 1. UV–Vis spectroscopy of SeNPs mycosynthesized using Fusarium graminearum.

crustosum was 275 and 270 nm, respectively [34, 35]. The first sign of SeNPs synthesis was the colour shift and UV-Vis spectroscopic detection of maximal SPR. The endophytic fungal isolate of *Fusarium graminearum* had the maximum colour intensity with an absorption peak of 273 nm, which aligns with the surface plasmon resonance (SPR) for SeNPs. In contrast, sodium selenite had a peak at 223 nm (Fig. 2), which demonstrates the genuine shift to 273 during selenium production.

XRD analysis was used to look into the crystallinity of mycosynthesised SeNPs (Fig. 3). The XRD pattern displayed the absorption peaks of (100), (101), and (102), which matched Bragg diffraction at 2θ values of 23.6°, 29.4°, and 45.51°. Crystalline structure of SeNPs was verified by comparing the acquired XRD pattern with those on JCPDS standard card No. 06-0362. The XRD pattern that was obtained agrees with those seen in previously published works on green synthesis of SeNPs [36].

FTIR showed eight peaks that occur at wavenumbers 3400, 1625.99, 1402.25, 1061.49, 1380, 864.11, 680.87, 486.6, and 406.98 cm^{-1} (Fig. 4). The presence of a significant and broad peak observed at 3400 cm^{-1} could potentially be attributable to protein and amino groups [37, 38]. Entophytic fungal strains exude the C=N moiety, which peaks at 2068 cm^{1}. The signal at 1402.25 cm^{-1} may have been the (O-H) group of carboxylic acid bending O-H [39, 40].

The FTIR chart of SeNPs shows various peaks, possibly due to biomass filtrate metabolites interacting with sodium selenite throughout the reduction and capping process. The peaks in the 400–800 cm^{-1} range are bending and stretching Se-O, forming cappings around SeNPs to prevent aggregation and agglomeration during the reaction with carbonyl groups [41].

FTIR analysis showed that enzymes, carbohydrates, and amino acids in fungal biomass filtrates reduced sodium selenite to biosynthesise the nanoparticles and form cappings that stabilised and prevented aggregation [42].

The size, form, and aggregation of fungal-mediated synthesised SeNPs, which have an

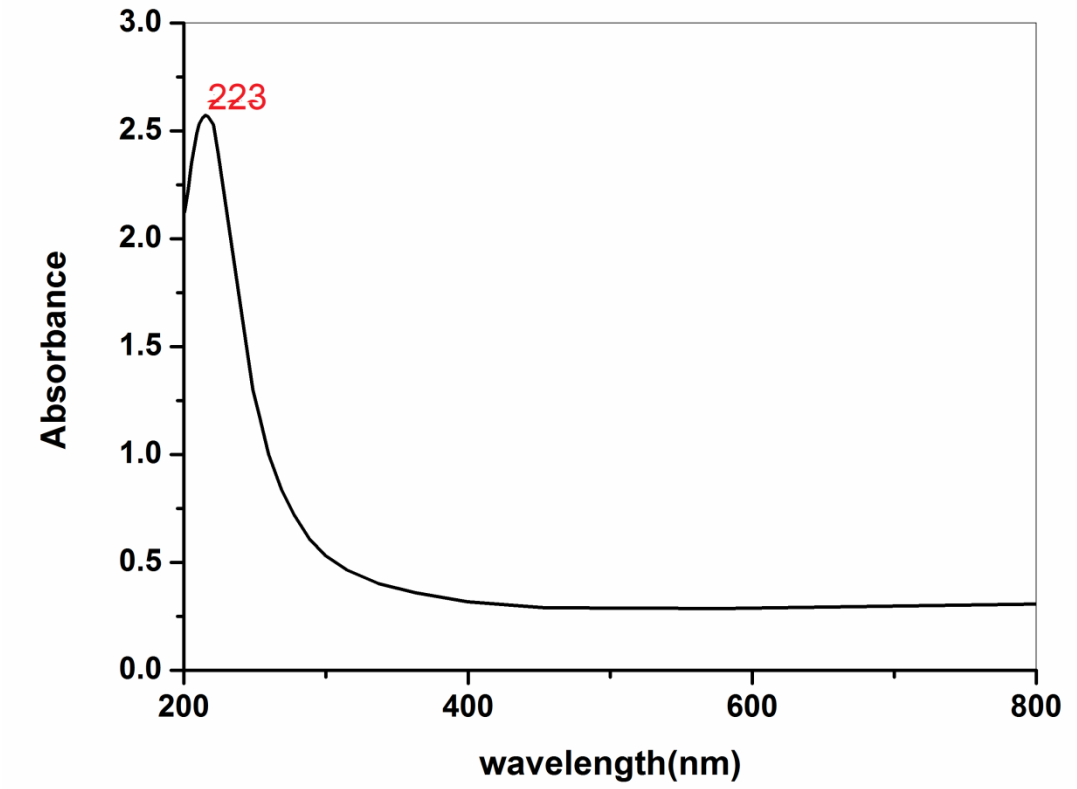

Fig. 2. UV–Vis spectroscopy of Sodium selenite.

impact on their biochemical processes, were examined by SEM. Figs. 5A and 5B show the spherical form of synthesised SeNPs, which are organised in a systematic manner with diameters 54–63 nm and, on average, 58.5 nm.

NP uses rely on the cappings, the surface

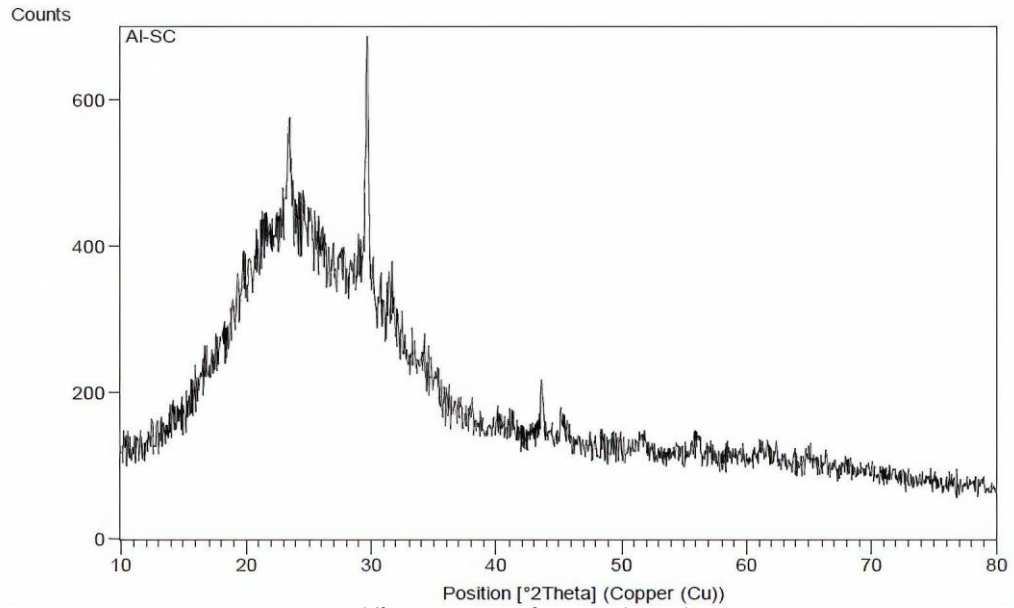

Fig. 3. X-ray diffraction pattern of mycosynthesised SeNPs.

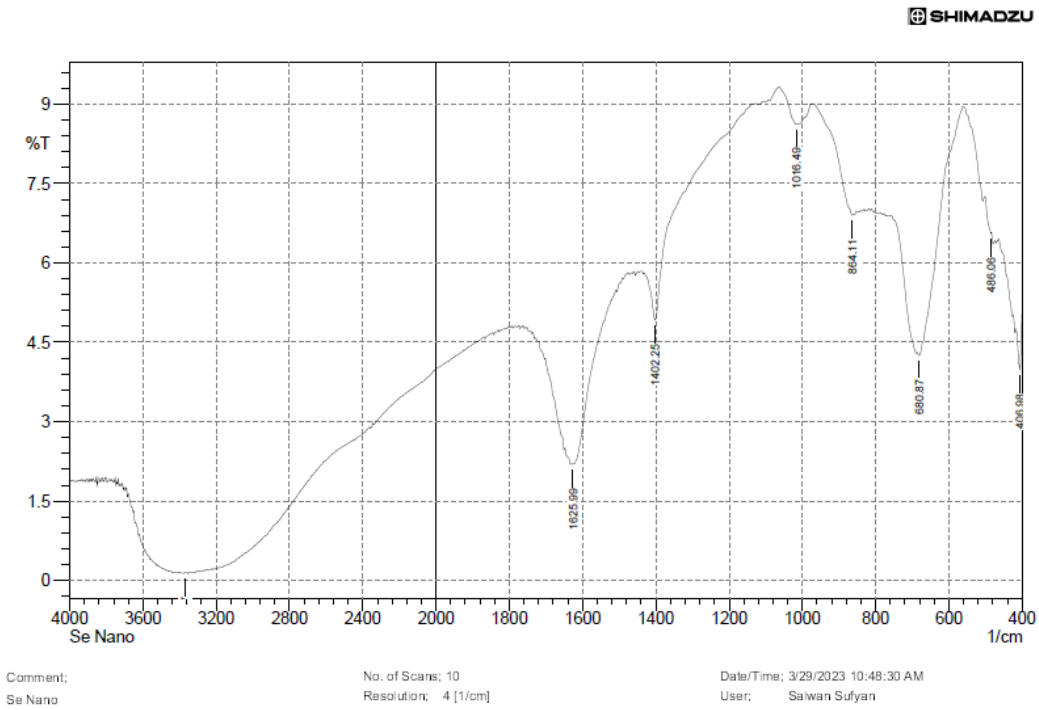

Fig. 4. FTIR spectrum of mycosynthesised SeNPs.

charge, the shape, the size, and the agglomeration process [43]. Activity increased when the size was reduced. SeNPs made from *Pantoea agglomeran* biomass filtrate had better antioxidant activity at lower sizes [44]. Also, NP shape affects their actions [45].

According to the EDX examination, it was determined that selenium (SeNPs) constituted around 26% of the sample, whereas carbon, calcium, magnesium, aluminium, and sodium were present in minimal quantities (Fig. 6).

DLS data (Fig. 7) showed that the synthesised SeNPs were 49.79 nm as a mean size with a polydispersity index (PDI) of 0.185, verifying the nanoparticle's nanoscale dimensions. An important NP size distribution index is the PDI [46].

Atomic Force Microscopy was used to measure and estimate SeNP sizes. A diameter of 83.23 nm was averaged from (40.22) to (74.87) nm (Fig. 8).

Shafiq et al. (2016) [7] and Aja et al. (2018) [47] described *Fusarium graminaerum* biosynthesised SeNPs in various sizes, which were sluggish to consume AFM.

The width ranged from 1 to 95.5 nm but was eventually 45.5 nm.

Antioxidant action of SeNPs

The stable free radical DPPH, at room temperature, exhibits a rich violet colour with a 517 nm, which represents the absorbed wavelength when dissolved in organic solvents. The inclusion of selenium nanoparticles (SeNPs) in the analysis resulted in a decrease in DPPH stability and a change in colour from violet to yellow, which can be attributed to the phenolic OH groups present [48].

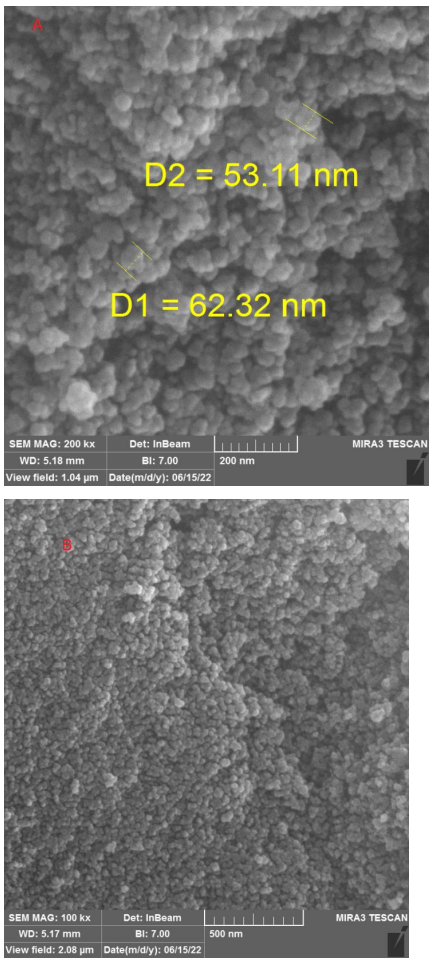

Fig. 5 (A&B). FE-SEM image of SeNPs.

The scavenging of DPPH was seen to be directly related to the concentrations of the SeNPs. Specifically, at concentrations of 0.01, 0.1, 1, 10, and 100 gm/ml of both SeNPs and Se salt, the DPPH free radicals' scavenging capabilities were found to be 17.2%, 38.3%, 62.04%, 85.55%, and 100%, respectively (Fig. 9). The observed antioxidant activity of selenium salt nanoparticles was measured to be 2.92%, 22.36%, 48.05%, 56.65%, and 100%, respectively. SeNPs2 demonstrated the most pronounced scavenging activity when present at a concentration of 100%.

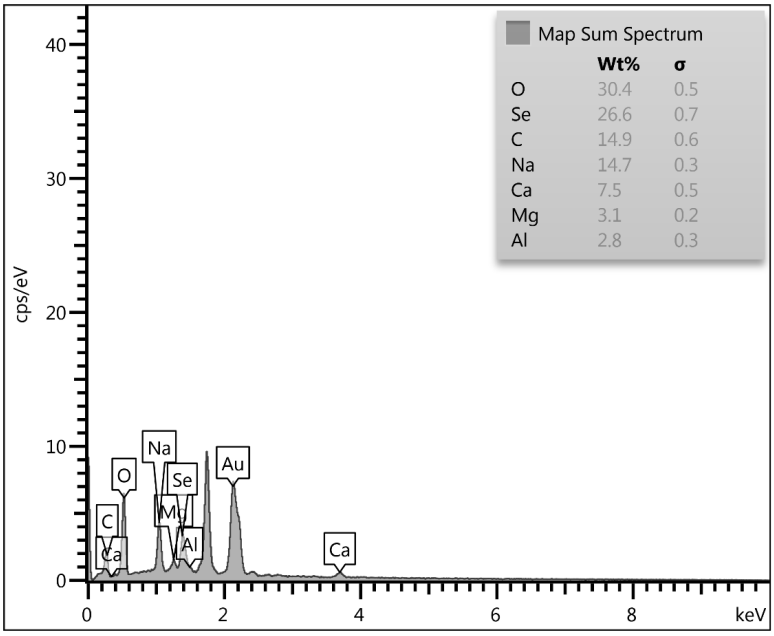

Fig. 6. EDX spectra for SeNPs.

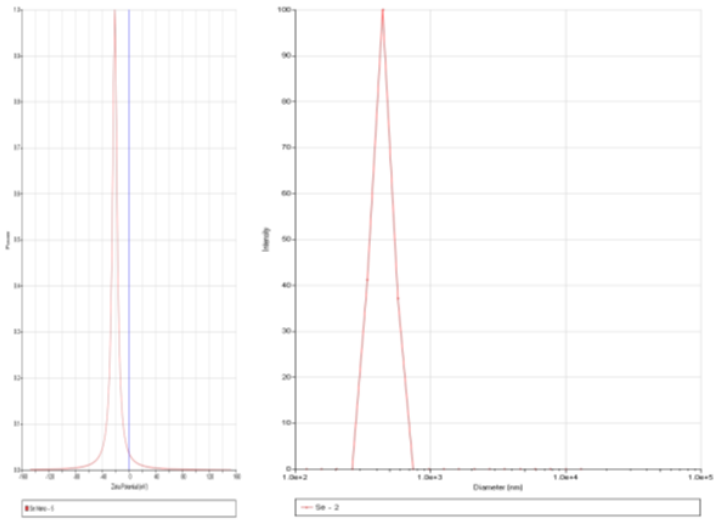

Fig. 7. Zeta potentials and dynamic light scattering analyses of SeNPs.

Assay for cytotoxicity and genotoxicity

Table 1 demonstrates that the mitotic index increased substantially as the concentration of SeNPs increased in a dose-dependent pattern. The mitotic index was highest at the concentration of 10 g/mL and lowest when 100 g/ml concentration was used in a solution of SeNPs. Evidently, the increased activity was caused by selenium NPs interfering with the normal mitotic cell cycle. However, AgNPs, Al_2O_3, and ZnO nanoparticles inhibit mitotic index dose-dependently [49]. Nanoparticles are responsible for structural or numerical changes in chromosomal behaviour [50], resulting in the fragmentation and recombination of chromosomal material. Some nanoparticles alter the viscosity of cytoplasm, resulting in aberrant spindle behaviour and chromosomal aberrations. TCA decreased with increasing selenium nanoparticle concentration up to 12 g/ml, and complete cell mortality was observed at 100 g/ml concentration after 24 hours.

Due to their sensitivity to these factors and their quantifiability, the Blastogenic Index (BI) and Mitotic Index (MI) are both valuable and

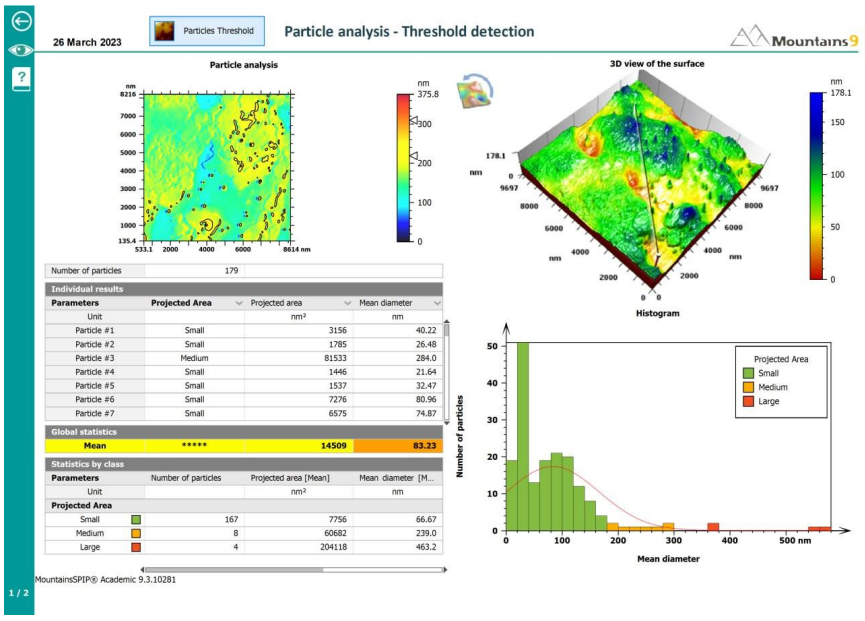

Fig. 8. AFM topography images of biosynthetic Ag-NP are from

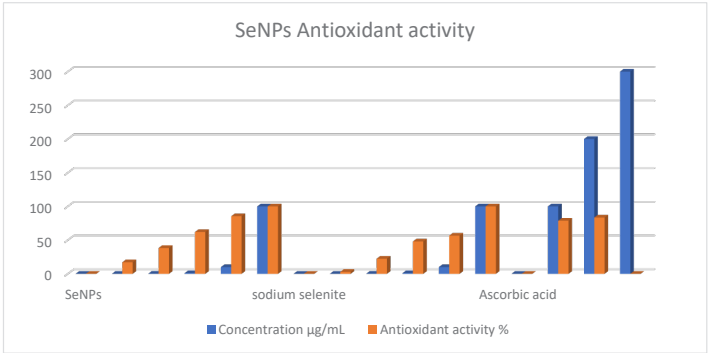

Fig. 9. Antioxidant effect of SeNPs, Sodium selenite and Ascorbic acid.

essential bioindicators for studying the impact of physical as well as chemical agents upon living plant, animal, and microorganism cells, specifically peripheral blood lymphocytes (PBL). Mitotic index (MI) and blastogenic index (BI) increased substantially when PBLs were exposed to varying concentrations of mycogenic selenium nanoparticles, as shown in Table 1. Simultaneously, the number of Total Chromosomal Aberrations (TCAs) decreased progressively. The genotoxicity and DNA damage associated with mycogenic selenium nanoparticles may be attributable to the distinctive characteristics of mineral nanoparticles, such as their high surface area, minuscule size, shape, coatings, and surface charge. When nanoparticles interact with biomolecules, reactive oxidative stress (ROS), including hydroxyl radicals, hydrogen peroxide, and the superoxide radical (O_2), is generated. Cell membrane disruption and disruption of protein, enzyme, and DNA synthesis can be caused by reactive oxygen species (ROS) (Fig. 10) [51, 52].

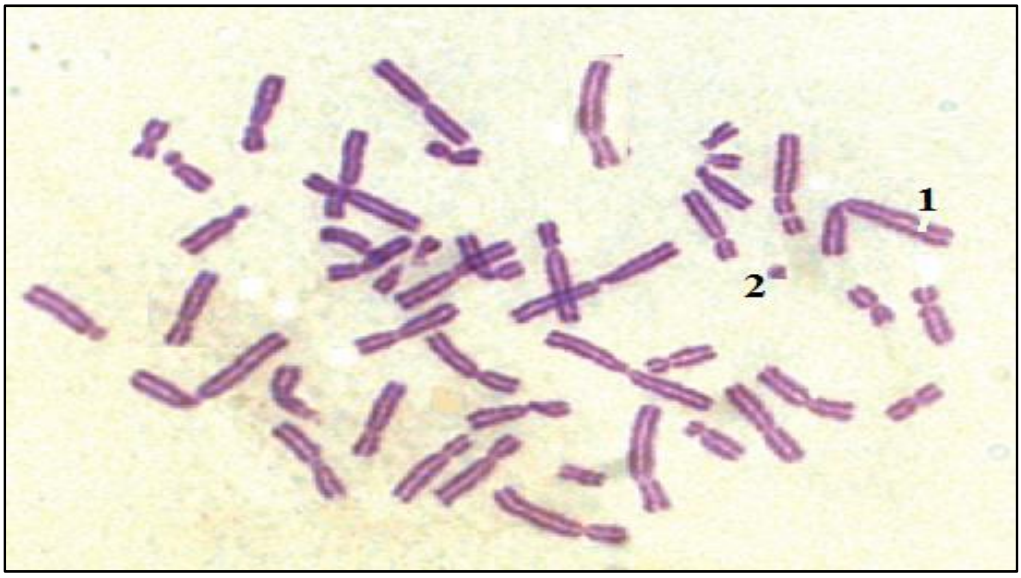

Fig. 10. Chromosomal aberrations.

Table 1 Chromosomal analyses of SeNPs prepared by *Fusarium graminearum* peripheral blood lymphocytes.

SeNPs				$SeNPs_2$			
Concentration µg/mL	BI	MI	TCA	Concentration µg/mL	BI	MI	TCA
0.0	68.13	1.77	0.12	0.0	68.13	1.77	0.12
0.01	71.82	2.28	0.11	0.01	70.22	1.85	0.10
0.1	87.28	3.71	0.07	0.1	75.66	2.13	0.08
1	92.73	5.87	0.03	1	81.13	3.15	0.02
10	96.24	7.11	0.0	10	83.26	4.21	0.0
100	28.36	0.0	0.0	100	0.0	0.0	0.0
0.25 MMC	18.26	0.12	2.2	0.25 MMC	18.26	0.12	2.2

MI= Mitotic Index
BI= blastogenic Index
TCA= Total Chromosomal Aberrations

CONCLUSION

It may be inferred that the use of endophytic fungi fusarium for the biosynthesis of SeNPs is a straightforward, expeditious, and efficient method with a diameter of 53–62 nanometers; furthermore, the utilisation of fungal cell filtrate renders it even more environmentally sustainable and less detrimental to human health and the surrounding ecosystem. It has been proven that selenium nanoparticles (SeNPs) exhibit scavenging activity increased with concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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