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Evaluation of Callus Responses of *Solanum nigrum* L. Exposed to Biologically Synthesized Silver Nanoparticles

Emad A. Ewais[1,*], Said A. Desouky[2], Ezzat H. Elshazly[2]

[1]Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt
[2]Department of Botany, Faculty of Science, Al-Azhar University, Assiut, Egypt

Abstract Effect of biosynthesized silver nanoparticles (AgNPs) on growth, anatomy, and protein and DNA of *Solanum nigrum* callus was investigated *in vitro*. Three concentrations of aqueous silver nanoparticles conjugated with camel milk were used in the present study. Murashige and Skoog (MS) nutrient media supplemented with different concentrations of AgNPs, 0, 2, 4 and 8 mg/l, were evaluated for their effects on the callus induction from the leaf explants of *Solanum nigrum*. Compact calli with white and greenish colour were obtained after 10 days upon control culture (no AgNPs), whereas friable watery calli with white, greenish or yellowish colour were observed after 10-13 days upon culture supplemented with AgNPs. Interestingly, it was found that exposure to AgNPs increased callus fresh weight and the intensity of callus formation. These were associated with the deformity of callus morphology. Light microscopy observations showed that the nanoparticles damaged cell wall. This was clear with the high concentration of AgNPs. Variation in protein pattern were recorded between callus control (no AgNPs) and callus exposure to AgNPs. Genetic stability between callus control and callus under AgNPs exposure was interrelated.

Keywords *Solanum nigrum*, Silver nanoparticles, Callus anatomy, Protein and genetic stability

1. Introduction

The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles are being considered to be the fundamental building blocks of nanotechnology. Nanotechnology is interdisciplinary which includes physics, chemistry, biology, material science and medicine. Instead of using toxic chemicals for the reduction and stabilisation of metallic nanoparticles, the use of various biological entities has received considerable attention in the field of nanobiotechnology [1]. Biological methods are regarded as safe, cost-effective, sustainable and environment friendly processes for the synthesis of nanoparticles [2]. Silver nanoparticles have been successfully synthesized using various bacteria [3], fungi [4] and plants [5].

Nanoparticles are becoming an area of research interest due to their unique properties, such as having increased electrical conductivity, ductility, toughness, and formability of ceramics, increasing the hardness and strength of metals and alloys, and by increasing the luminescent efficiency of semiconductors [6]. Plants are an essential base component of all ecosystems and play a critical role in the fate and transport of AgNPs in the environment through plant uptake and bioaccumulation [7]. Silver nanoparticles (AgNPs) are currently one of the most widely commercially used nanomaterials [8]. The risk of nanomaterials usage represents their possible accumulation in the environment with subsequent entry into the food chain, which is closely connected with their accumulation in organisms [9]. Plants represent an important trophic level [10]. However, there are only limited studies focused on the effect of nanomaterials on plants. The first point of possible phytotoxicity consists in interactions between nanomaterials and soil components and microorganisms, which significantly modify uptake of nutrients by plants [11]. The second point is closely connected with the uptake of nanomaterials (root, foliar), and their accumulation and transport within plant body with subsequent interactions with biomolecules, such as nucleic acids, proteins including enzymes, and cell structures, such as cell walls and biomembranes [12]. In particular the cell wall of plant cells represents a crucial structure in nanoparticle uptake compared to animal cells [13]. Both positive and negative effects on plants have been demonstrated. Lu *et al.* demonstrated both positive and negative effects of nano-SiO_2 and nano-TiO_2 on nitrate reductase in soybean [14]. Enhancement of biomass production in spinach (*Spinacia oleracea*) after application of TiO_2 nanoparticles has been recorded in a study by Gao et al., [15].

An understanding of the interactions between nanoparticles and biological systems is of significant interest.

* Corresponding author:
ewais_e@yahoo.com (Emad A. Ewais)
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Studies aimed at correlating the plant cells responses such as morphological, anatomy, physiology and genetic stability at molecular level are under way. These fundamental studies will provide a foundation for understanding the interaction of nanoparticles with biological systems. So, in this study, we employed callus culture of *Solanum nigrum* as a model species to investigate the effect of biosynthesised silver nanoparticles (AgNPs) prepared using camel milk on some morphological and anatomy characters of callus and assessment of genetic stability at protein and DNA levels.

2. Materials and Methods

2.1. Plant Material

Seeds of *Solanum nigrum* L. were obtained from Botany & Microbiology Dept., Faculty of Science, Al-Azhar Univeristy, Assuit, Egypt.

2.2. Seeds Sterilization Using NaOCl 5.25%

Seeds In vitro germination using basal MS medium [16] containing 3% sucrose and **callus induction** from developing leaf explant using 3.0mg/l NAA and 5.0mg/l BA were done as described previously [17].

2.3. Synthesis of Silver Nanoparticles

Silver nitrate was purchased from Sigma-Aldrich (St. Louis, MO). Camel milk was procured from local market. Camel milk (4 mL) was mixed with 96 mL of 1 mM silver nitrate solution and the resulting mixture was incubated for 8 h in rotatory shaker (180 rpm) at room temperature according to the method described by Lee et al. [18]. Reduction of silver ions in the reaction mixture was monitored by change in colour of the reaction mixture from milky white to dark brown. The obtained nanomaterial diffusion) was freeze dried at -80°C and used for characterization studies. Autoclaved water was used as a control negative for this experiment. The surface morphologies and sizes of the AgNPs were examined using biological transmission electron microscopy (JEOL JEM 2010 transmission electron microscope) and X-ray diffractometer as Moharram et al. [19].

2.4. Longitudinal Section of Callus and Observation under Light Microscope

For anatomical study small portions of calls of all samples with age thirty days was fixed in FAA (formalin: acetic acid: 70% ethanol; 1: 1: 18, v/v) for 48 hrs. at room temperature. Following a standard dehydration in a graded ethanol series, samples were washed in changes of ethanol 70% to99.9% ethanol with a difference of 10% in concentration and three hrs. difference in time ate the first two changes and then two hrs. difference between the rest changes. Then sample clearing was carried by means of ascending concentrations of ethanol-xylene with concentrations 10% to 100%. Then samples were embedded in paraffin wax at 45-55ºC for 48 hrs. with changing paraffin wax each 12 hrs. [20]. Longitudinal sections, 12-15 µm thick, were cut with steel blade on IHC World KD-1508A rotary microtome, then sections were fixed on glass slides by Haupt's adhesive (1gm gelatine powder, 100 ml warm distilled water[note: soluble gelatine should be filtrated before glycerol add, 7.5 ml glycerol and small phenol crystal) [21]. Before sections stalk on slides they were gently heated on hotplate at 30-35°C. After minimum 12hr. the fixed sections were stained with Safranin O-Fast-green double stain. Safranin O solution prepared by dissolving 1gm of stain powder in100 ml ethanol 50% while Fast-green solution prepared by dissolving 1gm of stain powder in100 ml ethanol 95%, then stains solutions filtrated. After staining, sections mounted in Canada balsam [22]. Staining chart (NO.) shows: stage A) de-waxing, stage B) rehydration for Safranin O stain, stage C) dehydration for Fast-green stain and stage D) for loading sections with Canada balsam.

Observation and photomicrographs were achieved using XSZ-N107 Research Microscope fitted with Premiere MA88-900 digital camera Staining Chart (NO.) (Safranin O - Fast green).

2.5. Protein Electrophoresis (SDS-PAGE)

Total protein was extracted from callus control (no AgNPs) and callus exposure to different concentrations of AgNPs. Then, it was ground to fine powder with pestle and mortar. Ten mg of powered flour was homogenized thoroughly with 400 µl extraction buffer using vortex. The extraction buffer was prepared by dissolving 0.6 g Tris base, 0.2 g Sodium Dodecyl Sulfate (SDS) and 30 g of urea in 50 ml of double distilled water. One ml of β- mercaptoethanol was added and then the solution was diluted to 100 ml with double distilled water. The mix was kept overnight at 4°C and then centrifuged at 13000 rpm for 10 minutes at room temperature (Sigma 3K 18 Bench Top centrifuge). For qualitative analysis of protein, 20 µl of the extracted protein was boiled in a water bath for 3-5 min and loaded on Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) containing 12.5% resolving gel and 4% stacking gel [23] using 3 µl bromophenol blue as tracking dye. The samples were then loaded in equal amounts (15 µl) and a molecular weight marker standards (5 µl) (Prism Ultra Protein Ladder) were loaded at on to each well. Electrophoresis was carried out at 150 V and 25 mA until tracking dye reached to the bottom of the gel. The gels were resolving gel and 4% stacking gel (Lammeli, 1970) using 3 µl bromophenol blue as tracking dye. The samples were then loaded in equal amounts (15 µl) and a molecular weight marker standards (5 µl) (Prism Ultra Protein Ladder) were loaded at on to each well. Electrophoresis was carried out at 150 V and 25 mA until tracking dye reached to the bottom of the gel. The gels were destained in solvent composed of 40 ml of methanol, 10 ml glacial acetic acid and 50 ml distilled water. The gel was stained overnight in 25 ml of Coomassie Brilliant Blue (R-250) staining buffer. The gels were photographed and the

molecular weights of the polypeptide bands were estimated by correlating position of the molecular weight marker standards.

2.6. Random Amplified Polymorphic DNA (RAPD) Analysis

Approximately 100 mg of fresh callus (callus control, no AgNPs, or callus exposure to 4mg/l AgNPs) were immersed in liquid nitrogen for DNA extraction using DNeasy plant Mini Kit (QIAGEN Hilden, Germany). PCR reaction was performed in 30µl volume tubes contained dNTPs (2.5 mM), MgCl2 (25 mM), 3 µl Buffer, 2 µl Primer, 0.20 µlTaq DNA polymerase (5U/µl), 2 µl Template DNA and 16.80 µl distilled H2O in an automated thermal cycle (Techno 512) programmed for one cycle at 94°C for 4 min followed by 45 cycles of 1 min at 94°C, 1 min at 57°C, and 2 min at 72°C. The reaction was finally stored at 72°C for 10 min. Based on the clear and accurate amplified bands profiles, different six primers were selected (Table 1). The reaction products were analyzed by electrophoresis on 1.2% agarose gels, stained with ethidium bromide and photographed under UV transilluminator by digital camera with UV filter adaptor. The DNA ladder 100 bp (Pharmacia) was employed as molecular markers for bands molecular size. Each amplified band profile was defined by the presence or absence of bands at particular positions on the gel.

Table (1). List of the primer names and their nucleotide sequences used analysis of callus control (no AgNPs) and callus exposure to 4.0mg/l AgNPs

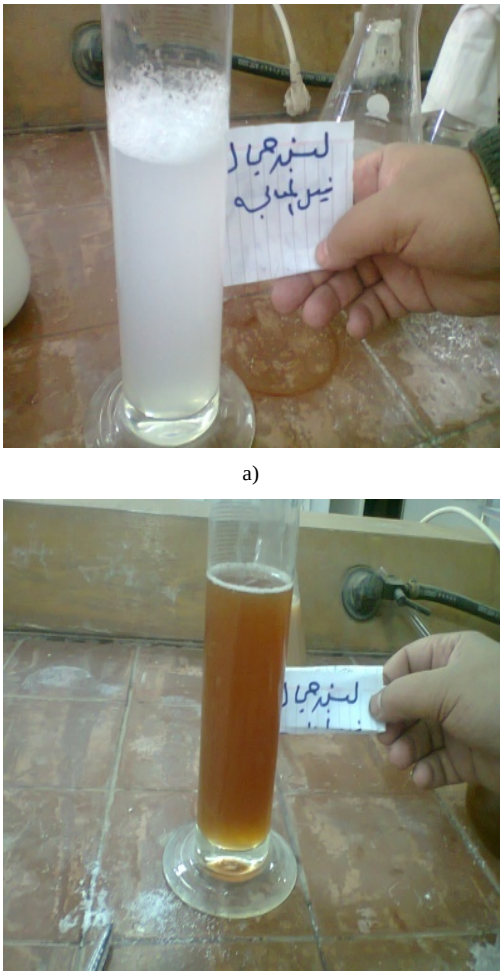
	Name	Sequence
1	OP-C04	5´ CCG CAT CTA C 3`
2	OP-C12	5´ CAG CAC CCA C 3`
3	OP-C15	5` GAC GGA TCA G 3`
4	OP-B11	5`GTA GAC CCG T 3`
5	OP-E15	5` ACG GCG TAT G 3`
6	OP-M01	5` GTT GT GGC T 3`

Assessment of genetic stability at protein electrophoresis (SDS-PAGE) and at DNA level (RADP) among callus control (no AgNPs) and callus exposure to AgNPs was estimated following the Jaccard's similarity matrix [24]. Profiles were considered different when at least one polymorphic band was identified. Bands were scored as'1' if it's present or '0' if it's absent based on standard marker using Community Analysis Package (CAP) software. The similarity coefficients were used to construct a dendrogram depicting genetic relationship using the unweighted pair group mean average (UPGMA) method [25].

3. Results and Discussion

3.1. Biosynthesized AgNPs

The present study is an attempt to synthesize AgNPs using camel milk. The colour of reaction mixture turned from milky white to dark brown after exposed to sunlight for 30 min of reaction, indicating $AgNO_3$ reduction. The intensity of the brown colour increased after 15 mint of incubation, giving it a darker look (Fig. 1). Scanning electron microscopy dispersive spectroscopy peak, approximately at 3ke V, confirmed the presence of silver nanoparticles. Scanning electron microscopy showed that nanoparticles are mostly circular with an average size of 30-90 nm (Fig. 2).



a)

b)

Figure (1). Showing colour changes (a) Camel milk before treatment; (b) camel milk after treatment with $AgNO_3$ showing brownish colour

3.2. Effect of Silver Nanoparticles (AgNPs) on Callus Growth

The results showed that AgNPs had a stimulating effect callus formation, while low and medium concentrations delayed the day of callus formation. It was observed that with increase AgNPs concentration, the degree of callus formation also increase (Table 2). In this respect, it was reported that the AgNPs concentration of 20, 40 and 60 ppm showed statically significant stimulation on shoot and root elongation of common bean and corn [26].

The obtained results showed that compact calli with white and greenish colour were observed after 10 days upon control culture (no AgNPs), whereas friable watery calli with white, greenish or yellowish colour were observed after 10-13 days upon culture supplemented with AgNPs (Figs. 3, 4, 5 and 6). The intensity of callus growth was found to be

extreme with MS medium supplemented with high concentration of AgNPs. This may be due to the structure of nanoparticles and their large surface which is closely connected with reactivity and modification possibilities. Support that, Krostofova et al., [27] who demonstrated the ability of magnetic nanoparticles to negatively affect metabolism and induced biosynthesis of protective compounds in a plant cell model represented by tobacco BY-2 cell suspension culture.

The intensity of callus growth was found to be extreme with MS medium supplemented with high concentration of AgNPs. Similar positive effect has been recorded by Gao et al., [15] who observed the enhancement of biomass production in spinach (*Spinacia oleracea*) after application of TiO_2 nanoparticles.

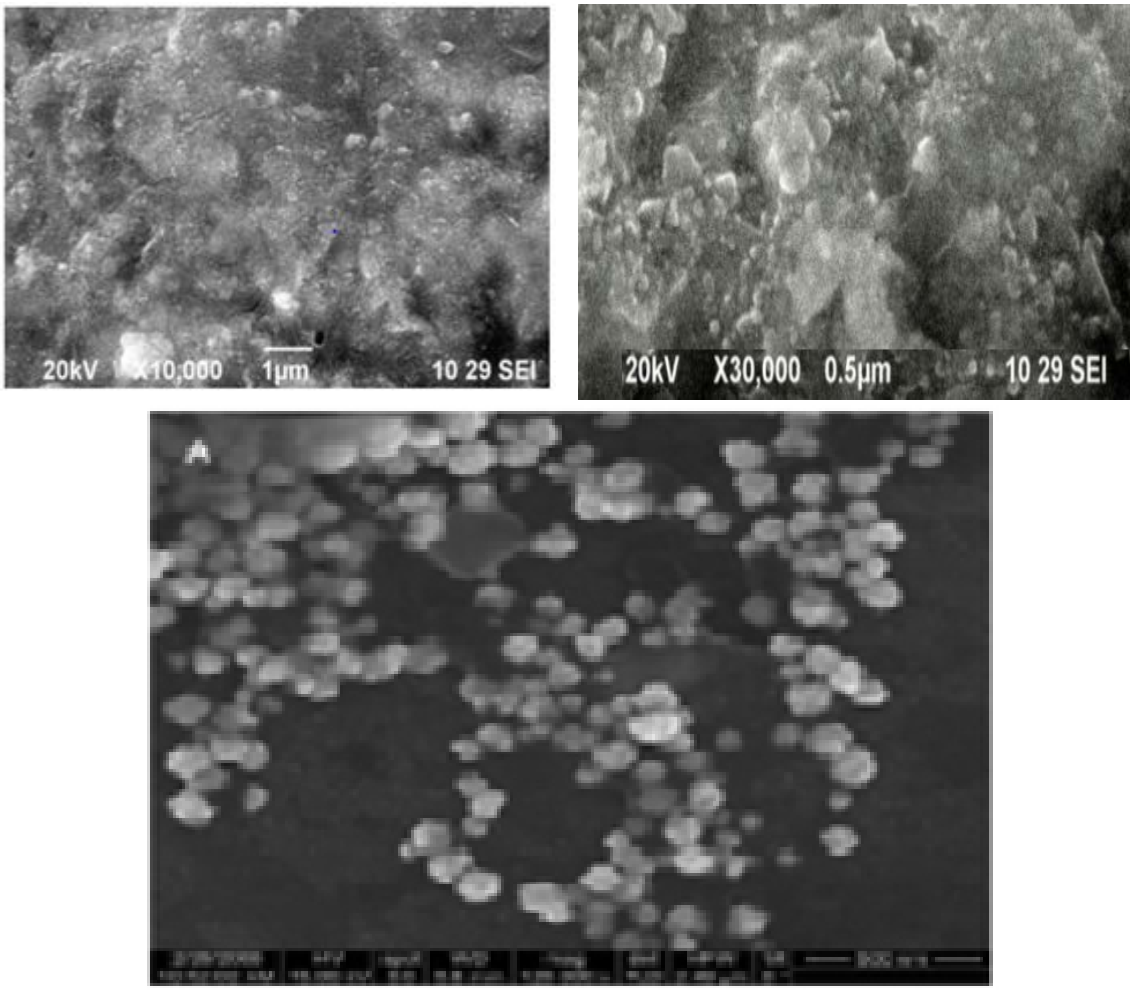


Figure (2). Particles are mostly circular in shape with the average size of 30–90 nm by using scanning electron microscope

Table (2). Effect of different concentration of silver nanoparticles (AgNPs) on callus growth of *Solanum nigrum* cultured in MS media

Exposure (AgNPs) mg/l	Percentage of callus Formation %	Day of callus formation	Callus colour Morphology	Mean F.wt. of callus (g/explant)	Degree of callus formation
Control (no SNPs)	81	10	Greenish Compact	3.06	++
2 mg/l	84	11	Yellowish, semi-compact	3.44	++
4 mg/l	85	13	Yellow-greenish Friable, Watery	3.58	+++
8 mg/l	89	10	White-green Friable	4.67	++++

Callus growth rating value = (++) moderate, (+++) profuse and (++++) extreme.

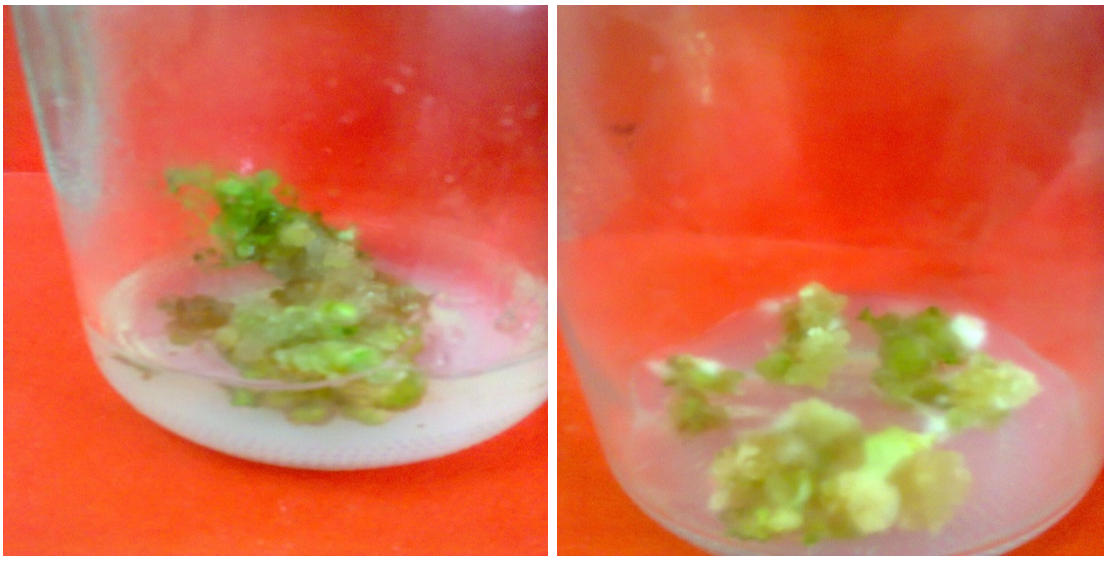


Figure (3). Compact callus with nodular structures from *Solanum nigrum* explants cultured MS control medium (with no AgNPs)

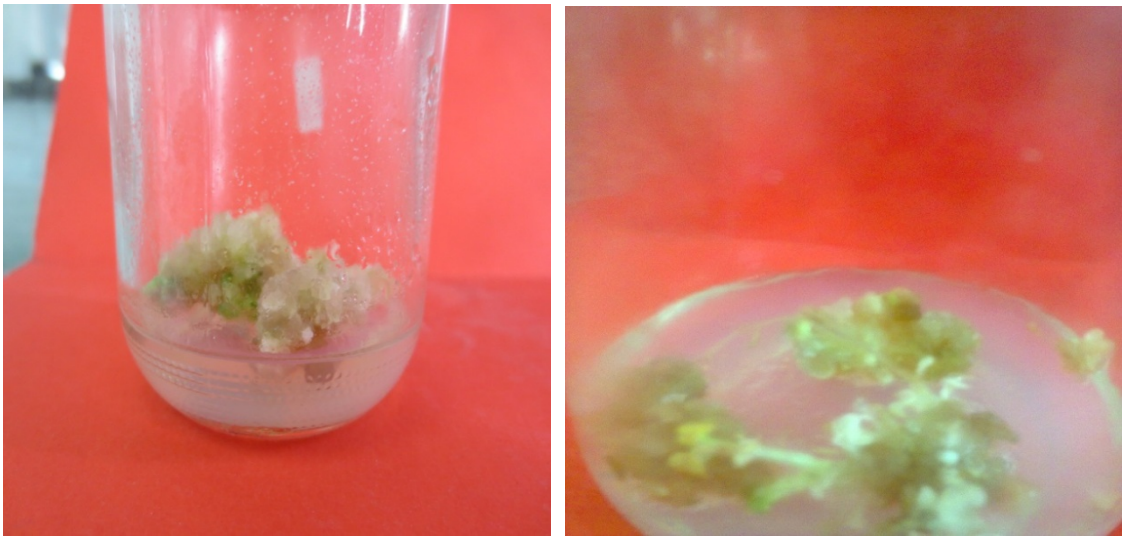


Figure (4). Semi-compact callus from *Solanum nigrum* explants cultured on MS medium supplemented with 2 mg/l AgNPs

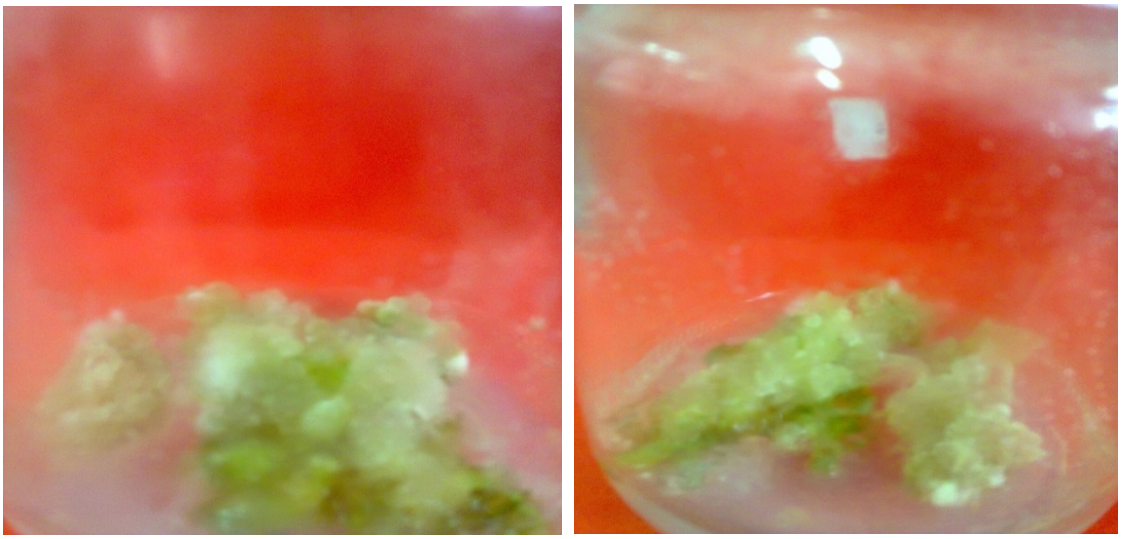


Figure (5). Friable callus from *Solanum nigrum* explants cultured on MS medium supplemented with 4 mg/l AgNPs

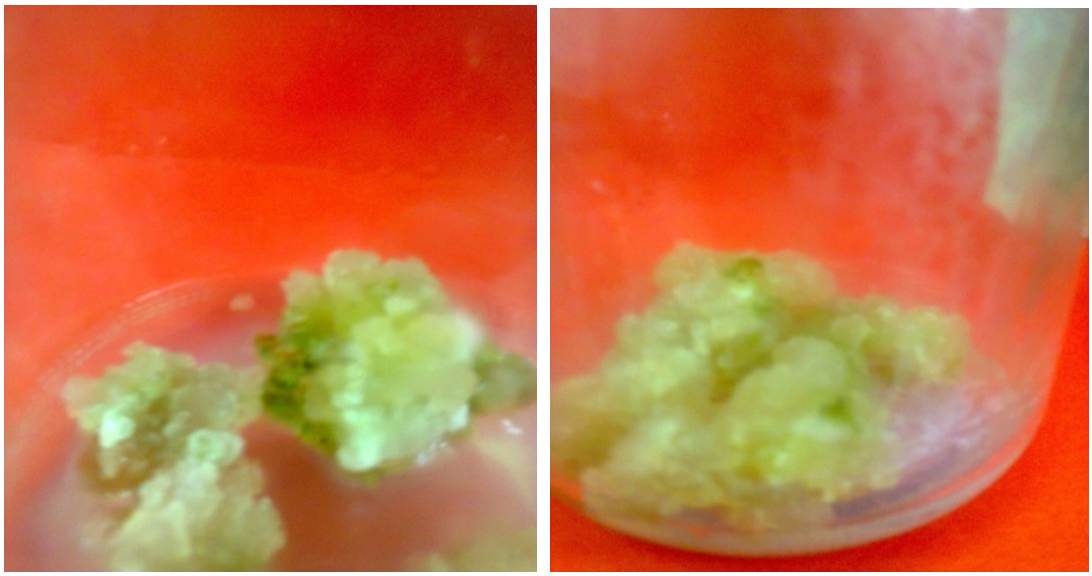


Figure (6). Friable, greenish white, callus from *Solanum nigrum* explants cultured on MS medium supplemented with 8 mg/l AgNPs

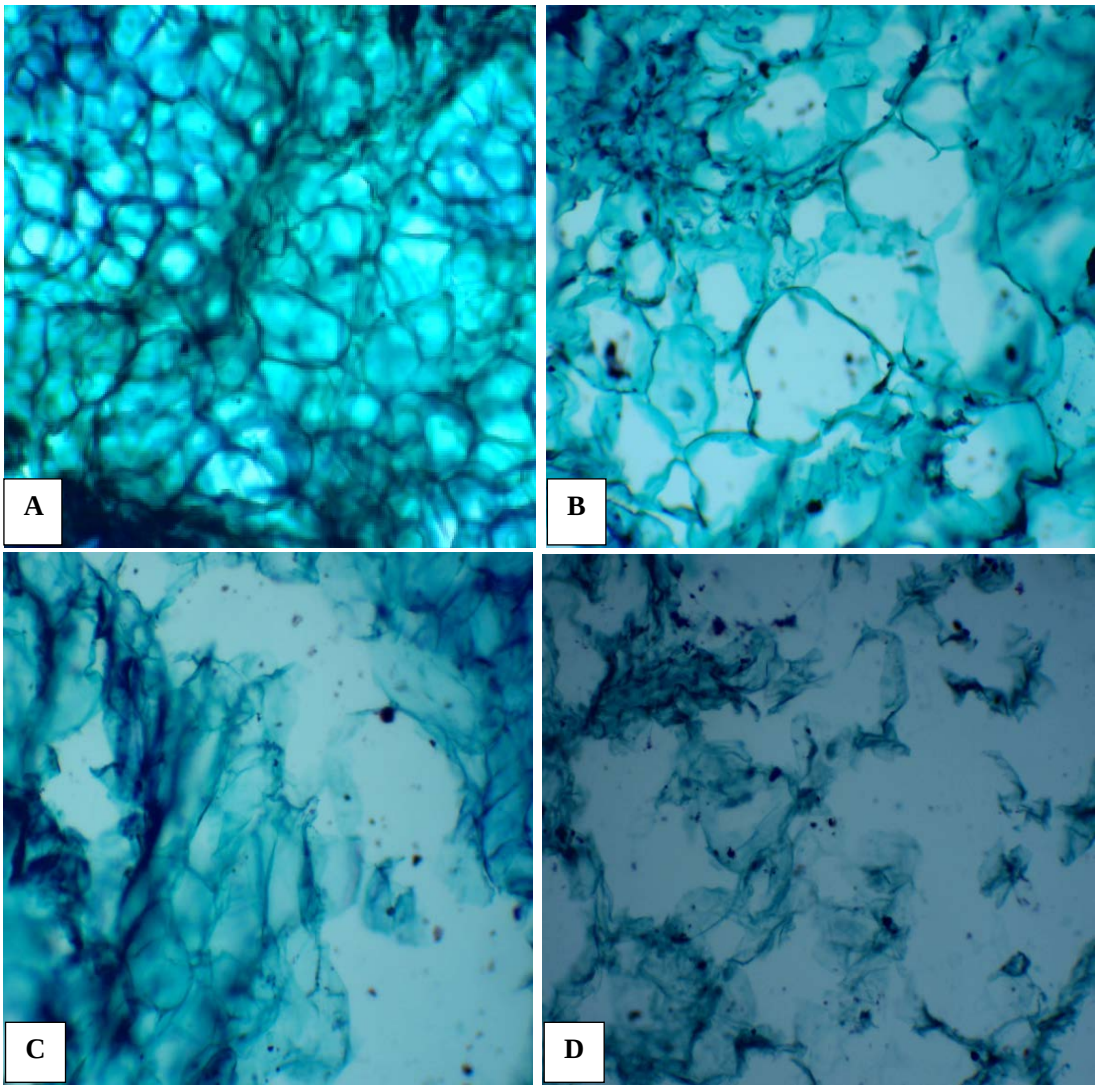


Figure (7). Light microscopic observation of longitudinal sections of callus of *Solanum nigrum* L. under treatments of control (A); 2 mg/L AgNPs (B); 4mg/L AgNPs (C) and 8mg/L AgNPs(D). (X100)

3.3. Effect of Silver Nanoparticles (AgNPs) on Callus Anatomy

It was observed (Fig. 7) that the deformity of callus structure of *Solanum nigrum* exposure to media containing AgNPs nanoparticles as compared to media without AgNPs (control). It was noticed that no damage for cell wall in callus of control (A), while damages were found in cell wall of callus exposure to AgNPs 2mg/l (B) and with increase in AgNPs, 4mg/l (C), the damage of cell wall also increase and it was dramatically with 8mg/l (D). These results coincide with those Harajyoti and Ahmed [28] who studied the phytotoxicity of silver nanoparticles solutions on *Oryza sativa*. They found that these nanoparticles had caused adverse effect on damage to external and internal portions of cells and breakage in cell wall of the roots. They added that the entry of particles is clearly seen by breaking the vacuoles. Accumulations of nanoparticles and its toxic effects on test plant species is totally depend upon the concentrations and exposure time.

The structural aberrations due to biologically synthesized silver nanoparticles was reported by Krishnaraj et al., [29] who concluded that no severe toxic effects were observed in silver nanoparticles treated plants in the morphological studies under scanning electron microscopy (SEM) while structural aberrations were observed in the light microscopic evaluation of root and stem anatomy.

Results of the present study showed that AgNPs were able to penetrate plant callus and dramatically affected cell wall and callus growth and support water uptake and accumulation inside the callus. In this respect, Khodakovskaya et al., [30] reported that the germination of tomato seeds was found to be dramatically higher for seeds that germinated on medium containing CNTs (10-40 μg/mL) compared to control. Analytical methods indicated that the carbon nanotubes (CNTs) are able to penetrate the thick seed coat and support water uptake inside seeds.

Plants represent an important trophic level [10]. However, there are only limited studies focused on the effect of nanomaterials on plants.

In particular the cell wall of plant cells represents a crucial structure in nanoparticle uptake compared to animal cells.

Based on the results obtained in our study, it can be concluded that uptake and translocation of metal nanoparticles is connected with changes in callus morphology and anatomy.

3.4. Assessment of Genetic Stability at Protein Level through SDS-PAGE Biochemical Marker

The protein profile system of *Solanum nigrum* revealed the biochemical variation and evolutionary relationship among callus control (no AgNPs) and callus under the treatment of AgNPs (2, 4 and 8mg/l) was demonstrated in figure (8). The molecular weights of detected bands for all samples ranged from 21 to 88 KDa. There were 12 bands detected at molecular weight 88, 82, 63, 52, 47, 38, 35, 33, 30, 25, 23, 21 KDa in the callus control (no AgNPs). Only 9 bands were detected in lane 2 (callus treated with 2mg/l AgNPs), whereas 3 bands with molecular weight 83, 63 and 25 KDa were absent. Callus treated with 4mg/l showed 13 bands, with two new bands at molecular weights at 66 and 27 KDa, in addition to disappear one band with molecular weight 47 KDa relative to that observed to control callus. Furthermore, Land 4 (callus treated with 8mg/l SNPs) showed the appearance of two new bands (77 and 57 KDa) and absence of two bands (66 and 63 KDa) as compared to control callus (Table 2).

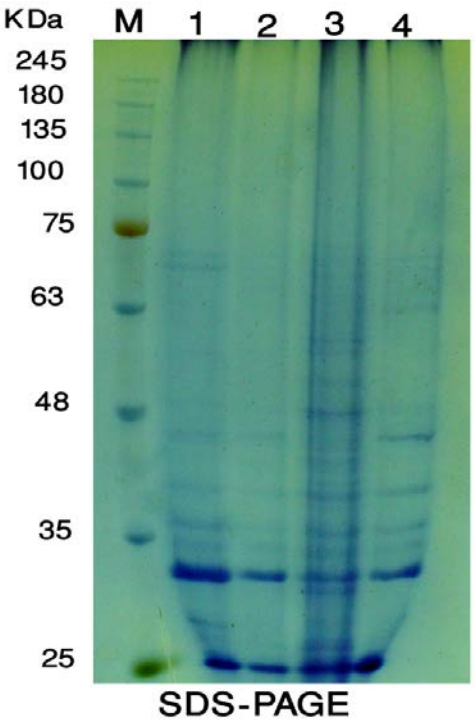


Figure (8). Protein profile of *Solanum nigrum* callus control (1); callus exposure to 2mg/L AgNPs (2); 4mg/L (3) and 8mg/L (4) using SDS-PAGE technique. Marker (M)

It was also observed (Table 3) that total number of bands detected with SDS-PAGE was forty six, while the number of polymorphic bands (including four unique bands) was eight. It was noted that the percentage of -polymorphism was 50% present. Proteins play many crucial roles in cells. They have structural, transport or catalytic (enzymes) function. In addition, there are peptides/proteins with special functions, such as glutathione and phytochelatins and enzymes involved in biochemical pathways connected with these compounds, which play crucial role in detoxification of both heavy metals and organic pollutants-xenobiotics [31, 32] Dissimilarity in protein profile between callus control and callus under treatment with different concentrations of AgNPs, especially at 4mg/l, may be based on the ability of AgNPs to induce protective cell mechanisms, such as biosynthesis of protective peptides/proteins and enzymes, which are involved in detoxification processes. A similar approach is reported by Salama [26] working on bean and corn, found that application of silver nanoparticles at the concentration of 20, 40 and 60 ppm caused an increase in

protein content of the two tested crop plants. At 60 ppm concentration the maximum significantly increase in protein (30% for common bean and 24% for corn) over control. At a dose of 100 ppm, significantly decrease in the protein (32% for common bean and 18% for corn) over control. The author mentioned that the increase in protein at certain concentration suggests the optimum dose limit for the growth of common bean and corn plants. However, the decrease in protein beyond this concentration suggests the toxic effect of AgNPs. In addition to this, Bekheta et al., [33] found that treating Gerbera plants with selenium led to appearance of new protein bands and disappearance of some protein bands. They recorded that the existence of such newly formed protein bands in the treated plants might be explained basing on the potentiality of the applied substance to trigger the expression of specific genes a long DNA molecule in the target cells, a process which appears to play a key role in regulating a cascade of biochemical reactions which might determine the ultimate appearance of growth patterns and yield of the produced plants.

The similarity coefficient values between the callus control without AgNPs and callus with different concentration of AgNPs (2, 4, 8mg/l) based on SDS-PAGE analysis were presented in (Table 4). The highest similarity value (0.75) was observed between callus control (no AgNPs) and callus exposure to 2mg/l AgNPs, followed by (0.71) between control and exposure to 8mg/l AgNPs, whereas 4mg/l AgNPs was given the lowest value (0.66) with control and (0.57) with 2mg/l AgNPs. The dendrogram of genetic distances among the callus control and callus treated with AgNPs at concentration 2, 4, 8 mg/l based on total soluble proteins (Fig. 9) showed that callus cultured on MS medium supplemented with 4 mg/l AgNPs has the highest difference in protein content than control (no AgNPs), followed by callus exposure to 8mg/l then to 2mg/l AgNPs. The changes in protein profile of callus of *Solanum nigrum* in response to SNPs indicated the action of nanoparticles at a molecular level of callus cells and acceptance the macroscopic anatomy deformed. Many investigations revealed that silver nanoparticles (AgNPs) exposure caused the alteration of some proteins related to endoplasmic reticulum and vacuole in seedlings of *Eruca sativa* indicating these two organelles as targets of the AgNPs action [34].

3.5. Detection of Genetic Stability at DNA Level Using RAPD (PCR) Molecular Marker

Random Amplified Polymorphic DNA (**RAPD**) marker was employed to detect level of genetic stability of callus of *Solanum nigrum* control (Lane 1) and callus treatment with 4.0mg/l AgNPs (lane 2). Six **RAPD** primers (OP-m01, OP-C15, OP-C12, OP-C04, OP-E15 and OP-B11) were selected according to their ability to detect distinct and clearly amplified products.

Table (3). Binary matrix showing the protein pattern of *Solanum nigrum* callus control without AgNPs (1) and callus wih 2mg/l (2), 4mg/l (3) and 8mg/l (4) of AgNPs based on SDS-PAGE marker

Band	Reference	MW	1	2	3	4	Frequency	Band Type
1	0.351	87.984	1	1	1	1	1	M
2	0.38	82.413	1	0	1	1	0.75	P
3	0.413	76.501	0	0	0	1	0.25	U
4	0.478	66.069	0	0	1	0	0.25	U
5	0.499	63.012	1	0	1	0	0.5	P
6	0.543	57.059	0	0	1	1	0.5	P
7	0.583	52.019	1	1	1	1	1	M
8	0.633	46.576	1	1	0	1	0.75	P
9	0.72	38.277	1	1	1	1	1	M
10	0.764	34.66	1	1	1	1	1	M
11	0.788	32.834	1	1	1	1	1	M
12	0.834	29.598	1	1	1	1	1	M
13	0.867	27.473	0	0	1	0	0.25	U
14	0.916	24.6	1	0	0	0	0.25	U
15	0.951	22.733	1	1	1	1	1	M
16	0.981	21.245	1	1	1	1	1	M
Total number of proteins			12	9	13	12		

Polymorphic band; M: Monomorphic band; U: Unique band, MW: Molecular Weight.

Table (4). Jaccard's similarity coefficient based on SDS-PAGE technique for *in* callus control (no AgNPs) and callus treated with AgNPs

Treatment	Control	2 mg/l AgNPs	4 mg/l AgNPs	8 mg/l AgNPs
Control	1.0			
2 mg/l AgNPs	0.75			
4 mg/l AgNPs	0.6667	0.5714		
8 mg/l AgNPs	0.7143	0.75	0.6667	

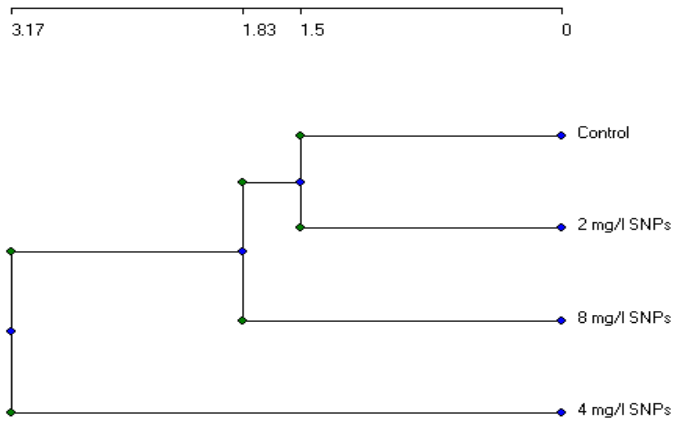


Figure (9). Relationships between callus control (no AgNPs) and callus follwoed AgNPs at different concentrations (2, 4, 8 mg/l) based on protein profile

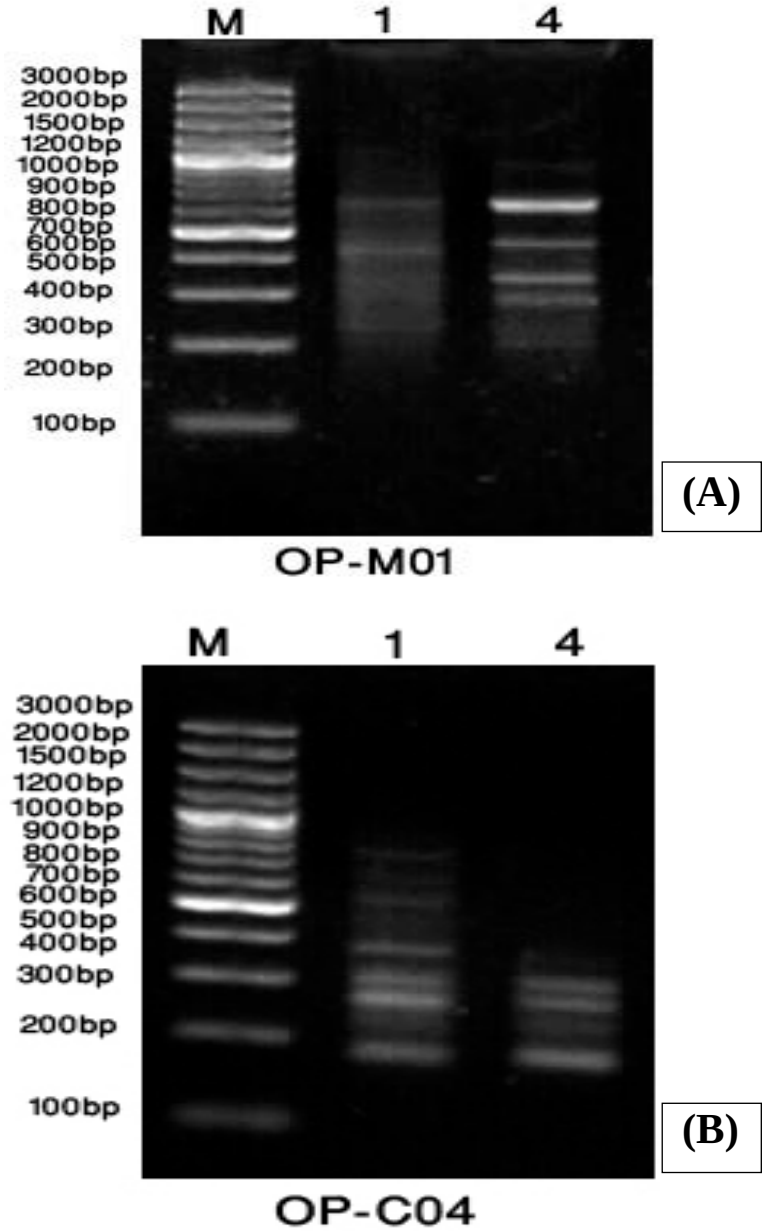


Figure (9). A band pattern produced by primer OP-MO1 (A) and primer OP-CO4 (B) for callus control (1) and callus exposure to 4mg/L AgNPs (4). Line (M) represents DNA marker

In this study, RAPD profiles were used to check genetic variation among of callus of *Solanum nigrum* L. under treatments of control, no AgNPs (Lane 1) and 4.0mg/l AgNPs (Lane 2).

Analysis of RAPD data (Table 5 & Fig. 9) showed presence of different levels of genetic variation. The total number of detected amplified fragments using six RAPD primers was 35, while the number monomorphic fragments were 19 (Table 2). RAPD OP-E15 produced maximum number of amplified products (7) and OP-CO4 and OP-B11 produced the least (5). The highest percentage of polymorphism (66.67%) was observed in OP-C12 primer followed by OP-E15 primer (133%). The lowest percentage of polymorphism (33.33%) was obtained in OP M-10 and OP C-15 primers.

Results in table (6) showed that the level of monomorphism was similar in both callus control and callus exposure to AgNPs, while monomorphism % was higher in callus control (no AgNPs). It was also observed the absence of polymorphism from both callus using six RALD primers.

Table (5). Percentage of polymorphism of amplified fragments produced using six RAPD primers

RAPD primers	TAF	MF	UF	Polymorphism (%)
OP M – O1	**6**	**4**	**2**	**33.33**
OP – C15	**6**	**4**	**2**	**33.33**
OP – E15	**7**	**3**	**4**	**57.14**
OP – C12	**6**	**2**	**4**	**66.67**
OP – CO4	**5**	**3**	**2**	**40.00**
OP – B11	**5**	**3**	**2**	**40.00**
Total	**35**	**19**	**16**	**45.71**

TAF: Total Amplified Fragments; **MF**: Monomorphic Fragment; **UF**: Unique Fragment. Polymorphism (%) = (No. of polymorphic (unque) bands ÷ Total nands) X 100

Table (6). Percentage of monoporphism in callus without AgNPs (Control) and callus exposure to AgNPs using six RAPD primers

Treatment SNPs	TAF	MF	Monomorphyism %
0.0 Callus (control)	**28**	**21**	**75**
4.0 mg/L SNPs	**30**	**21**	**70**

TAF: Total Amplified Fragment; MF: Monomorphic Fragment.

The obtained results showed genetic variations between callus control and callus exposure to AgNPs. Such result was harmony with those reported by Abdel-Azeem and Elsayed [35] working on *Vicia faba* seedlings, found that silver engineered nanoparticles affected mitotic indices as well as chromosomal morphology and disturbed chromosomes at metaphase and anaphase, laggards, fragments, bridges, chromosome stickiness and micronuclei. Recently, Supriyo et al., [36] evaluated the genotoxic potential of the silver nanoparticles with increasing concentrations using agarose gel electrophoresis of plasmid pZPY112. They observed plasmid incubated with 0.51 μg nanoparticles showing disappearance of the supercoiled plasmid band and appearance of relaxed circular and linear plasmid bands along with smaller fragmented DNA. Furthermore, plasmid incubated with 3.57 μg nanoparticles showing gradual degradation of the fragmented DNA bands; and in another lane, plasmid incubated with 5.1 μg nanoparticles showing more degradation of DNA.

4. Conclusions

Nanomaterials are considered to be one of the most important inventions of modern science [37]. In the present study, silver nanoparticles have been synthesized using safe, cost-effective, sustainable and environment friendly processes method (mixing 4 mL camel milk with 96 mL of 1 mM silver nitrate solution as previously mentioned). Their exceptionality is based on their constant physical properties, which are strictly dependent on their size, which varies from 1 to 100 nm. In addition, the interesting range of their properties is due to the large surface area that may be modified or functionalised for different biological applications [38]. These modifications can lead to higher water solubility or some targeting. Nanomaterials, especially nanoparticles, can be linked with different biologically active molecules, which can exactly direct them to specific sites within biomolecules including proteins and nucleic acids, sub-cellular, cellular, tissue and body structures [39]. Based on the results obtained in the present study, it can be concluded that uptake of metal nanoparticles (AgNPs) in vitro is connected with changes in *Solanum nigrum* callus morphology and anatomy, especially of biomass and deforming cell shape. Cell wall represents the main site of the interaction between nanoparticles and plant cell. This fact is probably connected with the effects on protein and nucleic acids metabolism that is clear with the variation observed in genetic stability of callus control (no AgNPs) and callus exposure to AgNPs.

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