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Biological Synthesis of Silver Nanoparticles using Ginger (*Zingiber Officinale*) Extract

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Abstract

Plant extracts are very cost effective and eco-friendly and thus can be used as an economic and efficient alternative for the large-scale synthesis of nanoparticles. We report on the use of ginger extract for the biosynthesis of silver nanoparticles. Bioactive silver nanoparticle synthesis was byreacting the ginger extract with aqueous silver nitrate solution at room temperature (27±2 °C) and at 60 °C. Formation of silver nanoparticles was confirmed by UV– visible spectroscopy, X-ray diffraction pattern and Scherrer's formula. Antibacterial activity of synthesized silver nanoparticles showed inhibitory activity against bacteriaviz., Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa, Bacillus cereus and Proteus vulgaris.Additionally the toxicity of the produced nanoparticles was checked for phyto and genotoxicity.

Keywords: *Antimicrobial activity; Mitotic index; Silver nanoparticles; Toxicity; XRD.*

1. INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles exhibit completely new and improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly. Nanocrystalline silver particles have found tremendous applications in the field of biomolecular detection, diagnostics, antimicrobials and therapeutics (Elechiguerra *et al*. 2005) and catalysis (Crooks *et al*. 2001). But, the concern for environmental contamination is sensitive as the chemical procedures involved in the synthesis of nanomaterial generates a large amount of hazardous byproducts. Thus, there is a need for green synthesis that includes clean, nontoxic and environment-friendly methods of nanoparticle synthesis (Mukherjee *et al*. 2001) with sustainable commercial viability. Green synthesis makes use of environmental friendly, nontoxic and safe materials (Sharma *et al*. 2009) like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles that offer numerousbenefits of being eco-friendly and compatible for pharmaceutical and other biomedical applications.

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The present study includes biological synthesis of silver nanoparticles using ginger extract and their characterization. Antibacterial, phytotoxicity and genotoxicity tests of the synthesized nanoparticles were also carried out.

2. EXPERIMENTAL DETAILS

2.1 Preparation of ginger extract

Ginger (*Zingiberofficinale*) extract wasused in the present study. The materials were collected from the Bangalore market, rinsed with sterile distilled water to remove any associated debris. These clean fresh materials were cut into fine pieces and grinded in a pestle and mortar (20 g of the sample in 100 ml of distilled water). The resulted infusion was filtered thoroughly using Whatmann No.1 filter paper.

2.2 Synthesis of silver nanoparticles

Solution was prepared by mixing 10 ml of pure extract to 90 ml of 1 mM aqueous solution of silver nitrate. Synthesis was carried out at room

temperature (27±2 ^{o}C) and at 60 ^{o}C with their respective controls.

2.3 UV -Vis spectroscopy analysis

The reduction of silver nitrate was monitored by measuring the absorbance.Spectral analysis was carried out using UV-VIS spectrophotometer (UV-1800; Shimadzu). The spectra between 190 and 1100 nm was scanned to find the absorbance peak.

2.4 X-ray diffraction measurements

X-Ray diffraction (XRD) measurements of the biosilver nanoparticle solution thus obtained were purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellets into 10 ml of deionized water and freeze-dried. 1 g of finely powdered sample was thoroughly mixed in mortar and pestle to get a fine homogenous mixture which was analyzed by XRD. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrerformula.The dried mixture was collected for determination of silver nanoparticles by a BRUKER D8 advance X-ray diffractometer (Jain *et al.* 2009).

2.5 Antagonistic effect by Agar Well Diffusion Method

The antibacterial activity of silver nanoparticles was tested by standard agar well diffusion method (Prabhu *et al*. 2010). Wells were made using sterile cork-borer under aseptic conditions. The inocula were prepared by diluting the overnight cultures with 0.9 % sodium chloride to 0.5 McFarland standards and were swabbed onto the plate. Synthesized solutions were loaded on marked wells with the help of micropipette under aseptic conditions and incubated at 37 ºC for 24 h. The zone of inhibition was measured and expressed in millimeters.

2.6 Effect of produced nanoparticles on seedgermination and Vigor index

The effect of the biosynthesized nanoparticles was studied by treating the *Phaseolusmungo*seeds. The seeds were rinsed thoroughly in sterile distilled water before treating. Germination test was carried out by the top of the paper method. 400 seeds for each treatment in triplicates were used for the study.Seeds were soaked in 20 % solution of synthesized nanoparticles and their respective controls for 3 h and plated onto the wet filter paper and incubated at room temperature (27 ± 2 ^{o}C) for seven days. Germination percentage and vigor index were calculated (Baki and Anderson, 1973).

2.7 Effects of produced nanoparticles on Mitotic Index

Onion bulbs were grown at room temperature (27 ± 2 ^{o}C) in glass beakers containing distilled water and solution containing biosynthesized nanoparticles (27 ^{o}C and 60^{o}C) for 48h. Two centimeter root meristems were cut and fixed in freshly prepared fixative (ethanol: glacial acetic acid :: 3:1) and stored at 4 ^{o}C.Theroot tip smears were prepared by hydrolyzing in 1N hydrochloric acid at 60 ^{o}C for 6-7 min. After hydrolysis, the root tips were thoroughly washed with water several times and stained with acetoorceine. Squash preparations were made with 45 % acetic acid and observed under the microscope (Sik *et al*. 2009). The mitotic index was used to determine the rate of cell division. Mitotic index was calculated by the formula:

Mitotic Index =No. of dividing cells/ total number of cells × 100

2.8 Statistical analysis

Experiments were conducted in triplicates and the results were presented as the Mean ± SD and the p value less than 0.05 were considered as statistically significant.

3. RESULT & DISCUSSION

Silver ion reduction was observed when 1mM silver nitrate solution was incubated with 20% aqueous extract of ginger roots at 27 ^{o}C and 60 ^{o}C. Initial stages of reduction showed change in colour from almost colourless to brown, which clearly indicated the formation of silver nanoparticles in the reaction mixture as shown in Fig. 1. The colourintensity increased with time of incubation. Control showed no change in colour under similar conditions. Color change could be observed as early as 30 min from colorless to faint yellow, indicating the formation of silver nanoparticles. As time elapsed the yellow colored solution eventually became dark brown by 3 h, which may be due to the increased concentration of nanoparticles as well as particle size. There was no significant change beyond 3 h indicating the completion of the reduction reaction.

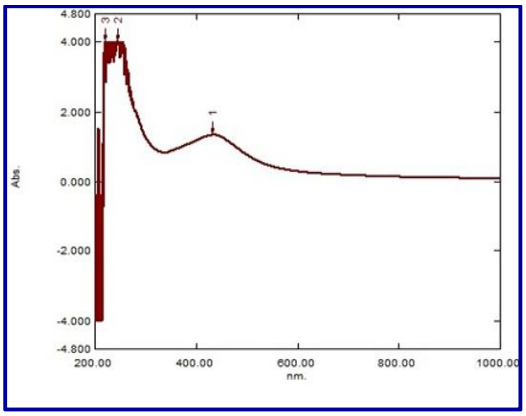
Physical appearance of the reaction mixture turning brown from colourless may be due to the

surface plasmon resonance of the silver nanoparticles, which is considered to be primary signature of nanoparticle formation. Change in the colour was similar in most of silver synthesized nanoparticles as in the use of anthoceros (Kulkarni *et al*. 2011), mushroom (Narasimha *et al*. 2011) and switchgrass (Mason *et al*. 2012).

Fig. 1: Biosynthesis of silver nanoparticles: Colour change observed during biosynthesis of silver nanoparticles using ginger extract at room temperature (a) (27 ± 2 °C) and at (b)60 °C

- A - Control without silver nitrate; B – silver nanoparticle before synthesis; C - silver nanoparticle after synthesis

UV-vis absorption spectrum of silver nanoparticles in the presence of ginger extract is shown in Fig. 2. The absorption peak is centered around 400–450 nm for ginger synthesized nanoparticles at room temperature (27 ± 2 °C) and 60 °C. At lower concentrations of the extract the SPR band is broad which may be due to the formation of particles with broad size distribution. The surface plasmon band in the silver nanoparticles solution is close to 410 nm for ginger extract at room temperature (Fig. 2a) and 450 nm for ginger extract at 60 °C (Fig. 2b). Similar report was recorded from *Eucalyptus hybrida* leaf extract which was centered around 412 nm (Dubey *et al*. 2009).



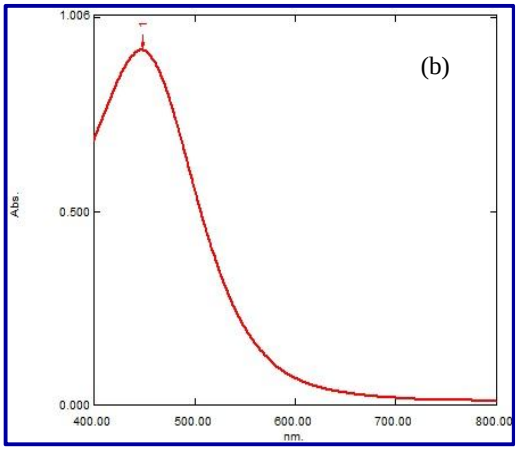


Fig. 2: UV-visible absorption spectra of silver nanoparticles synthesized by incubating 1mM silver nitrate solution with 2% ginger extract and incubated at room temperature (a) and at 60°C (b)

X-ray diffraction (XRD) patterns of nanoparticles were studied on a Bruker D8 ADVANCE X-ray powder diffractometer (Bruker AXS). The dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 20° to 100° at 2θ angles. The diffraction pattern in Fig. 3 corresponds to pure silver metal powder. The XRD pattern indicates that the

nanoparticles had a spherical structure. The obtained results illustrate that silver ions had indeed been reduced to Ag^{o} by ginger extract under reaction condition. All diffraction peaks correspond to the characteristic face centered cubic (FCC) silver lines (Mazumdar *et al*. 2011). Different diffraction lines were observed at 2θ angle 28, 29, 32, 34, 38, 47.5, 46, 59 and 64 respectively, but spectrum of nanoparticle exhibited three prominent Bragg reflections at around 32°, 38° and 47.5° respectively (Fig.3). The Average particle size can be calculated by using Scherrer's relation: $D= K\lambda / \beta \cos \theta$. Where K is the Scherrer constant with the value 0.94, λ is the wavelength of the X-ray, β-full-width at half-maximum and θ is the Bragg angle.

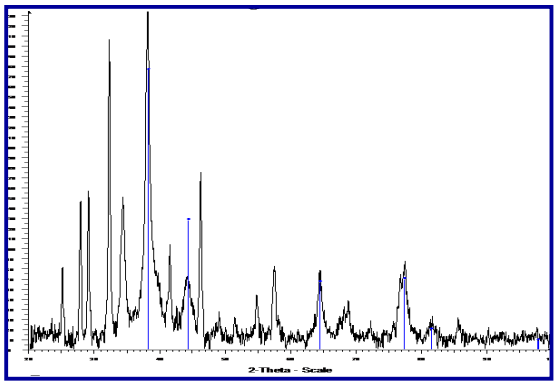


Fig. 3: X-ray diffraction patterns of silver nanoparticles synthesized using ginger extract. The silver nanoparticles synthesized by treating 20% ginger extract with 1 mM aqueous silver nitrate solution at 60 °C.

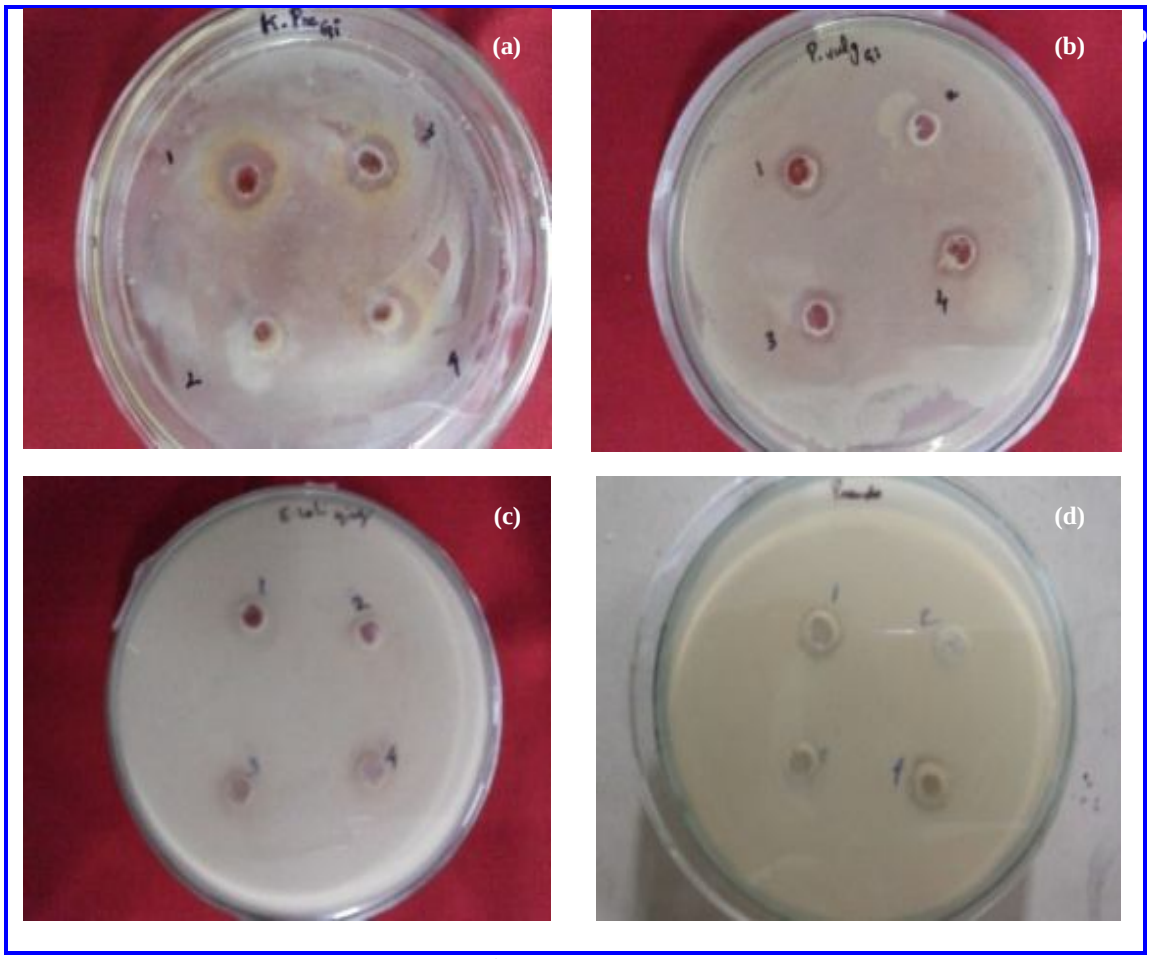


Fig. 4: Plates showing the antimicrobial activity of the synthesized silver nanoparticles against, *Klebsiella pneumonia* (a), *Escherichia coli* (b), *Proteus vulgaris* (c) and *Pseudomonas aeruginosa*(d). Well 1 - Silver nitrate; Well 2 – Ginger Extract; Well 3 – biosynthesizednanoparticle at room temperature; Well 4 – biosynthesized nanoparticle at 60 °C.

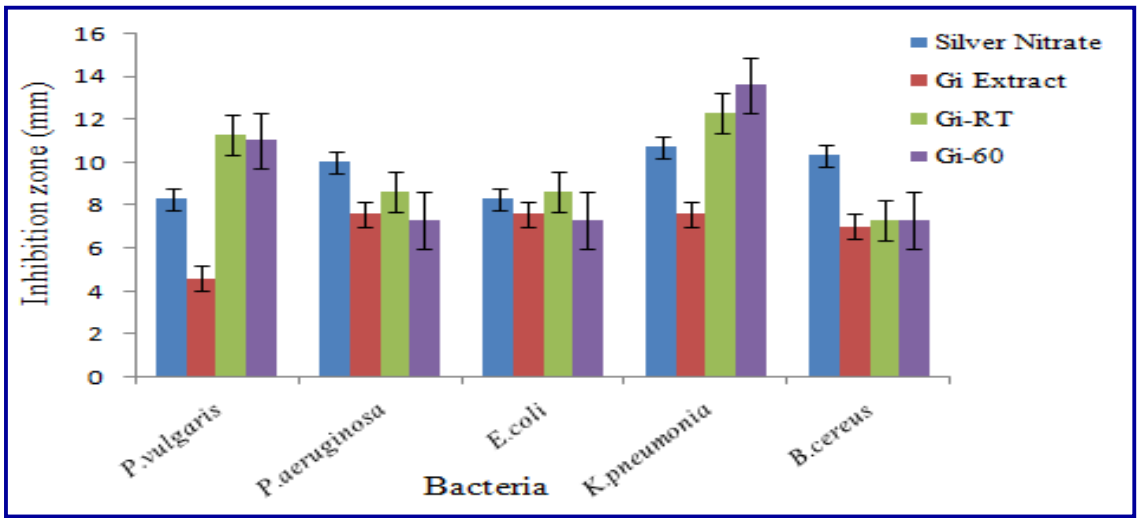


Fig. 5: Antibacterial activity of the synthesized silver nanoparticles against the test organisms was measured for diameter of zone of inhibition in millimeter by well diffusion method.

- Results are shown as Mean ± SE.Ginger Extract - control without nanoparticle; Gi- Ginger; Gi-RT – biosynthesized nanoparticle at RT; Gi-60 - biosynthesized ginger nanoparticle at 60 ^{o}C.

Calculations using Scherrer'srelation,showed that the average particle size was around 2.89 nm. The sizes of the deposits in our results is so small compared to previous studies using garlic extract (26.30 nm), which may be caused by different metal incubation conditions (HariPriyaa and Kumudini, 2014). The size of particles can be easily calculated from the graph (2θ= 307, 335, 177). Results confirmed production of silver nanoparticles. Similar results were observed in silver nanoparticles of 3-12 nm from peels of *Citrus sinensis* (Konwarh *et al.*, 2011).

The effects of the ginger-synthesized nanoparticles were tested for antimicrobial activity against pathogenic strains. The silver nanoparticles obtained from ginger extracts showed antimicrobial activity against five laboratory pathogens such as *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Proteus vulgaris*. Results of the zone of inhibition have been graphically represented in Fig. 5. It is evident that the nanoparticles synthesized are good candidates for their use as antibacterial agents. The diameters of the silver nanoparticle inhibition zones against different pathogenic cultures showed that ginger extract showed an inhibition zone which was comparatively less than the synthesized nanoparticles. The diameters of the silver nanoparticle inhibition zones against different pathogenic cultures were measured (Fig. 4). Control showed an inhibition zone which was comparatively less than the synthesized nanoparticles. Antibacterial activity of *E. coli* and *P. aeruginosa* showed similar results (Kheybari *et al*. 2010).

3.1 Toxicity of the produced nanoparticles was checked on green gram seeds

Biosynthesized nanoparticle samples were incorporated to the seeds to check germinations percentage. The study confirmed relatively lesser germination rate as well as significant growth of plumule and radical of *P. mungo* against control samples (Fig. 6).

Seeds treated with ginger extract at 27 ^{o}C and 60 ^{o}C showed germination percentage of 90 and 89 respectively. This slightly decreased after treatment with nanoparticles which showed 89 and 80 % which was not statistically significant (Fig. 6) indicating the ineffectiveness of nanoparticles on germination.

Shoot length of gingerextractat 27 ^{o}C and 60 ^{o}C was 2.1 and 0.9 cm respectively, whereas in nanoparticle-treated samples it was 4.5 and 0.5 cm which was comparatively higher has shown in Fig. 7.

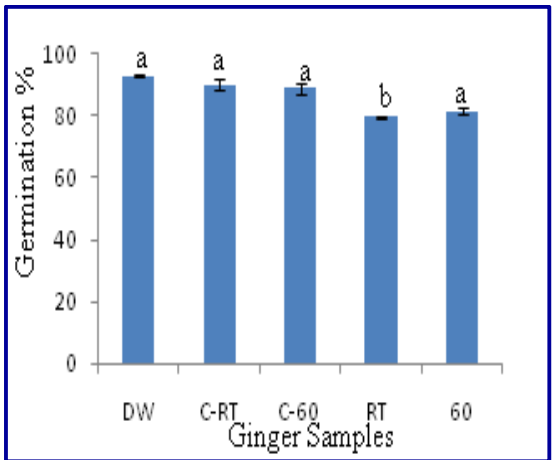


Fig. 6: Effect of treatment with silver nanoparticles synthesized using ginger extract at room temperature and 60 °C on seed germination of *Phaseolusmungo*

- Error bars indicates standard error. Different alphabets in each column shows that the data is significantly different fromeach other at $p \leq 0.05$

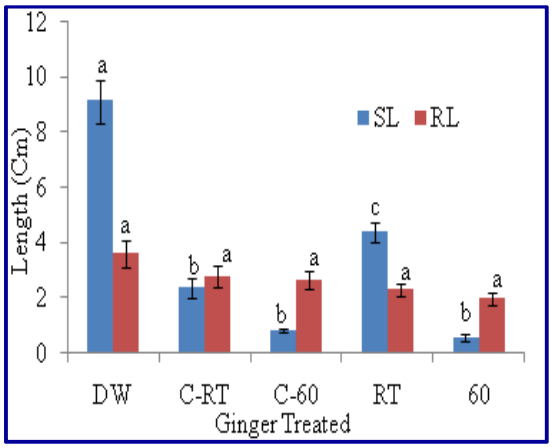


Fig. 7: Effect of treatment with silver nanoparticles synthesized using ginger extract at room temperature and 60°C on shoot and root length of *Phaseolusmungo* after seven days.

- Error bars indicate standard error; RL- Root length, SL- Shoot length. Different alphabets in each column shows that the data is significantly different from each other at $p \leq 0.05$

P. mungo seeds showed a vigour index of 459.18 for ginger extract at 27 °C and 304.25 at 60 °C as shown in Fig. 8. Seed treatment with synthesized nanoparticle at 27 °C showed higher vigor index when compared to its control whereas for at 60 °C treatment with synthesized nanoparticle was lower than its respective control.

Effect of synthesized nanoparticles on fresh and dry weights of germinated seedlingswas considered for the studies. Synthesized nanoparticles at 27 °C and 60 °C, with their respective controls were used. Results showed that fresh weight of synthesized nanoparticle at 27 °C which showed significant increase when compared to its respective control (Fig. 9). Dry weight of synthesized nanoparticles showed slight increase when compared to that of the controls.

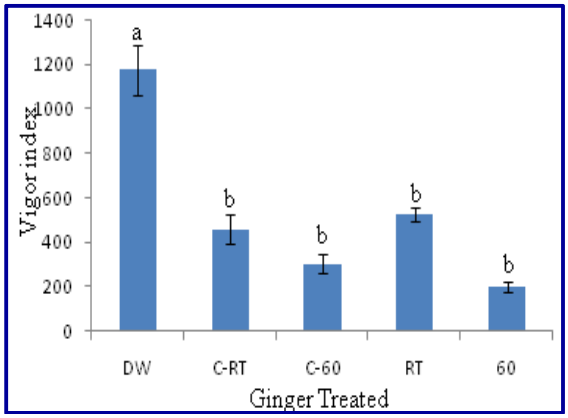


Fig. 8: Effect of treatment with silver nanoparticles synthesized using gingerextractat room temperature and 60 °C on vigor index of *Phaseolusmungo*

- Bars represent standard error.Different alphabets in each column shows that the data is significantly different from each other at $p \leq 0.05$

Mitotic indices of samples containing biosynthesized nanoparticles at 27 °C and 60 °C showed 16.02 and 15.37 respectively, which showed slight increase over their respective control as shown in Fig. 10.Effect of nanoparticle toxicity on onion root tip showed that all the stages of mitosis including prophase, metaphase, anaphase and telophase in all the tissues. But the number of cells showing mitosis varied between the nanoparticle-treated and control samples.The reduction in mitotic index may be caused by the effect of the silver nanoparticle on the microtubule (Macleod et al. 1969; Webster et al. 1969).

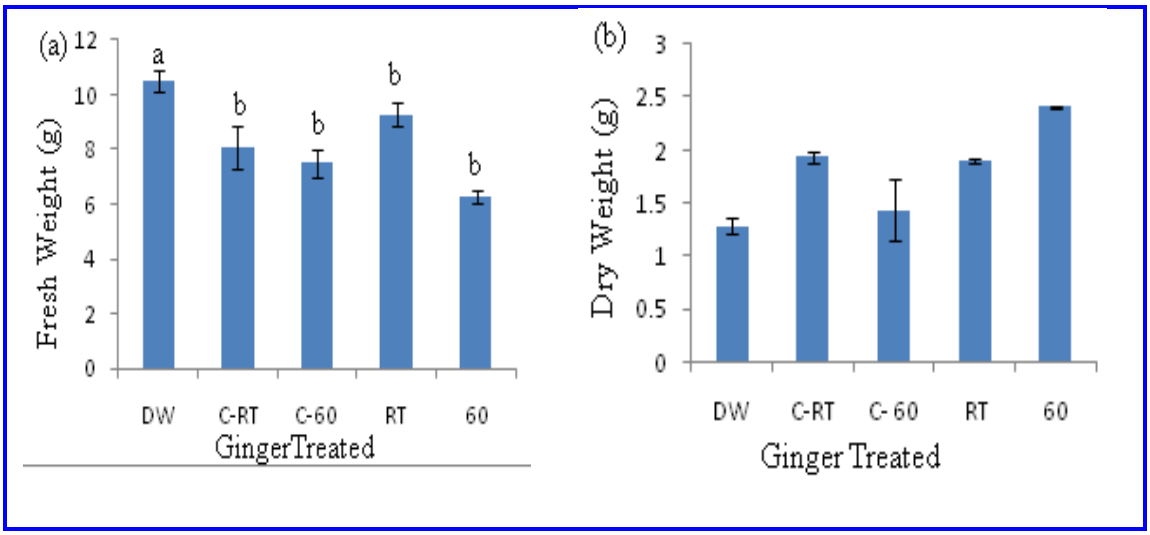


Fig. 9: Effect of treatment with silver nanoparticles synthesized using ginger extract atroom temperature and at 60 °C on the fresh (a) and dry weights (b) of *Phaseolusmungo*seedlings.

- Error bars represent standard error. Different alphabets in each column shows that the data is significantly different from each other at $p \leq 0.05$

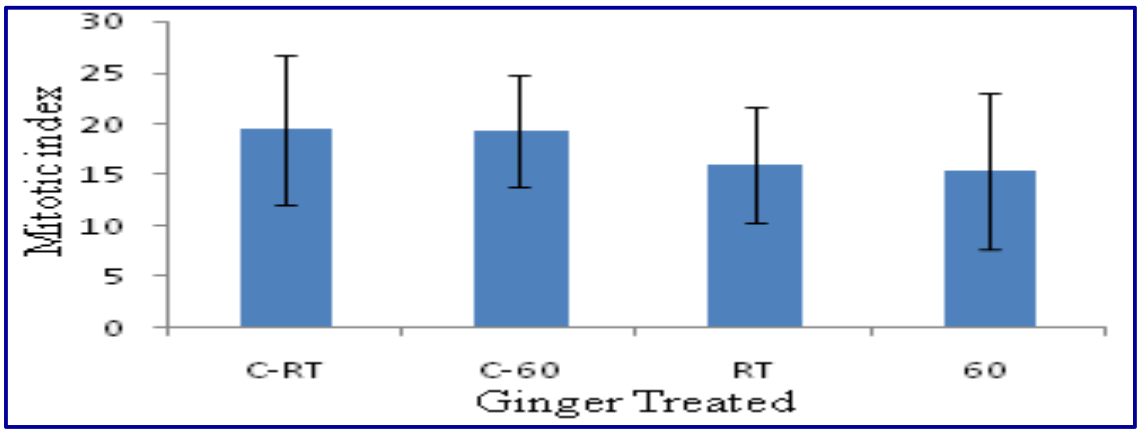


Fig. 10: Effect of silver nanoparticles on the mitotic index of the onion root-tip Meristems

- Error bars represent standard error

4. CONCLUSIONS

The environmental friendly method of biological silver nanoparticle synthesis can be potentially used in many applications including medicine. The benefits of the new green-synthetic protocols for generation of nanoparticles over the conventionally used processes include: first, only naturally occurring nonhazardous materials are used. Second, no hazardous waste is produced, third, reduced processing effort is required, fourth, the materials are more stable, are easily stored, and are easily transported and fifth, the materials can be more readily produced around the world using common and often biorenewable materials. Plants or their extracts can be efficiently used in the synthesis of gold and

silver nanoparticles as a greener route. Control over the shape and size of nanoparticles seems to be very easy with the use of plants. Such nanoparticles produced using plants have been used in various applications for human benefit. Elucidation of the mechanism of plant-mediated synthesis of nanoparticles is a very promising area of research.

It has been demonstrated that ginger extract is capable of producing silver nanoparticles and the silver nanoparticles are quite stable in solution. The biomedical applications of silver nanoparticle can be effective by the use of biologically synthesized nanoparticles which minimize the factors such as toxicity and cost and are found to be exceptionally stable. The synthesized nanoparticle was confirmed by color changes and it was characterized by UV-visible spectroscopy and X-ray diffraction which indicated that average size of the nanoparticle was 2.89 nm which was calculated using Scherrer's relation. The synthesized silver nanoparticles showed potential antibacterial activity against pathogenic strains. Effect of produced nanoparticles on *P. mungo*seed clearly indicates that they were slightly toxic. This was shown by slight decrease in germination percentage. But vigor index was almost similar to control. Toxicity on cell division as observed was also not significant.

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