4. RESULTS

4.1. SYNTHESIS AND CHARACTERIZATION OF BIOGENIC SILVER NANOPARTICLES FROM PLANT EXTRACT

4.1.1. Biogenic synthesis of silver nanoparticles (AgNPs)

In the present study, the formation of brownish orange color indicated the synthesis of AgNPs by using rhizomes as shown in (Plate 2b) of Alpinia calcarata Rosc. plant (Plate 2a). Extracts of dry powdered rhizomes were used to biologically synthesize AgNPs (Plate 2c). The formation of biogenic AgNPs was seen by color change in AgNO₃ solution to brownish orange as shown in (Plate 2e). (Plate 2d) shows that the AgNO₃ solution without addition of extracts of dry powdered rhizomes can be treated as control. This bioreduced aqueous component was further used for characterization purpose.

4.1.2. Characterization of biogenic silver nanoparticles

4.1.2.1. Ultraviolet-visible Spectroscopic studies

AgNPs were observed strongly in the range of 400 - 450 nm in visible region as shown in (Figure 1). In the present work, the biogenic AgNPs are rapidly formed (at pH 7) after the addition of Alpinia calcarata Rosc. extract, evident from the appearance of brownish orange color at 422 nm which is the characteristic wavelength of AgNPs with increase in absorbance at regular time intervals as depicted in (Figure 2).

4.1.2.2. Fourier Transform Infrared Spectroscopic studies

FT-IR spectrum of dry powder aqueous extract and synthesized AgNPs are shown in (Figure 3). The IR-spectrum of the AgNPs showed absorption bands at 1038, 1380, 1635 and 3430 cm⁻¹. The absorption bands at 1038 cm⁻¹ correspond to C-N stretching vibrations of the amine. The absorption bands at 1635 cm⁻¹ correspond to amide 1 band of proteins due to carbonyl stretch in proteins and absorption bands at 3430 cm⁻¹ are due to the O-H stretching in alcoholic compounds. The sharp band at 1380 cm⁻¹ is due to C-H stretching vibrations of aromatic and aliphatic amines.
4.1.2.3. **Powder X-Ray Diffraction studies**

XRD studies were carried out to identify the crystalline nature of the synthesized biogenic AgNPs. Diffraction peaks were observed at 2θ values of 38.2°, 44.4° and 64.1° that can be indexed to (111), (200) and (220) reflection planes of face centered cubic (fcc) as shown (Figure 4). The mean size of the biosynthesized AgNPs was determined by Debye-Sherrer formula and found to be in the range of 2-10 nm. The lattice parameter was calculated according to Bragg’s law and was found to be 4.077Å. The crystallinity index was calculated to be 1.3604.

4.1.2.4. **Electron Microscopy/ Energy Dispersive Spectroscopic studies**

Electron Microscopy studies were carried out to view the size of the synthesized biogenic nanoparticles. The HR-TEM image of synthesized AgNPs depicted in (Plate 3a) give clear indications regarding size, shape and size distribution of nanoparticles. The size of the synthesized biogenic AgNPs was found to be 2-20 nm. The SAED pattern of AgNPs reveals its crystalline nature (Plate 3b). From TEM images, (Plate 4) it can be seen that the AgNPs are capped with phytoconstituents of rhizome of *Alpinia calcarata* Rosc. The result of EDS gives a clear idea about the elements present in the nanoparticles (Figure 5). The strong signal of the Ag atoms indicates the crystalline property.

4.1.2.5. **Atomic Absorption Spectroscopic studies**

The concentration of silver in the synthesized biogenic AgNPs was determined using Atomic Absorption Spectrophotometer and the concentration of silver was recorded to be 24.89% of silver in 28 mg of synthesized silver nanopowder.

4.1.2.6. **Optimization of synthesized biogenic silver nanoparticles**

Different parameters were optimized for synthesizing AgNPs including pH, time, temperature, concentration of silver nitrate, concentration ratio of leaf extract and AgNO₃. There is no particles formation at acidic pH 3 and 5. Color formation was rapid at alkaline pH 9 but peak was shifted towards 500 nm. Agglomeration was observed at pH 11 immediately after adding the AgNO₃ into the reaction mixture (Figure 6). At neutral pH 7, the reaction was started as soon as the AgNO₃ was added into the reaction medium and the formation was observed within 30 min of incubation (Figure 7). The colorless solution was turned to brownish color which indicates the formation of AgNPs (Plate 2e). The characteristic absorption peak at 422 nm in UV–vis spectrum further
confirmed the formation of AgNPs. Temperature effect was also studied and found that the peak was found to be stable till 50°C. Thus AgNPs were synthesized at room temperature. However, distortion in the peak was seen above 60°C as seen in (Figure 8).

Different concentration of silver nitrate was optimized for the maximum synthesis of AgNPs. Interestingly, 5 mM concentration of AgNO₃ supported rapid formation whereas the peak got reduced at 4 mM and 3 mM concentrations (Figure 9). Similarly, different concentration ratios of leaf extracts and AgNO₃ solution were also optimized for maximum production of AgNPs. Interestingly 50 mL reaction medium containing 5 mL of plant extract and 5 mM concentration of silver nitrate solution turned to brownish orange color with in 30 min of incubation period, indicating rapid formation of AgNPs as shown in (Figure 10). Thus the optimized medium supported the maximum formation of AgNPs and the reaction occurred very rapidly.

To access the stability of AgNPs formed in the reaction solution at pH 7, UV–vis analysis study was carried out. This study clearly showed no alteration in the peak at 422 nm even after 2 months of incubation period, indicating strong stability of biosynthesized AgNPs. Therefore, it is clear that the optimization process played a pivotal role in the particles stability and aggregation.

4.2. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF BIOGENIC SILVER NANOPARTICLES

4.2.1. Zone of inhibition in bacteria

The zone of inhibition in bacterial growth by the biogenic AgNPs and AgNO₃ shows that this study is dependent on the concentration in medium as shown in (Table 2) and (Table 3). The biologically synthesized AgNPs exhibit excellent antibacterial activity against both Gram positive and Gram negative bacterial pathogens.

4.2.2. Changes in membrane permeability of bacterial cells

Membrane permeability test was performed to study the interaction of AgNPs on the bacterial cell surfaces. Ten milliliters of log phase bacterial cultures exposed to 10 ppm of AgNPs and AgNO₃ showed highest conductivity at 6 hr. While both Gram positive and Gram negative bacteria depict higher sensitivity to the AgNPs and AgNO₃
the conductivity is less for Gram positive bacteria compared to Gram negative bacteria as shown in (Figure 11).

4.2.3. Respiration activity of bacterial cells

Respiration activity of test pathogens was performed to elucidate the possible mode of action of AgNPs. In our present study, the respiration rate of AgNPs and AgNO₃ treated bacterial cells was decreased when compared with untreated bacterial cultures as shown for *S. aureus* (Figure 12).

4.2.4. Growth curves of bacterial cells exposed to different concentrations of biogenic silver nanoparticles

The growth curves of bacterial cells treated with AgNPs indicated that AgNPs could inhibit the growth and reproduction of bacterial cells. The growth curves of AgNPs treated *S. aureus* and *E. coli* cells are shown in (Figure 13) and (Figure 14). The bacterial growths of cells treated with 10 μg/mL AgNPs and AgNO₃ were inhibited. After 24 hr, almost all treated bacterial cells were dead. These findings indicate that the antibacterial activity of 10 μg/mL of AgNPs could not only inhibit bacterial growth but also could outpace the speed of reproduction of the bacterial cells.

4.2.5. Effect of AgNPs on protein leakage from bacterial cell membranes

It was found that AgNPs and AgNO₃ could enhance protein leakage by increasing the membrane permeabilities of Gram positive and Gram negative bacteria as shown in (Figure 15). After 6 hr of incubation, protein leakage from cells treated with AgNPs and AgNO₃ considerably increased; indicating that AgNPs can increase membrane permeability, however, there was no change in the amount of protein leakage from cells in the control group. Leakage from cells treated with AgNPs and AgNO₃ was significantly higher than that from cells in the control group. Furthermore, the initial protein leakage from the membranes of bacterial cells treated with AgNPs and AgNO₃ was almost the same as that from cells in the control group. Notably, higher amounts of proteins leaked through the Gram negative bacterial membranes compared to those through the Gram positive bacterial membranes, suggesting that the antibacterial sensitivity of the Gram positive was lower than that of the Gram negative bacteria.
4.2.6. Minimum inhibitory concentration

Synthesized AgNPs showed effective antibacterial activity against the test pathogens. MIC was recorded as the lowest concentration at which no visible growth of the test pathogens was observed. Among the different concentration of AgNPs tested 15 μg/mL proved to be MIC for *M. luteus* and *S. aureus* (Gram positive bacteria) whereas, in AgNO₃ 5 mg/mL was recorded as the MIC for the same. In *K. pneumonia* and *E. coli* (Gram negative bacteria) the MIC by AgNPs and AgNO₃ was seen at 5 μg/mL and was less. While both Gram positive and Gram negative depict higher sensitivity to the AgNPs and AgNO₃ the MIC is less for Gram positive bacteria compared to Gram negative bacteria as shown in (Figure 16) and (Figure 17).

4.2.7. Zone of inhibition by Well diffusion Assay against fungi

The antifungal activity of biosynthesized AgNPs was performed against few phytopathogenic fungi namely *Alternaria alternata*, *Curvularia lunata*, *Fusarium oxysporium*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Growth of *Fusarium oxysporium*, *Rhizoctonia solani* and *Sclerotium rolfsii* was greatly suppressed at 20 mg concentration AgNPs indicating strong antifungal activity of the synthesized AgNPs. The remaining plant pathogens *Alternaria alternata* and *Curvularia lunata* also showed, however, moderate antifungal activity against AgNPs as shown in (Table 4). However, the growth was greatly suppressed by 5 mg concentration of AgNO₃ in all fungal phytopathogens when compared to AgNPs as shown in (Table 5). (Plate 5a and 5b) shows the zones of inhibition in *Alternaria alternata* exposed to different concentrations of biogenic AgNPs and silver nitrate. Interestingly, AgNPs also exhibited antifungal activity against all the tested pathogens even at low concentration (5 mg/10 μL) and this is because of the stability of the synthesized AgNPs. *Candida albicans* showed an inhibition zone of 14 mm and 20 mm with 5 μg/mL of AgNPs and AgNO₃ as shown in (Plate 6a and 6b).

In both the cases, maximum level of inhibition zone was observed with the increasing concentration of AgNPs and AgNO₃. The results of the present study clearly revealed that the antifungal activity was increased with the increasing concentration of AgNPs. However, AgNO₃ formed bigger zones of inhibition in all fungal samples and concluded to be more toxic than AgNPs.
4.3. APPLICATION OF BIOGENIC SILVER NANOPARTICLES ON DENIM FABRICS

4.3.1. Evaluation of Antibacterial activity

Antibacterial activities of the treated fabrics were evaluated on *Escherichia coli* (ATCC 8739) as a Gram negative strain and *Staphylococcus aureus* (ATCC 6538) as a Gram positive strain by both qualitative method (AATCC Test Method 147-2004) and quantitative method (AATCC Test Method 100-2004). Biogenic AgNPs treated fabric sample showed a maximum inhibitory effect against *S. aureus* with a zone of inhibition of 11.5±0.9 mm followed by *E. coli* with a zone of inhibition of 3.5±1.7 mm. As shown in (Plate 7) it can be seen clearly that the inhibition zone of *S. aureus* (Plate 7a and 7b) is smaller than for *E. coli* (Plate 7c and 7d).

Quantitative evaluation of bacterial activity is done by counting the number of colonies grown. The untreated fabric shows 0% reduction in both Gram positive and Gram negative bacteria. In the AATCC 100-2004 test method, the fabric showed a very high percentage of reduction in bacteria at 99.52% and 92.04% against *S. aureus* and *E. coli*.

4.3.2. Wash Durability Test

Wash durability test carried out with the test fabrics showed that the significant antimicrobial activity was actively retained in the biogenic AgNPs treated fabrics up to 10 washes even after repeated washes cycles. The untreated control fabrics were not subjected to any wash durability test as it has no antibacterial activity. Treated fabric was found to be durable and retained antibacterial activity up to 10 washes for *S. aureus* and *E. coli* as shown in (Table 6). (Plate 8) shows the% bacterial reduction seen in *E. coli* by AgNP treated fabric. The antibacterial activity was retained up to 10 washes indicates that the rate of release was quite less.

4.3.3. Physical Properties

The surface density of AgNPs treated denim fabric increased from 355 g/ m² to 356.2 g/ m². However, there were no changes in the warp and weft linear density and warp linear density (Ne) and weft linear density (Ne) remained 7 and 12 respectively.
4.3.4. Mechanical Properties

The tensile strength of treated denim fabric changed from 520 lbs to 556 lbs, thereby there was an increase in the tensile strength. The treated fabric showed absorbency value of 18.90 sec compared to 9.69 sec in untreated fabric. There was a decrease in absorbency as indicated by increased time required for absorption of water drop due to surface coating which repels the water. Fabric handling property was checked properly before and after incorporating by AgNPs by feeling of touch. The stiffness of denim fabric was decreased which indicated an increase in fabric softness.

4.3.5. Atomic Absorption Spectroscopic studies

The initial amount of silver in the fabrics was determined by acid digestion and subsequent AAS analysis. The amount of silver found was 1083.61 mg/kg. The uncoated control fabric revealed no detectable silver, as expected.

4.3.6. Measurement of silver released into artificial sweat

Small pieces of each fabric sample were subjected to immersion in ISO artificial sweat formula (pH 5.5). Silver could not be detected in artificial sweat as it did not show any absorbance peak between 390 and 450 nm which is a characteristic region for silver as shown in (Figure 18).

4.3.7. FT-IR analysis

Fourier transform infrared was employed to examine the chemical composition of AgNPs treated and untreated denim fabric surface. So, in order to investigate the loading mechanism, FT-IR measurements were carried out on the samples over 1800 – 4000 cm\(^{-1}\), as shown in (Figure 19). The spectra of the AgNPs loaded denim fabric presented a higher transmittance intensity compared to untreated denim fabric, due to deposition of AgNPs on the surface of denim fibers.

4.3.8. Morphological studies using SEM and EDS

The surface topography observation of biogenic AgNPs finished fabric was carried out with a scanning electron microscope and was subjected to EDS analysis which shows that the main element is silver. The SEM image in (Plate 9a) demonstrates the smooth structure of the denim fabrics before coating with AgNPs. The treated fabrics showed AgNPs embedded on to the fabrics as shown in (Plate 9b), which is absent in
case of the untreated fabrics. It is clear that the AgNPs adhere not only to the surface, but also penetrate into the interstices of the yarn and fabric. This clearly indicates that the AgNPs are bonded well to the fabric surface. In addition, EDS peaks of silver were observed in these samples as shown in (Figure 20).

4.4. APPLICATION OF BIOGENIC SILVER NANOPARTICLES IN PHOTOCATALYTIC DYE DEGRADATION

4.4.1. Physiochemical characteristics of the textile dye effluent

The physicochemical characteristics of centrifuged textile dye effluent before and after the photocatalytic degradation along with recommended level of National Environmental Quality Standards (NEQS) are given in (Table 7). The textile effluent was highly alkaline in nature with pH was 8.1. The pH was reduced from 8.1 to 7.3 which is a neutral pH. Effluent was highly colored, showing the presence of high concentrations of unused dye and was reddish brown in color. However, the color changed to light pink after photocatalytic dye degradation. Electrical conductivity (EC) of the textile dye waste water samples was 891 μS/cm. However, after the photocatalytic textile dye degradation it reduced to 446 μS/cm. Total dissolved solids were much higher than the NEQS upper limits. The Total dissolved solids (TDS) was found to be 523 mg/L in the textile dye wastewater and reduced to 301 mg/L. BOD was 298 mg/L and COD was 712 mg/L and reduced to 147 mg/L and 392 mg/L respectively. Decrease in BOD and COD levels is suggestive of the fact that the process of bioremediation is in progress.

4.4.2. Photocatalytic dye degradation of commercial dyes and textile dye effluent

Photocatalytic degradation of commercial dyes and textile dye effluent was investigated using synthesized biogenic AgNPs by solar irradiation technique at different time intervals. (Plate 10) shows photocatalytic degradation of commercial textile dye effluent using biogenic AgNPs used in the current research. The characteristic absorption peak of various commercial dyes and textile dye effluent has been represented in (Table 8). Degradation of the commercial dyes and textile dye effluent at light intensity of 45000 - 49000 Lux was visualized by decrease in peak intensity within 60 min of incubation time. The degraded samples were used for determining the possible changes in the
absorption spectra of the dye in the UV -Vis range as shown in (Figure 21) to (Figure 27). A gradual reduction was also found in BOD and COD as shown in (Figure 28).

4.4.3. Effect of various ions in photocatalytic dye degradation

The efficiency of the photocatalysts increased under various ions as shown in (Table 9). It was found that the ions like chloride, sulphate and nitrate anions lead to an increase of the effectiveness of degradation whereas phosphate ions reduce the photocatalytic degradation.

4.4.4. Recycling of biogenic silver nanoparticles

Photo catalyst was recycled effectively which makes the process cost effective. For this reason, the catalyst was recycled four times. AgNPs were recovered by giving acid wash and again used to study its recyclability. The obtained results showed that the efficiency in reducing COD decreased from 96 to 58%.

4.4.5. Seed Germination Bioassay

In this study the germination of Phaseolus vulgaris Linn. seed in the textile dye effluent was only 0% whereas control germination was 100%. However, the degraded textile dye effluent proved to germinate 60% of the seeds as shown in (Plate 11).

4.5. APPLICATION OF BIOGENIC SILVER NANOPARTICLES IN WASTEWATER TREATMENT

4.5.1. Immobilization of biogenic silver nanoparticles

To prepare the AgNP beads, AgNPs were added to Sodium alginate and the obtained mixture was added drop wise into a CaCl₂ solution thereby forming visible, big and spherical beads with a diameter of 2.5 mm. The gel beads were formed instantaneously once the AgNPs and Sodium alginate mixture contacted with Ca²⁺ in the solution before their shape was distorted. The beads were then incubated for 9 hr. The AgNP beads obtained were brownish orange in color.

4.5.2. Studies on stability of AgNP beads

AgNP beads in diluted acidic and alkaline solutions were mechanically stable. But in strong acidic solutions, they tend to shrink. In concentrated solutions of sodium and potassium hydroxides they completely dissolve after 3 days leaving the AgNPs in the solution
4.5.3. Swelling studies

1 g of AgNP beads after absorbing water, swelled up to give a final weight of 1.685±0.112 g compared to Simple beads that gave a final weight of 1.255±0.079 g after swelling studies as shown in (Figure 29). Swelling of the calcium alginate beads in water lasted for about 120 min.

4.5.4. Antibacterial activity of the beads

The beads were evaluated for their antibacterial activity against *S. aureus* (Gram positive bacteria) and *E. coli* (Gram negative bacteria). Zones of inhibition were 11±2 and 15±1.5 mm respectively as shown in (Plate 12).

4.5.5. Biogenic AgNPs loaded in the beads

The amount of AgNPs loaded in AgNP beads was determined using Atomic absorption spectrophotometer and was found to be 571.21 mg/kg.

4.5.6. FT-IR analysis

FT-IR spectra of AgNP beads and Simple beads showed the bands around 3443, 1632, 1415 and 1039 cm⁻¹, indicating the stretching of O−H, COO⁻ (asymmetric), COO⁻ (symmetric) and C−O−C, respectively. In beads with aerobic sludge, the band located at 1630 cm⁻¹ is assigned to vibration of H₂O molecules as shown in (Figure 30).

4.5.7. Morphological characterization of beads

As seen, the beads in the wet state exhibited a spherical shape and a smooth surface with a diameter of about 2.5 mm. (Plate 13) shows the surface SEM pictures of the AgNP beads. Surface structure reveals that several wrinkles. AgNP beads were composed of a matrix structure, composed of porous channels which run throughout the bead. The surface of the dried beads was rough. Many large hollow pores or multiple small hollow pockets were observed. The surface structure clearly reveals AgNPs embedded in the matrix. It was evident that the contents of the beads were preserved. AgNP beads that were used in treatment of aerobic sludge swelled up and its surface showed bacterial adhesions.

4.5.8. Wastewater treatment

The wastewater treatment was seen by the changes in the weight of Simple and AgNP beads and physicochemical characteristics of aerobic sludge before and after the
aerobic sludge treatment by Simple and AgNP beads. The Simple and AgNP beads changed their weight from 2500 mg to 2631.27 and 2714.48 mg after treating the aerobic sludge.

4.5.9. Changes in physiochemical characteristics of the Aerobic Sludge

Analysis of treated aerobic sludge was done by the physiochemical characteristics shown in (Table 10). During this experiment, control tests which were only Simple beads were also tested. The aerobic sludge was highly alkaline in nature with pH 8.89. The pH was reduced from 8.8 to 8.2 by Simple beads. But AgNP beads reduced the pH to 7.1 which is a neutral pH. The aerobic sludge from blackish turbid changed to watery brown after treatment. Electrical conductivity (EC) of the aerobic sludge was 2160 μS/ cm. However, after the treatment, it reduced to 1152 μS/ cm. Total dissolved solids were much higher than the NEQS upper limits. The Total dissolved solids (TDS) was found to be 1512 mg/ L in the wastewater and reduced to 928 mg/ L. BOD was 660 mg/ L and COD was 1758 mg/ L and reduced to 317 mg/ L and 956 mg/ L respectively by AgNP beads.

4.5.10. Recycling of silver nanoparticles

Catalyst was recycled effectively which makes the process cost effective. For this reason, the catalyst was recycled four times. AgNPs were recovered by giving acid wash and again used to study its recyclability. The obtained results showed that the efficiency in reducing COD decreased from 83 to 66%. This is likely due to the fouling of the catalyst and loss due to repeated acid wash.

4.6. PHYTOTOXIC EFFECTS OF BIOGENIC SILVER NANOPARTICLES ON PLANTS

4.6.1. Seed viability

The seeds used in this study showed 100% seed germination.

4.6.2. Seed treatment and germination

Seeds were treated with two different concentrations of AgNPs and AgNO₃. Biogenic AgNPs and AgNO₃ treatments showed no significant effect on seed germination at 10 ppb and 100 ppb concentrations. Results also showed that 10 ppm and 100 ppm of AgNPs and AgNO₃ also do not effect in the germination. But, very high concentrations of
both the treatments retard seed germination. In this study, it was found that 8000 ppm of AgNO$_3$ show complete inhibition in seed germination, but even then there was 85% germination under AgNPs treatment. This treatment showed 87% and 73% seed germination at 400 ppm and 1500 ppm treatments. IC$_{50}$ in plant germination under AgNO$_3$ treatment was observed at 3000 ppm as shown in (Figure 31). This proves that biologically synthesized AgNPs caused less phytotoxicity in fenugreek seeds compared to AgNO$_3$ in seed germination. Germination Index decreased with the increase concentrations of AgNPs and AgNO$_3$ as shown in (Figure 32). As discussed earlier, there were 100% seed germinations in both the treatments; however difference was seen in Root elongation.

4.6.3. Estimation of plant growth and Morphological studies

Significant difference was observed among the treatments and there was a difference in growth in treated plants when comparison with control. (Plate 14) shows various stages of growth from seed germination, transfer of seedlings in the hydroponic solution and plant maturation.

4.6.3.1. Height and weight

It was observed that there was a huge difference in the height and weight of the plants during the entire treatment. The treated plants grew very minimal in height and weight compared to the control as shown in (Figure 33), (Plate 15) and (Figure 34). Root elongation was significantly inhibited in the seedlings exposed to AgNPs and AgNO$_3$. However, growth inhibition was more severe in seedlings exposed to 100 ppm than in seedlings exposed to 10 ppm of each treatment. However, further investigations showed that there had been a tremendous increase in difference in height and weight between the control and treated plants after 30 days.

4.6.3.2. Flowers, Leaves and Pods

It was observed that the total number of leaves and leaf length in control was much higher compared to the treated plants as shown in (Figure 35) and (Plate 16). AgNO$_3$ were found to be more toxic and more effective in the reduction of leaf growth. It was also observed that under stress, flowering began much earlier in the treated plants compared to the control. In AgNO$_3$ treated plants (D and E), flowering began between 37-43 days range. Under AgNP treatment (B and C), flowering began in 48-52 days.
range where as in control plants, flowering began only after 70 days as shown in (Figure 36). However, even after early flowering in treated plants, the formation of pods was very late when compared to control as shown in (Figure 37) and completed their life cycle early when compared to the Control. There was also a huge difference in the length of the pods as shown in (Plate 17).

4.6.4. Plant injury

4.6.4.1. Roots

In the present study, a significant reduction in the root and shoot length was found in the AgNPs and AgNO₃ treated plants but the formation of root hair was highly affected. As shown in (Plate 18), the root system in the treated plants was shorter than the control plants. The roots of the treated plants turned brown probably due to the phenolic compounds as shown in (Plate 19). The roots of the control plants grew longer and remained healthy throughout the experiment. Morphological details of the root system in 30 days exposed plants from different treatment groups were observed under microscope. Root apices appeared swollen and irregularly curved in treated seedlings as shown in (Plate 20). A localized thickened zone was observed from the root apex, corresponding to an increased diameter of cross-sections.

4.6.4.2. Leaves

The leaves in the treated plants showed less chlorophyll content and tremendous injury and turned yellowish, brownish and whitish in color as shown in (Plate 21) depicting the adverse symptoms of distortion, chlorosis, interveinal chlorosis, white spots and necrosis. The leaves of AgNO₃ treated in lower and higher concentration, were found to be curled and shrunken. The leaves turned pinkish leaves and growth was highly retarded. Leaves of AgNPs treated plants turned pale green initially and later yellow.

4.6.5. Developmental botany

The extended BBCH scale is a system for a uniform coding of phenologically growth stages of monocotyledonous and dicotyledonous plants. (Table 11) shows the extended BBCH scale for control plants and plants treated with 10 ppm and 100 ppm concentrations of AgNP and AgNO₃. As shown there was no difference in BBCH scale in “Germination” both the control and treated plants. There was no difference in the development of the leaves in the plants in
“Leaf development” stage. Treatments were given to the plants from the 11th day after they were transferred to the hydroponic solution. The solutions were refreshed once in every three days and treated with 10 ppm and 100 ppm concentrations of AgNP and AgNO₃.

There were variations seen during the “Formation of side shoots” in the control and treated plants. 9 or more side shoots were visible in the control plants, but the treated plants showed only 3 side shoots. The inflorescence emergence was first seen in plants treated with 100 ppm and 10 ppm AgNO₃ followed by 100 ppm and 10 ppm AgNPs and finally the control plants. The inflorescence emergence in 100 ppm AgNO₃ treated plants was almost 33 days prior to the control plants. Similar observations were seen in “Flowering” and during “Development of fruit”. (Plate 22) shows various developments in the control and treated plants on 60th day. However, in the “Development of fruit” the length of pods varied in 5 samples. Control plants showed complete growth in pod length, but the plants treated with 10 ppm AgNPs showed 40% growth in pod length. Plants treated with 10 ppm AgNO₃ and 100 ppm AgNPs showed 20% growth. The least growth in pod length (20%) was shown by plants treated with 100 ppm AgNO₃. “Ripening of fruit and seed” was faster in the treated plants compared to the control plants.

4.6.6. Phytochemical analysis

The changes in the metabolites contents of the control and treated plants caused by both AgNPs and AgNO₃ treatments were studied by estimating total protein, carbohydrate, phenol, flavonoids, chlorophyll and proline in various parts of the plant such as root, stem and leaves. These contents showed greater variations in various organs of the plant.

Protein content in the treated and control plants were estimated using the method of Bradford, 1976. Using the standard plot of Bovine albumin serum (y = 0.0055x + 0.0042, R² = 0.9933), the protein contents of the leaves, stem and roots were found and has been shown in (Figure 38), (Figure 39) and (Figure 40). The protein content in different organs of the plants treated with AgNO₃ and AgNPs were lesser initially, however, they later increased under stress.

Carbohydrate content in the treated and control plants were estimated using the method of Dubios et al., 1954. Using the standard plot of D glucose (y = 0.0118x +
0.0255, $R^2 = 0.9906$), the carbohydrate contents of the leaves, stem and roots were found and has been shown in (Figure 41), (Figure 42) and (Figure 43). The carbohydrate content like protein in different organs of the plants treated with AgNO$_3$ and AgNPs increased later under stress.

The total phenols in the samples were estimated using the method of Ainsworth and Gillespie, 2007. Using the standard plot of Gallic acid ($y = 0.0106x + 0.0139$, $R^2 = 0.9918$), the phenol contents of the leaves, stem and roots were found and has been shown in (Figure 44), (Figure 45) and (Figure 46). Analysis of total phenol content in the plants treated with AgNPs and AgNO$_3$ showed a shift towards secondary metabolism. Silver in both the forms increased the total phenol contents in the plant organs however AgNPs recorded lesser phenol content than the plants treated with AgNO$_3$. The effects were more pronounced in leaves and roots of AgNO$_3$ treated plants. Interestingly, reduction in the protein and carbohydrate levels after 13th day of treatment coincided with a significant increase in the phenolic content.

The total flavanoids in the samples were estimated using the method of Chang et al., 2002. Using the standard plot of Quercetin ($y = 0.0005x + 0.0133$, $R^2 = 0.9915$), the flavonoid content in the leaves, stem and roots were found as shown in (Figure 47), (Figure 48) and (Figure 49). The amount of flavonoids in the treated plants decreased with increased concentration of AgNPs and AgNO$_3$.

The total chlorophyll (a, b) in the samples was estimated using the method of McKinney, 1941. The total chlorophyll content in the leaves of T. foenumgraecum after treatment with different concentrations of AgNP and AgNO$_3$ has been shown in (Figure 50). Chlorophyll is a significant biomarker that reflects the status of plant growth. Both chlorophyll a and b decreased in the treated plants.

Proline is reliable indicator to the biotic as well as abiotic stress. Proline accumulates in plant species under a broad range of stress conditions. In the present study, the proline content was significantly higher in plants treated with both AgNPs and AgNO$_3$ compared with the control. Using the standard plot of proline ($y = 0.016x + 0.01246$, $R^2 = 0.9906$), the proline contents of the leaves, shoot and roots were found ranging as shown in (Figure 51). Plants grown with AgNPs and AgNO$_3$ showed rapid accumulation of proline. As compared with control (0.9 mg/ g fresh weight of proline),
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Plants showed an increase up to 1.1 and 1.5 mg/g proline under AgNPs and AgNO₃ treatments at 10 ppm concentrations on the 10th day. At higher concentrations of 100 ppm of each treatment, proline content was enhanced up to 1.4 and 1.6 mg/g proline under AgNPs and AgNO₃ treatments on the same day. Proline concentration further increased in all the treatments compared to the control and reached 6.9 and 5.8 mg/g proline under AgNO₃ treatments on 70th day where as under the AgNPs treatment, an observation at 2.9 and 3.8 mg/g proline was seen.

4.6.7. Enzyme assays

Antioxidant systems such as Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POX) and Ascorbate Peroxidase (APOCH) provide protection against toxic effects of reactive oxygen species (ROS) such as \( \cdot \)O₂ and H₂O₂.

Elevated levels of CAT and POX activities were recorded from leaf samples of plants subjected to silver treatment. CAT is an important oxidative enzyme. The CAT levels in leaves, stem and roots under various treatments have been shown in (Figure 52), (Figure 53) and (Figure 54). Maximum levels of CAT activity were recorded in the leaves of AgNO₃ treated plants throughout the study while in AgNPs treated group the CAT activity was less. Peroxidase also referred as non-specific Peroxidase or guaiacol-peroxidase, catalyses the reduction of hydrogen peroxide with a concurrent oxidation of a substrate. The POX levels in leaves, stem and roots under various treatments have been shown in (Figure 55), (Figure 56) and (Figure 57). The level of POX increased in the treated plants when compared to the Control as shown.

Similarly, the SOD levels in leaves, stem and roots under various treatments have been shown in (Figure 58), (Figure 59) and (Figure 60). As shown here, increased levels of SOD (Superoxide Dismutase) were also seen in the treated plants. The activities of Ascorbate Peroxidase (APOCH) also increased under stress in the treated plants of *Trigonella foenumgraecum* in leaves, stem and roots under various treatments as shown in (Figure 61), (Figure 62) and (Figure 63). All the enzymes showed an increase in leaves under AgNPs and AgNO₃ treatments, significant levels were seen till the 10th day. Similar results were seen in stem and roots also suggesting less ROS generation implying less toxicity to plants.
4.6.8. Atomic Absorption Spectroscopic studies

Atomic absorption spectroscopic studies of the roots and leaves in AgNPs treated plants showed 0.73 and 0.46 μg/ g of fresh weight of silver in 10 ppm AgNPs treated plants and 1.48 and 0.97 μg/ g of fresh weight of silver in 100 ppm AgNPs treated plants while in stem sample of the same treatment group, silver estimate was below the detection level. Increased levels of silver estimates were recorded in the roots, stem and leaves of AgNO₃ treated plants such as 5.87, 1.62 and 3.53 μg/ g of fresh weight in 10 ppm of AgNO₃ treated plants and 11.32, 3.41 and 7.34 μg/ g of fresh weight in 100 ppm AgNO₃ treated plants.

4.6.9. Plant anatomical studies

Anatomical features of both the stressed and unstressed plants based on transverse sections of the root and the shoot were studied. Plants of both the treatment groups showed alterations in the stem and root anatomy. Anatomical investigations of the transverse sections of roots exposed to AgNPs and AgNO₃ showed disappearance of the characteristic air chambers and partition filaments as shown in (Plate 23). The root epidermis was degraded in the treatment exposed to AgNPs and AgNO₃ and the root exodermis and endodermis were thickened. Treatments accelerated the maturation of the cell wall in endodermis and exodermis. The tracheary elements of the xylem and cortical parenchyma of roots exposed to contaminated soil presented thicker walls than the control. In case of stem sections, partial filaments disappeared. Characteristic changes were observed in xylem ring size and shape: remarkable variations in AgNPs and AgNO₃ treatments were observed and central extensions of xylem elements were more pronounced in them as shown in (Plate 24).
**Plate 2:** (a) Morphology of Alpinia calcarata *Rosc.* (b) Dry rhizomes (c) Powdered dry rhizomes (d) Synthesized biogenic silver nanoparticles (AgNPs) and Silver nitrate (AgNO₃) (e) Green synthesis of biogenic silver nanoparticles at different time intervals

**Figure 1:** Synthesized biogenic silver nanoparticles observed strongly in the range of 400 - 450 nm in the visible region in UV spectra
Figure 2: UV spectrum of biogenic silver nanoparticles formed at characteristic wavelength at 422 nm with increase in wavelength at regular time intervals

Figure 3: Fourier Transform Infrared spectrum of biogenic silver nanoparticles
**Figure 4:** X-Ray Diffraction pattern of biogenic silver nanoparticles showing characteristics peaks centered indexed to the crystalline planes (111), (200) and (220) of face centered cubic silver.

**Plate 3:** (a) High Resolution Transmission Electron Microscopy studies of biogenic silver nanoparticles (b) SAED pattern of biogenic silver nanoparticles

Figure 5: Energy Dispersive Spectrum of biogenic silver nanoparticles revealed the presence of high amount of silver
Figure 6: UV spectrum of optimization of biogenic silver nanoparticles under different pH

Figure 7: UV spectrum of optimization of biogenic silver nanoparticles at different incubation timings
**Figure 8**: UV spectrum of optimization of biogenic silver nanoparticles at different temperatures

**Figure 9**: UV spectrum of optimization of biogenic silver nanoparticles at different concentrations of silver nitrate solution
Figure 10: UV spectrum of optimization of biogenic silver nanoparticles at different concentration ratios of plant rhizome extract and silver nitrate.
**Figure 11:** Change in membrane permeability of bacterial cells under 10 μg/mL concentration of biogenic silver nanoparticles and silver nitrate treatment.

**Figure 12:** Respiration activity of *Staphylococcus aureus* under 10 μg/mL concentration of biogenic silver nanoparticles and silver nitrate treatment.
Figure 13: Growth curves of *Staphylococcus aureus* bacterial cells exposed to 10 μg/mL concentration of biogenic silver nanoparticles and silver nitrate.

Figure 14: Growth curves of *Escherichia coli* bacterial cells exposed to 10 μg/mL concentration of biogenic silver nanoparticles and silver nitrate.
**Figure 15:** Effect of 10 μg/mL concentration of biogenic silver nanoparticles and silver nitrate on protein leakage from bacterial cell membranes.

**Figure 16:** Minimum inhibitory concentrations (MIC) of bacterial cells exposed to different concentrations of biogenic silver nanoparticles.
Figure 17: Minimum inhibitory concentrations (MIC) of bacterial cells exposed to different concentrations of silver nitrate

Plate 5: Zones of inhibition in *Alternaria alternata* fungi exposed to different concentrations of (a) biogenic silver nanoparticles and (b) silver nitrate
Plate 6: Zones of inhibition in *Candida albicans* exposed to different concentrations of (a) biogenic silver nanoparticles and (b) silver nitrate
Plate 7: Antibacterial activity seen in (a) *Staphylococcus aureus* in untreated fabric (b) *Staphylococcus aureus* in biogenic silver nanoparticles treated fabric (c) *Escherichia coli* in untreated fabric (d) *Escherichia coli* in biogenic silver nanoparticles treated fabric

Plate 8: Percentage of bacterial reduction seen in *Escherichia coli* by biogenic silver nanoparticles treated fabric
Figure 18: UV spectrum showing no detection of biogenic silver nanoparticles in ISO artificial sweat formulation (pH 5.5) due to the absence of absorbance peak of 390 to 450 nm which is characteristic region of silver

Figure 19: Fourier Transform Infrared Spectra analysis of untreated fabric and biogenic silver nanoparticles treated fabric
Plate 9: Scanning Electron Microscope image of the fabric morphology of (a) untreated fabric (b) fabric treated with biogenic silver nanoparticles

Figure 20: Energy Dispersive Spectrum of fabric treated with biogenic silver nanoparticles showing the presence of high amounts of silver
Plate 10: Photocatalytic degradation of commercial textile dye effluent using biogenic silver nanoparticles in 60 min

Figure 21: UV spectra showing the photocatalytic degradation of commercial dye effluent using biogenic silver nanoparticles in 60 min
**Figure 22:** UV spectra showing the photocatalytic degradation of Brown Direct dye using biogenic silver nanoparticles in 60 min

**Figure 23:** UV spectra showing the photocatalytic degradation of Procion Fuchsia MX-8B dye using biogenic silver nanoparticles in 60 min
**Figure 24:** UV spectra showing the photocatalytic degradation of Procion Navy Blue MX-2G dye using biogenic silver nanoparticles in 60 min

**Figure 25:** UV spectra showing the photocatalytic degradation of Reactive Orange 16 dye using biogenic silver nanoparticles in 60 min
**Figure 26:** UV spectra showing the photocatalytic degradation of Reactive Yellow 17 dye using biogenic silver nanoparticles in 60 min

**Figure 27:** UV spectra showing the photocatalytic degradation of Procion Bright Turquoise MX-G dye using biogenic silver nanoparticles in 60 min
Plate 11: Seed germination in *Phaseolus vulgaris* Linn. (a) Control (b) in raw commercial textile dye effluent (c) in treated commercial textile dye effluent using biogenic silver nanoparticles

**Figure 28:** Reduction in BOD and COD levels in commercial textile dye effluent after photocatalytic degradation using biogenic silver nanoparticles with in 60 min
Figure 29: Swelling studies in biogenic AgNP beads and Simple beads

Plate 12: Antibacterial activity shown by Simple beads and biogenic AgNP beads in (a) *Staphylococcus aureus* (b) *Escherichia coli*
Figure 30: Fourier Transform Infrared analysis of biogenic AgNP beads and Simple beads before and after treating aerobic sludge.

Plate 13: Scanning Electron Microscope images showing the morphological features of biogenic AgNP bead.
**Figure 31:** Seed Germination in *Trigonella foenumgraecum* Linn. seedlings under various concentrations of biogenic silver nanoparticles and silver nitrate

**Figure 32:** Germination Index (%) on day 2 and day 4 in *Trigonella foenumgraecum* Linn. seedlings under A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Plate 14: Various plant growth stages (a) Viable seeds (b) Seed germination (c) 4 days seedlings (d) 7 days seedlings (e) Transfer of 10 days seedlings in hydroponic solution (f) 20 days old plants (g) 30 days old plants (h) 50 days old plants (i) 70 days old plants

Figure 33: Effects on plant height under various treatments for 120 days A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Plate 15: Morphological difference in plant heights in 40 days, 70 days and 90 days plants under various treatments (from left to right: Control, 10 ppm biogenic silver nanoparticles treated plants, 100 ppm biogenic silver nanoparticles treated plants, 10 ppm silver nitrate treated plants, 100 ppm silver nitrate treated plants)
Figure 34: Effects on plant weight under various treatments for 120 days
A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 35: Effects on total number of leaves under various treatments for 100 days
A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Plate 16: Morphological difference in leaves under various treatments
(a) Control (b) 10 ppm biogenic silver nanoparticles (c) 100 ppm biogenic silver nanoparticles (d) 10 ppm silver nitrate (e) 100 ppm silver nitrate

Figure 36: Effects on total number of flowers under various treatments for 80 days A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
**Figure 37:** Effects on total number of pods under various treatments for 110 days. **A** (Control), **B** (10 ppm biogenic silver nanoparticles), **C** (100 ppm biogenic silver nanoparticles), **D** (10 ppm silver nitrate), **E** (100 ppm silver nitrate).

**Plate 17:** Morphological difference in length of pods under various treatments. (a) Control (b) 10 ppm biogenic silver nanoparticles (c) 100 ppm biogenic silver nanoparticles (d) 10 ppm silver nitrate (e) 100 ppm silver nitrate.
Plate 18: Morphological difference in root length in 30 days old plants under various treatments (a) Control (b) 10 ppm biogenic silver nanoparticles (c) 100 ppm biogenic silver nanoparticles (d) 10 ppm silver nitrate (e) 100 ppm silver nitrate

Plate 19: Browning of treated roots under various treatments (a) Control (b) 10 ppm biogenic silver nanoparticles (c) 100 ppm biogenic silver nanoparticles (d) 10 ppm silver nitrate (e) 100 ppm silver nitrate
Plate 20: Light microscope images of root apices seen in under the effect of various treatments (a) Control (b) 10 ppm biogenic silver nanoparticles (c) 100 ppm biogenic silver nanoparticles (d) 10 ppm silver nitrate (e) 100 ppm silver nitrate

Plate 21: Leaf injury seen under the effect of various treatments on 40th, 70th and 90th day in (a), (f), (k) Control (b), (g), (l) 10 ppm biogenic silver nanoparticles (c), (h), (m) 100 ppm biogenic silver nanoparticles (d), (i), (n) 10 ppm silver nitrate (e), (j), (o) 100 ppm silver nitrate
Plate 22: *Trigonella foenumgraecum* Linn. plants showing morphological growth and development on 60th day under various treatments (left to right: control, 10 ppm biogenic silver nanoparticles, 100 ppm biogenic silver nanoparticles, 10 ppm silver nitrate, 100 ppm silver nitrate)
Figure 38: Total Protein in plant leaves over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 39: Total Protein in plant stem over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
**Figure 40:** Total Protein in plant roots over a period of 90 days under various treatments: A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate).

**Figure 41:** Total Carbohydrates in plant leaves over a period of 90 days under various treatments: A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate).
**Figure 42:** Total Carbohydrates in plant stem over a period of 90 days under various treatments  
A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

**Figure 43:** Total Carbohydrates in plant roots over a period of 90 days under various treatments  
A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
**Figure 44:** Total Phenol in plant leaves over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

**Figure 45:** Total Phenol in plant stem over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 46: Total Phenol in plant roots over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 47: Total Flavonoids in plant leaves over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 48: Total Flavonoids in plant stem over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 49: Total Flavonoids in plant roots over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 50: Total Chlorophyll in plant over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 51: Proline in plant over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 52: Catalase activity in plant leaves over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 53: Catalase activity in plant stem over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 54: Catalase activity in plant roots over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 55: Peroxidase activity in plant leaves over a period of 90 days under various treatments A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 56: Peroxidase activity in plant stem over a period of 90 days under various treatments  A (Control),  B (10 ppm biogenic silver nanoparticles),  C (100 ppm biogenic silver nanoparticles),  D (10 ppm silver nitrate),  E (100 ppm silver nitrate)

Figure 57: Peroxidase activity in plant roots over a period of 90 days under various treatments  A (Control),  B (10 ppm biogenic silver nanoparticles),  C (100 ppm biogenic silver nanoparticles),  D (10 ppm silver nitrate),  E (100 ppm silver nitrate)
**Figure 58:** Superoxide Dismutase activity in plant leaves over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

**Figure 59:** Superoxide Dismutase activity in plant stem over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 60: Superoxide Dismutase activity in plant roots over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 61: Ascorbate Peroxidase activity in plant leaves over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Figure 62: Ascorbate Peroxidase activity in plant stem over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)

Figure 63: Ascorbate Peroxidase activity in plant roots over a period of 90 days under various treatments  A (Control), B (10 ppm biogenic silver nanoparticles), C (100 ppm biogenic silver nanoparticles), D (10 ppm silver nitrate), E (100 ppm silver nitrate)
Plate 23: Anatomical studies of transverse sections of stem of *Trigonella foenumgraecum* Linn. exposed to 40 days of various treatment in 40x resolution (a) Control (b) 10 ppm biogenic silver nanoparticles (c) 100 ppm biogenic silver nanoparticles (d) 10 ppm silver nitrate (e) 100 ppm silver nitrate

Plate 24: Anatomical studies of transverse sections of root of *Trigonella foenumgraecum* Linn. exposed to 40 days of various treatment in 40x resolution (a) Control (b) 10 ppm biogenic silver nanoparticles (c) 100 ppm biogenic silver nanoparticles (d) 10 ppm silver nitrate (e) 100 ppm silver nitrate