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ORIGINAL ARTICLE

Assessment of Nanotoxicity of Silver Nanoparticles on Pea (*Pisum sativum*) grown under *ex situ* conditions

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There has been an expanding interest for eco friendly synthesis of silver nanoparticles that don't have so much toxic impacts on crops. Silver nanoparticles have a wide scope of utilizations, for example, catalysis, hardware, photonics, optoelectronics, detecting, agriculture and drugs. In this study, the biologically synthesized and characterization of silver nanoparticles have become the prime areas. Green synthesis of nanoparticles using plant extracts is being explored globally owing to the absence of disadvantages associated with conventional methods. This study reports the synthesis of silver nanoparticles using the extract of *Bambusa vulgaris* (Bamboo), *Azadirachta indica* (Neem) leaves, characterization of the synthesized by using techniques such as Ultraviolet-Visible (UV-Vis) spectroscopy confirmed the synthesis of nanoparticles, Fourier Transform Infrared Spectroscopy (FTIR),Scanning Electron Microscopy (SEM) and EDX studies revealed the characteristics of the nanoparticles synthesized. Also under this, we examined the effects of silver nanoparticles (AgNPs) on pea plants in the terms of silver accumulation, production of reactive oxygen species (ROS), Quantification of Cell Death under *ex situ* conditions.

Key words: silver nanoparticles, reactive oxygen species, Bambusa vulgaris, accumulation

Recently, nanotechnology has become a seriously investigated region and nanoproducts are broadly picking up employments, particularly in gadgets, medical care, makeup and medication(Nel *et al.,*2006). NPs have pulled in much consideration for their likely uses in plant development improvement, controlled arrival of agrochemicals, and yield insurance (Nair *et al.,*2010; Khot *et al.,* 2012). The positive or negative impacts of AgNPs on plants relies upon the size, shape, surface covering, term of introduction, plant species, and formative stage (Gupta *et al.,*2020; Nair *et al.,*2016; Remédios *et al.,* 2012). Inhibition of plant development, decrease in seed germination rate, expanded chromosomal abnormalities and oxidative harm, have been accounted for in numerous plants when presented to AgNPs (Verma *et al.,*2018; Tripathi *et al.,*2017; Oukarroum *et al.,* 2013;Patlolla *et al.,* 2012). Nonetheless, it is hazy whether the harmfulness of AgNPs is identified with explicit impacts brought about by the nanoparticles, Ag particles, or both (Cui *et al.,* 2014).

Plants, as a basic segment in the climate, are basic to biological system capacity and food gracefully trustworthiness. In view of the discoveries of late research center examinations, abiotic and oxidative stresses brought about by NPs presentation in plants were depicted at both physiological and biochemical levels(Khan *et al.,* 2020; Rossi *et al.,* 2017;Asli *et al.,* 2009; Dimkpa *et al.,* 2012').A typical finding from plant nanotoxicity contemplates is that abundance measures of ROS are delivered upon NP (CuO NPs, Ag NPs, CeO_2 NPs) presentation to earthbound plant species, for example, wheat (*Triticum aestivum L*.), rice (*Oryza sativa L*.), onion (*Allium cepa L*.), and (*Zea mays*) corn (Panda, *et al.,* 2011; Mirzajani *et al.,* 2013.) ROS could actuate harm of basic natural atoms, for example, lipids, proteins, and DNA (Gill *et al.,* 2010)

By and large, obviously metal-based NPs can make harmfulness biota in the climate. Despite the pathway of metal based NPs are delivered or released in the climate, potential dangers should be completely described to keep away from negative effect on ecological and human wellbeing. In the last 10 years, a lot of examination zeroed in on the vehicle and destiny of NPs in plants, creatures, and microorganisms has been done. In this investigation of the pertinent writing, we try to audit (Nel *et al.,* 2006).the impacts of NPs on higher plants at physiological, biochemical, and sub-atomic levels; (Ali *et al.,*2020).

MATERIALS AND METHODS

Collection of different types of plant sources from different locations of Meerut, UP

Plants resources such as *Bambusa vulgaris* (Bamboo), *Azadirachta indica* (Neem), were used for the synthesis of silver nanoparticles.

Silver nanoparticles synthesis and characterization

For the arrangement of extract, effectively reviled new green leaves of each plant were utilized with measure of 25 gms. What's more, these leaves blended in with 200 ml of distil water after that combination was heated for 30 minutes at that point separated with Whatman No.1 filter paper (25 μm). Fluid extract was put away at 4°C for additional utilization. This technique for silver nanoparticles arrangement previously utilized by V. Parashar, *et al* 2009.The strategy for biosynthesis of silver nanoparticles through plants extract was at that point utilized by Kalaiarasi, *et al.,* (2013). As per the Kalaiarasi, *et al.,* (2013), aqueous solution of silver nitrate two milimolar (2mM) was ready for the amalgamation of AgNPs. Boiled leaves concentrate of plants (10 ml) added to the silver nanoparticles (90 ml) answer for decrease on Ag particles saved for the 28^0C temperature at shaker (150 rpm) for overnight in dark conditions. After brooding period, the test shading change from light to dim earthy colored which is demonstrates the unions of silver nanoparticles. The bio-change was regularly checked outwardly after time spans (0 hr, 4 hrs, 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs). further characterization was done by FESEM (facilitated from Indian Institute of Technology, Kanpur), EDX (facilitated from Indian Institute of Technology, Kanpur) ,FTIR(facilitated from Indian Institute of Technology, Bombay).

Plant material and treatments

Pea plants (*Pisum sativum*) were used as host plants and leaves of pea plants were utilized for experiments. All tests (AgNPs (T1 and T2) and

(C)control plants) were led in triplicate. After a month, *ex situ* developed plants were collected for the estimation of different parameters.

Nano-toxicity evaluation in pea plants

For the determination of level of accumulation of nanoparticles and evaluation of toxicity caused in plants; following methods were used:

Silver content measurement in pea plant

The Pea (*Pisum sativum*) plants treated with AgNPs (T1 and T2) and control plants (C) were eliminated from the soil at 90 DAT and washed with deionized water to eliminate any Ag holding fast to the plant surface. The edible segments of plants (food or feed) for example leaf and natural product tests were dried at 70°C for 48 hrs and ground.A combination of 30% nitric acid (HNO_3), 30% hydrogen peroxide (H_2O_2) was set up in 3:1(v/v) proportion and 50 mg of each ground tests were processed in the blend by warming at 120°C for a hr.After cooling, the reviews were sifted however channel paper and the volume of blends were made up to 10 ml with deionized water.These tests were dissected by Atomic Absorption Spectrophotometer (AAS) to set up the µg/g grouping of Ag in the samples (figure.1). The office was benefited from Advance Research and Analytical Services, Ghaziabad. (Homaee and Ehsanpour ,2016 were likewise utilized this strategy for estimation of Ag in the patato plants in his exploration).

Quantification of Cell Death

To assess cell viability, the strategy proposed by Baker and Mock (1994) was utilized. New leaves of C, T1 and T2 plants were gathered, and lowered in 0.25% (w/v) Evans blue for 5 min.Leaves were washed with refined water and hatched in 1 mL of half (v/v) ethanol containing 1% (w/v) sodium dodecyl sulfate (SDS). The test was conveyed in triplicate. The absorbance of the concentrates was estimated by spectrophotometer at 600 nm against Evans blue standard arrangements. Cell suitability was communicated as Evans blue take-up (figure:2).The information were examined by ANOVA including LSD utilizing SPSS programming.

RESULTS

Biosynthesis of silver nanoparticles

The progressive extension in shade of the plants separate after the extension of 2mM $AgNO_3$ showed the improvement of AgNPs. The shade of the reaction mixes turned earthy colored dark because of the arrangement of AgNPs. Figure 3&4 shows the association of silvernanoparticles from *Bambusa vulgaris* (Bamboo), *A. indica* (Neem) (figure: 3&4).

UV Vis analysis

In this investigation study, first thing AgNPs were portrayed by UV-Vis two crease column spectrophotometer (Lasany LI-295). All spectra were recorded at room temperature, in a quartz cell with 1 cm optical way, to know the engine lead of nanoparticles. The inspecting range for the models was 200-800 nm. The spectrophotometer was furnished with "UV prov programming" to record and analyze the data. The standard amendment of the spectrophotometer was finished by using a reasonable reference. The case of AgNPs was analyzed at 0, 4, 12, 24, 48, 72, 96, and 120 hrs. The optical tops for AgNPs were seen between the extents of 400 to 540 nm. All the replications of treatment tests showed at 96 hr were given in figures 5&6.

FESEM analysis

In this assessment study, dried instances of AgNPs were set up by putting two drops (20µl) of the nanoparticle plan on aluminum foil and after that dry these models setting in a hot air oven at 50°C for 24 hrs. The FESEM office was profited by Advance Imaging Center, Indian Institute of Technology (IIT), Kanpur (UP, India). The photos were taken at Everhart-Thornley Detector (ETD) mode at 15 kV electron high strains (EHT). Scanning electron microscopy images of the lyophilized silver nanoparticles showed mostly spherical particles of a size below 100 nm as shown in (figure:7&8).The particles were vigilant, non-smooth, round in nature, and polydispersed. Examinations of SEM micrograph furthermore revealed nanoparticles with a few monoclinic non-round structures. The nanoparticles size in went from 20 to 75 nm joined from *Bambusa vulgaris* (Bamboo) and *A. indica* (Neem).

This office was additionally benefited from Advance Imaging Center, IIT, Kanpur (UP, India). The EDX report shows the EDX range of AgNPs. EDX range demonstrated pinnacles of silver (Ag) and aluminum (Al). EDX examination demonstrated the optical assimilation top at 3 keV. The EDX ranges of AgNPs of samples are given in figures 9& 10, where X-hub is indicating the vitality in keV and Y-hub is connoting power check.

EDX analysis

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FTIR analysis

FTIR technique was used for characterization of silver nanoparticles. The FTIR facility was availed from Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Bombay to recognize the organic, inorganic, biomolecule residues along with nanoparticle formation, which may come along via reducing agent on to the surface of AgNPs. Absorption bands for *B. vulgaris* AgNPs were found to be at 3368.49 cm^{-1}2932.99 cm^{-1},1614.73 cm^{-1}, 1517.26 cm^{-1}, 1450.12cm^{-1}, 1368.06 cm^{-1}, 1228.62 cm^{-1}, 1173.70 cm^{-1}1059.81 cm^{-1}, 981.37 cm^{-1}, 912.12 cm^{-1}, 835.59 cm^{-1}, 764.43 cm^{-1}, 565.69 cm^{-1}, 517.52 cm^{-1}s (Fig.11).

Absorption bands for *A. indica* AgNPs were found to be at 3388.75 cm^{-1}, 2931.79 cm^{-1}, 1613.03 cm^{-1},1516.92 cm^{-1}1449.90 cm^{-1}1384.07 cm^{-1}, 1239.47 cm^{-1}1173.54 cm^{-1}, 1058.93 cm^{-1}, 980.60 cm^{-1},912.06 cm^{-1}836.03 cm^{-1}, 565.99 cm^{-1}, 517.81cm^{-1} (Fig.12).

Quantification of silver content

The reports of nanoparticle collections in leaves during both the preliminaries are given in figure no.13&14.The qualities spoke to here are determined after duplication with weakening element. Despite the fact that, the amassing of both the nanoparticles in particular T1 (AgNPs 1: *B. vulgaris*), T2 (AgNPs2: *A. indica*) are in traceable amount but, T1 (AgNPs 1: B. vulgaris), demonstrated overall greater accumulation in treated plants. The greatest accumulation recorded in the T1 plants of trial 2. The outcomes recommend that there is extremely slight aggregation of nanoparticles in the medicines examined by Atomic Absorption Spectrophotometer (AAS).

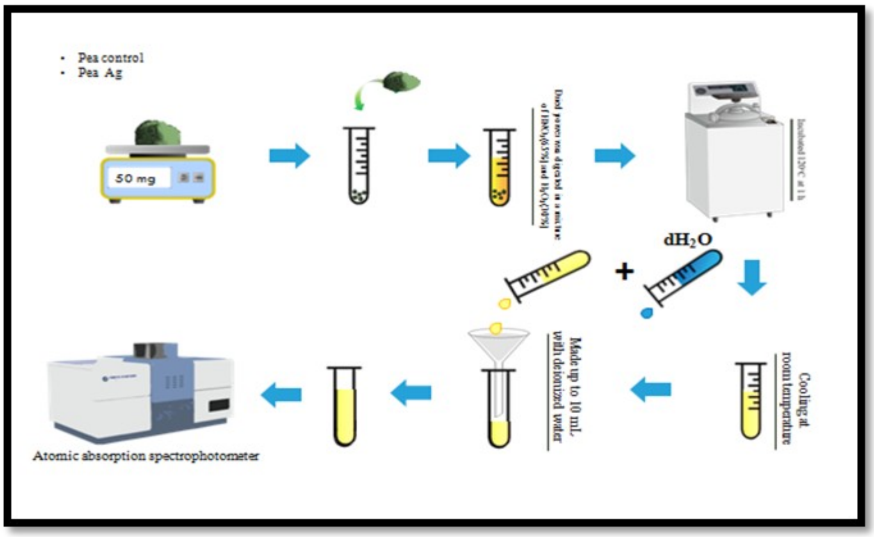

Figure 1. Diagrammatic representation of method for the measurement of Ag content in Pea (*Pisum sativum*) plant.

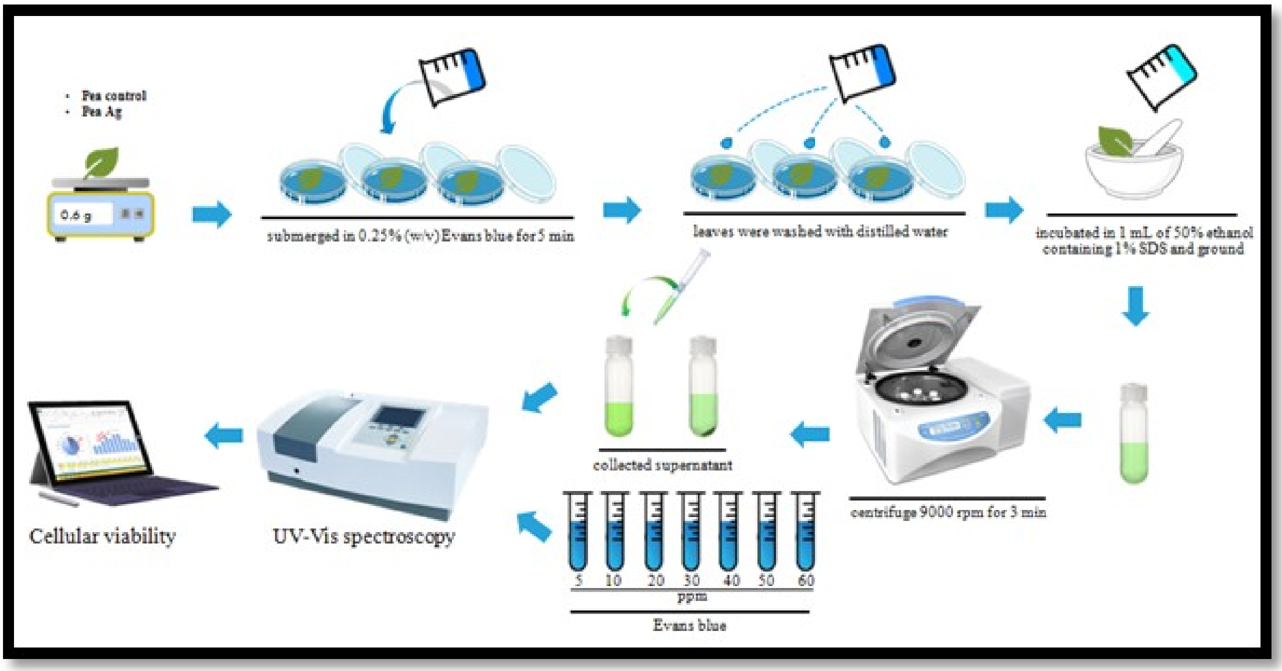

Figure 2. Evans blue tests for quantification of Cell Death in Pea (*Pisum sativum*) leaves.

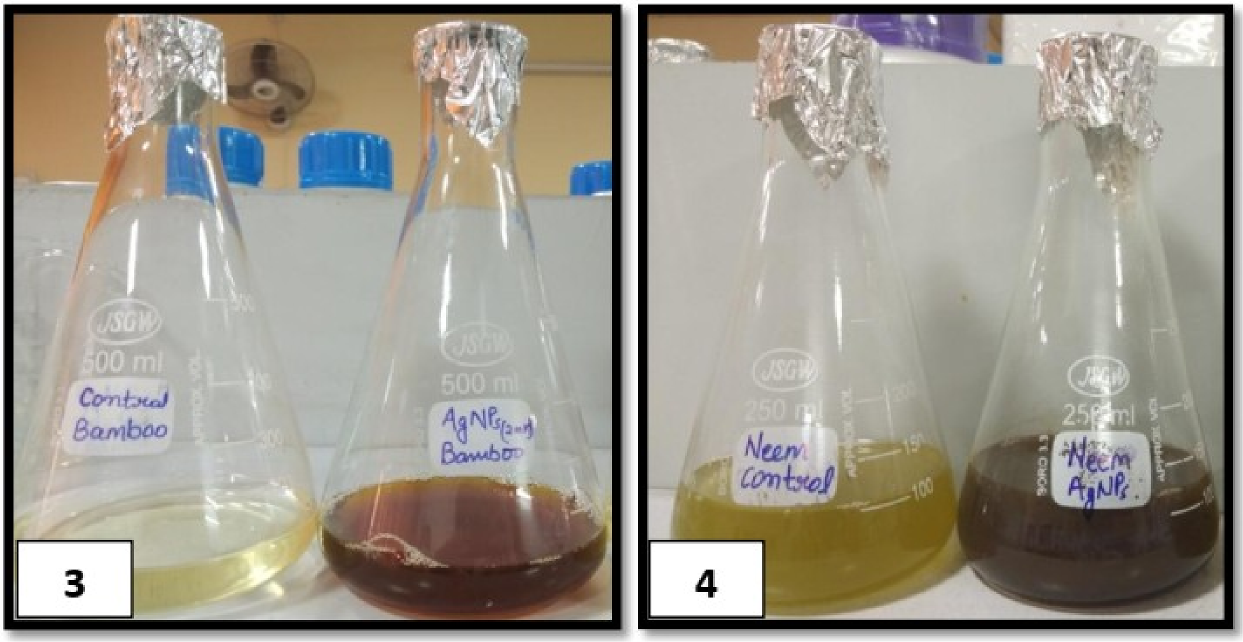

Figure 3 & 4: Shows the development of AgNPs in leaves concentrates and difference observed in the color of control and test flasks of *B. vulgaris* (Bamboo) and control and test flasks of *Azadirachta indica* (Neem) were utilized for unions of silver nanoparticles after 96 hrs.

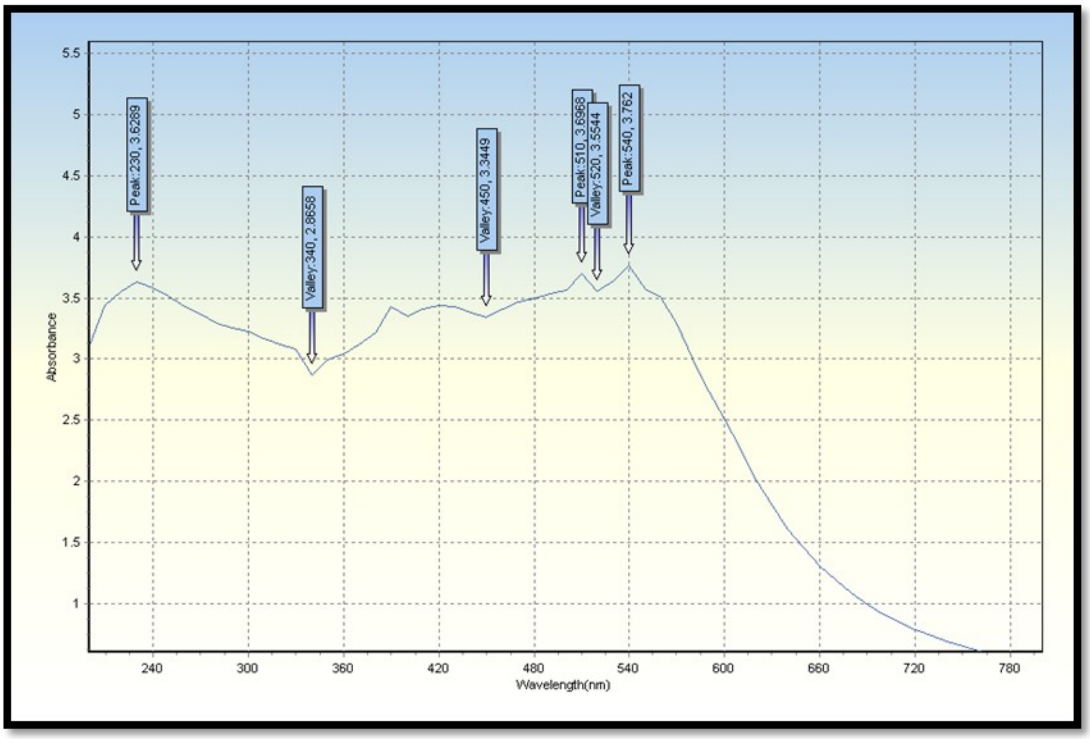

Figure 5. UV-Vis spectral reports of leaves extract *B. vulgaris* (Bamboo) were used for syntheses of silver nanoparticles.

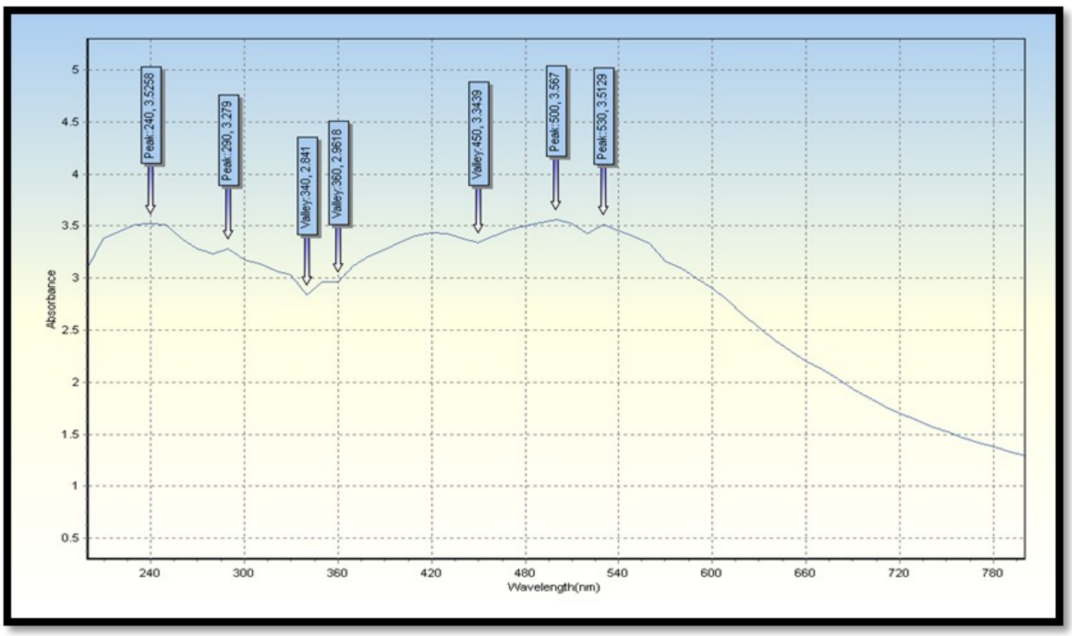

Figure 6. UV-Vis spectral reports of leaves extract *A. indica* (Neem),were used for syntheses of silver nanoparticles.

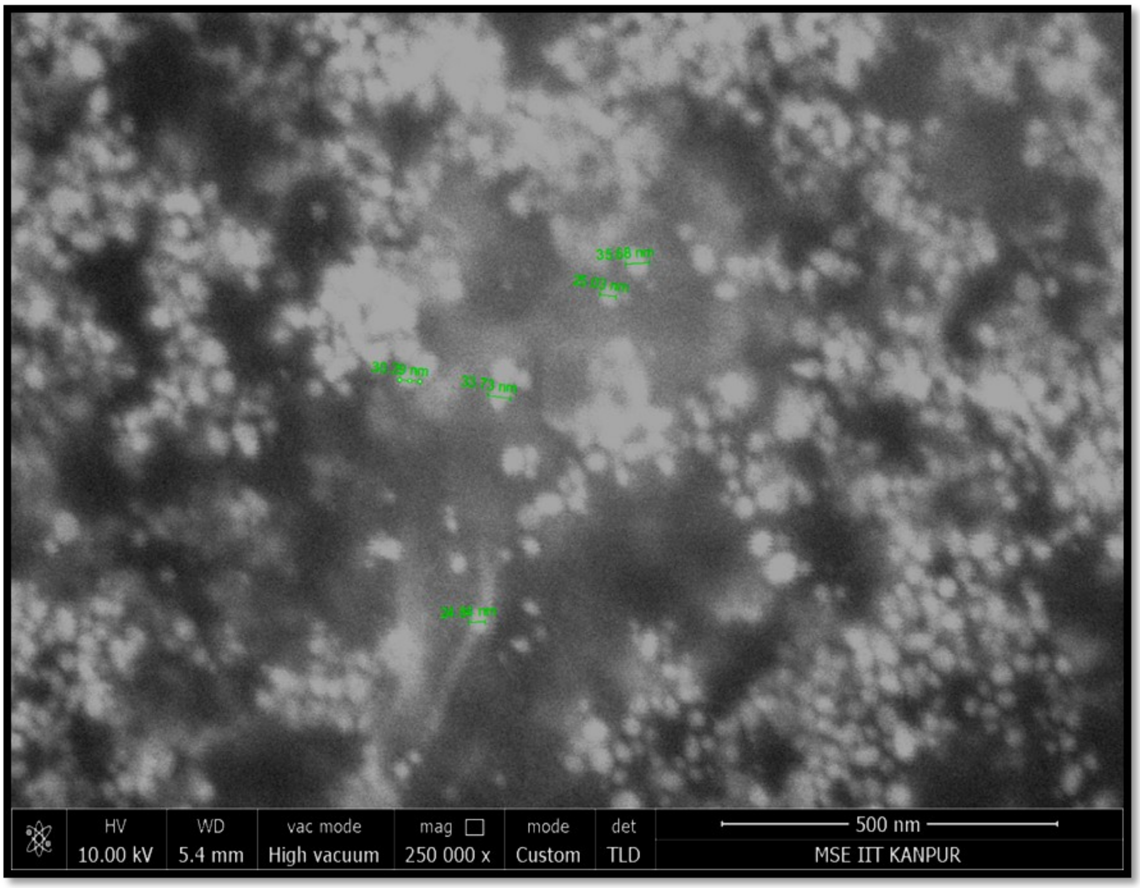

Figure 7. Shows the FESEM image of AgNPs synthesized from *B. vulgaris* leaves extract.

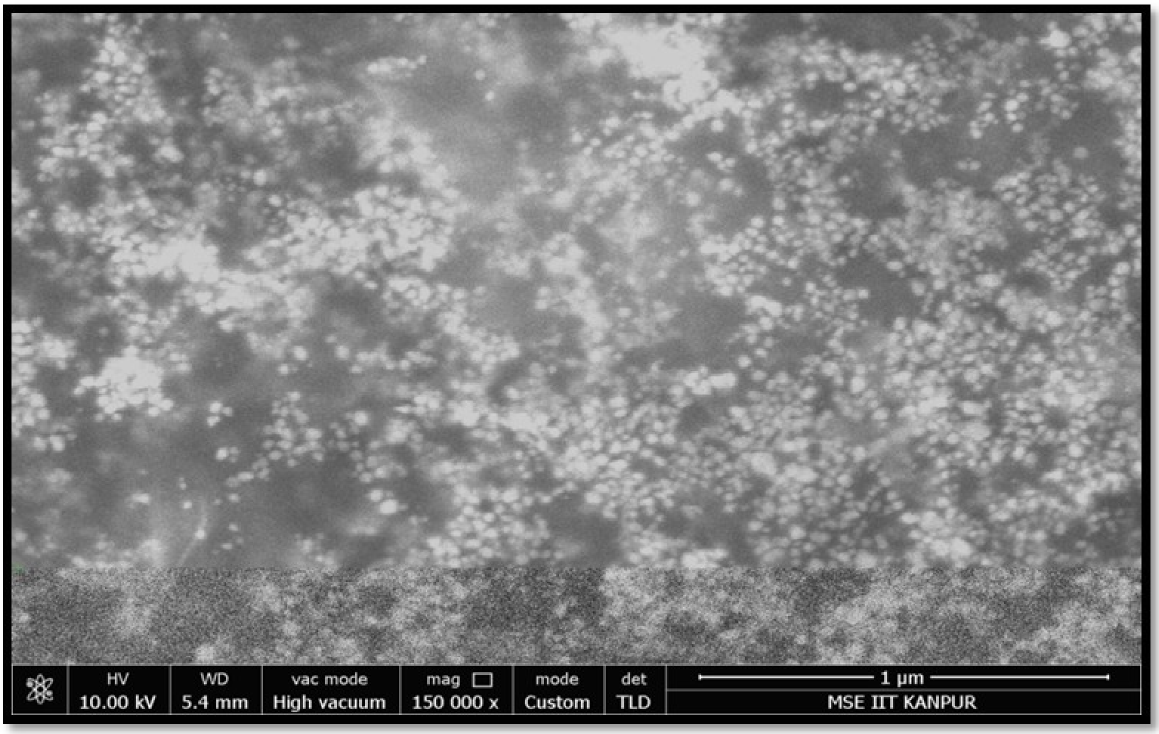

Figure 8. Shows the FESEM image of AgNPs synthesized from *A. indica* (Neem),leaves extract.

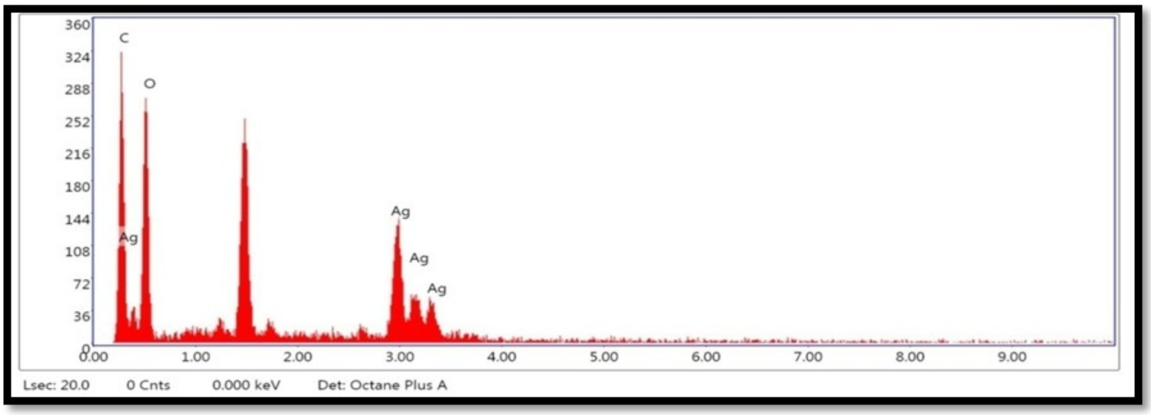

Figure 9. Shows the EDX analysis graph of AgNPs synthesized from *B. vulgaris* leaves extract.

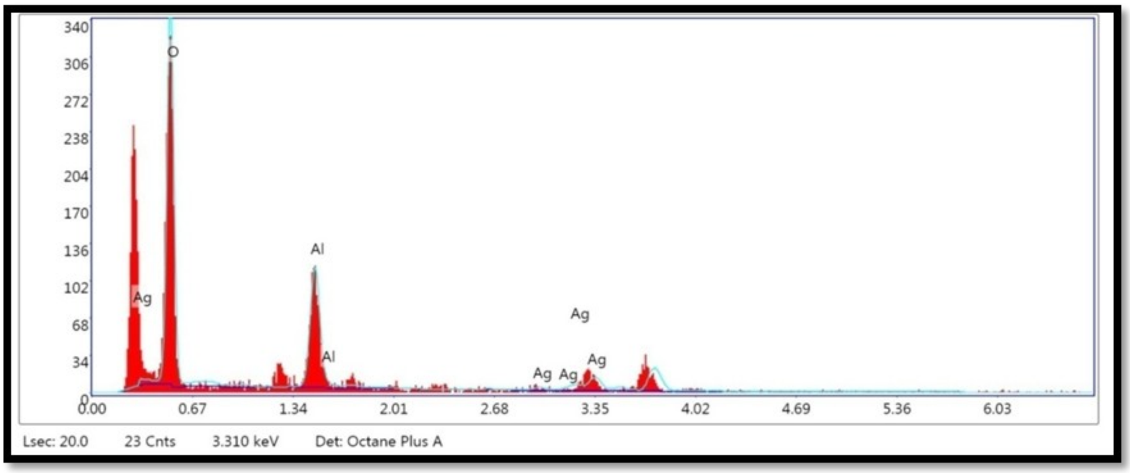

Figure 10. Shows the EDX analysis graph of AgNPs synthesized from *A. indica* (Neem), leaves.

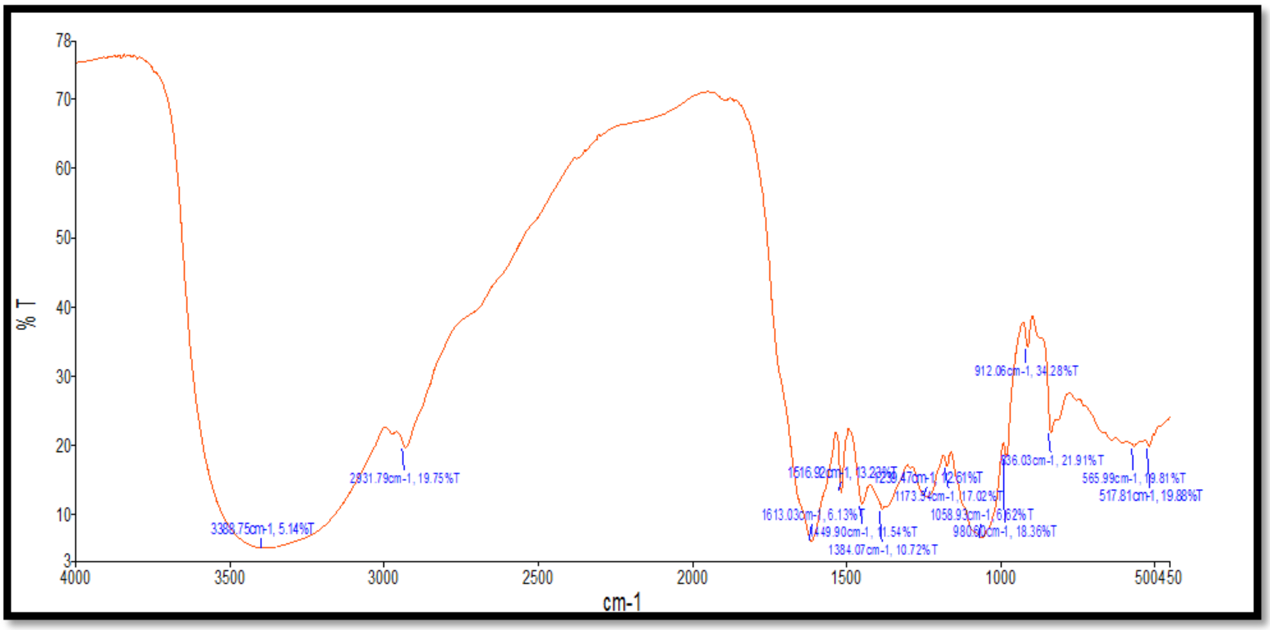

Figure 11. Shows the FTIR spectra of AgNPs synthesized from *B. vulgaris* leaves extract.

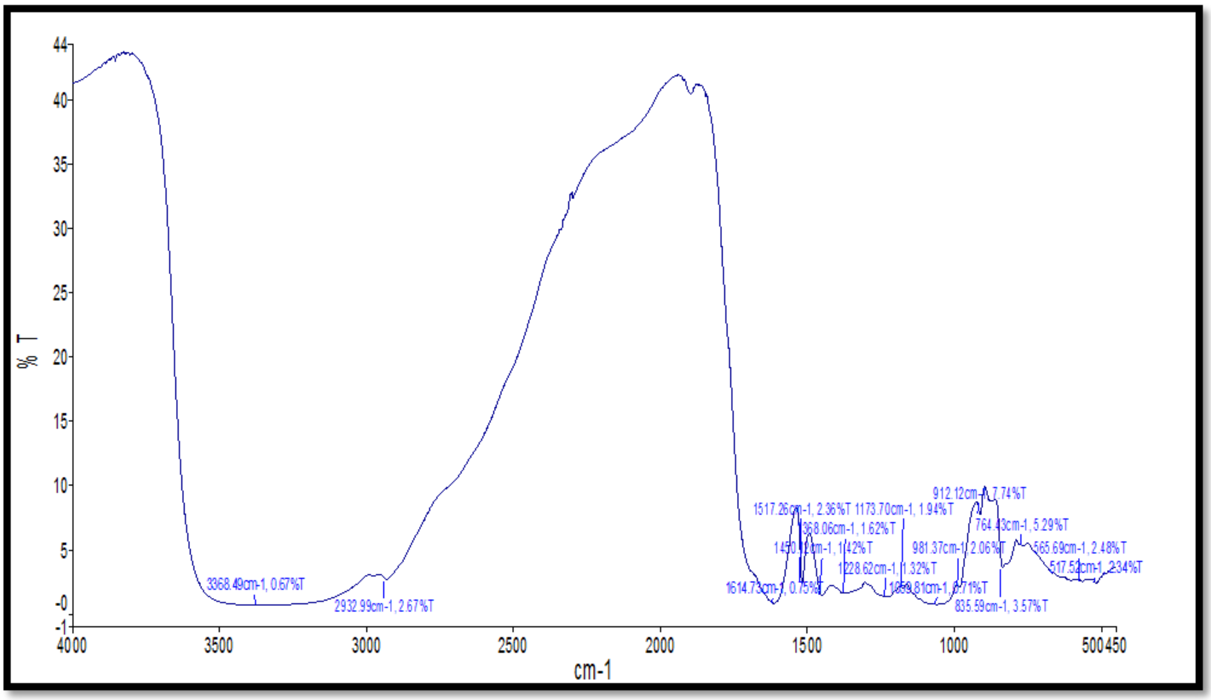

Figure 12. Shows the FTIR spectra of AgNPs synthesized from *A. indica* leaves extract.

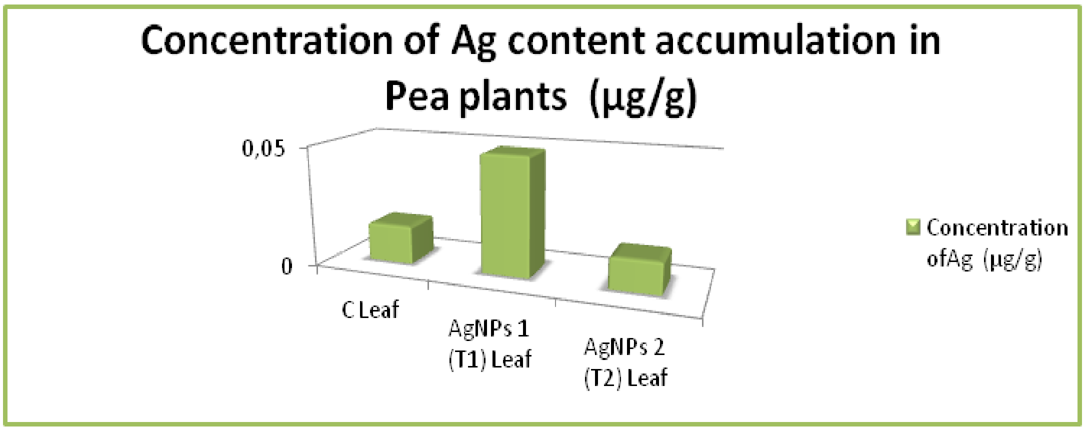

Figure 13. Accumulation quantity of silver content in C, T1 and T2 Pea (*Pisum sativum*) plant study groups during Trial 1.

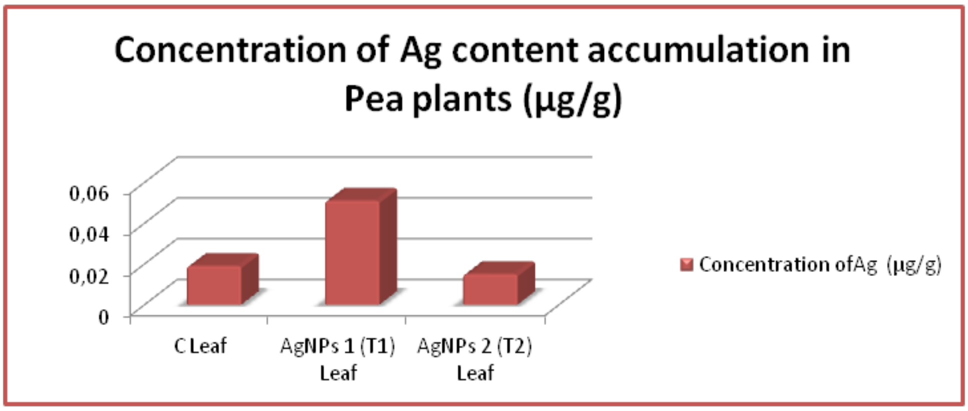

Figure 14. Accumulation quantity of silver content in C, T1 and T2 Pea plant study groups during Trial 2.

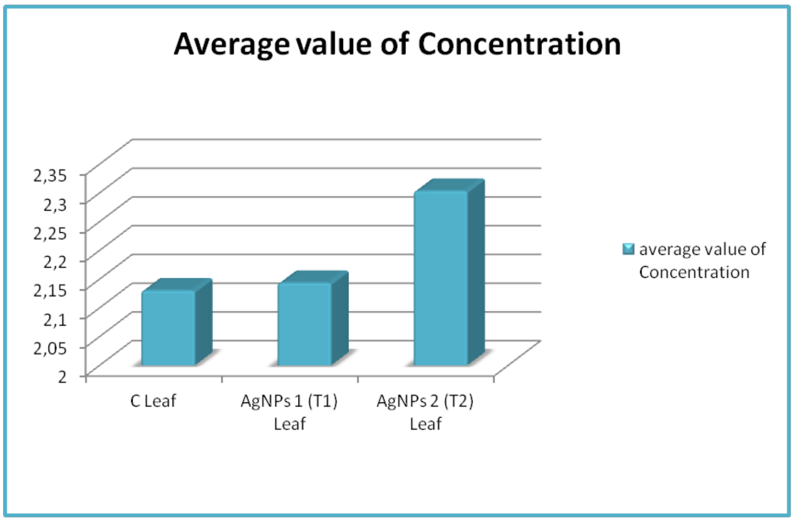

Figure 15. Amount of Evans blue dye trapped in different samples in trial 1 plants at 90 DAT, where, average value of concentration of three replicates.

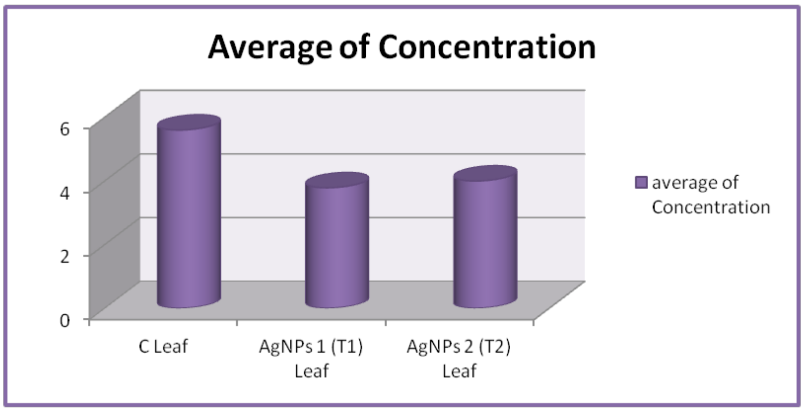

Figure 16. Amount of Evans blue dye trapped in different samples in trial 2 plants at 90 DAT, where, average value of concentration of three replicates.

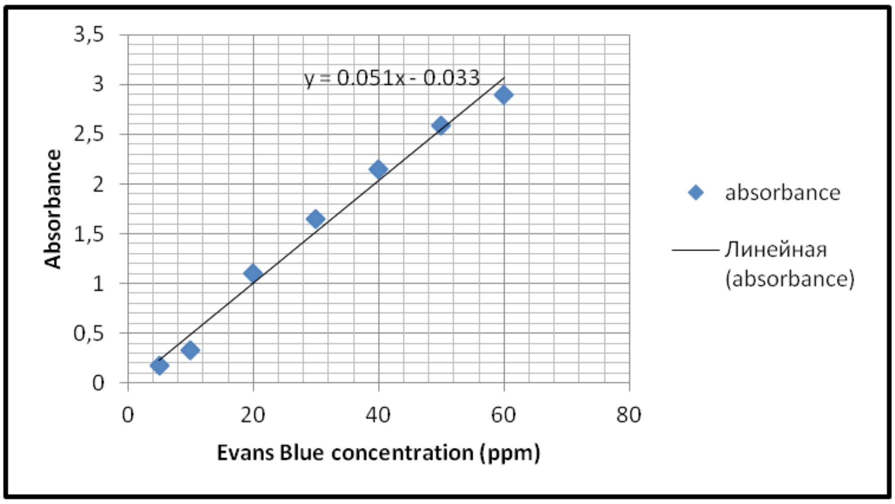

Figure 17. Evans Blue standard linear graph recorded during testing samples of trial 1 at RT.

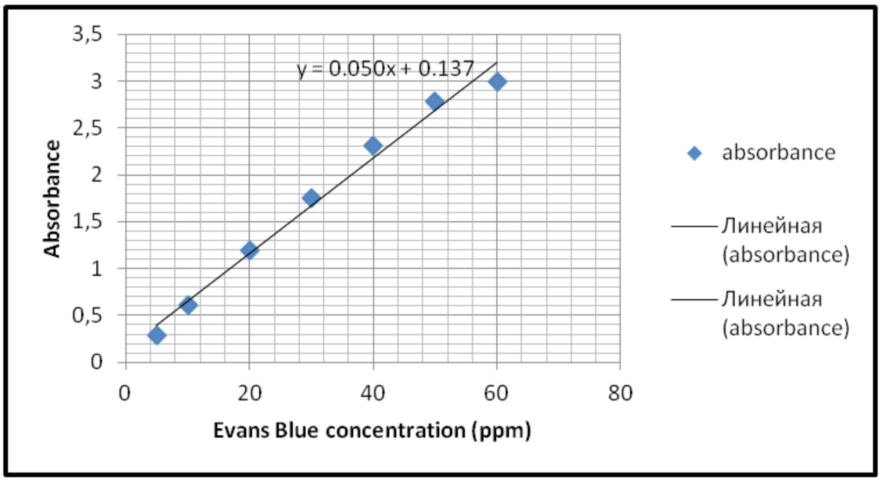

Figure 18. Evans Blue standard linear graph recorded during testing samples of trial 2 at RT.

Result of quantification of cell death

The absorbance estimations of C, T1 and T2 tests of both the trials were recorded and Evans blue fixation was determined (figure no.15&16) with reference to standard graph (figure 15 and16). The Evans blue level was found to be most elevated in C in the trial 2 followed by T2 and in trial 1, Evans blue capture was found to be most in T2 test followed by T1 and C. The outcomes propose that C demonstrated more cell demise as compared to T1 and T2. Likewise, there was least cell harm recorded in the T1 tests in trial 2. It could be closed by the outcomes that nanoparticles are by one way or another giving better in general upkeep to the plants and opposing cell harm and demise that may have caused because of biotic or abiotic stress.

DISCUSSION

This study investigated the biosynthesis of AgNPs and characterization of silver nanoparticles using the aqueous leaf extract *Bambusa vulgaris* and *A. indica*. Biosynthesis of silver nanoparticles from fluid leaf concentrates of *Cucumis prophetarum* (Hemlata *et al.,* 2020), *Cucumis sativus* (Senthil *et al.,* 2010) and Cucumis Melo (Babulreddy *et al.,* 2013) has been accounted earlier. Furthermore the Synthesis of metal nanoparticles utilizing different plant separates were accounted for previously (Udayasoorian et al 2011, Rai *et al.,* 2009; Ingle *et al.,*2008; Kim *et al.,* 2007).

UV−visible spectroscopy is one of the most broadly utilized procedures for auxiliary portrayal of nanoparticles (Harmsen *et al.,* 2017). The presence of an absorbance top at around 420 nm obviously demonstrates the development of AgNPs in the arrangement because of surface plasmon reverberation (SPR) electrons present on the nanoparticle surface (Bilal *et al.,* 2017).

SEM and FESEM picture shows agglomerates of little grains and some scattered nanoparticles (Guzmán *et al.,* 2009). EDX instrument generally remains fixed with FESEM and it is used to research the components present inside an example. It affirms the component of nanoparticle test with its volume rate and besides gives bits of knowledge concerning some other contaminant part that might be connected with the perfect metal nanoparticles (Rao *et al.,* 2013; Bakar *et al.,* 2012; Goudarzi et al., 2016; Zhang, *et al.,* 2017; Hemmati *et al.,* 2019). The pinnacle comparing to aluminum is clear as the example smear was set up on the aluminum foil base. For the most part metallic silver nanocrystals show run of the mill optical assimilation top roughly at 3 keV because of surface plasmon reverberation (Gomathi *et al.,* 2020).

FTIR spectroscopic examination affirmed that carbonyl gathering structure amino corrosive buildups and proteins has the more grounded capacity to tie metal, might shape a layer covering the metal nanoparticles (covering of silver nanoparticles) to forestall agglomeration and accordingly settle the medium (Khan *et al.,* 2018; Chokshi *et al.,*2016; Paul *et* *al.,* 2015; Saware *et al.,* 2014; Balaji *et al.,* 2009; Mandal *et al.,* 2005).

Plant toxicities studies were carried out to see the impacts AgNPs on pea plant.The results of toxicity on pea (*Pisum sativum*) plant were found similar in both control and treated plants and the concentration was very low.Plant toxicities studies were carried out to see the impacts AgNPs on pea plant.The results of toxicity on pea (*Pisum sativum*) plant were found similar in both control and treated plants and the concentration was very low. *Ex situ* approaches are advantageous in nanotoxicology examination since they can create a similar exploration results rapidly and financially without utilizing costly creature models (Sharifi *et al.,* 2012). *Ex situ* moves toward that make unambiguous and quantitative consequences of toxicity are esteemed for fundamentally surveying the evaluated biocompatibility of nanoformulations. The dependability of *ex situ* screening examinations to give an *in vivo* harmfulness forecast of nanoformulations in the lungs of rodents (Sayes *et al.,* 2007). *Ex situ* strategies are commonly important to recognize properties of NPs that can feature their toxicity as well as create a grouping of NP harmfulness for unthinking investigations**.**

CONCLUSION

Nanoparticles are being widely studied for their potential use as , nanofertilizers, nanopesticides and nanofungicides etc (Kah et al, 2018; Shukla et al, 2020a; Shukla et al, 2020b; Singh et al, 2020) and this is raising concern about the possible nano-toxicity that might be caused on plants and environment due to the use of nanoparticles. The present study concludes that application of nanoparticles on plants does not cause any noticeable toxicity to the plant; hence, it is safer to be used as crop growth enhancer or protecting agent.

CONFLICTS OF INTEREST

All authors have declared that they do not have any conflict of interest for publishing this research.

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