RESEARCH PAPER

Evaluation of Biosynthesized Silver Nanoparticles for Growth Enhancement of *Amaranthus blitum* and Their In Vitro Cytotoxic Activity on Chang Liver Cells

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How to cite this article: Esha Dwivedi, Priya Govindarajan, M. Thangamani, K. Sujatha, V. Sabapathi Mouleeshuwarapprabu R. Evaluation of Biosynthesized Silver Nanoparticles for Growth Enhancement of Amaranthus blitum and Their In Vitro Cytotoxic Activity on Chang Liver Cells. Int J Drug Deliv Technol. 2026;16(79s):574-597. DOI: 10.25258/ijddt.16.79s.61.

INTRODUCTION

Nanotechnology is gathering information of atom in nano scale range, with consideration to physical, catalytic, magnetic and optical properties (Sadik *et al*., 2009). However, the concentration of usage chronically exposes soil microbes and micro fauna, as well as the plants themselves, to level of chemical reactivity that may be toxic. (Azam JAFARI *et al*., 2015).

Nanotechnology deals with materials have dimensions in the nanometer range (<100 nm). They may consist of single, isolated nanoparticles, of assemblies of such nanoparticles, of thin films which are two-dimensional nanostructures, of composites in which the constituents are of nanosize dimensions (nanocomposites). (Turk J Biol *et al*., 2002). Nanosized structures created by lithography and etching as for example semiconductor devices, or of structures created by self-organization ofindividualnanostructures. Nanoparticles are seen as solution to many technology and environmental challenges. (W. Kulisch *et al.,* 2002)

By bio- synthesis methods of NPs reduced hazardous to the global efforts (Dahl et al,). Implantation of these sustainable processes should adopt the fundamental principles of green chemistry (Alivistoes *et al*., 2006). These principles emphasize on maximizing the efficiency of chemical processes without compromising safety concern of the products.The physical, chemical and biological properties of the nanoparticle change in fundamentally from the properties of both individual atoms/molecules and of the corresponding bulk material.Diverse chemical natureof nanoparticles, including: metals, metal oxides, non-oxide ceramics, carbon and biomolecules. (Khalil FARHADI *et al*., 2003). Nanoparticles have several different morphology forms such as spheres, cylinders, platelets, tubes, etc. Consequently, they are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. The various diversity of the nanoparticles arising from their wide chemical nature, shapes and morphologies, the medium in which the particles are present, the state of dispersion of the particles and most importantly, the several possible surface modifications the nanoparticles can be subjected to make this an important active field of science. (Abbasalizadeh S *et al.,* 2015)

"Nano" means one-billionth, thus nanotechnology deals with materials measured in a billionth of a meter. A nanometer is 1/80,000 the diameter of a human hair or approximately ten hydrogen atoms wide. Nanotechnology is the science of very small things. But nanotechnology is not just involved with small things. Nanotechnology is a multi-disciplinary science. It includes knowledge from biology, chemistry, physics and other disciplines. (Joseph and Morrison, 2006) defined Nano technology as the manipulation or self-assembly of individual atoms ,molecules or molecular clusters into structures to create materials devices with new or vastly different properties. Nanotechnology is science, engineering, and technology conducted at the nano-scale, which is about 1 to 100 nanometers. (Wang, L. J *et al*., 1999)

Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. The synthesis of nanomaterials of specific composition and size is a burgeoning area of materials science research. The properties of these materials in applications as diverse as catalysis, sensors and medicine depend critically on the size and composition of the nanomaterials. New routes to the preparation of these materials extend the choice of properties that can be obtained. Several synthetic techniques that usually employ atomic, molecular, and particulate processing in a vacuum or in a liquid medium are in use. Most of the techniques are capital intensive, as well as inefficient in

materials and energy use. Hence, there is an ever-growing need to develop clean, nontoxic, and environmentally benign synthetic procedures. Consequently, researchers have used biological synthesis, since this technique provides particles with good control over the size distribution. The main reason for this may be that the processes devised by nature for the synthesis of inorganic materials on nano- and micro- scales have contributed to the development of a relatively new and largely unexplored area of research based on the use of microbes in the biosynthesis of nanomaterials. Among the microorganisms, prokaryotic bacteria have received the most attention in the area of bio-synthesis of nanoparticles. Microbial resistance against heavy metal ions such as Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb or U has been explored for bioleaching processes of ores and for biological metal recovery systems. (Sakihama Y, *et al*., 2002)

Early studies reveal that Bacillus subtilis 168 is able to reduce Au^{3+} ions to produce octahedral gold particles of nanoscale dimensions (5–25 nm) within the bacterial cells by incubation of the cells with gold chloride solution under ambient conditions. Silver is highly toxic to most microbial cells and can be used as biocide or antimicrobial agent. Nevertheless, it has been reported that several bacterial strains are silver resistant and may even accumulate silver at the cell wall to as much as 25% of the dry weight biomass, thus indicating their use for industrial recovery of silver from ore materials. (Chandlee JM *et al*., 2006)

The silver resistant bacterial strain Pseudomonas stutzeri AG259 accumulates silver nanoparticles in the size range, 35–46 nm, along with some silver sulphide, in the cell. The exact reaction mechanisms leading to the formation of silver nanoparticles by the silver resistant bacteria is yet to be elucidated. In this study, we have made an attempt to corroborate the microbial reduction of water soluble Ag+ to Ag^{o} using an airborne bacteria (Bacillus sp.) present in the atmosphere. (Krishnaraj C *et al*., 2010)

The leaves of *Amaranthus blitum* contain about 3.88% protein, 1.1% fat, 9.38% carbohydrate, 3.2% ash, 323mg Ca, 8.3mg Fe, they are very rich in Vitamins A & C, rich in vitamin B1. It is edible and has various medicinal applications. A fluid extract of the plant is used as an astringent internally in the treatment of ulcerated mouths and throats, externally as a wash for ulcers and sores. The juice of the roots is used externally to relieve headaches. The plant has a folk reputation for being effective in the treatment of tumours and warts. Amaranth is the common name for the domesticated species of the genus Amaranthus (family Amaranthaceae). It is one of the oldest food crops in the world (Gigliolacamaggio, *et al.,* 2012). Amaranthus is one of the most promising plant genus and it consists of approximately 70 species 40 of which are native to the Americans 17 are mainly vegetable species three are grain while others are weedy (Andreas *et al.,* 2011).

Amaranth is a multipurpose crop whose leaves and grains are tasty and of high nutritional value, additionally it can be cultivated as an ornamental plant (Venskutonis & Kraujalis *et al.,* 2013). The genus Amaranthus has received considerable attention in many countries because of the high nutritional value of some species that are important sources of food, either as vegetable or grain (Srivastava *et al.,* 2001). Vegetable amaranth has been used in China for over 400 years, it is commonly found in the Caribbean and Africa, grain amaranth was cultivated and revered by the Aztecs of Mexico, the Mayas of Central America and the Inca of South America (Brien & Price *et al*., 2008).Amaranth plants grow as weed and are available abundantly during the rainy season in Haryana State. Its use in the United States is limited to canned imports for ethnic uses, primarily in the New York City area (Singh *et al*., 2001). In Kenyan rural areas, amaranth is known as a traditional vegetable which can grow in open fields. It exhibit the highest diversity of species exploited as traditional vegetables (Wambugu & Muthamia *et al.,* 2009).

Amaranth is mostly grown for its edible leaves which are a regular food component of most local community diets in the country; however, some of the species have edible seeds. It is one of the few 2 plants whose leaves are eaten as a vegetable while the seeds are used in the same way as cereals. There is no distinct separation between the vegetable and grain types since the leaves of young plants grown for grain can be eaten (Kariuki *et al*., 2013). Previously there has been aggressive promotion of exotic types of vegetable resulting to abandonment of indigenous vegetable. However recently, due to the realization of their high nutritive and medicinal value and low input requirement, there has been intensified awareness creation resulting in increased production, consumption and marketing of these vegetables (Wambugu & Muthamia *et al.,* 2009). In Kenya, the volume of production of vegetable amaranth have increased over the last few years in response to the growing urban vegetable demand (Onyango&Imungi *et al.,* 2007). Vegetable amaranth is found in many supermarkets and green grocers‟ stores in the urban centers of Kenya. More than 90% of the supply of the vegetables to these outlets is normally from farms that are within the environs of the urban center. (Moraa *et al.*, 2008).

Nano agriculture involves the employment of Nano particles in agriculture these particles will impart some beneficial effects to crops. The emergence of nanotechnology opens up potential novel applications in agriculture and biotechnology. Nanoparticles tagged to agrochemicals or other substances could reduce the damage to other plant tissues and the amount of chemicals released into the environment. Nanoparticles mediated plant transformation has the potential for genetic modification of plants for further improvement. Specifically, application of Nanoparticles technology in plant pathology targets specific agricultural problems in plant–pathogen interactions and provide new ways for crop protection. The application of Nanoparticles in the scope of life sciences is their use as ‘smart’ delivery Systems. The detection of plant diseases and analysis of Nanoparticles introduced into plants and to assess the use of

such Nanoparticles in selected plant tissues. The results open a wide range of possibilities for using Nanoparticles in general plant research and agronomy. (Jacob *et al*., 2005).

REVIEW OF LITERATURE

Metal nanoparticles can potentially be used as tools for engineering biological redox reactions. Present study underlines the effect of silver metal nanoparticles (at 0, 25, 50,100, 200 and 400 ppm) on the growth and antioxidant status of 7-day-old Brassica juncea seedlings. Fresh weight, root and shoot length, and vigor index of seedlings is positively affected by silver nanoparticle treatment. It induced a 326 % increase in root length and 133 % increase in vigor index of the treated seedlings. Improved photosynthetic quantum efficiency and higher chlorophyll contents were recorded in leaves of treated seedlings, as compared to the control seedlings. Levels of malondialdehyde and hydrogen peroxide decreased in the treated seedlings. Nanoparticle treatment induced the activities of specific antioxidant enzymes, resulting in reduced reactive oxygen species levels. Decrease in proline content confirmed the improvement in antioxidant status of the treated seedlings. The observed stimulatory affects of silver nanoparticles are found to be dose dependent, with 50 ppm treatment being optimum for eliciting growth response. Present findings, for the first time indicate that silver nanoparticles promote the growth of B. juncea seedlings by modulating their antioxidant status. (Priyadarshini Sharma *et al*., 2012).

Interactions of biologically synthesized silver nanoparticles on hydroponically grown Bacopa monnieri (Linn.) Wettst. plant growth metabolism were documented. Estimates of protein, carbohydrate, total phenols, in addition antioxidant enzymes, catalase and peroxidise were assayed in various parts of the plants grown in hydroponic solution. The silver nanoparticles used in this study were synthesized by treating AgNO3 with aqueous leaves extracts of Acalypha indica Linn., a medicinal herb as a source of reductants. Enhanced peroxidase and catalase activity, simulated the stress conditions induced by the silver nitrate treatment. No severe toxic effects were observed in silver nanoparticles treated plants in the morphological studies under scanning electron microscopy (SEM) while structural aberrations were observed in the light microscopic evaluation of root and stem anatomy. Further, the uptake of silver in the root and stem tissues of B. monnieri (Linn.) Wettst. was confirmed using atomic absorption spectrophotometer (AAS). (Krishnaraj C *et al*., 2012)

The green synthesis of CuO NPs was carried out by using *Morus alba* leaf extract and accumulation of NPs more actively by tomato plants as compared to cauliflower was possibly due to the difference in root morphology. The use of plants or their extracts in the synthesis of copper nanoparticles for various human applications. (M. G. H. Zaidi *et al.,* 2017).

The green synthesis of CuO NPs was carried out by using *Morus alba* leaf extract and accumulation of NPs more actively by tomato plants as compared to cauliflower was possibly due to the difference in root morphology. Transmission electron microscopy (TEM) and energy dispersive X-ray (EDX) analyses confirmed that silver nanoparticles. (Jouzani GS *et al*., 2017).

The study by (Neha Upadhyay, Shivesh Sharma *et al*., 2017), discussed on differential impact of AgNPs and $AgNO_3$ on *Brassica* seedlings, their mode of action, and reasons for their differential impact and could be implied in toxicological research for designing strategies to reduce adverse impact of AgNPs and $AgNO_3$ on crop plants and mechanistic advances in the synthesis of AgNPs from medicinal plant extracts and discussed the recent improvements achieved in the use of biogenic AgNPs as cancer theranostic agents.

No severe toxic effects by treating with Cu Nps aqueous leaves extracts of Acalyphaindica Linn and enhanced peroxidase and catalase activity were observed in silver nanoparticles treated plants in the morphological studies. Effective antibacterial activities shown by Cu Nps for agriculture leading to the impact of biosynthesized Cu has been studied on a few plant species at their very early growth stages under this manuscript by (Shobha G, Vinutha Moses *et al*., 2014) which reviews the biosynthesis, characterization and risks of Cu Nps.

The presence of soluble organics in the plant extracts was mainly responsible for the silver ions reduction to nano-sized silver particles and on silver nanoparticles synthesis from various medicinal plants, various methods of characterization and it's biological application exhibiting anti-bactericidal and anti-fungicidal activities making them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food, and textiles was explained by (**Arpita Roy *et al*., (2015).**

Metal nanoparticles can potentially be used as tools for engineering biological redox reactions that underlines the effect of silver metal nanoparticles (at 0, 25, 50, 100, 200 and 400 ppm) on the growth and antioxidant status of 7-day-old Brassica juncea seedlings. Fresh weight, root and shoot length, and vigor index of seedlings is positively affected by silver nanoparticle treatment. (M. G. H. Zaidi *et al.,* (2017).

This study was undertaken by (J.J. Schlager et al., 2005), to address the current deficient knowledge of cellular response to nanosized particle exposure. The study evaluated the acute toxic eVects of metal/metal oxide nanoparticles proposed for future use in industrial production methods using the in vitro rat liver derived cell line (BRL 3A).

Green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers in the concern of Indian flora has yet to divulge innumerable sources of cost-effective non-hazardous reducing and stabilizing compounds utilized in preparing AgNPs. The study by (Mantosh Satapathy *et al*., 2014), investigates an efficient and sustainable route of AgNP preparation from aqueous $AgNO_3$ using leaf extracts of three plants, *Musa*

balbisiana (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (black tulsi).

Use of Gold-nanoparticles in enhancing growth and yield of *B. juncea*, under actual field conditions and present a viable alternative to GM crops for ensuring food security. Various growth and yield related parameters, including plant height, stem diameter, number of branches, number of pods, seed yield etc. were positively affected by the gold nanoparticle treatment.(Michalak A.*et al*., 2008)

The increasing commercial production of engineered nanoparticles (ENPs) has led to concerns over the potential adverse impacts of these ENPs on biota in natural environments. Silver nanoparticles (AgNPs) are one of the most widely used ENPs and are expected to enter natural ecosystems. The effects of AgNPs on germination and growth of eleven species of common wetland plants and plant responses to AgNP exposure in simple pure culture experiments (direct exposure) and for seeds planted in homogenized field soils in a greenhouse experiment (soil exposure) was examined by (Justin P. Wright *et al.,* 2012).

The increasing applications of different nanomaterials in the myriad of nano-enabled products and their potential for leaching have raised considerable environmental, health and safety (EHS) concerns. The systematic studies investigating potential anomalies in the morphology and anatomy of crop plants are scarce reported on the developmental responses of two agriculturally significant crop plants, maize (*Zea mays* L.) and cabbage (*Brassica oleracea* var. *capitata* L.), upon *invitro* exposure to nanoparticles of citrate-coated silver (Citrate–nAg) and zinc oxide (nZnO). Analyses involve histology of the primary root morphology and anatomy metal biouptake, moisture content, rate of germination, and root elongation. Comparative toxicity profiles of the ionic salts ($AgNO_3$ and $ZnSO_4$). The structural changes in maize primary root cells upon exposure to Citrate–nAg, nZnO, $AgNO_3$, and $ZnSO_4$, possibly due to metal biouptake, suggesting potential for functional impairments in the plant growth and development. Lok (R.Pokhrel, Brajesh Dubey *et al., 2013)*

MATERIALS AND METHODS

Collection and Preparation of Soil Sample:

The Soil sample was collected from roadside tress. The sample (approximately 4 g each) was collected using some clean, dry and sterile polythene bag along with sterile spatula. The sample was transferred to lab. Under sterile conditions, 1 g of each soil sample was added to 5 mL of nutrient broth and incubated at 35°C for 24 hours. (Aslim *et al*., 2001)

Isolation of *Bacillus* sp.

After the incubation period, 0.1 mL of the supernatant of each tube containing suspension of soil and culture media were inoculated in nutrient agar plates by streaking at 30°C for 24 hours. The plates were examined and the suspected Bacillus colonies were obtained. (Aslim *et al*., 2001)

Biosynthesis and Characterization of the Silver Nanoparticles (AgNPs):

To obtain microbial biomass, small amount (one loop) of the grown colonies were picked up and inoculated into each of Erlenmeyer flasks containing 100 mL of the liquid LB medium. The Erlenmeyer flasks were incubated in a rotary shaker incubator, with stirring speed of 150 rpm for 24 h at 40°C. At the end of each incubation period, the liquid medium was centrifuged at 10,000 ×g for 15 min for removing cell debris. The cell-free supernatant obtained in the previous step was poured into separate Erlenmeyer flask; flask was supplemented with the silver nitrate ($AgNO_3$) salt at 10mM concentration. The Erlenmeyer flask containing the supernatant mixed with the 10mM of silver nitrate, were incubated for 24 h at the same condition that was considered for the microbial biomass growth. To confirm the extracellular formation of the AgNPs, bioreduction of the silver ions in the medium was monitored using changes in the color from white to dark brown. The supernatant that was exposed with the 10mM of silver nitrate, was air dried and the remaining powder was scrapped out for the subsequent characterization of the silver nanoparticles. The morphological characterization and distribution of the nanoparticles was scanned using UV, SEM, XRD and FTIR. (Shaligram NS, Shashi S *et al*., 2003)

UV-Vis Analysis of silver nanoparticle:

The optical property of AgNPs was determined by UV-Vis spectrophotometer (Perkin-Elmer,Lamda 35,Germany). After the addition of AgNO3 to the Nutrient broth of Bacillus sp., the spectras were taken in different time intervals up to 24Hrs. between 300nm to 800 nm. Then the spectra was taken after 24Hrs. of AgNO3 addition. (Jain N, Bhargava A *et al.,* 2011)

SEM Analysis of silver nanoparticle:

The morphological features of synthesized silver nanoparticles from neem plant extract were studied by Scanning Electron Microscope (VEGA3 TSCAN). After 24Hrs. of the addition of AgNO3 the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 30 kV. (Panwar J *et al.,*2011)

XRD Analysis of silver nanoparticle:

The phase variety and grain size of synthesized Silver nanoparticles was determined by X-ray diffraction spectroscopy (Philips PAN analytical). The synthesized silver nanoparticles were studies with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s. Different phases present in the synthesized samples were determined by X′ pert high score software with search and match facility. The particle size of the prepared samples were determined by using Scherrer′s equation as follows □□≈0.9□□□□cosθ Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and B

is the full width at half maximum of the peak in radians. (Tarafdar J C *et al*., 2011)

FTIR Analysis of silver nanoparticle:

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer **(**perkin-Elmer LS-55- Luminescence spectrometer**)**. The solutions were dried at 75°C and the dried powders were characterized in the range 4000–400 cm-1 using KBr pellet method. (Majudmar S *et al.,* 2011).

Collection of seeds (*Amaranthus blitum*):

Bio-synthesis experiments were conducted with selected species of *Amaranthus blitum*. They were chosen as a part from being the most commonly grown crops and also represent the two major plant system. The growth matrix used was fertile soil. The seeds of *Amaranthus blitum* representing an important taxonomical and functional set of species were chosen for study. All seeds were purchased from an organic store (Ambattur, Chennai) and kept in the dark at 4°C until use. (Jyothsna Yasur *et al.,* 2013)

Effect of silver nanoparticle in *Amaranthus blitum*:

Experimental trays were prepared by adding biologically synthesized nanosilver at the varying concentrations of 100,200 and 500 mg and marked (respectively, as NP100, NP200 and NP500) and control (without nanosilver).1g of seeds were weighed and soaked under 10ml of distilled water for the seeds to sprout after 24 hrs. Fertile soil was collected and transferred to 4 individual trays to allow seed germination of *Amaranthus blitum.* The biosynthesized silver nanoparticles were exposed to the soil at varying concentrations. The nanoparticle exposed soil was sprinkled with uniformly weighed sprouted seeds of *Amaranthus blitum* (200 mg) for the surface exposure of seeds and the nanoparticles to the soil. (Priyadarshini Sharma *et al*., 2011). The effect of biosynthesized silver nanoparticle on seed germination was studied in *Amaranthus blitum* by performing:

- Growth level measurement.
- Estimation of chlorophyll.
- Estimation of protein.
- Estimation of carbohydrate.
- Cytotoxicity analysis.

Growth level measurement in *Amaranthus blitum*:

The growth of seeds germinated was measured and noted periodically. The germinated seedlings of comparatively high growth were selected to be analyzed for measuring the level of growth. The effect of nanoparticles in seeds varies with different silver nanoparticle exposure (100,200 and 500µg concentrations) and Control seeds with no nanoparticle exposure in *Amaranthus blitum*. The AgNPs on varying concentrations shows significant effect over the germination of seeds and level of growth. (Pusa Jai Kisanet *et al.,* 2006*)*

Estimation of chlorophyll in *Amaranthus biltum*:

The germinated seedlings (along with root, shoot and leaves) were plucked out from the soil to be taken as sample and collected for further proceedings. The germinated seedlings (along with root, shoot and leaves) were cut up into small pieces and ground in mortar and pestle in the presence of acetone of 500µl concentration in order to the sample to be grinded. All the concentrated extracts were made up to about 6 ml and stored in eppendorfs for further use. The sample was allowed to stand in a refrigerator in the dark at 4° C for about 30 min before being cleared by centrifugation and the pellet discarded. The extract was then pelleted by centrifugation and the pellet was discarded. Extraction of chlorophyll by soaking the sample in solvents overnight was not employed because it provides an opportunity for chlorophyllase to convert chlorophylls to chlorophyllides. Spectrophotometric readings were made using UV–visible spectrophotometer and OD value noted at the absorbance of 620nm and 680nm. (Raymond, Ritchie J *et al*., 2005)

Estimation of protein in *Amaranthus blitum*:

Total free protein content present in the germinated seedlings were estimated colorimetrically according to the Lowry's method.. Plant sample was centrifuged in DW (1 g/10 ml) at 10,000 rpm for 10 mins. The supernatant collected was then used for protein estimation. To the above 0.2ml supernatant, 0.8 ml DW and 5 ml of alkaline copper sulphate reagent (analytical reagent) was added. The solution was incubated at room temperature for 10 mins. Now 0.5ml Folin Ciocalteau reagent was added 62 to the above solution and then the solution was incubated for 30 min. The optical density (absorbance) was measured at 660 nm in a spectrophotometer for the colour development. The standard curve was prepared by using 0.2, 0.4, 0.6, 0.8 and 1.0 ml of BSA solution. (Sakihama Y*et al*., 2002)

Estimation of carbohydrate in *Amaranthus blitum*:

Anthrone method was used for total carbohydrates determination given by Hedge and Hofreiter (1962). 100 mg of the plant sample was hydrolysed with 5 ml of 2.5N HCl for three hours in a boiling water bath, cooled at room temperature and neutralized with sodium carbonate. The volume was made upto 100 ml and centrifuged to collect the supernatant. To the 1ml of supernatant, 4ml of anthrone reagent was added and heated for 8 mins. in a boiling water bath. Now rapidly cooled it and monitor the green to dark green colour at 630nm using spectrophotometer. The standard curve was prepared by using 0.2, 0.4, 0.6, 0.8 and 1.0 ml of glucose solution. A standard graph was drawn by plotting the concentration of the standard on the X axis versus absorbance on the Y axis. From the graph the amount of carbohydrate present in the sample tube was calculated. The unit of total carbohydrates is expressed in the terms of percentage. (Dubois, M *et al.,* 2000)

Cytotoxicity assays:

The media suitable for cytotoxicity assay was prepared. Minimal Essential medium (MEM) was taken and 9.5grams of MEM dissolved in 900ml of pre-sterilized

double distilled water mixed well and closed. Sodium hydrogen carbonate was prepared by taking 3.75 grams to be dissolved in 50ml of pre-sterilized double distilled water mixed well and closed. Both contents were sterilized at 15 lbs, 121 °C, for 15 min. Allow to cool at room temperature. Add 10 ml of L-glutamine and antibiotics streptomycin 1ml, penicillin G 100 µl and amphotericin B 20 µl. Check pH and adjust to 7.2 to 7.4 and Syringe filter all the contents. Store it at 4ºC. L-glutamine of 0.3grams was weighed and kept. Dissolve glutamine to 10ml of pre-sterilized double distilled water mix well. Antibiotics – streptomycin, penicillin G and amphotericin B. 1mg of each was weighed. Dissolve each antibiotic in 1ml of pre-sterilized double distilled water mixed well. Fetal Calf Serum (10%) was syringe filtered and added to required amount of media(serum media) Syringe filter all the contents. Mix all the Ingredients and add antibiotics streptomycin 100 µl (1mg stock). Distribute in 5mL aliquots. Store at -20 °C. (Burchette RJ *et al*., 2000)

Sub culturing of cells

Take the culture flask and discard the medium. Gently rinse the cells with MEM medium. Add 4ml of TVPG (prewarmed to 37°C) over the cells. Allow TPVG to act for 1-2 minutes. Discard the TPVG and incubate for 5-10 mins. Add 5ml of 10% MEM serum medium. Break off the cell clusters by gently pipetting back and forth with pipette (Passaging the cells). Add 20ml of growth medium to the Culture flask and transfer the cells into 96 well plates.

MTT assay

The cytotoxicity activity of samples on chang liver cell was determined by the MTT assay. Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 24hrs at 5 % CO_2 incubator. View the images under Inverted microscope 40X and take the photos. After removal of the sample solution and 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. (Boess *et al*., 2003)

RESULTS AND DISCUSSSION

ISOLATION OF *Bacillus spp.*

The soil bacteria may represent a survival mechanism where organisms can eliminate competition and colonize a niche. *Bacillus* strain isolated from soil was active against most Gram-positive, but not Gram-negative bacteria. The isolated bacterial strain was identified as *Bacillus sp.*

SYNTHESIS OF SILVER NANOPARTICLES:

Silver nanoparticles are being extensively synthesized using the biological source of bacterial species. This method of nanoparticles synthesis involves no toxic chemicals and termed as green chemistry procedure. *Bacillus sp.* was used for the synthesis of silver nanoparticles. The aqueous $AgNO_3$ solution turned to brown color after 3 days of incubation in dark, indicating the formation of AgNPs in the reaction solution. (Fig.2)

CHARECTERIZATION OF SILVER NANOPARTICLES:

UV-Vis spectroscopy Analysis:

The dispersions of silver nanoparticles display intense colors due to the Plasmon resonance absorption. Therefore, metallic nanoparticles have characteristic optical absorption spectrum in the UV–visible region. As shown in figure 1, UV– visible spectrum of AgNPs was strong, broad peak and located between 300 and 800 nm. Treatment of bacterial cell free supernatant with different doses of gamma radiation before and after mixing with silver nitrate solution showed that the cell free supernatant irradiated after mixing with AgNO3 solution was more effective than that of the cell supernatant irradiated before mixing with AgNO3 solution in the formation of silver nanoparticles. The increase in the synthesis of silver nanoparticles measured by formation of silver nanoparticles with a peak appears at 190 nm Figure 4. UV-Vis spectra of silver nanoparticles were synthesized by B. thuringiensis. The peak of the above spectra was found at 421nm and this peak is due to Surface Plasmon Resonance (SPR) property of silver nanoparticles. The peak corresponding to irradiation dose of 0.75 kGy showed the maximum absorbance, after which the decrease in the Surface Plasmon Resonance band intensity by increasing the irradiation dose more than 0.75 kGy was observed. The concentration of silver nitrate added strongly affects the reaction. As shown in Figure (3a), the absorbance intensity affects the cell free supernatant.

SEM Analysis:

SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes of several µm in case of 30:1, 120:1 & 240:1 ratios where as in 60:1 ratio the size is of several nm (Fig.3c).The size of the prepared nanoparticles was more than the size of nanoparticle which should be; i.e.; between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles. The result showed that the particles were of spherical shape in case of 30:1, 60:1, and 120:1 ratios but sheet shape in case of 240:1 ratio. The shape varies due to the concentration increased in 240:1 ratio. The morphological features of synthesized silver nanoparticles were studied by SEM analysis shown in (fig.3c). SEM analysis suggested that most of the particles are spherical in shape.

XRD Analysis:

The phase variety and grain size of synthesized Silver nanoparticles was determined by X-ray diffraction spectroscopy (Philips PAN analytical). The synthesized silver nanoparticles were studies with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s. Different phases present in the synthesized samples were

determined by X′ pert high score software with search and match facility. The particle size of the prepared samples were determined by using Scherrer′s equation as follows □□≈0.9□□□□cosθ Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians. XRD spectrum of synthesized silver nanoparticles (Fig.3b) shows distinct diffraction peaks around 29.53° . These sharp Bragg peaks might have resulted due to crystalline nature of silver nanoparticles.

FTIR analysis:

It was observed from the FT-IR spectrum of AgNPs that the bands at 3346.87 cm–1 (correspond to a primary amine NH band), 2126.13 cm-1 1636.3 cm-1 and 647.685 cm-1 (correspond to a secondary amine NH band and primary amine CN stretch vibrations of the proteins, respectively) as shown in Figure (10). The positions of these bands were close to that reported for native proteins. The FT-IR results indicate that the secondary structures of proteins were not affected as a 56 consequence of reaction with Ag+ ions or binding with AgNPs. The band at 1388.5 cm–1 is assigned to methylene scissoring vibration from the protein in the solution. This evidence suggests that the release of extracellular protein molecules from bacteria (fig.3d).

Growth level measurement of *Amaranthus blitum*:

The effect of nanoparticles in seedlings varies with different silver nanoparticle exposure (100,200 and 500µg concentrations) in *Amaranthus blitum*. A significant effect of AgNPs in the seedlings was observed at varying concentrations and respective growth in root and shoot length as shown in (Tab.1) based on different concentrations of nanoparticles. The seedlings showed a positive response towards AgNPs at 200mg concentration of nanoparticle treatment.

The level of growth of the seedlings was compared by measuring the growth of *Amaranthus blitum* at exposure of nanoparticles at varying concentrations in (Control, 100mg, 200mg and 500mg) samples. On the 18th day of plant growth, the seeds grown under nanoparticle exposure of 200µg concentration were found to show the highest growth of 3.9cm. (fig.9)

Estimation of chlorophyll in *Amaranthus blitum*:

Absorption spectroscopy was used to ensure that the samples of *Amaranthus blitum* (Control, NP 100, NP 200, NP 500) employed at different nanoparticle exposure contain varying concentrations of chlorophyll which have characteristic absorption bands at 663 and 645nm. The amount of Chlorophyll A present in the sample NP 200 was found to be 7.22mg. The amount of Chlorophyll B present in the sample NP 200 was found to be 15.09mg. The total chlorophyll content present in the sample NP 200 was found to be 22.29mg. (Tab.2). The sample of NP 200 was found to contain higher concentration of total Chlorophyll content by calculating the amount of Chlorophyll a, Chlorophyll b and total Chlorophyll content when compared to other samples which proves the higher concentration of chlorophyll in the NP 200 sample. (fig.10)

Estimation of protein in *Amaranthus blitum*:

The amount protein present in the germinated seedlings was found to vary at varying concentrations of silver nanoparticle exposure to *Amaranthus blitum* was studied under UV spectrophotometric analysis at 540nm. Using standard graph the amount of protein present in the plant was calculated. The varying amount of protein present in the germinated seedlings under different concentrations of nanoparticle exposure was identified by Lowry's method.

Amount of Protein in *Amaranthus blitum* (µg) = Concentration of Standard BSA × 100

Volume of test sample

The amount of protein present in the sample of NP 200 was found to be 170 µg. The NP 200 sample was found to contain comparatively high amount of protein compared to other nanoparticle exposed samples at different concentrations and less amount of protein when compared to the control sample which proves the higher concentration of protein in the control sample. (Fig.11)

ESTIMATION OF CARBOHYDRATES:

The amount of Carbohydrate in the germinated seedlings at different concentrations of nanoparticle exposure was identified by Anthrone method which proves the higher concentration of protein in the control sample.

Amount of Carbohydrate (µg) = Concentration of glucose × 100

Volume of test sample

The amount of carbohydrate present in the sample of NP 200 was found to be 02.092mg. The NP 200 sample was found to contain higher amount of Carbohydrate compared to other seedlings which proves that the seedling grown under nanoparticle exposure of 200mg was found to show higher concentration of Carbohydrate. (fig.12)

CYTOTOXICITY ASSAYS:

The bacterial strains of *Bacillus* sp. and synthesized nanoparticles from *Bacillus* sp. are easily available and possess less toxicity as compared to the conventional chemical fertilizers. Increasingly, study of their fate and impact in the normal cell lines is becoming important due to the disadvantages of the silver nanoparticle induced plant, being toxic to the cells. The cell viability can be compared between plant samples exposed to different nanoparticle concentrations by cytotoxicity assays. The purpose of this investigation was to evaluate potential toxicity involved in nanoparticle toxicity. The current study aimed to investigate the toxicity of nanoparticles in an in vitro model of chang liver cells. These cell lines have been well tested for their relevance for toxicity studies. MTT reduction in a dose dependent manner over a 24 hrs exposure duration. Cell survival was measured by means of the colorimetric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) assay. Viable cells were determining the concentration

required for the inhibition of viability (IC50) to be determined graphically. (fig.12). The effect of the samples on the proliferation of chang liver cells was expressed as the % of cell viability.

% cell viability = A540 of treated cells / A540 of control cells × 100%

CALCULATION:

The silver nanoparticles present in the samples showed more toxicity at higher doses of 100µg/ml. Overall, the data based on MTT assays suggest that Ag nanoparticle showed less toxicity at 10 µg/ml concentration, whereas Ag nanoparticle at 50 µg/ml was moderately toxic. Silver nanoparticle exposure at 100 µg /ml was found to be highly toxic when compared to other samples of varying nanoparticle concentrations. In the absence of Ag nanoparticle, displayed no toxicity. (fig.13) Dose dependent cytotoxicity was observed in AgNPs treated Chang Liver cells and the increase in concentration of AgNPs showed increased cytotoxicity in Chang Liver cells. Effect of biosynthesized AgNPs on growth of normal cell line (chang liver cell line) did not exhibit significant cytotoxicity at their lower concentration of 10 and 50µg/mL and it increases with its increased concentration, at 100 µg/mL. A large number of in vitro studies indicate that AgNPs are toxic to the mammalian cells.

LIST OF TABLES

Tab.1 GROWTH LEVEL MEASUREMENT of *Amaranthus blitum*:

CONCENTRATION OF NANOPARTICLE	MEASUREMENT OF GROWTH			
	.3rd day	6 h day	1 th day	18 th day
Control	3.1	36	39	41
100	3.0	34	36	38
200	3.1	37	41	46
500	3.3	35	39	32

Tab. 2 ESTIMATION OF CHLOROPHYLL in *Amaranthus blitum*:

	OPDICAL DENSITY	
CONCENTRATION OF NANOPARTICLE	**645nm**	**663nm**
Control	0.77	0.83
100	0.70	0.64
200	0.81	0.74
500	0.49	0.43

Tab. 2a ARNON'S METHOD OF CHLOROPHYLL ESTIMATION:

Sample	A663	A645	Chlorophyll a	Chlorophyll b	Total chlorophyll
Control	0.83	0.77	8.47	13.75	22.20
100	0.70	0.64	6.25	15.04	19.27
200	0.81	0.74	7.22	15.09	22.29
500	0.49	0.43	4.14	9.21	13.35

Tab. 3 ESTIMATION OF PROTEIN in *Amaranthus blitum*:

SAMPLES	OPTICAL DENSITY (660nm)	CONCENTRATION OF PROTEIN (µg)
CONTROL	0.23	220
100	0.15	140

200	0.18	170
500	0.13	120

Tab.4 ESTIMATION OF CARBOHYDRATES in *Amaranthus blitum*:

SAMPLE	OPTICAL DENSITY (660nm)	CONCENTRATION OF CARBOHYDRATE (mg)
CONTROL	1.34	2.061
100	0.94	1.446
200	1.36	2.092
500	0.87	1.338

Tab.5 CYTOTOXICITY ASSAY FOR SILVER NANOPARTICLE INDUCED *Amaranthus blitum IN CHANG LIVER CELL LINE*:

Tab .5a CONTROL SAMPLE:

S.No	Concentration (µg/ml)	Absorbance (540nm)	% cell Viability
	100	0.27	22.6
	50	0.69	57.9
	10	1.05	88.2
	Control cells	1.19	100

Tab.5b NP 100 SAMPLE:

S.No	Concentration (µg/ml)	Absorbance (540nm)	% cell Viability
	100	0.11	9.2
	50	0.41	34.4
	10	0.97	81.5
	Control cells	1.19	100

Tab.5c NP 200 sample:

S.No	Concentration (µg/ml)	Absorbance (540nm)	% cell viability
	100	0.07	5.8
	50	0.59	49.5
	10	0.96	80.6
	Control cells	1.19	100

Tab.5d NP 500 sample:

S.No	Concentration (µg/ml)	Absorbance (540nm)	% cell viability
	100	0.03	2.5
	50	0.31	26.0
	10	0.70	58.8

	Control cells	1.19	100

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Fig.1 NUTRIENT BROWTH WITH Bacillus sp.

Fig.2 NUTRIENT BROTH WITH $AgNO_3$ & Bacillus sp.

Before Incubation: **After incubation:**

Fig.3 CHARATERIZATION OF SILVER NANOPARTICLES:

Fig.3a UV visible spectroscopy analysis:

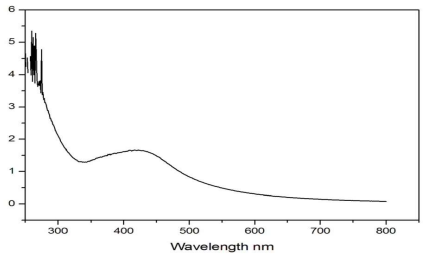
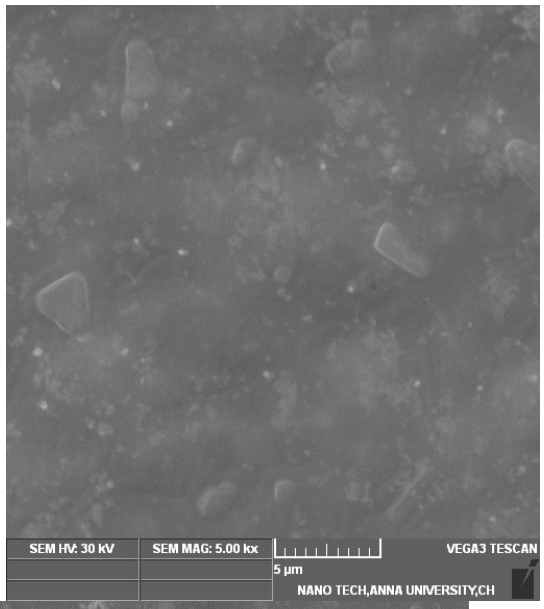
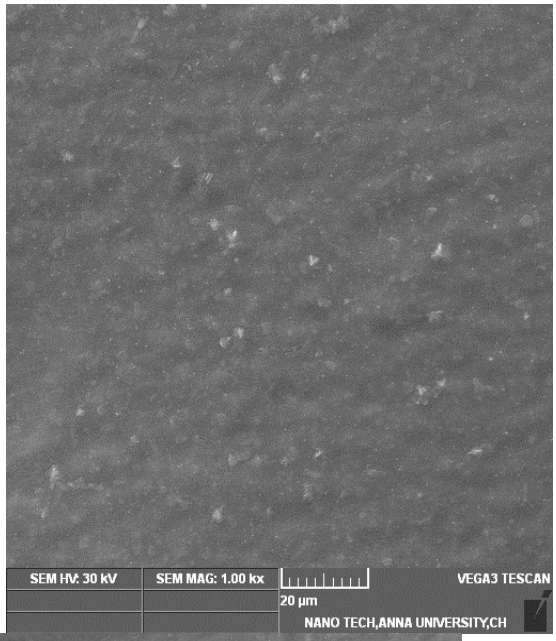


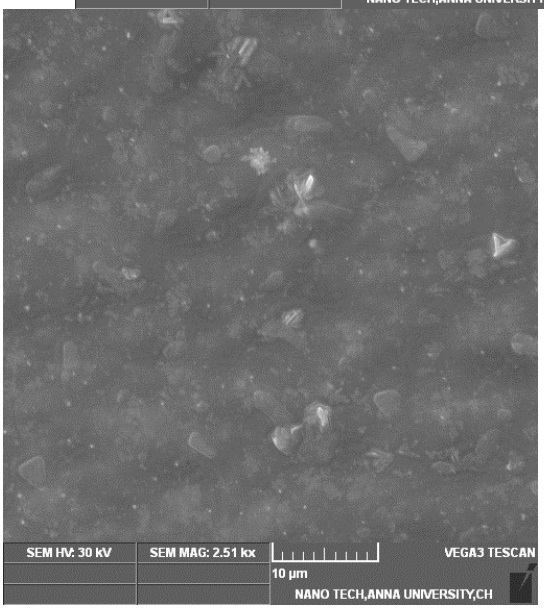
Fig.3b FTIR analysis:

Fig. 3c SEM ANALYSIS:

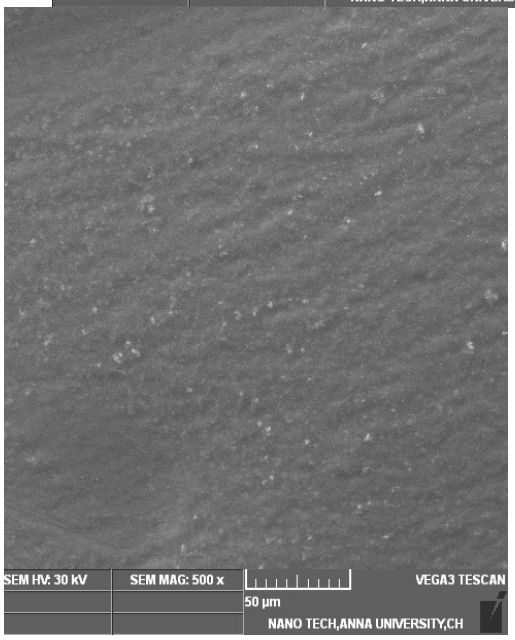
5µm magnification: **10µm magnification:**







20µm magnification:



50µm magnification:

Fig 3d XRD analysis:

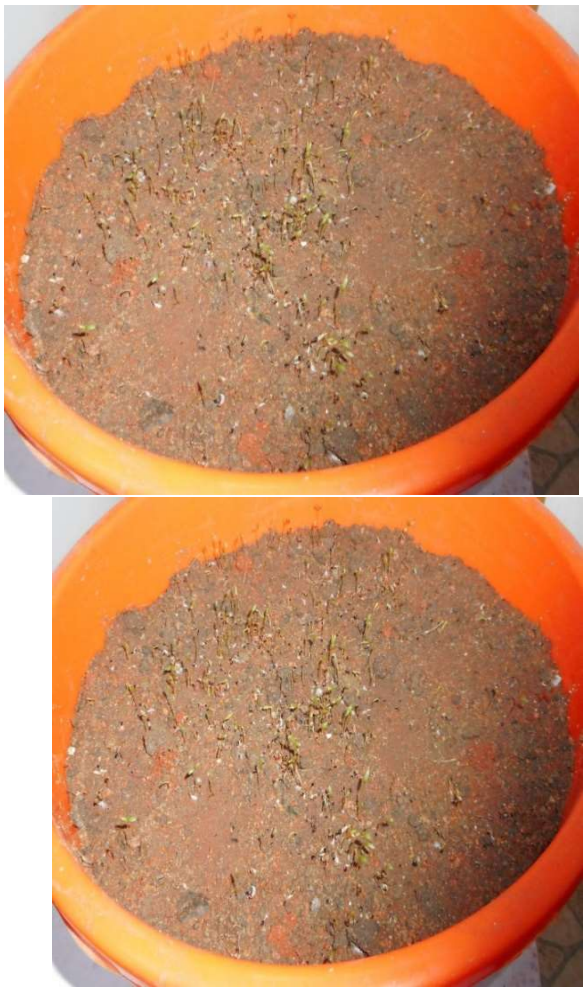
Fig.4 GROWTH OF *Amaranthus blitum* AT DIFFERENT CONCENTRATIONS OF NANOPARTICLE EXPOSURE:

Fig 4a DAY 1:

CONTROL: **100mg CONCENTRATION:**

200mg CONCENTRATION: **500mg CONCENTRATION:**

Fig. 4b Day 6: Control: **100mg CONCENTRATION:**



200mg CONCENTRATION: 500mg CONCENTRATION:

Fig.4c Day 12:
Control: 100mg CONCENTRATION:

200mg CONCENTRATION: **500mg CONCENTRATION:**

Fig 4d Day 18:
Control: **100mg CONCENTRATION:**

200mg CONCENTRATION: **500mg CONCENTRATION:**

Fig 5 ESITMATION OF CHLOROPHYLL in *Amaranthus blitum*:

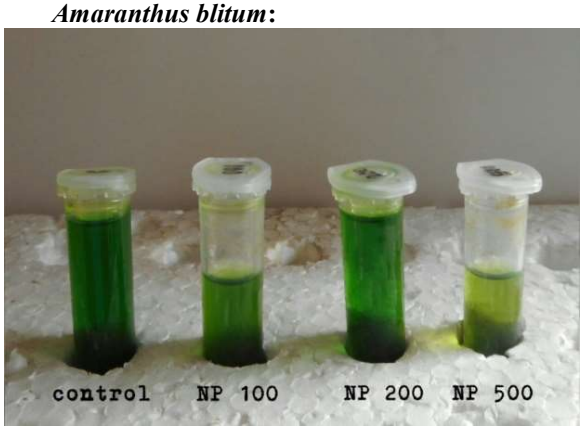
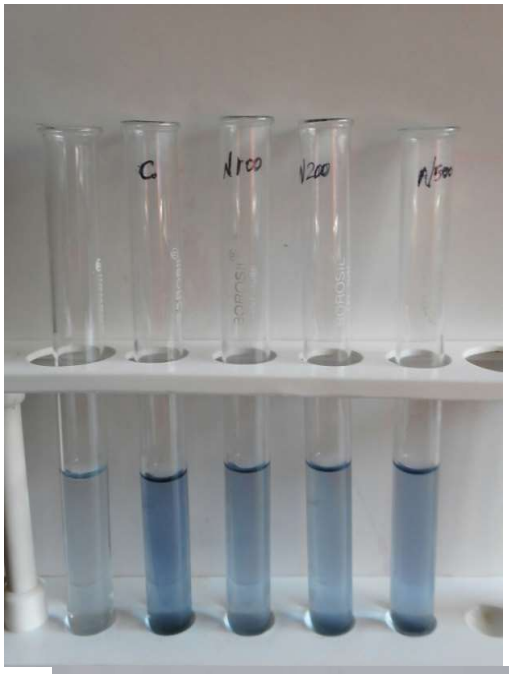


Fig.6 ESTIMATION OF PROTEIN *Amaranthus blitum*:

SAMPLE: **STANDARD:**



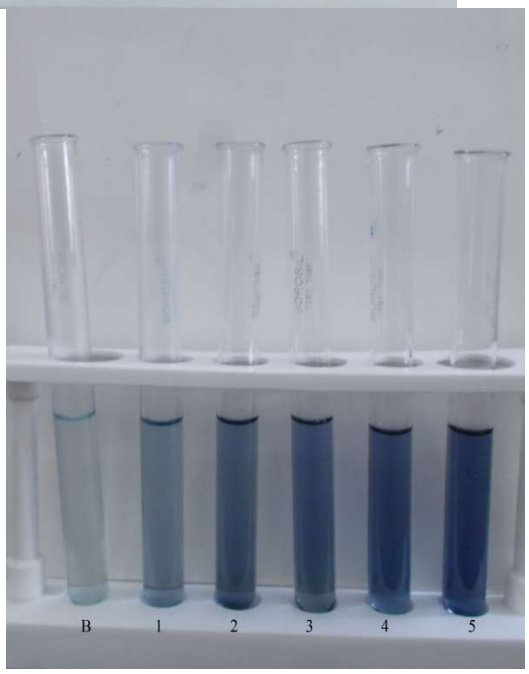
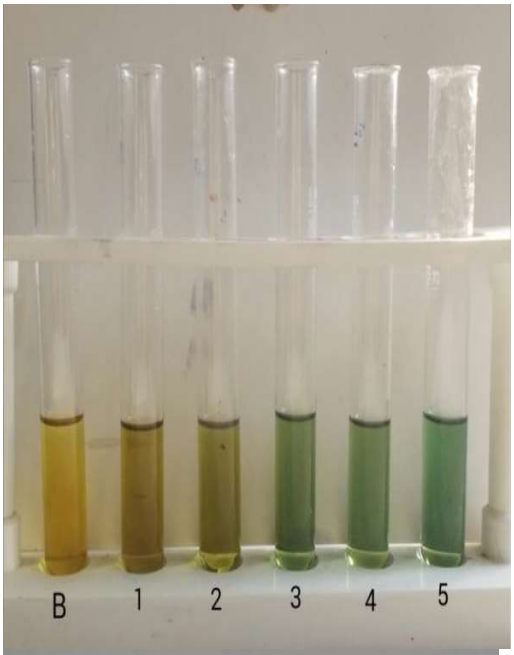


Fig.7 ESTIMATION OF CARBOHYDRATES in *Amaranthus blitum*:
SAMPLE: STANDARD:



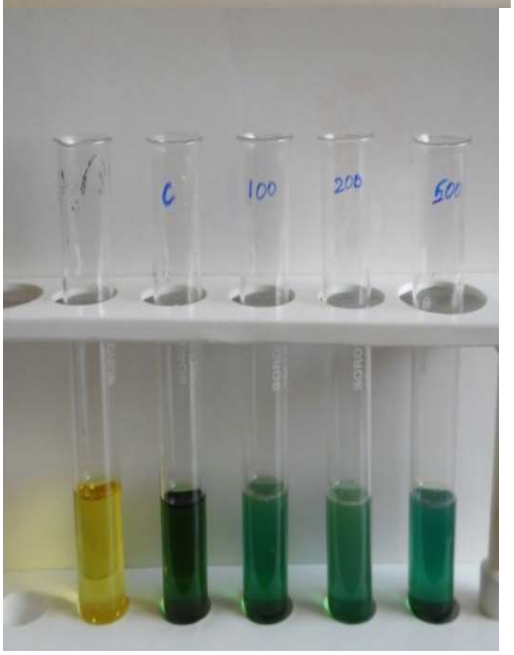
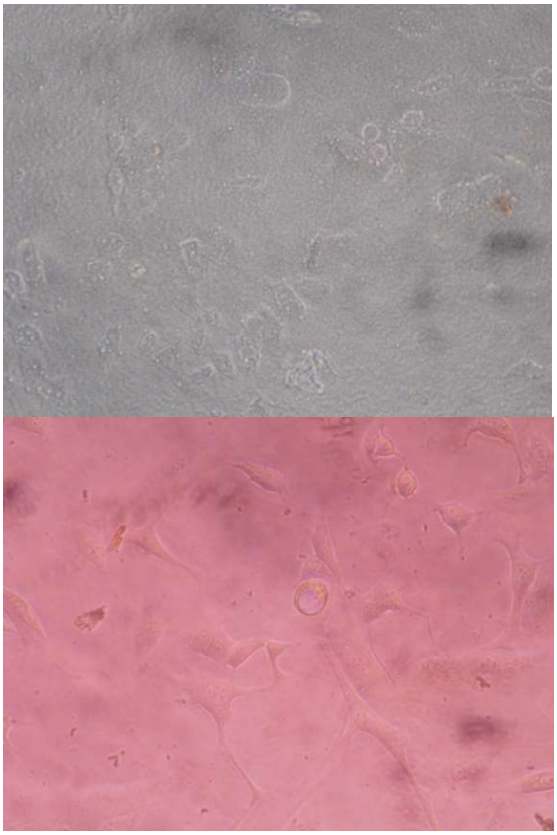


Fig.8 CYTOTOCICITY ASSAY FOR *Amaranthus blitum* IN CHANG LIVER CELL LINE:
Fig8.1 CONTROL SAMPLE:
100 µg concentration: 50 µg concentration:



10 µg concentration: Chang Liver control cells

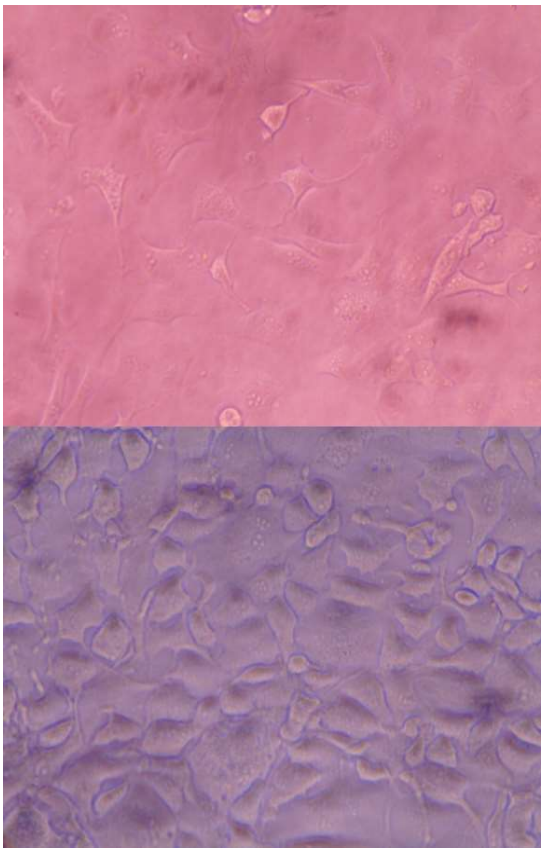
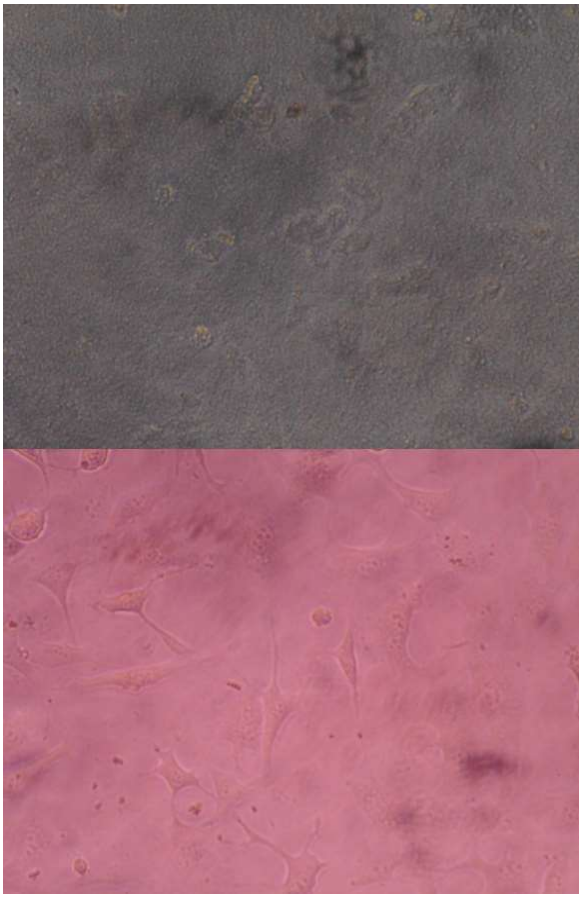


Fig 8.2 NP 100 sample:
100 µg concentration:
50 µg concentration:



10 µg concentration: **Chang Liver contol cells:**

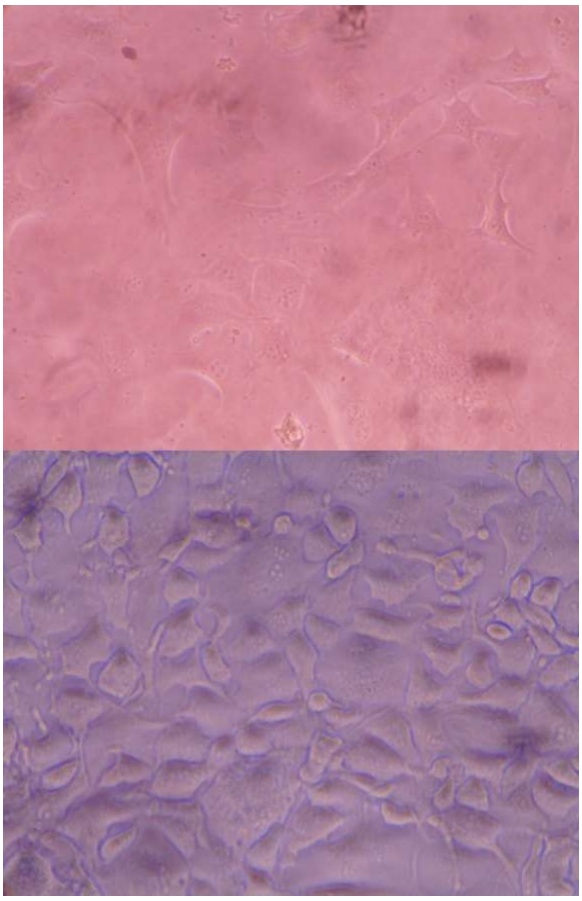
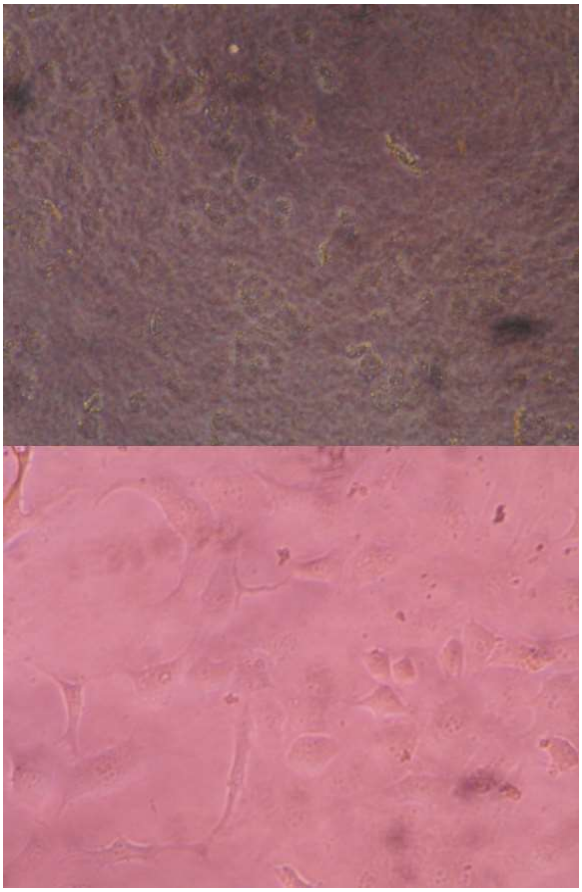


Fig.8.3 NP 200 SAMPLE:
100 µg concentration:
50 µg concentration:



10 µg concentration: **Chang Liver contol cells:**

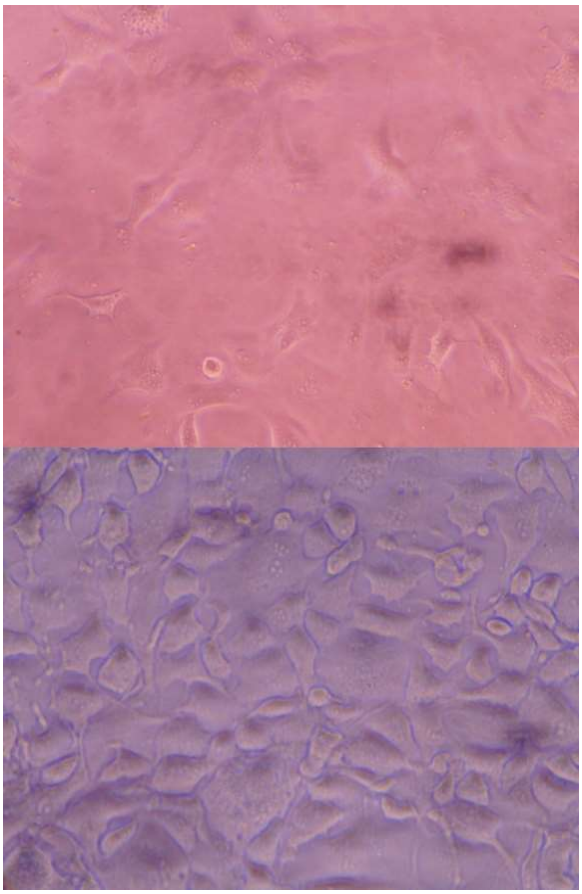
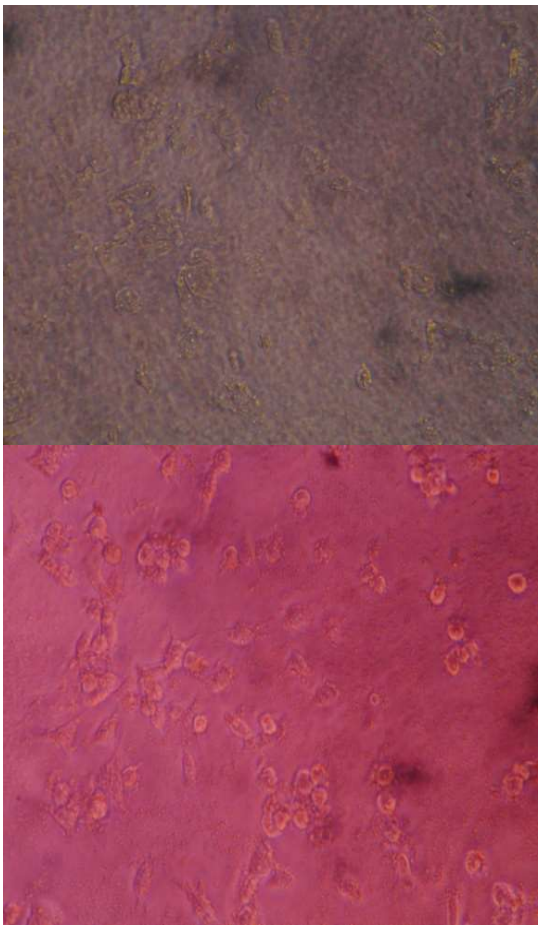


Fig. 8.4 NP 500 SAMPLE:
100 µg concentration:
50 µg concentration:



10 µg concentration:
Chang Liver contol cells:

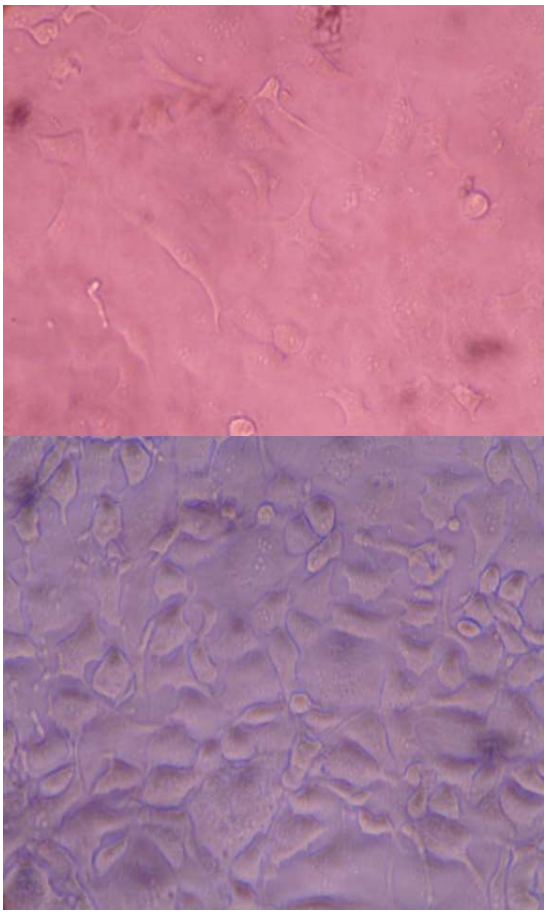
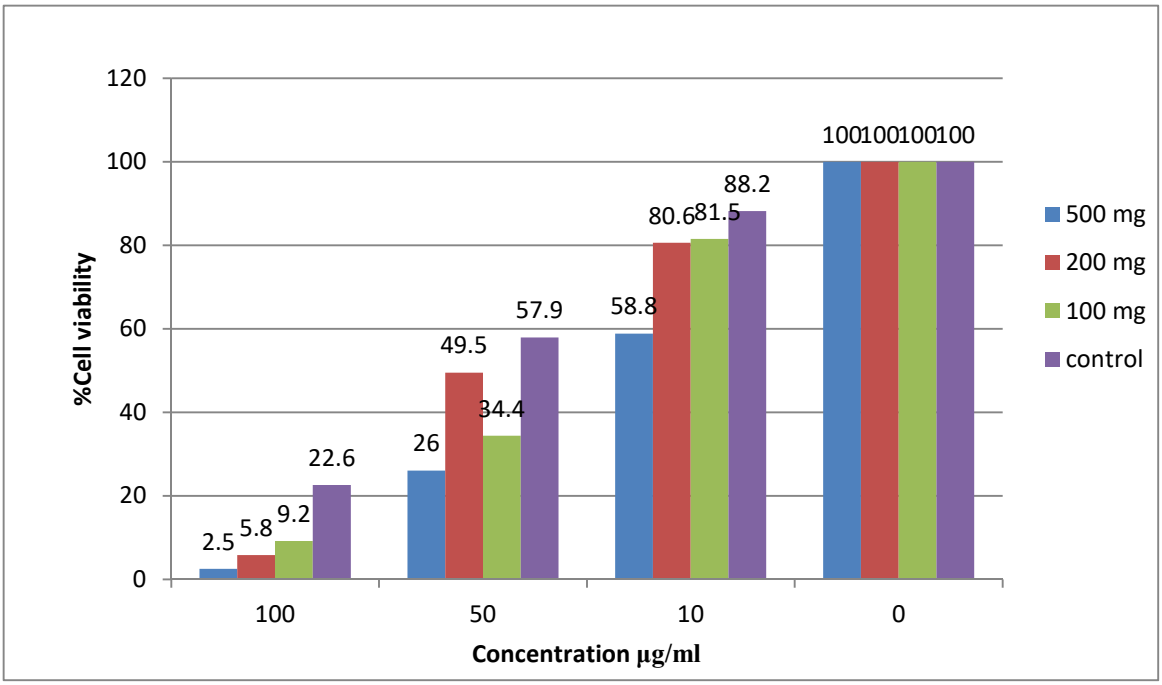


Fig.9 GROWTH LEVEL MEASUREMENT in *Amaranthus blitum*:
Fig 10ESTIMATION OF CHLOROPHYLL in *Amaranthus blitum*:

Fig.10 ESTIMATION OF PROTEIN in *Amaranthus blitum:*
Fig.11 ESTIMATION OF CARBOHYDRATE IN *Amaranthus blitum*:
Fig 12 PERCENTAGE OF CYTOTOXICITY IN SILVER NANOPARTICLE INDUCED *Amaranthus blitum*:



DISCUSSION

Atmospheric bacteria were grown on nutrient agar substrate containing 3.5 mM AgNO3 (Aldrich) under aerobic conditions at 30 C. A single colony was isolated. The isolated bacteria was identified as Bacillus sp. through their morphology and biochemical studies were conducted by (Mansour Amin *et al.,* 2015). Biological synthesis of AgNPs was carried out following Song et al. [25] method. Typically, 10 mL of aqueous extract was added to 190 mL of 1 mM aqueous silver nitrate solution for the reduction of Ag+ ions (Raman Sukirtha *et al.,*2011). Similar to the study by (Mansour Amin *et al.,* 2015), the bacterial species isolated from soil sample was added to 5 mL of nutrient broth and incubated at 35°C for 24 hours. The Erlenmeyer flasks with Bacillus sp. were incubated in a rotary shaker. The Erlenmeyer flask containing the supernatant mixed with 10mM of silver nitrate, were incubated for 48 hours for the biosynthesis of silver nanoparticles. The nanoparticle of silver nitrate was synthesized from biological source of Bacillus species and further proceeded for characterization by UV-visible determination, XRD analysis, FTIR analysis and SEM analysis.

The hydroponic plant of B. monnieri seeds were taken for the study the seeds were exposed to different concentrations of AgNPs and $AgNO_3$ (10 ppb, 100 ppb, 10 ppm and 100 ppm) suspended in water to study its effect on seed germination and another method of nanoparticle exposure to hydroponic culture where three groups of B. monnieri seedlings were raised in cotton beds and a week old uniform seedlings from each group is transferred to hydroponic system. Group 1 plants comprises of seedlings raised with 10 ppm of AgNPs supplemented nutrient medium (Hoagland's medium) while group 2 plants were supplemented with 10 ppm of $AgNO_3$ and group 3 seedling grown in normal nutrient medium served as control. Based on the seed germination results, 10 ppm was chosen for treatment in this study. The results of the seed germination study clearly revealed that AgNPs with various concentrations (10 ppb, 100 ppb, 10 ppm, 100 ppm) did not affect the seed germination while $AgNO_3$ treatment exhibited marked retardation with increasing concentration in the study conducted by (C. Krishnaraj et al.,).

Bulk exposure of nanosilver was compared with commercial silver in maize seeds (SiO2, MW 60.08, 40–150 mesh) for field study. The required greenhouse field was prepared in the area of 15 9 10 m of each plots containing sandy loam soil (pH 7.0 ± 0.5) in the agriculture land at Tiruchengode, Tamil Nadu, India. Experimental plots were prepared by adding nanosilver at the concentrations of 5, 10, 15, and 20 kg ha-1 (hereafter termed, respectively, as N5, N10, N15, and N20), bulk silver at 15 and 20 kg ha-1 (hereafter termed, respectively, as B15 and B20), and control (without silver source). Then, the plots were thoroughly plowed to spread the particles. Maize seeds (Zea mays L., TIP TOP) collected from M/s. Rasi seeds Pvt. Ltd, Attur, India, were washed twice with distilled water. Triplicate plot was prepared and then, 25 maize seeds were sown in each plot at a seed interval of 30 cm and appropriate temperature and humidity were maintained. higher chlorophyll content is achieved at N15 and N20 (0.045 and 0.047 lg mL-1, respectively) than other regimes of silica treatments. (Suriyaprabha R *et al*., 2012)

After the seedlings were grown in the four regimes of nanosilver and bulk silver amended soil, the changes in physiological parameters were observed. During this study, the seed germination percentage of each treatment plot was

evaluated. Further, the growth characteristics were measured in terms of stem height, number of shoots and roots, stem diameter, root length, and leaf area (third leaf) after 20 and 40 days of maize growth by collecting samples in a randomized block design (Suriyaprabha R *et al*., 2012). After 20 days, the growth characteristics of maize remains the same in all treated plots including micro silica treatments and plant does not show substantial increment with an increasing concentration of silica sources in the growth characteristics as the mentioned parameters especially stem height and leaf area have not differed significantly with each other

Similar procedures were followed in the growth of seeds using silver nanoparticles where the seeds of *Amaranthus blitum* were collected and germinated in fertile soil to allow seed germination. Experimental trays were prepared by adding nanosilver at the concentrations of 100,200 and 500 mg (respectively, as NP100, NP200 and NP500) and control (without nanosilver). The biologically synthesized silver nanoparticles from Bacillus species were exposed to the soil at varying concentrations (100,200,500mg) and a control soil with no nanoparticle exposure by the natural germination of seeds in soil where the soil to be taken in 4 individual trays with different concentration of nanoparticles by the surface exposure of nanoparticles to 200mg of seeds individually.

The effect of biosynthesized silver nanoparticle on seed germination was primarily studied by periodic growth level measurement. The seed grown under biosynthesized silver nanoparticle exposure at 200mg concentration was found to show better seed germination These results were contrary to the earlier investigations where the AgNPs nanoparticle exposure did not affect the growth of seedlings in the experimentation done by (C. Krishnaraj et al.,).The effect of chemically synthesized silver nitrate on plant metabolism were tested by phytochemical assays (Protein, Carbohydrate and total phenol content) and enzyme assays (protease, catalase) were further proceeded with morphological and anatomical analysis by Priyadarshini Sharma *et al.,*(2012). In a study conducted by (Suriyaprabha R *et al*., 2012), The total protein content of 20-day-old maize leaves was determined according to Bradford method using bovine serum albumin as the standard (Bradford 1976). Chlorophyll was extracted with 80 % acetone and the ratio of chlorophyll a to chlorophyll b in the leaf samples was determined spectrophotometrically (U-2900; Hitachi, Japan) (Arnon 1949). Similar to the previous study, the effect of biologically synthesized silver nanoparticle was tested by performing phytochemical tests of estimation of protein by Lowry"s method, estimation of chlorophyll a, chlorophyll b and total chlorophyll content by by the modified Arnon method. Leaf disks of 7 mm diameter were soaked in acetone and DMSO (1:1v/v) solution for 12 h. The Chla, Chlb and total chlorophyll concentrations (milligram per gram fw) in the leaf tissues were calculated and estimation of carbohydrates by Anthrone method. In this study the nanoparticle exposure to seedlings at 200mg concentration were about to show higher amount of protein, carbohydrates and chlorophyll compared to other nanoparticle concentrations.

In a study by (Kaliyamurthi Satyavani et al 2012), showing a simple, rapid and green synthesis of silver nanoparticles from *S.monoica* of size 31 *nm*, at a concentration of 500 *nM* had cytotoxic effects on Hep-2 tumor cells under *in vitro* conditions. Cell viability was evaluated by the MTT colorimetric technique with slight modification. Briefly, in each well 200 *µl* of MTT [3-(4, 5-dimetheylthiazol-2)-2, 5 diphenyl tetrazolium bromide] without phenol red, yellowish in color solution (5 *mg/ml* in PBS), was added to each well. The plates were incubated for 6-7 *hr* in 5% CO_2 incubator for reduction of MTT by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. After five *hr* of treatment, the silver nanoparticles were found to be cytotoxic to tumour cells at a concentration of 500 *nM* and higher. Silver nanoparticles at 500 *nM* decreased the viability of Hep-2 cells to 50 % of the initial level. Longer exposures resulted in additional toxicity to the cells. The resulting intracellular purple formazon solubilized the MTT crystals by adding and quantified by spectrophotometric mean and then the supernatants were removed.

In a study of invitro cytotoxicity of sgreen synthesized silver nanoparticle by (Raju Vivek et al., 2012) MCF-7 and HBL cells were maintained in DMEM and McCoys 5a medium, supplemented with non-essential amino acids. Cells were cultured at 37 ◦C in a humidified atmosphere containing 5% CO2 in a CO2 incubator. Cells were cultured and □1× 104 cells/wells were seeded into 96 well tissue culture plates and incubated for 48 h. Both MCF-7 and HBL 100 cells were treated with series of 10–100 _g/mL concentrations of green synthesized AgNPs. The treated cells were incubated for 24 h and 48 h for cytotoxicity analysis. The cells were then subjected for MTT assay. The stock concentration (5 mg/mL) of MTT-3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide, a yellow tetrazole) was prepared and MTT was added in each AgNPs treated wells and incubated for 4 hrs. Purple color formazone crystals were observed and these crystals were dissolved with dimethyl sulphoxide (DMSO), and read at 620 nm in a multi well ELISA plate reader (Thermo, Multiskan). OD value was subjected to sort out percentage of viability.

The dose dependent cytotoxicity was observed in biosynthesized AgNPs treated MCF-7 cells. Fifty percentage of cell death, which determines the inhibitory concentration (IC50) value of biosynthesized AgNPs against MCF-7 cells holds at 50 µg/mL in 24 h and 30 µg/mL in 48 h. However the cytotoxicity effect of biosynthesized AgNPs against HBL-100 did not exhibit significant cytotoxicity at lower concentration and cytotoxicity increases with increasing concentration by 80

µg/mL in 24 h and 60 µg/mL in 48 hrs. A large number of in vitro studies indicated that the AgNPs are toxic to the mammalian cells. Indeed, our results also provide conclusive evidence for cytotoxic effect of biosynthesized AgNPs against breast cancer MCF-7 cell line compared with HBL-100 normal breast cell line. (Raju Vivek et al., 2012).

The report of cytotoxicity of biosynthesized silver nanoparticles by (Raman Sukirtha., et al 2011) on dose dependent cytotoxicity was observed in AgNPs treated HeLa cells and the increase in concentration of AgNPs showed increased cytotoxicity in HeLa cells. Fifty percent of cell death which determines the LD50 value of biosynthesized AgNPs against HeLa cells holds at 300 µg/mL concentration. Effect of biosynthesized AgNPs and 5-FU on growth of normal cell line (HBL 100) did not exhibit significant cytotoxicity at their lower concentration and it increases with its increased concentration, at 750 µg/mL and 1000 µg/100 mM, respectively. A large number of in vitro studies indicate that AgNPs are toxic to the mammalian cells.

The current study aimed to investigate the toxicity of nanoparticles in an in vitro model derived from rat liver cells BRL 3A. These cell lines have been well characterized for their relevance for toxicity studies. MTT reduction in a dose dependent manner over a 24 hrs exposure duration. However, the nanomaterials showed more toxicity at higher doses of 500mg/ml. Cell survival was measured by means of the colorimetric MTT assay. Overall, the data based on MTT assays suggest that Ag showed less toxicity at 10 mg/ml concentration, whereas Ag nanoparticle at 50 mg/ml was moderately toxic. Ag nanoparticle exposure at 100 mg /ml was found to be highly toxic when compared to other nanoparticle concentrations. In the absence of Ag nanoparticle, displayed no toxicity. Similar to the results of previous experiments conducted, the final cytotoxicity tests to be carried out by MTT assay in Chang liver cells where the nanoparticle present in the germinated seedlings affects the cell viability by exposure of samples (control, NP 100, NP 200, NP 500) at different concentrations. Dose dependent cytotoxicity was observed in AgNPs treated BRL 3A cells and the increase in concentration of AgNPs showed increased cytotoxicity in Chang Liver cells. Effect of biosynthesized AgNPs on growth of normal cell line (Chang Liver cell line) did not exhibit significant cytotoxicity at their lower concentration of 10 and 50µg/mL and it increases with its increased concentration, at 100 µg/mL. A large number of in vitro studies indicate that AgNPs are toxic to the mammalian cells.

SUMMARY AND CONCLUSION

As being non-toxic and environment friendly, the green route of nanoparticle biosynthesis from *Bacillus species* proving its dominance over conventional and hazardous methods of using chemical fertilizers on plants in the present study. Main significance of the present work is the comparison of the different concentrations of bio-synthesized silver nanoparticles to be induced to a plant and concluding with the best concentration of silver nanoparticle exposure to the plant to be effective for enhancing the growth of the plant and showing better results in its invitro cytotoxicity studies. The surface exposure of silver nanoparticle concentration of 200mg to the plant was found to show better results in phytochemical and cytotoxicity studies in the plant of *Amaranthus blitum*. The biologically synthesized AgNPs interaction on *Amaranthus blitum* mimics mild stress condition on exposure of higher concentration of nanoparticle affected samples in chang liver cell line. Future aspects of this study of AgNPs application to control several plant diseases by the mild stress produced, to be beneficial to plants in protecting them against pathogen attacks and disease control and mandates further study in the future.

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