



# Studies on Nano Toxicity Effect of Gold Nanoparticles on *M. incognita* and Tomato Plants Growth and Development

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## Abstract

Plant parasitic nematodes are one of world major agriculture pest, causing in excess of 157 billion dollars in worldwide damage annually. This study has provided evidence that gold nanoparticles have great utility for management of root knot nematodes in tomato crop. Effect of gold nanoparticles on *M. incognita* J2 was remarkable under the direct exposure in water, after three hours of incubation of *M. incognita* with GNPs showed the 100% mortality. Lesser survival rate of *M. incognita* in soil treatment showed strong nematicidal effect of gold nanoparticles. Subsequently the pot experiment had shown the beneficial effects of gold nanoparticles for intensively managing the root knot nematode. Pot experiment not only showed us that GNPs were lethal to root knot nematodes were also induces growth of tomato plants and didn't have any kind of negative impact of plant growth. In our study GNPs were found to be safe and lethal to *M. incognita*.

**Keywords:** *M. incognita*; Gold nanoparticles; Toxicity

## Introduction

Biogold nanoparticles synthesized by microbes are result of various molecular strategies to overcome the metal stress by decreasing redox state of metal electron shuttle which can be extracellular or intracellular, leading to conversion of gold metal ions to nanoparticles of defined shape and size [1]. The biosynthetic approach is particularly significant over the chemical synthesis leading to generation of toxic substances and expenditure of heavy metal and the nanoparticles have a tendency to clump together and are rendered useless nanoparticles produced by physical method are unstable and tend to agglomerate [2,3]. Advantage of nanoparticles synthesized by various living systems is a coating with peptide or protein which tends to uniform charge distribution all over the surface of nanometal resulting in repulsion between them [4]. Thus bio-gold nanoparticles are extremely stable even after months. Bacteria have utmost ability to pop in metal ions and conglomerate within cell without any harm. Biogold nanoparticles were first synthesized by Beveridge and Murry [5] by *Bacillus subtilis* followed by Nair et al. [6], who investigated lactic acid bacteria leading to synthesis of 20 nm to 200 nm and are called nanocrystals. In present study a bacterial isolate *Bacillus licheniformis* strain GPI-2 was isolated from pebbles samples of a local gold mine near Khaltunala latitude (31.21515044oN), which synthesized gold nanoparticles of 20 nm to 35 nm size and hexagonal as well spherical shape. This study was undertaken to evaluate the efficiency of gold nanoparticles synthesized by *Bacillus licheniformis* GPI-2 to control significant plant root nematode (*Meloidogyne incognita*).

*Meloidogyne incognita* is a nematode belonging to family Heteroderidae and is commonly known as root-knot nematode as it prefers to attack the root of its host plant. It has worldwide distribution and numerous hosts. When *M. incognita* attacks the roots of host plants, it sets up a feeding location, where it deforms the normal root cells and establishes giant cells. The roots become gnarled or nodulated, forming galls, hence the term "root-knot" nematode (Pline et al., 1988).

Tomato (*Solanum lycopersicum* L) is an important vegetable crop, and its cultivation occurs worldwide. Yield loss due to root-knot nematodes (*Meloidogyne spp.*) on tomato range from 40% to 46% in India [7]. Management of *Meloidogyne incognita* is carried out by methods such as soil fumigation, nematicide seed treatments, postemergence nematicide application, and cultivars partially resistant to *M. incognita*. Tomato Plants infected with *Meloidogyne spp.* show typical symptoms of root galling. Keeping this in view present study was carried out to evaluate the nematicidal effect of biogold nanoparticles on *M. incognita*.

## OPEN ACCESS

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**Table 1:** Biochemical tests of the isolated strain *Bacillus licheniformis* GPI-2.

Positive tests	Negative tests
Arginine dihydrolase	Lysine decarboxylase
Hydrolysis of esculin	Ornithine decarboxylase
Beta galactosidase	Tryptophan
Phenyl alanine deaminated	Deaminase, hydrolysis of urea,
Degradation of tyrosine acid production from the following	Degradation of tyrosine acid production from the following were found to be negative
Glycerol	Acid production from D and L fucose
Salicine	Methyl beta xyloside
Starch	D arabinose
Glycogen	D and L arabitol
Lactose	Adonitol
D mannose	Sorbose
Maltose	Lsyxose
Ribose	
Sorbitol	

## Material and Methods

### Isolation of bacterial isolate

The bacterial isolate used in this study was isolated from a gold mine of Khaltunala using serial dilution technique in nutrient agar after incubation at 37°C for 48 hrs. The bacteria were characterized morphologically, and biochemically and finally molecular using 16S rDNA technology [8]. Qualitative determination of gold nanoparticles synthesizing ability of *Bacillus licheniformis* was determined which was depicted by color change from pale yellow to red wine color.

### *In vitro* synthesis of gold nanoparticles by indigenous *Bacillus licheniformis* strain GPI-2

Extracellular biosynthesis of gold nanoparticles was carried out using supernatant of *Bacillus licheniformis* strain GPI-2, treated with 1 mm gold chloride solution followed incubation at 37°C and to achieve maximum gold nanoparticles activity, time range of 0 to 240 hrs was investigated.

### Characterization of gold nanoparticles was carried out using *Bacillus licheniformis* strain GPI-2

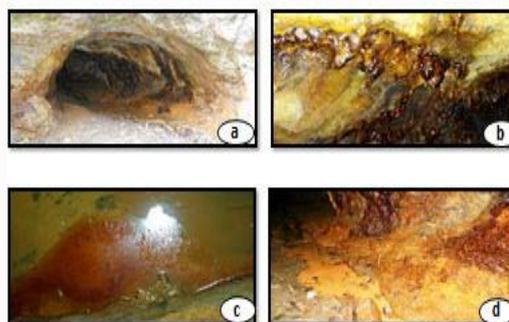
**UV-vis spectroscopy:** Biosynthesis of gold nanoparticles indicated via visual observation of color change of the culture filtrate and confirmed by UV visible spectroscopy.

**Fourier transform infrared (FTIR):** Microcup was washed with 100% absolute ethanol. 10 µl samples was filled in a 2 mm internal diameter microcup and loaded onto the FTIR set at 26°C ± 1°C. The samples were scanned in the range of 4,000 to 400 cm<sup>-1</sup> using a Fourier transforms infrared spectrometer (Thermo Nicolet Model 6,700, Waltham, MA, USA). The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

**Transmission electron microscope:** A drop of the sample was applied to a carbon coated copper grid. After about 1 min, the excess solution was removed using blotting paper and the grid was air dried before analysis.

### Direct vulnerability of *Meloidogyne incognita*

**Germination of tomato seeds:** To study the nematicidal effect

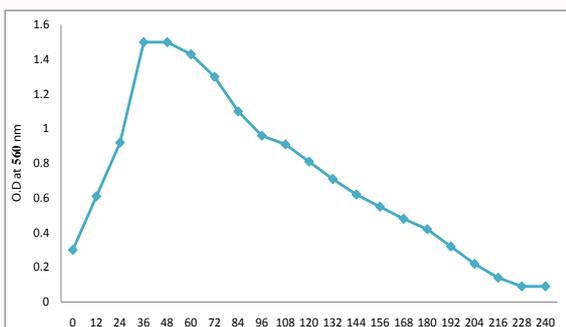


**Figure 1:** Different sites of samples collection of gold mine Khaltunala.

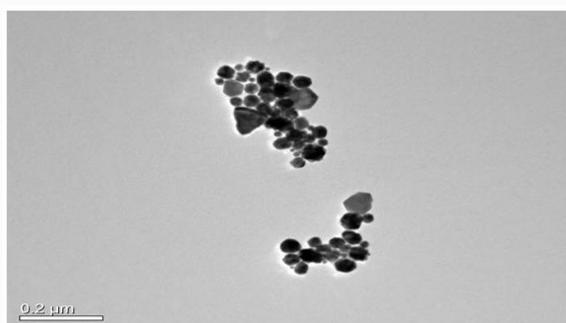


**Figure 2:** Biosynthesis of gold nanoparticles by *Bacillus licheniformis* GPI-2.

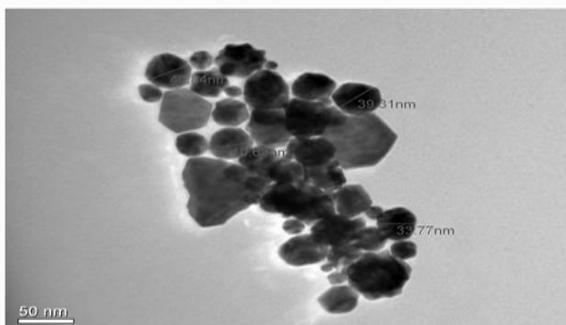
the biogold GNPs prepared as above *Solanum lycopersicum* were prepared from departmental of vegetable science DR YSP UHF Nauni. Thus seeds were shown in pot tray containing autoclaved sand and top soil (1:2) for germination. The seedlings after reaching two leaf stages were transplanted into plastic pots containing similar mixture as above. In this manner 60 seedlings were transplanted into 60 pots and these were maintained in green house to be used for further bioassay of nematode egg hatch and survival.



**Figure 3:** UV-Vis absorption spectra of *in vitro* synthesized gold nanoparticles after incubation of *Bacillus licheniformis* GPI-2 with 1 mM gold chloride for a time period of 0 to 240 hrs at pH: 6.8.



**Figure 4a:** Characterization of gold nanoparticles through transmission electron microscope showing the different morphology of gold nanoparticles.



**Figure 4b:** TEM image of gold nanoparticles showing different size of gold nanoparticles.

**Meloidogyne incognita:** Culture of *Meloidogyne incognita* was maintained on the tomato plants. They were maintained as a greenhouse stock culture on tomato cv. Rachna. Infected plants were uprooted, and the entire root system was dipped in water and washed gently to remove adhering soil. Egg masses of *M. incognita* were collected using forceps, disinfected with a 0.4% solution of sodium hypochlorite for three minutes then rinsed three times with sterilized water. Egg masses were placed in an autoclaved tissue grinder and gently crushed, then pipetted onto an autoclaved 25  $\mu\text{m}$  aperture sieve and rinsed with sterilized water. A subset of the collected eggs was used for the egg hatch bioassay whilst the remainder was incubated at 25°C until hatched in order to obtain J2. The fresh nematode inoculum (2.00 J2 ml<sup>-1</sup>) was used for *in vitro* and pot experiments.

Vulnerability of *M. incognita* to gold nanoparticles in water J2 of *M. Incognita* isolated from tomato plants were used for this direct

vulnerability assay to gold nanoparticles was done to get insight of nematocidal activity of gold nanoparticles. 30 nematodes were added to 3 ml of solution containing 0,100,200,300,400,500  $\mu\text{l}$  of colloidal solution of GNPs with five replication treatments and incubated at room temperature 25°C. Nematode mortality rate was measured with inverted microscope. After every 30 mins samples were checked for the mortality and their rate was recorded to determine the effective dose required for affecting nematodes. Healthy nematodes were defined as those were curled where as vulnerable or unhealthy nematodes were defined as those that appeared stiff or straight bodies.

### Soil treatment with gold nanoparticles

Soil was inoculated with *M. incognita*. The water saturation level of the 50 cm<sup>3</sup> soil sample was predetermined to be 25 ml. The total was homogenized divided into 50 cm<sup>3</sup>, placed into plastic container and then saturated with 25 ml. gold nanoparticles solution at 0,300,600,900,1200,1500  $\mu\text{l}$ . The samples were arranged in a completely randomized design with five replicates and incubated at room temperature for one to ten days. After the designated exposure time nematodes were extracted after from samples using Baermann tray system. After 48 hrs submergence in water, the samples were then poured into sieve filtered *M. incognita* were counted using an inverted compound microscope.

### Pot plant experiment

Pot plant experiment with gold nanoparticles was carried out. Pots containing soil which was already infected with *M. incognita* culture and seedling of tomato were transplanted into pots and these pots were again inoculated with fresh culture of *M. incognita* followed by application 0  $\mu\text{l}$  to 1500  $\mu\text{l}$  colloidal solution of GNPs to the pot plants. Plants wilting and others symptoms for diseases were checked and plant shoot lengths were recorded to effect of GNPs on plants physiological processes and growth parameters. Treatments varied from C, T-1,T-2,T-3,T-4 and T-5 i.e 0,300, 600, 900, 1200,1500  $\mu\text{l}$  of biogold nanoparticles synthesized by *Bacillus licheniformis* strain GPI-2.

### Estimation of photosynthetic pigments

Photosynthetic pigments (chlorophyll a) in leaves were assayed according to Hiscox and Israelstam [9]. The extraction was made from 100 mg of fresh sample in acetone (80%) in the dark at the room temperature and was measured with a UV/VIS spectrophotometer (Shimadzu UV-160, Kyoto, Japan) ( Salem and Amari, 2013).

### Measurements of total leaf conductance and transpiration rate

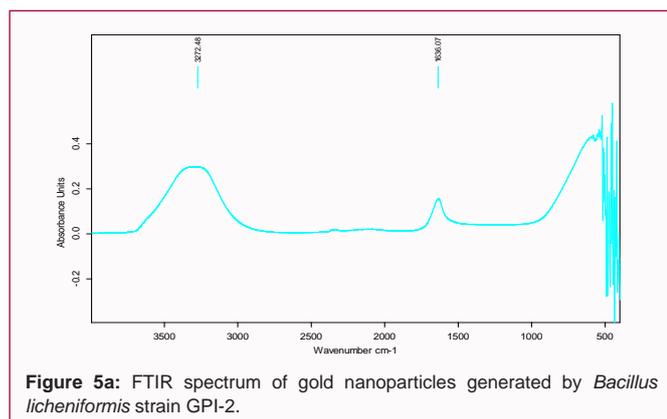
Total leaf conductance and transpiration rate of the tomato leaves were measured using LiCor, 6400XT, Lincoln, NE, USA.

## Results

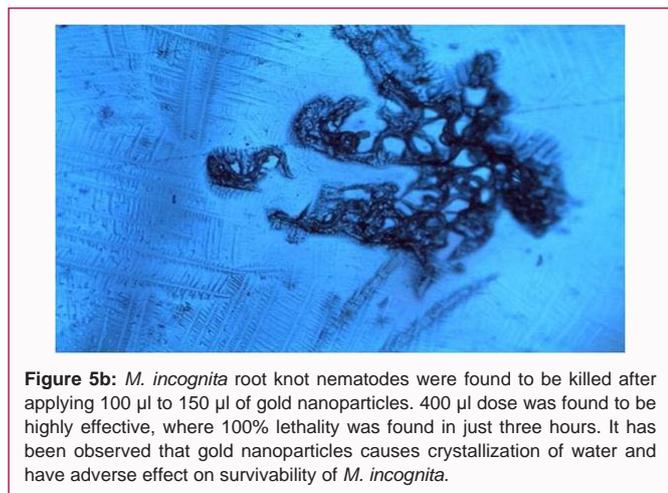
Isolation of gold nanoparticles synthesizing bacteria was carried out from different samples viz. yellow soil, pebbles, biofilm and stalagmites collected from a local gold mine using nutrient agar medium at 37°C (Figure 1). This was identified as *Bacillus licheniformis* GPI-2 after morphological, biochemical and molecular characterization using 16S rDNA technologies.

### *In vitro* synthesis of gold nanoparticles by indigenous *Bacillus licheniformis* strain GPI-2

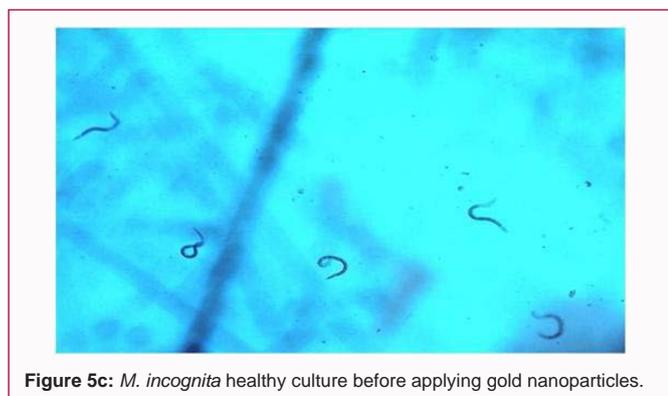
Extracellular biosynthesis of gold nanoparticles was carried out using supernatant of *Bacillus licheniformis* strain GPI-2, treated with 1 mM gold chloride solution and incubated at 37°C for a time period



**Figure 5a:** FTIR spectrum of gold nanoparticles generated by *Bacillus licheniformis* strain GPI-2.



**Figure 5b:** *M. incognita* root knot nematodes were found to be killed after applying 100  $\mu$ l to 150  $\mu$ l of gold nanoparticles. 400  $\mu$ l dose was found to be highly effective, where 100% lethality was found in just three hours. It has been observed that gold nanoparticles causes crystallization of water and have adverse effect on survivability of *M. incognita*.



**Figure 5c:** *M. incognita* healthy culture before applying gold nanoparticles.

of 0 to 240 hrs. Biosynthesis absorption spectra of gold nanoparticles which was indicated by color change of solution from yellow to red wine (Figure 2) and was further confirmed spectrophotometrically. The gold nanoparticles synthesis activity increased with the increase in incubation period up to 36 hrs and removed stable up to 48 hrs (Figure 3) and the time of incubation course and increase in formation of gold nanoparticles took place up to 36 hrs and remained stable up to 48 hrs and then the values declined up to 240 hrs.

#### Characterization of *in vitro* synthesis of nanoparticles by *Bacillus licheniformis* strain GPI-2

The TEM image (Figure 4a and 4b) clearly showed discrete gold nanoparticles in the size range of 20 nm to 45 nm which were mostly triangular, irregular and hexagonal indicating that it was possible to synthesize gold particles of nano dimensions with satisfactory level

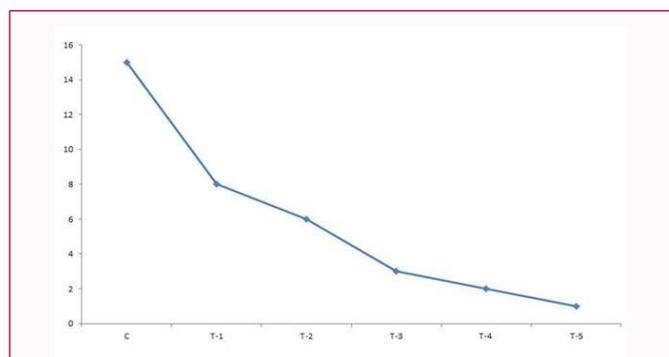
of monodispersity. FTIR measurements were carried out to identify the possible biomolecules protein responsible for the capping and efficient stabilization of the gold nanoparticles synthesized by *Bacillus licheniformis* GPI-2. FTIR spectrogram has shown presence of four peaks 3280.18, 2380.99, 2109.12 and 1636.32 (Figure 5a). High sensitivity to small variations in molecular geometry and hydrogen bonding patterns makes the amide I band uniquely useful for the analysis of protein secondary structural composition and conformational changes [10]. The FTIR spectra reveal the presence of different functional groups. Wave number between 3235  $\text{cm}^{-1}$  and 3280  $\text{cm}^{-1}$  indicates for hydrogen bond lengths between 2.69Å to 2.85Å. Alkynes C-C triple bond stretch is found at 2109  $\text{cm}^{-1}$ . Peak at 1636  $\text{cm}^{-1}$  corresponds to the N-H bend of primary amines due to carbonyl stretch. Amide I is most intensive absorption band in protein. It is primarily governed by the stretching vibration of the C=O (70% to 80%) and CN stretching groups (10% to 20%) frequency 1600  $\text{cm}^{-1}$  to 1700  $\text{cm}^{-1}$ . In the amide I region (1700  $\text{cm}^{-1}$  to 1600  $\text{cm}^{-1}$ ), each type of secondary structure gives rise to a somewhat different C=O stretching frequency due to unique molecular geometry and hydrogen bonding pattern. N-H Stretch of primary and secondary amines, amides. Amide A is with more than 95% due to N-H stretching vibration. This mode of vibration does not depend on the backbone conformation but is very sensitive to the strength of a hydrogen bond. Peak maximum around 1650  $\text{cm}^{-1}$  corresponds to proteins alpha helical structure. Half width of alpha helix band depends upon on the stability of the helix. When half width of about 15  $\text{cm}^{-1}$  then we have more stability of helix and transition free energy of more than 300 cal/ml. Amide I absorption is primarily determined by the backbone conformation and independent of the amino acid region, its hydrophilic or hydrophobic properties and charge. Average frequency of the main compounds is about 1629  $\text{cm}^{-1}$ . Arginine amino acid role was found at 1636  $\text{cm}^{-1}$  in gold nanoparticles synthesis through results of FTIR.

#### Direct vulnerability of *Meloidogyne incognita* to gold nanoparticles in cavity block experiment

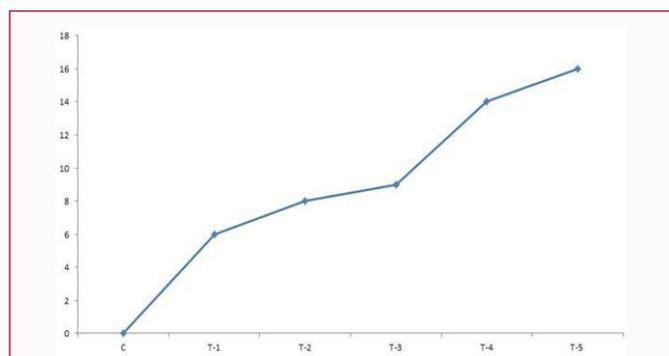
Direct vulnerability of *Meloidogyne incognita* to gold nanoparticles in water was evaluated and it was found that gold nanoparticles increased the mortality of *Meloidogyne incognita* effectively and efficiently. After one hour of application of GNPs mortality was initiated and 100% mortality has been achieved after three hours. It was observed that after application of gold nanoparticles crystallization of water occurred even at room temperature followed by clumping or aggregation of these nematodes leading to mortality. T-5 treatment consists of 500  $\mu$ l of biogold nanoparticles found to be highly effective to cause the maximum mortality where as in case of control no mortality has been found (Figure 5b and 5c).

#### Soil treatment with gold nanoparticles

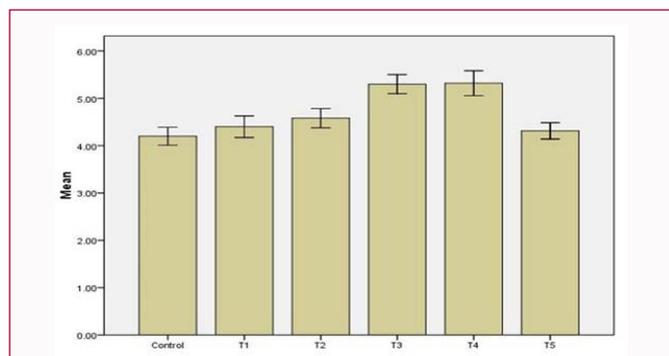
Enumeration of *M. incognita* extracted from soil samples was found to be reduced significantly treated with gold nanoparticles in repeated laboratory experiment. Exposure time of 6 hr to 8 hr did not affect the number of *M. incognita* and during this time there was no significant effect of GNPs on *M. incognita* population in soil. There was an interaction between exposure time and GNPs. Initially there was linear relation of time with number of unhealthy nematodes. As exposure time increased death of nematodes increased drastically. Number of *Meloidogyne incognita* extracted from soil samples were reduced when the soil samples were treated with GNPs with different concentration. 0, 300, 600, 900, 1200, 1500  $\mu$ l GNP's. Most effective treatments were 1200  $\mu$ l and 1500  $\mu$ l because they have maximum mortality rate (Figure 6). GNPs reduced the number of J2 recovered



**Figure 6:** Number of *Meloidogyne incognita* extracted from soils samples were reduced when the soil samples were treated with GNPs with different concentration. 0, 300, 600, 900, 1200, 1500  $\mu$ l GNP's. Most effective treatments were 1200  $\mu$ l and 1500  $\mu$ l because they have maximum mortality rate.



**Figure 7:** *M. incognita* mortality caused after foliar spray of gold nanoparticles application. It has been found that higher doses of GNPs were highly effective to prevent infection *M. incognita* to tomato plants at T<sub>4</sub>, T<sub>5</sub>.

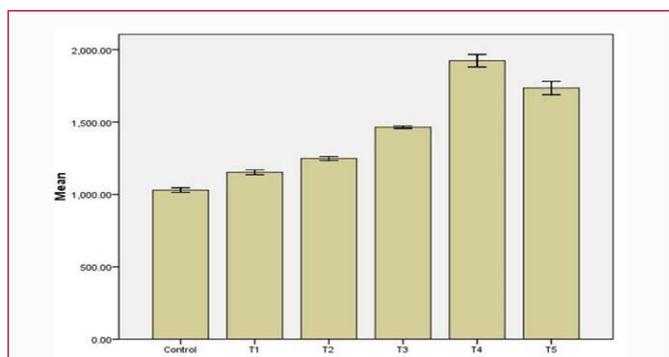


**Figure 8:** Gold nanoparticles mediated growth profile of tomato plants up to one week. Gold nanoparticles caused an increase in plants height, as compare to the untreated seedlings.

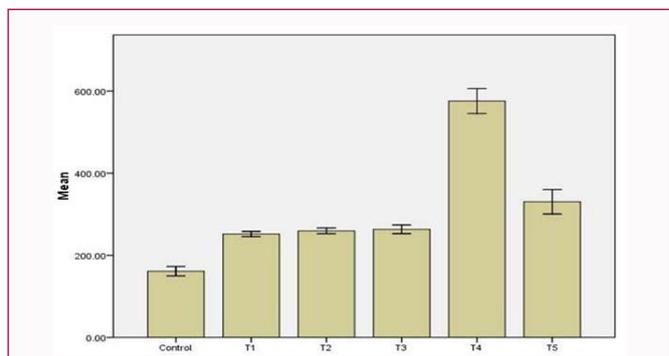
after 1<sup>st</sup> to 4<sup>th</sup> day of exposure of gold nanoparticles.

**Evaluation different concentration of the biogold nanoparticles on *M. incognita* infected tomato seedlings**

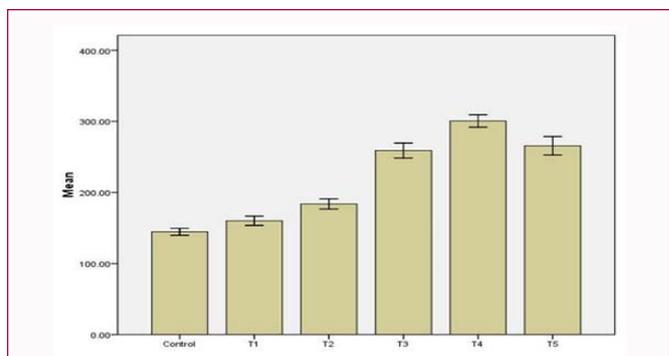
Tomato seedlings were raised from seeds purchased from vegetable department of UHF Nauni. Tomato seedlings were infected with live culture of *M. incognita* followed by application of different concentration of biogold in case of control no GNPs were applied but in T-1, T-2, T-3, T-4, T-5 GNPs were applied. Which ranges from 0  $\mu$ l to 1500  $\mu$ l of GNPs. Higher doses of GNPs were highly effective to prevent the infection *M. incognita* to tomato plants; it also found that gold nanoparticles have increased the shoot growth of tomato plants (Figure 7). GNPs application in pot plants has shown higher



**Figure 9:** Effect of gold nanoparticles on chlorophyll component on tomato plants, it has been identified that T<sub>4</sub> treated plants with 4 ml of GNPs showed higher chlorophyll content than others.



**Figure 10:** Effect of GNPs on transpiration rate, transpiration rate found to be higher in case of T<sub>4</sub> plants.



**Figure 11:** Effect of gold nanoparticles on conductance rate of tomato plants. T<sub>4</sub> plants have shown higher conductance than other treated as well control plants.

resistance to *M. incognita* infection whereas control plants found susceptible to *M. incognita* infection.

**Plant growth promoting**

In comparison to control plants, seeds were pre-soaked with various concentrations of gold nanoparticles; GNPs significantly increases the seed germination and increased shoot length (Figure 1 and 8). The degree of increase appeared to depend mainly on the concentration used. Maximum growth has been seen in case of T4 plants and it has been found that smaller size nanoparticles were more efficient for inducing growth; it may be due to GNPs penetrated roots of tomato plants that increase uptake of minerals and nutrient from soil. As roots are not protected by ant hard covering like seeds, penetration of gold nanoparticles are associated with particle size of GNPs provides much better results.

### Photosynthesis, transpiration, conductance

In our study we have observed that gold nanoparticles treated tomato plants have higher chlorophyll content as compared to untreated control plants and our other experiments like conductance and transpiration also have similar effect on tomato plants. In case of photosynthesis T4 treatment of GNPs provided best result among all treated and untreated plants (Figure 9-11).

### Effect of GNPs on nutrient uptake

Numerous studies have shown that uptake of nutrient elements is affected by size of nanoparticles and nature of species. Smaller the size, larger the surface area so their capacity to take more payload was increased movement of nutrients (macro elements and microelements) foster by these nanoparticles. Where they move inside roots they carry payload of different macro elements and microelements which significantly increase the growth of roots, shoots and make better availability of nutrient of plants than untreated control plant.

## Discussion

Among the ambience of natural resources, prokaryotic bacteria have received attention for synthesis of gold bionanoparticles. The reason for bacterial preference for gold nanoparticles synthesis is the relative ease of manipulation. Microbes in general and bacteria in particular are preferred because of ease of downstream processing [11]. Both gold thiosulfate and gold chloride have been reportedly used for the accumulation of gold by bacteria. The bacteria get killed, resulting in the release of organic molecules causing further precipitation of gold [12]. The reduction and precipitation of gold involves periplasmic hydrogenases or cytoplasmic hydrogenases. Cytochrome C3 could be the complementary mechanism of gold reduction in bacteria. In the present investigation, gold chloride ( $\text{HAuCl}_4$ ), which is very hygroscopic and highly soluble in water, has been used successfully for formation of gold bionanoparticles. In the present study, for bioprospecting of gold nanoparticles producing bacteria- local gold mine and four hot water springs of Himachal Pradesh were investigated. Nangia et al. [13] also described isolation of gold nanoparticles synthesizing bacteria *Stenotrophomonas maltophilia* from Singhbhum gold mines (Jharkhand). Birader and Lingappa [14] were also successful for isolation of gold nanoparticles synthesizing *Bacillus sp.* from Hatti region of Karnataka. Some strains of *Arthrobacter* genera with ability to synthesize gold nanoparticles have been isolated from basalt rock from Georgia [15], Correa-Llanten et al. [16] have stated the isolation of gold nanoparticles synthesizing *Geobacillus sp.* from Deception island, Antarctica.

Present investigation has provided evidence that gold nanoparticles have highest rate of mortality and were effective for management of root knot nematodes [17] reported J2 of *M. incognita* were exposed to AgNPs in water at 30 to 150  $\mu\text{l/ml}$ , 99% nematodes became inactive in 6 hrs. Taha et al. [18] evaluated the silver nanoparticles effect on EPN dependent on nano Ag, concentration and exposure time. They have found significantly effect on EPNs reproductivity at two concentration 500 ppm and 1000 ppm. Abdellatif et al. [19] reported effective control of root Knot nematodes *T. turbinata* and *U. lactuca* similar to chemical control in egg plant.

Taha evaluated nematicidal effect of silver nanoparticles on J2 *M. incognita* in laboratory and in screen house. When *M. incognita* population was exposed to AgNP in water at 20 ppm/ml, 40 ppm/ml, 200 ppm/ml, 500 ppm/ml and 1500 ppm/ml achieved 96.5% mortality after 72 hrs with 1500 ppm. Concentration of 200 ppm caused 52%

mortality at third day and 1500 ppm was found to be most effective dose while other doses were ineffective. Ardakani et al., 2016 studied effectiveness of AgNPs on *M. incognita* in case of tomato plants, dose of 800  $\text{mg/ml}^{-1}$ , 400  $\text{mg/ml}^{-1}$  and 200  $\text{mg/ml}^{-1}$  of AgNP were highly significant to immobility and to mortality of J2 *M. incognita* and  $\text{TiO}_2$  nanoparticles showed 4.3% and 2% mortality when applied 800  $\text{mg/ml}^{-1}$  and 400  $\text{mg/ml}^{-1}$  concentration. Abdellatif et al. [19] studied influence of silver nanoparticles on *M. javanica* reproduction and growth. They have observed that AgNPs treatment was equally effectiveness as chemical treatment (Vydate 24% L), resulted in reduction of egg-masses number per root system. Concentration of 17 mg, 100  $\text{mL}^{-1}$  of *U. lactuca* with silver nanoparticles most effective in reduction of *M. javanica* population (69.44% J2s in soil, number of females of *M. javanica* in roots reduced to 84.51% when treated with silver nanoparticles.

### Plant growth promoting

In the present investigation we have found that gold nanoparticles significantly increased the seed germination and increased shoot length of tomato seedlings in comparison to control plant 21.67% increase in the shoot length have been found in case of T4 treated plants and it has been found that smaller sized nanoparticles were more efficient for inducing growth. Jasim et al., 2016 investigated the influence of silver nanoparticle fenugreek (*Trigonella foenumgraceum* L) seedlings. They have assessed the effect of silver nanoparticles leaf number, root length, shoot length and met weight as well on the diosgenin content. Hojjat et al. [20] studied the influence of silver nanoparticles lentils seeds. They have found that seed germination index, root length, shoot length and other parameters were affected by silver nanoparticles. They have observed that accumulation and uptake of nanoparticles as dependent on the exposure concentration.

Yesmeen et al. [21] investigated the effect of silver, copper and iron nanoparticles on germination and seedling vigour index of wheat seeds. They have concluded that seeds treatment and incubation time affect the seedling growth such as root and shoot growth. Seedling vigour increased when seeds were soaked in nanoparticles and incubated in distilled water. Pandey et al. [22] studied toxicity of silver nanoparticles on *Brassica juncea* var. varuna. Silver nanoparticles induce slight increase in root; shoot length, chlorophyll content and protein content. In our study we have observed that gold nanoparticles treated tomato plants have higher chlorophyll content as compared to untreated control plants and our other experiments like conductance and transpiration also have similar effect on tomato plants. In case of photosynthesis  $T_4$  treatment of GNPs provided best result among all treated and untreated plants. Nubia et al. [23] examined the impact of silver nanoparticles on the physiology and nutritional quality of radish sprout and suggested that nAg could significantly affect the growth, nutrient content and macromolecules conformation in radish sprouts. when the seedling were exposed to 500  $\text{mg/L}$  had 901 mg AG/kg dry weight and significantly less Ca, Mg, B, Cu, and Zn compared with control. Their also revealed that changes occur in lipids, proteins and structural components of plants cell such as lignin, pectin and cellulose.

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