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ARTICLE

# Effects of Green Silver Nanoparticles on Soil Quality and Induced Germination: A Future Alternative Fertilizer or Environmental Toxicant?

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ABSTRACT

Silver nanoparticles (AgNP) synthesized from It influenced the inherent soil properties like bulk density (BD), water holding capacity (WHC), available N, P, K, urease, phosphatase activity and TOC. The apparent increment WHC, N, P, K, urease, and phosphatase in soil were observed whereas reduction of BD was noticed. Due to application of nanosolutions the pH of the soil shifted towards neutrality from 0 to 60 days. Moreover, they also did not have any toxicity upon plant as well as earthworm ecosystem.

## 1. Introduction

Silver nanoparticles (AgNP) are among the most widely utilized engineered nanoparticles (Anjum et al., 2013)[2]. Since long, silver is known to show severe toxicity to numerous types of microorganisms; and, silver in nano form has been reported to be more lethal to microorganisms as compared to other forms (Morones et al., 2005). As a result, potential antimicrobial activities of AgNPs against all classes of microorganisms (even at very low concentrations) endorse their notable applicability in biomedical research. In recent past, few workers have observed promising role of AgNPs as effective and alternative pesticides to the conventionally applied synthetic organic compounds (Jagtap et al., 2013; Babu et al., 2014)[12]. In view of using AgNPs as agrochemicals, increment in accumulation of these materials in soil is an eventual fate; which should results into alteration of soil physico-chemical properties (dispersibility, dissolution rate, surface area, surface chemistry, size, agglomeration, transformation, ionic strength, and charge etc). This in turn may determine the stability and transportation of nanoparticles in soil (Tourinho et al., 2012; Anjum et al., 2013)[20][2]. Moreover, extensive application of AgNPs in the consumer based industries leads them to dispose as a waste material in the soil environment (Ben-Moshe et al., 2013)[6].

A differential retention of silver nanoparticle in suspension of natural soil with respect to the contents of clay particles has been reported earlier (Cornelis et al. 2012)[9]. However, presence or absence of organic matter (surfactants or humic acid) may also greatly influence the mobility of silver nanoparticle (Anjum et al., 2010)[3]. Moreover, considerable shift in soil pH was observed in both acid and alkaline soils due to high exposure of silver nanoparticles (Benoit et al., 2013)[7]. On the other hand, few

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workers claimed that AgNPs have no detrimental effects on physico-chemical properties of soil .Therefore, considerable amount of research gaps can be identified in regard to time and concentration of exposure and the interaction of AgNPs with soil properties. Moreover, the effects of AgNPs on soil macro-animals like earthworm are yet to be studied thoroughly.

Here in this study we applied various concentrations of a *Mentha arvensis* leaf extract mediated green silver nanoparticles (AgNP) to soil. The changes in physico-chemical properties of soil were assessed along with solubility study of ions in the respective treated soil. Moreover, the toxicity of AgNP was assessed with two earthworm species: *Eisenia fetida* and *Metaphire postheuma*. Seed germination assay was also performed to determine the efficacy of AgNP on probable plant growth.

## 2. Materials and methods

### 2.1 Source of AgNP and treatment combinations

*Mentha arvensis* mediated nanoparticles were procured from the department of Chemical Sciences, Tezpur University for the purpose of this experiment. Required concentrations of the nanoparticle (10, 25, 50, 75, 100 mg $kg^{-1}$) were obtained through serial dilution method from the stock solution. Treatment combinations were listed below:

Control-Soil without AgNP

$AgNP_{10}$-10 mg $kg^{-1}$ concentration of AgNP, $AgNP_{25}$-25 mg $kg^{-1}$ concentration of AgNP

$AgNP_{50}$-50 mg $kg^{-1}$ concentration of AgNP, $AgNP_{75}$-75 mg $kg^{-1}$ concentration of AgNP

$AgNP_{100}$-100 mg $kg^{-1}$ concentration of AgNP

### 2.2 The experimental setup

#### 2.2.1 Lab scale soil incubation study

Typical alluvial soil samples were collected from nearby vicinity (order: typic endoaquept) (Napaam, Tezpur, Assam). Consequently the collected soil samples were air dried, removing the plant parts and breaking the clods. Sieving was debarred to maintain the natural condition of the composite soil. Afterwards the prepared soil samples were poured into cone shaped porous earthen vessels with a volume of 2L and dimension 0.45m × 0.25m (height × diameter). Required concentrations of nanosolutions were applied in each vessel as listed above. Each treatment combination was replicated thrice and the study was continued for 60 days. Proper ambience temperature was maintained within 25-30°C. Sprinkling of water was done at 2-3 days interval to ensure the natural condition of the treated soil. Periodically samples were collected at 0, 30 and 60 days and analysis of various physico-chemical properties was done.

#### 2.2.2 Physico-chemical changes of the experimental soil samples

Water holding capacity (WHC), bulk density (BD), pH, available nitrogen (Avl N), available phosphorus (Avl P), exchangeable potassium (Avl K), total organic carbon (TOC) were analyzed following Page et al., 1982 . Additionally the efficiency of nanoparticles on some vital soil enzymes (urease and phosphatase) was analyzed through standard protocoals (Tabatabai and Bremner, 1969; 1972) [18][19].

#### 2.2.3 Effect of AgNPs on periodical solubility of ions

Soil samples from the lab scale soil incubation study were collected and utilized to assess the potential influence of AgNPs on periodical solubility of ions. Composite soil samples were prepared for experimental setup following the steps: breaking the clods, removing plant parts etc. as mentioned in earlier experiment. Afterwards 10g of the prepared soil samples were treated with required concentrations of AgNPs i.e., 10, 25, 50, 75 and 100 mg $kg^{-1}$ and poured into erlenmeyer flasks of 250 ml capacity. Each flask containing the treated samples were replicated thrice to maintain complete randomization process. Subsequently, distilled deionized water was mixed in each flasks at 1:10 ratio with the substrate (10 g soil substrate: 100 ml deionized water) and reacted at 120 rpm for 7, 14 and 21 days in a mechanical shaker at room temperature (25-30° C). Filtration was done at each sampling period with Whatmann No. 1 filter paper. Samples are analyzed for $PO_4^{3-}$, $NO_3^-$, $SO_4^{2-}$, $Cl^-$, total alkalinity, pH by following the standard methodologies (Page et al., 1982)[16].

#### 2.2.4 Phytotoxicity: Seed germination assay

10 number of seeds of *Vigna radiata* were placed in tissue papers per petriplates. 5 ml of nano solutions of required concentrations (100, 75, 50, 25 and 10 mg $kg^{-1}$) were added in each plate. Subsequently the inoculated seeds were maintained at 25°C in dark condition. The number of germinated seeds, length of plumule and radical were measured after 48 hours of incubation. The measurement of relative root growth (RRG), relative seed germination (RSG) and germination index (GI) were done following Karak et al., 2014.

$$RSG\,(\%) = \frac{Number\ of\ seeds\ germinated\ with\ AgNP\ nanosolutions}{Number\ of\ seeds\ germinated\ with\ distilled\ water} \times 100$$

$$RRG\,(\%) = \frac{Mean\ root\ length\ of\ seeds\ receiving\ AgNP\ nanosolutions}{Mean\ root\ length\ of\ seeds\ receiving\ distilled\ water} \times 100$$

$$GI\,(\%) = \frac{RSG \times RRG}{100}$$

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#### 2.2.5 Earthworm population and health analysis

The toxicity of nanoparticles was tested upon earthworm species *Eisenia fetida* and *Metaphire postheuma*. Urine free cowdung collected from a nearby farm were used as a substrate material. Juvenile, non-clitellated specimens of earthworms with an average length of 2–3.5cm and weighing about 200-250mg were collected from the vermiculture unit of the department of Environmental science, Tezpur University, Assam (India). Afterwards, the earthworms were cleaned with water and kept in a moist filter paper for overnight @ 25°C for gut evacuation; afterwards used for incubation study. A 50 ml stock solution of different concentrations of the nanoparticle (100, 75, 50, 25 and 10mg $kg^{-1}$) was mixed with the substrate. 20 worms per kg of substrate were added in each earthen vessel.

Efficacy of AgNP nanosolutions on earthworm population and health were enumerated by taking earthworm count, body weight and length measurement at 10 days interval and continued up to 60 days.

#### 2.2.6 Statistical analysis and graphical representation

One way ANOVA was conducted in SPSS 16.0 software and analyzed the variations between different treatment combinations. However, least significance difference (LSD) test was also performed to understand the efficiency of nanoparticles. Graphical representation was done with the help of Sigma plot 10 and MS excel.

## 3. Results and discussion

### 3.1 Changes in BD, WHC, pH, available N, available P, available K, urease and phosphatase activity, total organic carbon (TOC) of lab scale soil study

The inherent soil properties are represented in Table 1. Data on changes in BD, WHC, available N, P, K, urease, phosphatase activity and TOC are presented in Figure 1. Bulk density of the soil particles temporally decreased in the lower concentrations of nanosolutions (10, 25 and 50mg $kg^{-1}$). However in higher doses of nanosolutions (100 and 75 mg $kg^{-1}$) slight increment was observed. Incorporation of nanosolutions in soil @ 10 mg $kg^{-1}$ followed by 25 and 50 mg $kg^{-1}$ elevated the WHC of the substrate after 60 days of incubation (Figure 1) ($P_{0.05}$=0.000, LSD=0.54). We recorded significant reduction in BD under lower concentrations of AgNP (50, 25 and 10 mg $kg^{-1}$). This may be due to increase in soil porosity caused by AgNP by forming stable aggregates in the soil. Depending on the ionic strength of the media AgNPs can form stable aggregates (Zhang and Zhang, 2014)[22]. Simultaneously, WHC of the soil also increased significantly under these concentrations. This is interesting, because WHC is directly related to the porosity of the soil. This could be due to the higher surface area created by the nanosized particles in soil, which in turn considerably improved soil structural aggregation.

**Table 1.** Inherent properties of the test soil

| Parameters | mean±stdev |
|---|---|
| pH | 5.08±0.1 |
| BD (g /cc) | 0.64±0.05 |
| WHC (%) | 49.5±0.87 |
| TOC (%) | 0.18±0.03 |
| Available K (mg $kg^{-1}$) | 150±1.74 |
| Available P(mg $kg^{-1}$) | 40.5±1.25 |
| Phosphatase (µ g $g^{-1}$ $h^{-1}$) | 156.7±0.57 |
| Available N (mg $kg^{-1}$) | 305.4±2.5 |
| Urease (µ g $g^{-1}$ $h^{-1}$) | 12.86±0.58 |

Inherently the soil pH was acidic in nature (Table 1). However, due to application of nanosolutions the pH shifted towards neutrality from 0 to 60 days ($P_{0.05}$=0.000, LSD=0.095). Maximum increment in pH was depicted from 10 mg $kg^{-1}$ followed by 25 and 50 mg $kg^{-1}$ in the later period of the study, this may be due to the neutral characteristics of AgNP (Barua et al., 2013)[4]. N availability remarkably increased under lower concentrations of the AgNP treatments during the study period. N augmentation was recorded from 10 mg $kg^{-1}$ followed by 25 and 50 mg $kg^{-1}$ ($P_{0.05}$=0.000). However, 100 and 75 mg $kg^{-1}$ depicted slight increment decrement in terms of N availability compared to the lower concentrations of the study. Similarly, considerable increase in soil urease activity was observed in 10 mg $kg^{-1}$ followed by 50 and 25 mg $kg^{-1}$. However, such improvement in urease activity was not observed in 100 and 75 mg $kg^{-1}$ of AgNP application ($P_{0.05}$= 0.000, LSD=0.39). Urease is one of the most important soil enzymes that regulate N mineralization in arable soils. Interestingly, in the present study significantly high urease activity under lower doses of AgNP treatments were recorded. Improvement in soil pH, enhanced urease activity coupled with favourable physical environment (additional porosity) probably increased N availability in soil.

Temporal augmentation was observed in available K content in AgNP treated soils during the study ($P_{0.05}$=0.000, LSD=1.74). At later period of the study the status of available K was in the order: 10 mg $kg^{-1}$>25 mg $kg^{-1}$>50 mg $kg^{-1}$>100 mg $kg^{-1}$>75 mg $kg^{-1}$. However in the present study, higher K mineralization in nanoparticle treated soil could be due to the improvement in particle size distribution and granular stability (evidenced from the data on bulk density) provided by the added nanomaterials.



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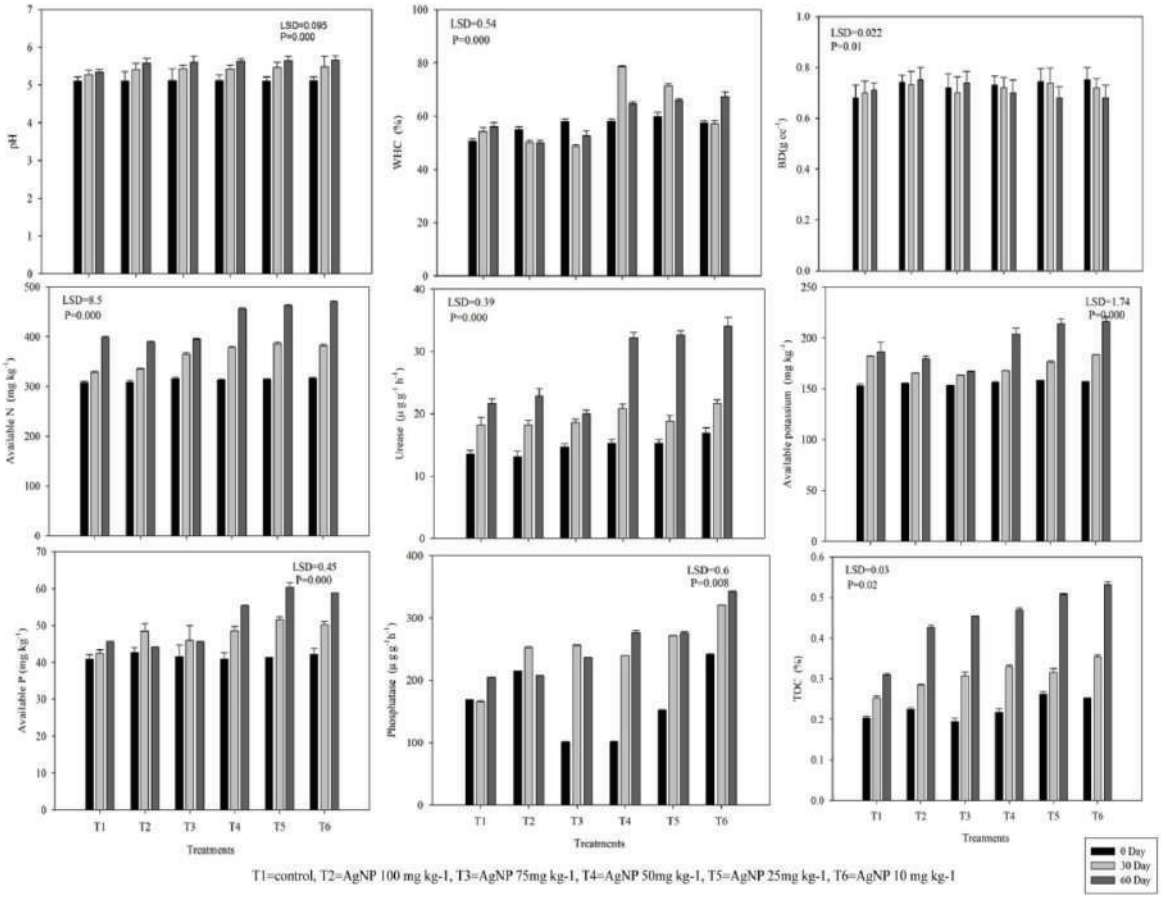


**Figure 1.** Changes in pH, WHC, BD, available N, available P, available K, urease and phosphatase activity, total organic carbon (TOC) of soil incubation study due to application of different combinations of AgNP nanosolutions

P availability dramatically enhanced in lower concentrations of AgNP treatments i.e., 25 mg $kg^{-1}$ followed by 10 and 50 mg $kg^{-1}$. Whereas in case of 100 and 75 mg $kg^{-1}$ P availability was increased upto 30 days, after that reduced P availability was observed at 60 days of incubation period. Enzyme phosphatase has a significant role in enhancement of phosphorus availability in soil solutions. Here in this study phosphatase availability was observed in the order: 10 mg $kg^{-1}$>50 mg $kg^{-1}$>25 mg $kg^{-1}$>75 mg $kg^{-1}$>100 mg $kg^{-1}$. Positive effect of AgNPs on soil phosphatase activity may be one of the factors that enhances available P content in soil solution.

The TOC content of the experimental soil was initially very low in nature. Overall due to application of AgNP, TOC content gradually increases @ 10, 25 and 50 mg $kg^{-1}$ from 0 to 60 days. However, TOC change was very marginal in case of 100 and 75 mg $kg^{-1}$ during the study. At the end of the study period TOC level of soil treated with AgNP was in this order: 10 mg $kg^{-1}$> 25 mg $kg^{-1}$>50 mg $kg^{-1}$>75 mg $kg^{-1}$>100 mg $kg^{-1}$ ($P_{0.05}$=0.02, LSD=0.03). Similar impacts of nanomaterials on stability of soil organic matter content have been reported earlier (Xie et al., 2008; Johnson et al., 2009)[21][13]. Ag has been reported to have a high affinity for reduced S group (thiol) of soil organic matter and can form S-Ag-S bonds (Bell and Kramer, 1999)[5]. These types of organo-metallic complexation might have decreased organic matter degradation or mineralization and thus enhanced sequestration of C in soil.

### 3.2 Changes in pH, alkalinity, chloride, sulphate, phosphate, nitrate ions in solubility study

Figure 2 depicted the changes of pH, alkalinity, chloride, sulphate, phosphate, nitrate ions in solubility study. A temporal escalation was observed in the pH from 10, 25 and 50mg $kg^{-1}$ (0 and 21 days) ($P_{0.05}$=0.000, LSD=0.262), yet a sudden drop was found to occur in the 14th day. pH varied significantly in our experiment although there was an overall rise in pH up to 50 mg $kg^{-1}$concentration of AgNP inoculated soils. Such changes in pH significantly influence mobility of trace elements in soil (Reddy et al., 1994; Bhattacharyya et al., 2011)[17][8]. Much escalation in alkalinity was comprehended in 50mg $kg^{-1}$ in 7 and 14 days with inconsequential drop in the 21th day. The alkalinity was found to be constant in case of 25, 10mg $kg^{-1}$ of AgNP. An upsurge was observed in chloride concentration

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in 10 and 25 and 50mg $kg^{-1}$ at the 21st day (LSD=2.47, P=0.000).

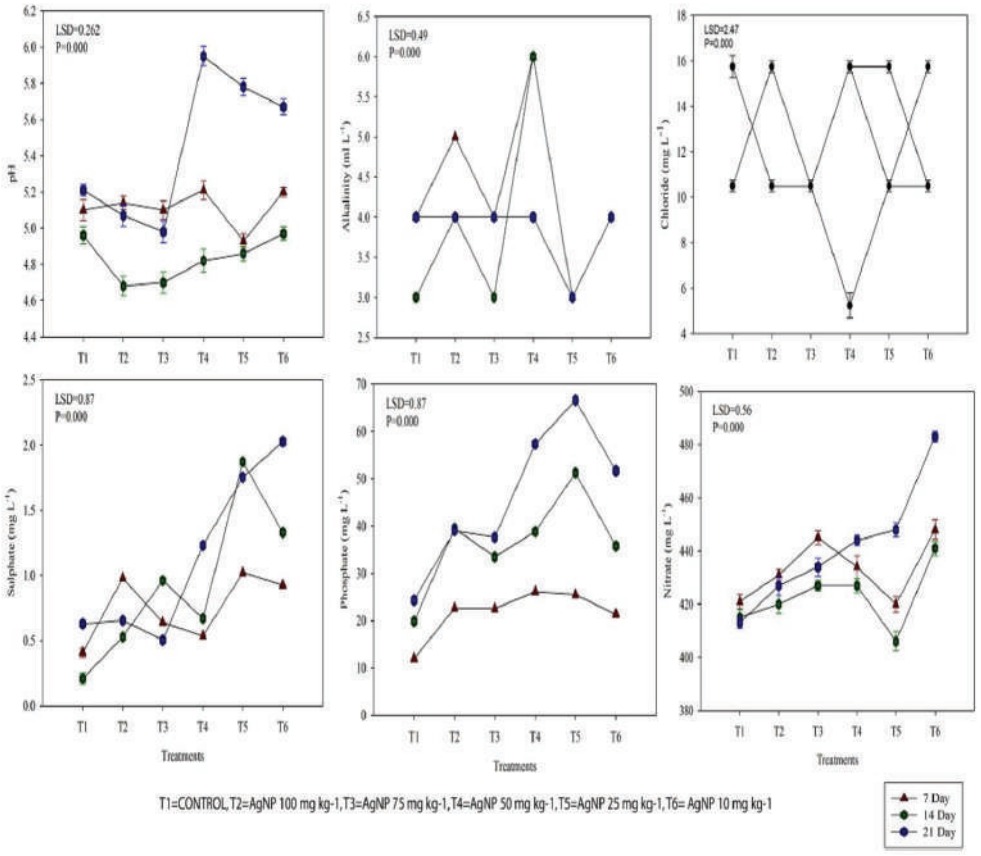


**Figure 2.** Changes in pH, alkalinity, chloride, sulphate, phosphate, nitrate ions in solubility study due to application of AgNP

Substantial augmentation was observed in phosphate solubility in 10, 25 and 50mg $kg^{-1}$ AgNP treatments from 7th to 21th day; nevertheless a slight increment in phosphate solubility was observed in 100 & 75mg $kg^{-1}$ in the later period of the study ($P_{0.05}$=0.000, LSD=0.87).

Abundant increase in sulphate solubility was found to occur in 10mg $kg^{-1}$ AgNP followed by 25 and 50mg $kg^{-1}$ at 21 days ($P_{0.05}$=0.000, LSD=0.87). Nitrate solubility was higher in 10, 25 and 50mg $kg^{-1}$ of AgNP treatments at 21 days; unlike 100 and 75mg $kg^{-1}$ which have lesser nitrate N compared to initial value ($P_{0.05}$=0.000, LSD=0.56).

### 3.3 Phytotoxicity: Effect of AgNP on seed germination

AgNP provided minimal inhibition of seed germination and growth compared to the control one (Figure 3). The germination index followed the order: 10 mg $kg^{-1}$>25mg $kg^{-1}$>75mg $kg^{-1}$>50 mg $kg^{-1}$>100 mg $kg^{-1}$ (P=0.000, LSD=0.487). Whereas, the relative seed germination of *V. radiata* of AgNP treated seeds was in the order: 10 mg $kg^{-1}$>25 mg $kg^{-1}$=75 mg $kg^{-1}$=50 mg $kg^{-1}$=100mg $kg^{-1}$ (P=0.000, LSD=0.826).This figure present a probable effect of nanoparticles on agricultural crops. From the figure it is comprehensible that lower concentrations of the nanoparticles are providing better environment in germination of the seeds. Primarily 10 mg $kg^{-1}$ of AgNP depicted most significant effect in RSG (111%) than the rest of the treatments. It was conspicuous that high doses of AgNP showed equal amount of RSG (100%). In case of RRG the efficacy was in the order: 10 mg $kg^{-1}$> 25mg $kg^{-1}$>75 mg $kg^{-1}$> 50mg $kg^{-1}$> 100mg $kg^{-1}$. Here also lower doses provide better germination condition for *Vigna radiata*. It is noteworthy to describe that 10 mg $kg^{-1}$ AgNP provide most significant effect followed by 25 mg $kg^{-1}$. In some previous studies positive effect of AgNP on seed germination was reported by some researchers (Abdel-Azeem et al., 2013; Najafi et al., 2013)[1][15]. Nanoparticles have a general tendency to form complex in solutions and thus remain in dispersed state. In addition, the seed coats are selectively permeable to AgNPs and possess good antimicrobial property. All these factors together might have indirectly created a favorable condition for the plant seeds.



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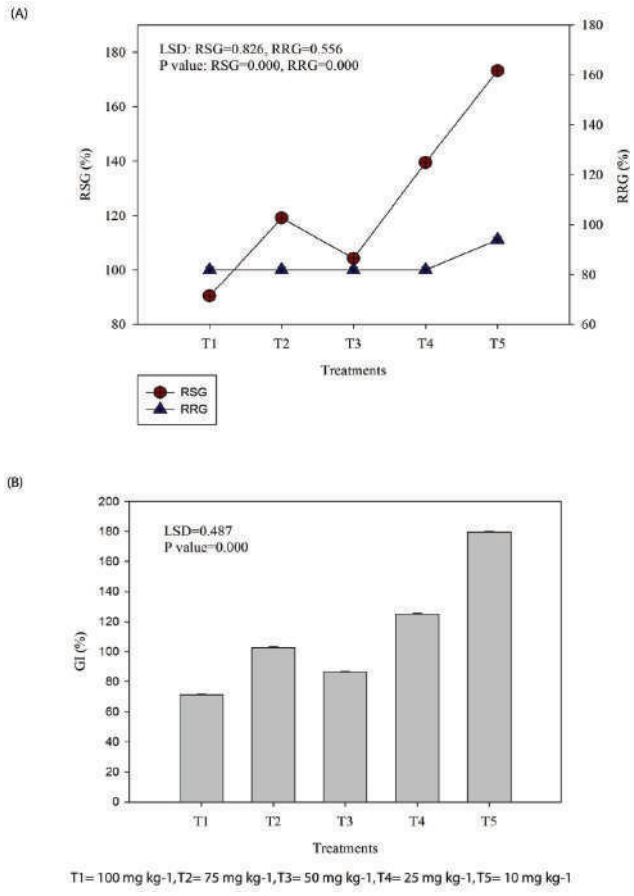


**Figure 3.** Effect of AgNP on RSG, RRG and GI of V. radiate seeds

### 3.4 Effect of AgNP on earthworm proliferation and changes in morphology

Table 2 and 3 represented the data of earthworm count, body weight and length measurement. Drastic mortality was observed in the population of *P. excavates* after 10 day of the incubation and total reproductive failure was examined in both higher and lower concentrations of AgNP. The growth and fecundity of *E. fetida* and *M. postheuma* substantially reduced with interemittent mortality when exposed to higher than 75 mg $kg^{-1}$ of AgNP during the period of incubation.However, reduction in proliferation rate and body weight of these two species was not evidenced under low concentration treatments of AgNP (10, 25 and 50 mg $kg^{-1}$) till 60 days. The survivality rate of *E. Fetida* was more prominent than *M. Postheuma* in AgNP treated substrate. 10 mg $kg^{-1}$ treated feedstock depicted the most positive outcome in terms of earthworm count in *E. fetida*. However, in *M. Postheuma* 25 mg $kg^{-1}$ feedstock provided the most viable environment for earthworm propagation.

Body weight and length enumeration was considered to observe the feasibility of feedstock mixture for earthworm growth. Body weight and length for *E. Fetida* prominently enhances @ 10 mg $kg^{-1}$ treated substrate. Moreover for *M. Postheuma* 25 mg $kg^{-1}$ was the most suitable substrate for earthworm growth.

The general idea about nanoparticle is that they have harmful effect on organisms and ecosystem. Previous results reported that AgNP had adverse effects on growth and proliferation of earthworm species *E. fetida* (Heckman et al., 2011)[11]. However, this research revealed that low dose AgNP exposure may not cause severe helath hazard to earthworms if the duration of exposure is not too long. This may be due to the fact that incorporation of nanomaterials causes increase in porosity in the substrate (Barua et al., 2013)[4]. As earthworms can absorb oxygen from their surrounding environment through their moist skin and they are mostly susceptible to anaerobic environment (Barua et al., 2013)[4]. Hence, enhancement in porosity probably ensured adequate circulation air to sustain normal growth and proliferation of the earthworm speices. Our findings are in good agreement with previous report (Barua et al., 2013)[4].

## 4. Conclusion

The present study revealed that silver nanoparticles prepared through the leaf extract of Mentha arvensis cause not rigorous detrimental effect to the soil environment. This also showed that lower concentrations of AgNPs (10, 25 and 50 mg $kg^{-1}$) did not hamper the growth and proliferation of earthworms, the nature's chemical managers. Seed germination assay also depicted feasibility of lower concentrations in *V. radiata* seeds growth. The solubility experiment demonstrated enhancement of plant essential $PO_4^{3-}$ and $NO_3^-$ in the lower concentrations of the AgNPs; this indicated the probability of better growth of plants, which we can assume through seed germination assay data. Moreover, the results proved that the soil quality improved substantially without any detectable hindrance from lower range of AgNPs.

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**Table 2.** Earthworm count, body weight and length measurement of *Eisenia fetida incubated* in AgNP treated substrate.

| | Earthworm count | | | | | | Body weight (g) | | | | | | Body length (cm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc | 10 D | 20 D | 30 D | 40 D | 50 D | 60 D | 10 D | 20 D | 30 D | 40 D | 50 D | 60 D | 10 D | 20 D | 30 D | 40 D | 50 D | 60 D |
| $AgNP_{100}$ | 16±1 | 25±1 | 15±1 | 20±1 | 25±1 | 28±1 | 0.3±0.2 | 0.4±0.1 | 0.8±0.3 | 1.3±0.2 | 1.1±0.3 | 1±0.3 | 3.5±0.7 | 4.5±0.5 | 6.5±0.5 | 8.1±0.9 | 8.5±0.5 | 8.8±0.5 |
| $AgNP_{75}$ | 25±1 | 21±1 | 19±1 | 34±1 | 26±1 | 24±1 | 0.6±0.1 | 0.9±0 | 1.2±0.3 | 1±0.5 | 0.8±0.1 | 0.9 ±0.2 | 3.8±0.5 | 4.2±0.7 | 5.8±0.9 | 6.5±0.5 | 7.8±1 | 9.3±0.7 |
| $AgNP_{50}$ | 20±1 | 20±1 | 19±1 | 54±1 | 87±2.6 | 77±1 | 0.3±0.1 | 1.2±0.2 | 1.3±0.2 | 1.2±0.2 | 1.4±0.1 | 1.72±0.3 | 3.7±0.7 | 5.2±0.7 | 6.7±0.1 | 8.5±0.5 | 9.4±0.9 | 10.9±0.9 |
| $AgNP_{25}$ | 20±1.7 | 21±1 | 36±1 | 53±1 | 75±1 | 89±1 | 0.4±0.1 | 1±0.5 | 1.2±0.4 | 1.1±0.4 | 1.54±0.1 | 1.72±0.1 | 3.3±0.7 | 5.3±0.7 | 7.8±0.9 | 8.7±0.7 | 9.8±0.5 | 11.5±0.5 |
| $AgNP_{10}$ | 20±1 | 20±1 | 45±1 | 58±1 | 101±1 | 95±1 | 0.1±0.1 | 1.5±0.5 | 1.1±0.4 | 1.3±0.2 | 1.64±0.5 | 1.8±0.2 | 3.5±0.7 | 6.1±0.8 | 8.5±0.5 | 9.5±0.5 | 11.5±0.9 | 12.5±0.5 |
| Control | 20±1 | 14±1 | 18±1 | 45±1 | 25±1 | 28±1 | 0.2±0.1 | 1.1±0.4 | 1.0±0.4 | 1.2±0.2 | 1.2±0.1 | 1.08±0.2 | 3.3±0.2 | 5.8±0.7 | 7.3±0.7 | 8.5±0.5 | 9.2±0.7 | 9.8±0.9 |
| L.S.D at P <0.01 | 0.97 | 0.82 | 0.82 | 0.82 | 1.03 | 0.89 | 0.08 | 0.25 | 0.25 | 0.21 | 0.19 | 0.17 | 0.52 | 0.56 | 0.59 | 0.54 | 0.59 | 0.52 |

**Table 3.** Earthworm count, body weight and length measurement of *Metaphire postheuma* due to application of AgNP

| | Earthworm count | | | | | | Body weight (g) | | | | | | Body length (cm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. | 10 D | 20 D | 30 D | 40 D | 50 D | 60 D | 10 D | 20 D | 30 D | 40 D | 50 D | 60 D | 10 D | 20 D | 30 D | 40 D | 50 D | 60 D |
| $AgNP_{100}$ | 20±1 | 11±1 | 14±1 | 18±1 | 23±1 | 19±1 | 1.2±0.3 | 1.1±0.3 | 1±0.4 | 0.8±0.2 | 1±0.5 | 1±0.4 | 3.05±0.7 | 4.5±0.5 | 6.8±0.5 | 7.7±0.9 | 9.1±0.5 | 8.5±0.7 |
| $AgNP_{75}$ | 21±1 | 13±1 | 17±1 | 22±1 | 26±1 | 18±1 | 0.4±0.1 | 1.3±0.2 | 1.1±0.2 | 0.9±0.1 | 1.1±0.3 | 1.1±0.3 | 3.1±0.5 | 4.6±0.7 | 5.1±0.9 | 5.9±0.5 | 6.4±1 | 8.3±0.7 |
| $AgNP_{50}$ | 21±1 | 19±1 | 23±1 | 40±1 | 35±1 | 45±1 | 1.2±0.3 | 1.1±0.2 | 0.8±0.2 | 0.9±0.1 | 1.4±0.2 | 1.2±0.3 | 3.8±0.7 | 4.5±0.7 | 5.8±1.1 | 6.5±0.5 | 7.1±0.9 | 9.4±0.5 |
| $AgNP_{25}$ | 21±1 | 21±1 | 29±1 | 48±1 | 70±1 | 58±1 | 1.2±0.2 | 1.4±0.1 | 1.1±0.3 | 1.1±0.3 | 0.8±0.2 | 1.29±0.2 | 3.3±0.7 | 5.9±0.7 | 7.9±0.9 | 9.3±0.7 | 9.7±0.5 | 10.4±0.7 |
| $AgNP_{10}$ | 22±1 | 20±1 | 24±1 | 41±1 | 44±1 | 51±1 | 0.3±0.2 | 0.9±0.1 | 1.5±0.3 | 1.6±0.2 | 1±0.5 | 1.3±0.2 | 3.8±0.9 | 5.5±0.7 | 8.5±0.5 | 9.7±0.5 | 10.5±0.9 | 11.8±0.5 |
| Control | 20±1 | 23±1 | 20±1 | 37±1 | 15±1 | 21±1 | 0.3±0.2 | 1.7±0.1 | 0.8±0.2 | 0.8±0.2 | 1±0.5 | 0.9±0.1 | 3.3±0.3 | 4.8±0.7 | 7.3±0.2 | 8.8±0.5 | 9.2±0.7 | 9.5±0.7 |
| L.S.D at P <0.01 | 0.82 | 0.89 | 0.82 | 0.97 | 1.03 | 0.82 | 0.18 | 0.15 | 0.20 | 0.26 | 0.34 | 0.20 | 0.55 | 0.59 | 0.53 | 0.52 | 0.50 | 0.48 |

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